T.D.B/9



# Phylogeny, Phylogeography and Colonization Patterns of Reptiles in Indian Ocean Islands

Sara Rodrigues Passos Rocha

U.PORTO
FACULDADE DE CIÊNCIAS
UNIVERSIDADE DO PORTO

U.PORTO
FC FACULDADE DE CIÊNCIAS
DEPARTAMENTO DE BIOLOGIA
BIBLIOTECA
0311

# Phylogeny, Phylogeography and Colonization Patterns of Reptiles in Indian Ocean Islands

Sara Rodrigues Passos Rocha

Dissertação apresentada à Faculdade de Ciências da Universidade do Porto para a obtenção do grau de Doutor em Biologia

Porto, 2010

U.PORTO
FC FACULDADE DE CIÊNCIAS
DEPARTAMENTO DE BIOLOGIA
BIBLIOTECA
0311

U.PORTO
FC FACULDADE DE CIÊNCIAS
UNIVERSIDADE DO PORTO

*Aos meus pais,*
*Ao maninho*

DECLARAÇÃO

Na elaboração desta dissertação, e nos termos do nº 2 do Artigo 8º do Decreto-lei nº 388/70 da República Portuguesa, os resultados de trabalhos já publicados foram totalmente aproveitados e fazem parte integrante de alguns capítulos desta dissertação. Em todos estes trabalhos, a candidata participou na obtenção, interpretação, análise e discussão dos resultados e na elaboração das suas formas publicadas.

A Faculdade de Ciências da Universidade do Porto foi a instituição de origem da candidata, tendo o trabalho sido realizado sob orientação do Doutor David James Harris, Professor Convidado da Faculdade de Ciências da Universidade do Porto e investigador do Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO) e co-orientação do Doutor David Posada Gonzaléz, Professor Associado do Departamento de Bioquímica, Genética e Inmunología da Universidade de Vigo. O trabalho laboratorial foi realizado na Facultad de Biología da Universidad de Vigo e no CIBIO.

Este trabalho foi apoiado pela Fundação para a Ciência e a Tecnologia através da atribuição de uma bolsa de doutoramento de referência SFRH/BD/17541/2004.

STATEMENT

In this dissertation, and in compliance with nr. 2 of article 8 of law nr. 388/70 of the Portuguese Republic, the results of published works were totally used and included in some of the chapters. In all these works, the candidate participated in obtaining, interpreting, analyzing and discussing the results and in writing the published forms.

Faculdade de Ciências da Universidade do Porto was the home institution of the candidate, and the work was directed by Dr. David James Harris, Invited Professor of the Faculdade de Ciências da Universidade do Porto and researcher at Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO), and co-directed by Dr. David Posada Gonzaléz, Associate Professor at the Departamento de Bioquímica, Xenética e Inmunoloxía da Universidade de Vigo. The laboratory work was performed in Facultad de Biología, da Universidad de Vigo and in CIBIO.

This work was financially supported by a PhD grant from Fundação para a Ciência e a Tecnologia PhD, reference SFRH/BD/17541/2004.

AGRADECIMENTOS - ACKNOWLEDGEMENTS - AGRADECIMIENTOS – AGRADECEMENTOS *

Não é exagero dizer que este trabalho teria sido completamente impossível sem a contribuição de muita gente a quem nem sempre há o espaço para referir. De facto, esta tese não é apenas fruto da minha dedicação ou da dos autores dos vários trabalhos nela contidos, mas sim de um conjunto muito maior de pessoas e instituições a quem me dá agora um enorme prazer poder agradecer. Se uns trabalharam directamente para a obtenção dos resultados aqui apresentados, outros foram não menos importantes na criação de condições para que várias partes do trabalho pudessem ser realizadas, tanto a nível profissional, como pessoal.

À Fundação para a Ciência e Tecnologia, agradeço a concessão de uma bolsa de Doutoramento (SFRH/BD/17541/2004) e do projecto de investigação PTDC/BIA-BDE/65745/2006.

Aos meus orientadores devo a oportunidade de realizar este projecto, assim como o seu contributo em várias fases da sua concepção, análise e escrita.

To James I have to thank the support and the encouragment I received, back since I was an undergraduate student, and that led to my application for a PhD grant and ultimately to this project. The fact is that having not been for it, I would never had ventured in the Indian Ocean Islands and their history. I am thankful for that and for the opportunity it represented for me to make part of excellent research groups and projects, to learn and to be in touch with so many exciting works and people. Thank you for the confidence you deposited in me and in my ability to carry on this work. Thank you also for being our cooker and such a nice company over fieldwork, making it even more amusing than it already would be.

A David, quiero agradecerte la amabilidad con que me recibiste en Vigo, aportando todos los recursos necesarios para que yo pudiese trabajar. Te agradezco también, inmensamente, el modo en que siempre me has tratado: la proximidad, el constante buen humor y la receptividad para aclarar conceptos y resultados han sido muchas veces motivantes y determinantes. He aprendido mucho contigo. Te agradezco además la preocupación por mi formación y el apoyo para que aprovechase siempre las mejores oportunidades de aprender.

Thank you to both of you for the revision of this thesis.

A Paloma, tengo que agradecerte que me hayas recibido en tu laboratorio, preocupándote por si todo estaba yendo bien, y siempre disponible para ayudar. Gracias por haberme hecho un hueco y haberme hecho sentir tan bien ahí. Gracias también a Armando por haberme recibido en XB2.

My deepest acknowledgement goes to a number of people in the Seychelles that made this work possible. Thanks to the Seychelles Bureau of Standards (SBS) and to the Ministry of Environment and Natural Resources for the permits to carry out fieldwork. To Dr. Denis Matatiken and Wilna Accouche I acknowledge their help in dealing with the burocracies of fieldwork permits. Thank you to Justin Gerlach from Nature Protection Trust of Seychelles (NPTS) by his early input in this work providing important information and comments. They were very important and motivating. Thank you to the people at the Seychelles Island Foundation (SIF) for their interest in the work we were developing, and to SIF, to the Marine Parks authority and to the Island Conservation Society (ICS) for having supported our activities in

the field. Thank you to Gérard Rocamora for making the fieldtrip to Aride possible. Thank you so much for the help of so many local people during fieldwork: Mr. Lindsay Chong-Seng and Vallee du Mai wardens, to Christopher Kaiser and Terrence Valentin, to Aride Warden Sally Mills and Aride Rangers, to Fregate Island Private (especially to Lucia Russell), to workers at La Veuve Reserve on La Digue, to Greg Wepener, Paul L. and Linda Vanherck at North Island, to Mr. Gilles Saout at Grande Soeur, to Jock Henwood, Kevin and San-Marie Jollife, and Gary Ward at Cousine, to Mr. Glenny Savy at ICS and to many more persons whose names I can't remember anymore. A special acknowledgement is due to Nancy Bunbury and Christopher Kaiser-Bunbury, for their valuable advices and friendship. What a great coincidence and pleasure having met you!

I sincerely hope this is just the beginning of many other works together.

To Miguel Vences I have to acknowledge his always enthusiastic input in many aspects of this work, and the confidence he also deposited in me and in the results I produced. Your amazing dedication and motivation are inspiring and I hope to have been up to them. To Frank Glaw, Phillipe-Sebastian Gehring and Herbert Rosler I most sincerely thank your contribution and such smooth way work was always carried out with you.

Ao Miguel Carretero devo também um agradecimento especial. Não apenas por também teres feito parte deste projecto desde o início, pelo empenho que lhe dedicaste, e pela rapidez e eficácia com que sempre respondeste a todos os meus (muitos) pedidos de ajuda, mas acima de tudo pela tua atitude para com o trabalho e para com os estudantes que vais acompanhando. A tua motivação e entusiasmo pelo que fazes são evidentes e contagiantes. O profissionalismo com que o fazes, marcante. Foi sempre um prazer trabalhar contigo. Espero poder continuar e saber estar à altura de o fazer. Obrigada ainda pela fantástica companhia na viagem a Aldabra. Visto assim de longe até já nem me lembro de quanto tempo passamos a tentar que o estômago não se desse conta do balanço do barco.

À Ana Perera e à Raquel Vasconcelos tenho imenso que agradecer. Obrigada por toda a energia e competência com que se dedicaram ao trabalho de campo. O vosso empenho foi muito importante e tornou possivel grande parte do trabalho aqui apresentado. Não fossem voçês tão mais competentes que eu em toda a logistica do dia e estava o caso mal parado! Obrigada ainda por terem feito todo o trabalho sempre com uma boa disposição contagiante. À Ana (e ao Pedro) tenho ainda que agradecer os inúmeros mapas e figuras e a paciência com que lidaram com a falta da minha para tais coisas.

À Andreia, agradeço a energia posta nos pequenos cágados. Lamento que não tenha corrido da melhor maneira!

Nada teria sido o mesmo, claro, sem o fantástico acolhimento de *nuestros hermanos*. Tantas vezes me senti realmente previlegiada por poder contar com um grupo tão grande de gente tão grande. Ainda sinto.

Juan, gracias por tu interés y por tu cariño. Por tu habilidad para transformar en divertidos incluso los días en que nada parecía funcionar en el laboratorio. Por la motivacion y las conpeticiones de "work-endurance". Tengo que confesar que nunca he ganado. Me he dormido y he dejado el ÉSkype encendido. Gracias por seguir cuidándonos desde lejos. Te echamos mucho de menos. José Luis, gracias por los primeros tours por Vigo. Por el inolvidable *Mari*. Una pena que te hayas marchado, pero al final bien, que me dejaste las pipetas y la mesa. Gra, gracias por teres estado sempre aí, polo coidado e empeño que pos no máis mínimo que fas. Aínda que non o creas, si que tornabas os meus días moito máis

divertidos. Gracias por todo o que me tes ensinado e pola túa paciencia coas miñas parvadas. Ó fin nunca conseguiches ensinarme galego, pero iso é culpa miña seguro! Pili, Piluchi, muchas gracias a ti también por cuidar de que todo nos fuese bien en el lab. ¡Qué gusto tener a alguién así! Gracias por las tantas veces que me has ayudado a bajar las muestras a tiempo y por las otras tantas que descubría que me habías dejado geles listos antes de bajar. Nunca será demasiado repetirlo: ¡gracias! A Cuca, ¡uno tiene que darle las gracias por existir! Me faltan las palabras... A Mónica, que en el fondo no tan fondo es un cacho de pan! (esto fue Gra quien me dijo que se dice así, aunque me parece mal! Em português seria um "coração mole" e toda a gente sabe que o pão em Espanha, é duro... pero si lo dice Gra, yo me lo creo!). A Vicky y a Mateus, por tantas cosas que no se pueden ni medir ni decir. Ao Mateus ainda por ser o gajo-dos-computadores perfeito. Obrigada pela paciência! Não deve ser fácil e espero algum dia poder retribuir. A Marco, porque tampoco debía ser fácil ser el único chico en un laboratorio en el que las 3 chicas estaban terminando la tesis. A Andrés porque al final nunca me has pegado. Gracias también por ser mi transportadora personal entre Vigo y ese sitio del que ni quieres acordarte que existe. A Antonio, porque tener a alguien a quien no entiendes ni la mitad de lo que dice es siempre un desafío interesante. A María, por seguir tratándome bien a pesar de que le haya intentado destrozar el coche nada más llegar. A Silvia, Chus, Lucía, Raquel, Angél, Miguel A., Leo M., Ramón, Carlos, Emilio; por hacer siempre tan agradable el tiempo que compartimos.

Ao Miguel Fonseca por seres tão grande inspiração na dedicação, tranquilidade e qualidade com que progrides no teu trabalho. Obrigada por muitas pequenas e grandes coisas que tornaram possivel o meu e também por teres partilhado o teu comigo. Foi um previlégio poder contar com a tua companhia ao longo destes anos. Obrigada por várias atitudes que me devolveram a motivação quando esta falhava. Creio que em grande parte a ti devo a energia para embarcar no voo seguinte.

To all the out-of-the-lab friends in Vigo. Aitor, Vagelis, Pepe, Adi, Fiona, Rebeca, José, Sabine, Marcelo, Raúl, Reiko. It was great to be able to share this time with you. Thank you for being so many times everything I just needed to have around.

A Chello tengo muchísimo que agradecer. Tanto, que ni siquiera consigo pensar ahora cómo habría sido Vigo sin ti. Bueno, yo siempre soy un poco dramática en mis declaraciones, pero es cierto que también tú fuiste muchas veces justo lo que yo necesitaba. Gracias por haber cuidado tan bien de mí. Gracias por haber sido la mejor compi de piso que una puede querer, por haber sido mi alarma personal tantas mañanas, por la playlist siempre lista, por los pulpos y otras esquisiteces culinarias, por dejar raramente que se acabase la Guinness en la nevera. ¡Ahora saca tú las ganas de acabar! It's time for courage!

Já lá vão alguns anitos desde que cheguei ao CIBIO, e torna-se muito dificil distinguir o pessoal do profissional. A verdade é que também no CIBIO, mais do que um local de trabalho, encontrei amigos e pessoas excepcionalmente interessadas e dedicadas, e sempre dispostas a partilhar um pouco do seu valioso tempo para ajudar os outros. Não vos sei agradecer o suficiente e dizer o quão importante isso foi na minha construcção científica e pessoal. Muito mais do que do meu próprio trabalho, bebi do vosso e do vosso entusiasmo, dedicação e talento. Ao Professor Nuno Ferrand quero agradecer o ter-me recebido no CIBIO e permitido formar parte de tão excepcional grupo. Quero agradecer a todos, sem excepção, com quem ao longo destes anos fui partilhando laboratório e arredores. Em particular, quero agradecer à

Catarina, Rui, Xana, Cardia, Armando, Racas, Sequeira, Zef, Miguel, Rita, Helena, Tex, Raquel Vasconcelos, Zé Carlos, Xico, Ana Perera, Pedro Tarroso, Antigoni, Ricardo, Stuart, Gonçalo, Paula, Vasco e Bárbara, Filipe, Catarina Rato e Raquel Xavier.

À Catarina, ao Rui, ao Ricardo, à Xana, ao Cardia, ao Zef, ao Teixas e ao Stuart, quero agradecer a preciosa ajuda na análise e discussão de dados, o apoio, todas as dicas e sugestões fundamentais, e ainda a revisão de alguns manuscritos. Obrigada por terem sempre encontrado o tempo necessário no meio das vossas também atarefadas agendas. À Catarina, além de todas as outras coisas, tenho que agradecer preciosa ajuda com o IM e o ter-me deixado ocupar o seu computador durante tanto e tanto tempo com as intermináveis corridas.

Ao Rui e ao Stuart, agradeço ainda o abrigo sempre disponivel na treehouse.

Ao Ricardo e ao Rui, obrigada pelas constantes (e eficázes) doses de motivação e relativização. Foi muito importante poder contar convosco. Obrigada por não me deixarem sentir tão longe nesta temporada final de desterro. Ao Ricardo, obrigada pela preocupação, pelos checkpoints telefónicos, pelos e-mails de ânimo matinal. Na verdade o que eu preciso é mimo. Espero de alguma forma ter retribuído. Agora é que estamos mesmo quase lá!

Ao Vasquinho, a bem gira capa desta tese, e a rapidez com que a fizeste.

À Sarita e à Sandra e a Nieves y Merchi. No meu caso as pessoas imprescindíveis, hiper-competentes e sempre de boa disposiçao vieram também a dobrar! Obrigada!!!

Agradeço ainda a todos os meus amigos. Uns estão perto, outros longe, espalhados pelo mundo. Obrigada também a voçês por encontrarem sempre um modo de me fazer chegar o vosso carinho. À Mariana, obrigada por estares sempre aí à distancia de um clique, mesmo do outro lado do charco.

Aos meus pais quero agradecer o apoio fundamental que sempre representaram. Também para voçês os últimos anos foram um peso muito maior do que o que eu gostaria que tivessem sido. Obrigada por nem assim terem questionado as minhas escolhas ou a minha determinação. Obrigada por terem estado aqui para me dar a mão. Obrigada por me terem ensinado, com o vosso exemplo, a ser trabalhadora e a tentar fazer as coisas como é certo, e não como é mais fácil. Ao Pai, obrigada pelo talento e carinho com que tratas da cozinha da família ;D

Ao Andrézito, obrigada também pelo teu esforço e pelo talento com que nos brindas todos os dias. Obrigada por não deixares de acreditar em mim, mesmo face a tanta evidência contrária. O teu carinho, mesmo de longe, foi determinante.

Ao Luís e à Dulce, agradeço por de tantos modos terem também tornado este (chamemos-lhe sprint) final mais fácil e bonito.

À Tia Lúcia, obrigada pela tradução de última-hora do resumo para francês.

* este texto não está escrito conforme nenhum dos acordos ortográficos

**CONTENTS**


| | | |
|---|---|---|
| Resumo | | 15 |
| Summary | | 19 |
| Resumé | | 23 |

**CHAPTER 1**

| | | |
|---|---|---|
| **GENERAL INTRODUCTION** | | **27** |
| **1.1** | **Islands as Privileged Systems for the Study of Evolution** | **29** |
| **1.2** | **The Western Indian Ocean Islands Biodiversity Hotspot** | **30** |
| *1.2.1* | *Geological Evolution of Western Indian Ocean Area:* | |
| | *Gondwana Fragmentation and Islands Emergence* | *31* |
| *1.2.2* | *Sea-Level Fluctuations and their Role on Indian Ocean Biota Evolution* | *36* |
| | *The Mascarenes Plateau and the "Asiatic flavour" of Malagasy Region Biota* | *37* |
| | *Stepping Stones between Africa and Madagascar?* | *40* |
| | *Sea Level Fluctuations in the Seychelles* | *41* |
| *1.2.3* | *The Ocean Currents* | *43* |
| **1.3** | **The Origins of Western Indian Ocean Biota** | **44** |
| *1.3.1* | *Madagascar* | *44* |
| *1.3.2* | *Mascarenes* | *45* |
| *1.3.3* | *Comoros* | *46* |
| *1.3.4* | *The Seychelles* | *47* |
| **1.4** | **Diversification of Western Indian Ocean Biota** | **49** |
| *1.4.1* | *Patterns of Diversification within the Seychelles* | *49* |
| **1.5** | **Tools and Recent Advances** | |
| | **on Phylogeography Inference Methodologies** | **51** |
| **1.6** | **Objectives and Organization of the Thesis** | **55** |
| **1.7** | **References** | **61** |

**CHAPTER 2**

**PHYLOGENETIC RELATIONSHIPS, COLONIZATION PATTERNS AND SYSTEMATICS OF *Phelsuma* DAY-GECKOS** 69

Paper I. Phylogenetic affinities of Comoroan and East African day geckos (genus *Phelsuma*): multiple natural colonisations, introductions and island radiations.
Rocha S, Posada D, Carretero MA, Harris DJ (2007)
*Molecular Phylogenetics and Evolution*, 43, 685-692.

Paper II. Multigene phylogeny of Malagasy day geckos of the genus *Phelsuma*.
Rocha S, Vences M, Glaw F, Posada D, Harris DJ (2009)
*Molecular Phylogenetics and Evolution*, 52, 530-537.

Paper III. Phylogenetic systematics of day geckos, genus *Phelsuma* based on molecular and morphological data (Squamata: Gekkonidae).
Rocha S, Rosler H, Gehring PS, Glaw F, Posada D, Harris DJ, Vences M (2010)
*Zootaxa*, 2429, 1-28.

**CHAPTER 3**

**PHYLOGENETIC RELATIONSHIPS, GENETIC DIVERSITY AND ISLANDS POPULATIONS STATUS OF THREE WIDESPREAD WESTERN INDIAN OCEAN GROUPS: *Gehyra, Hemidactylus* AND *Mabuya spp.*** 119

Paper IV. Cryptic variation and recent bipolar range expansion within the Stumped-Toed Gecko *Gehyra mutilata* (Wiegmann, 1834) across Indian and Pacific Ocean Islands.
Rocha S, Ineich I, Harris DJ (2009)
*Contributions to Zoology*, 78, 1-8.

Paper V. On the diversity, colonization patterns and status of *Hemidactylus* spp. (Reptilia: Gekkonidae) from the Western Indian Ocean Islands.
Rocha S, Carretero M, Harris DJ (2010)
*Herpetological Journal*, 20, 83-89.

Paper VI. Genetic diversity and phylogenetic relationships of *Mabuya spp* (Squamata: Scincidae) from Western Indian Ocean Islands.
Rocha S, Carretero M, Harris DJ (2010)
*Amphibia-Reptilia*, 31, 375-385.

**CHAPTER 4**

**DIVERSITY AND DIVERSIFICATION ON THE SEYCHELLES ARCHIPELAGO:**
***Urocotyledon Phelsuma* AND *Mabuya spp.*** **153**

Paper VII. One or more? Cryptic diversity within the endemic Prehensile-tailed gecko *Urocotyledon inexpectata* across the Seychelles islands: patterns of phylogeographic structure and isolation at multilocus level.
Rocha S, Harris DJ, Posada D.
*Submitted to Biological Journal of the Linnean Society* 155

Paper VIII. The time frame of diversification of day-gecko genus Phelsuma on the Seychelles and its driving mechanisms.
Rocha S, Posada D, Harris DJ
*Submitted to Systematic Biology* 177

Paper IX. The evolutionary history of *Mabuya* skinks from the Seychelles islands: introgressive hybridization, morphological evolution and patterns of geographic structure.
Rocha S, Perera, A. Silva A, Posada D, Harris DJ
*Submitted to Molecular Ecology* 209

**CHAPTER 5**

**GENERAL DISCUSSION** **255**

**5.1 Phylogenetic Relationships, Colonization Patterns and Systematics of day-geckos of the genus *Phelsuma*** **258**

*5.1.1 Phylogenetic Relationships and Systematics Revision* *258*

*5.1.2 Time Estimates and the Age of Phelsuma: a Recent or Not-So Recent Group?* *260*

| | | |
|---|---|---|
| *5.1.3* | *Are "Old" (Paleocene-Miocene) Estimates Compatible with Island Ages and the Geological History of the Region?* | *262* |
| *5.1.4* | *Using Islands as Calibration Points* | *264* |
| **5.2** | **Phylogenetic Relationships, Genetic Diversity and Islands Population Status of Three Widespread Western Indian Ocean Groups** | **266** |
| *5.2.1* | *Antropogenically Shaped Patterns of Distribution* | *266* |
| *5.2.2* | *The Endemic Lineages of the Aldabra Archipelago* | *267* |
| *5.2.3* | *The M. maculilabris Species-Complex from Western Indian Ocean Islands* | *269* |
| *5.2.4* | *The Use of Molecular Data to Distinguish Human-Related from Natural Patterns* | *270* |
| **5.3** | **Diversity and Diversification on the Seychelles Archipelago** | **271** |
| *5.3.1* | *Fieldwork surveys and Improving Distribution Data Across the Seychelles* | *271* |
| *5.3.2* | *Common Phylogeographic Patterns Across the Granitic Seychelles* | *272* |
| | *The major influence of distance in the genetic structure of organisms: the northern islands - southern islands phylogeographic break* | *272* |
| | *The southern affinities of Fregate populations* | *272* |
| | *Additional structure within both northern and southern clades* | *273* |
| | *Main divergences within each group are pre-Pleistocenic* | *274* |
| *5.3.3* | *Similar Patterns and Divergence Ages in Other Taxa* | *274* |
| *5.3.4* | *Seychelles Reptile Fauna: a stronger African or Asian "flavour"?* | *275* |
| *5.3.5* | *The usefulness of multilocus approaches* | *277* |
| **5.4** | **Concluding Remarks** | **278** |
| *5.4.1* | *Final Considerations and Major Conclusions* | *278* |
| *5.4.2* | *Future Perspectives* | *281* |
| **5.5** | **References** | **282** |
| **6** | **Appendix** | **287** |

RESUMO

Arquipélagos oceânicos são normalmente "hotspots" de biodiversidade e constituem excelentes laboratórios naturais para estudos evolutivos. As ilhas do Oceano Índico Ocidental são disso um bom exemplo: distribuídas pela parte ocidental do Oceano Índico e pela costa oriental de África, vários arquipélagos vulcânicos (Comoros, Mascarenhas), graníticos (Seychelles) e coralinos (Aldabra) rodeiam a ilha de Madagáscar e em conjunto formam um "hotspot" de biodiversidade em que os valores de endemismo são geralmente acima dos 90%, para vários grupos.

Obter um catálogo detalhado desta biodiversidade e entender a sua história evolutiva é essencial quer para o conhecimento dos padrões históricos de diversificação na região, assim como para a construção de estratégias eficazes de conservação. Esta tese teve como objectivo contribuir para um melhor conhecimento da distribuição, biogeografia e padrões filogeográficos de alguns grupos de répteis nas ilhas do Oceano Índico Ocidental, o uso desse conhecimento para clarificar a sistemática dos grupos em questão e a distinção entre padrões de distribuição naturais e padrões de influência antropogénica.

Em primeiro lugar, estudamos as relações filogenéticas e padrões de colonização de um dos grupos mais abundantes da região; as osgas do género *Phelsuma*. Com base em dados de múltiplos marcadores e numa amostragem quase completa a nível de espécies, mostramos que este género diversificou em Madagáscar, de onde colonizou os arquipélagos circundantes e clarificamos as relações filogenéticas dentro deste grupo, definindo oito clados principais. Demonstramos ainda que os grupos de espécies das ilhas Mascarenhas e Seychelles tiveram origem em eventos únicos de colonização (seguidos de diversificação *in-situ*), ao contrario das ilhas Comoros que foram colonizadas várias vezes e também mais afectadas por dispersões antropogénicas. Revimos ainda a sistemática deste género, propondo a sua organização em 11 grupos monofiléticos e descrevemos caracteres fenotípicos que caracterizam estes grupos.

Em seguida, exploramos a diversidade genética e relações filogenéticas em três grupos de lagartos com distribuições amplas pelos diferentes arquipélagos da região e para os quais as relações entre populações de diferentes ilhas e se seriam de origem nativa ou introduzidas, não eram claras. Usando marcadores mitocondriais, mostramos a existência de diversidade críptica na espécie *Gehyra mutilata*, uma espécie com uma ampla distribuição nas ilhas dos Oceanos

Índico e Pacifico, e mostramos ainda que a distribuição insular desta espécie é muito recente, e pelo menos nas ilhas do Oceano Índico provavelmente resultante de factores antropogénicos. Também para as quatro espécies do género *Hemidactylus* presentes nestas ilhas, mostramos que a sua distribuição insular é muito provavelmente na sua maioria resultante de factores antropogénicos. Os resultados obtidos apontam para a provável origem antropogénica de todas as espécies de *Hemidactylus* que ocorrem nas Comoros e nas ilhas graníticas do arquipélago das Seychelles, sendo que as únicas colonizações naturais parecem ter sido as de *H. mabouia* para Madagáscar e daí para o arquipélago de Aldabra. Demonstramos ainda que variação críptica existe em todas as espécies de *Hemidactylus* na sua área de distribuição nativa.

Através de uma amostragem abrangente no arquipélago das Comoros e ilhas costeiras africanas, clarificamos ainda as relações filogenéticas entre diferentes populações de *M. maculilabris*, *M. comorensis* e *M. striata*, três espécies de escincídeos para as quais o conhecimento era claramente escasso. Confirmou-se a distinção entre *M. maculilabris* e *M. comorensis*, mostrando que *M. comorensis* colonizou o arquipélago das Comoros naturalmente e ainda que as populações desta espécie em Madagáscar, assim como na ilha de Moçambique, resultam de introduções humanas. Diferenciação críptica é ainda descrita em *M. maculilabris* e *M. striata*, apesar da distribuição insular desta ultima espécie ser claramente muito recente, não existindo diferenciação relativamente às populações continentais analisadas.

Finalmente e com base em múltiplos marcadores mitocondriais e nucleares, estudamos a diversidade, estrutura genética e história evolutiva de três grupos de lagartos endémicos no arquipélago das Seychelles. Mostramos que diferenciação críptica e altos níveis de subestruturação genética existem em *Urocotyledon inexpectata*, uma espécie aparentemente mais abundante que o anteriormente pensado; argumentamos sobre a possibilidade da diferenciação simultânea de cada subespécie das espécies do género *Phelsuma* nas Seychelles, salientando o possível papel de determinantes ecológicos e diferentes pressões selectivas nos diferentes graus de diferenciação morfológica dentro de cada espécie e mostramos ainda que hibridação e introgressão massiva aconteceram entre *M. wrightii* e *M. sechellensis*, o que explica a parafilia destas espécies ao nível do DNA mitocondrial.

Apesar da história evolutiva única de cada grupo, alguns padrões comuns são passíveis de salientar, tais como i) a existência de uma estruturação genética marcada entre os grupos de ilhas norte e sul, indicando uma grande influencia da distancia na estruturação e distribuição destas espécies; ii) a maior proximidade filogenética entre as populações da ilha de Fregate e as

das ilhas do sul; iii) a presença de estruturação adicional dentro de cada grupo geográfico principal, e iv) a idade pré-Pleistocénica da principal diferenciação dentro de cada grupo de espécies. Os padrões observados podem agora ser usados para enquadrar e formular hipóteses biogeograficas para o arquipélago das Seychelles, a ser testadas noutros taxa.

SUMMARY

Oceanic island settings are usually remarkable biodiversity hotspots and exciting "natural laboratories" where to study evolutionary phenomena. The Western Indian Ocean islands are a good illustration of this: scattered across the Western Indian Ocean, off the southeast coast of Africa, several volcanic (the Comoros, the Mascarenes), granitic (the Seychelles) and coralline (Aldabra) archipelagos surround the largest island of Madagascar and together form the "Indian Ocean Islands" biodiversity hotspot, where endemism values across different groups are usually above 90%.

Comprehensively cataloguing this biodiversity and understanding its evolutionary history is paramount to both the knowledge of historical patterns of diversification in the region and to the building of effective conservation frameworks. This thesis aimed to contribute to a better understanding of the distribution, phylogeographic patterns and evolutionary history of selected groups of reptiles from the Western Indian Ocean islands, to use this knowledge to clarify the systematics of these groups, and to distinguish natural from possibly anthropogenic related events.

We started with the study of the phylogenetic relationships and colonization patterns of a major group of Western Indian Ocean islands' lizards; the day-geckos of the genus *Phelsuma*. Based on data from multiple loci and an almost complete sampling of the genus, we obtained strong evidences that suggest that this genus diversified in Madagascar, from where it colonized surrounding archipelagos, and clarified its intrageneric relationships, defining at least eight major clades. We further established that while single colonizations originated the extant Mascarenes and Seychellois species, the Comoros archipelago was colonized several times, and was more affected by human-mediated introductions. In addition, we reviewed the intrageneric systematics of these geckos and proposed its organization into 11 monophyletic species groups, replacing previous attempts of dividing the genus into phenetic species assemblages. We also provided an overview of selected phenetic characters that characterize these groups. The time frame of diversification of this group remains controversial but may be much older than previously suggested.

Secondly, we explored genetic diversity and phylogenetic relationships across three

groups of lizards with widespread distributions across the different archipelagos and for which the relationships between populations from different islands and their (native or human-mediated) origin was, in most cases, not clear. To distinguish natural from human-mediated patterns is an essential step for understanding the biogeographic history of a region and before diversification processes can be tackled. Using mtDNA markers we showed that cryptic diversity exists within *Gehyra mutilata*, a species that occurs across Indian and Pacific Ocean islands, and also that its insular distribution is very recent and, at least across the Indian Ocean islands, most probably the result of human-aided dispersal. Also, for the four species of the genus *Hemidactylus* present across the Western Indian Ocean islands we propose that most of their insular range may actually be the result of very recent (and probably human-aided) dispersal events. Our results indicated that most probably all *Hemidactylus* species occurring in the Comoros and in the granitics Seychelles are not native, and that instances of natural colonization seem to be only the ones of *H. mabouia* to Madagascar and from there to the coralline archipelago of Aldabra. We further demonstrated cryptic variation in all species in their native range. With a comprehensive sampling across the Comoros archipelago and East African coastal islands we clarified the phylogenetic relationships between different populations of *Mabuya maculilabris*, *M. comorensis* and *M. striata*; three species of skinks for which inter-islands relationships and status across the Western Indian Ocean region were particularly problematic. We confirmed the distinctiveness between *M. maculilabris* and *M. comorensis*, showing that *M. comorensis* colonized the Comoros archipelago naturally and argue that the Malagasy populations of this species result from human-mediated introductions from Moheli (Comoros) as well as *M. maculilabris casuarinae* (in the Mozambique island). Cryptic variation was further reported within *M. maculilabris*, and *M. striata*, although the Western Indian Ocean range of this latter species is clearly very recent and insular populations show no differentiation from the continental ones.

Finally, we investigated the diversity, structure and factors promoting the diversification within the Seychelles archipelago of three endemic groups of lizards. Based on multiple nuclear and mtDNA loci and using model-based methods we investigated the genetic diversity, structure and evolutionary history of these groups. We showed that cryptic variation and high levels of genetic structure exist within *Urocotyledon inexpectata*, a species apparently more abundant than previously thought. We argue that divergence within each of the Seychellois *Phelsuma* species may be simultaneous (contrary to previously arguments of their asynchronous

divergence) and highlight the possible role of ecological determinants and differential selective pressures in the morphological diversification within each group. Finally, our results indicate that hybridization and massive mtDNA introgression happened between *M. wrightii* and *M. sechellensis*, explaining the current mtDNA paraphyly of these species.

Despite each group's unique evolutionary history, a few common patterns are depicted such as i) a shared northern-southern phylogeographic structure, indicating a major influence of distance in shaping these species genetic structure; ii) the closest affinity of Fregate populations to the southern clades; iii) the presence of additional structure within each (northern and southern) group, and iv) main pre-Pleistocenic divergences within each clade. The observed patterns can now be used to frame biogeographic hypotheses within the Seychelles archipelago that can be further tested across other taxa.

RESUMÉ

Les archipels océaniques sont normalement des "hotspots" de biodiversité et constituent d'excellents laboratoires naturels pour des études évolutives. Les îles de l'Océan Indique occidental en sont la preuve : distribuées par la partie occidentale de l'Océan Indique et par la côte orientale d'Afrique, plusieurs archipels volcaniques (Comores, Mascarenhas), granitiques (Seychelles) et de corail (Aldabra) entourent l'île de Madagascar, et, ensemble forment un « hotspot » de biodiversité, dans lequel, les valeurs de l'endémisme sont normalement au dessus des 90%, pour plusieurs groupes.

Obtenir un catalogue détaillé de cette diversité et comprendre son histoire évolutive est essentiel, soit pour la connaissance des étalons historiques de diversification dans la région, soit pour les stratégies efficaces de conservation. Cette thèse a eu comme objectif, celui de contribuer pour une meilleure connaissance de la distribution, de la biogéographie et des étalons phylogéographiques de reptiles dans les îles de l'Océan Indique occidental, l'usage de cette connaissance pour clarifier la systématique des groupes en question et la distinction entre des étalons de distribution naturels et des étalons d'influence anthropogénique.

D'abord, on á étudié les rapports phylogénétiques et les étalons de colonisation d'un des groupes plus abondants dans la région ; les geckos du genre *Phelsuma*. Ayant comme base les données de multiples marqueurs et dans un échantillonnage presque complète au niveau des espèces, on a montré que ce genre a diversifié au Madagascar, d'où il a colonisé les archipels environnants et on a clarifié les rapports phylogénétiques dedans ce genre, en définissant huit clades principaux.

On a démontré encore que les groupes d'espèces des îles Mascarenes et Seychelles ont eu son origine dans des cas uniques de colonisation (suivis de diversifications in-situ), au contraire des îles Comores qui ont été colonisées plusieurs fois et ont été aussi plus affectées par des dispersions anthropogéniques. On a encore revu la systématique de ce genre, en proposant son organisation en 11 groupes monophylétiques et on a décrit les caractères phénotypiques qui caractérisent ces groupes.

Ensuite, on a exploité la diversité génétique et les rapports phyllogénétiques en trois groupes de lézards avec de larges distributions par les différents archipels de la région et pour lesquels les rapports entre populations de différentes îles et il s'agissait de population d'origine native ou y

introduites, n'étaient pas nettes. En utilisant marqueurs mitochondriaux, on a montré l'existence de diversité cryptique dans l'espèce *Gehyra mutilata*, une espèce avec une large distribution dans les îles des Océans Indique et Pacifique, et on a encore montré que la distribution insulaire de cette espèce est très récente au moins dans les îles de l'Océan Indique, probablement en résultat de facteurs anthropogéniques.

On a aussi montré pour les quatre espèces du genre *Hemidactylus* présentes dans ces îles que sa distribution insulaire est très probablement, dans sa majorité résultante de facteurs anthropogéniques. Les résultats obtenus indiquent une probable origine anthropogénique de toutes les espèces de *Hemidactylus* qui apparaissent dans Les Comores et dans les îles granitiques de l'archipel des Seychelles, étant que, les seules colonisations naturelles semblent avoir été celles de H. mabouia pour Madagascar et de là pour l'archipel d Aldabra. On a encore démontré quelle variation cryptique existe dans toutes les espèces de *Hemidactylus* dans son aire de distribution native.

À travers un échantillonnage englobant dans l'archipel des Comores et des îles côtières africaines, on a encore clarifié les rapports phylogénétiques entre différentes populations de *M. maculilabris*, *M. comorensis* et *M. striata*, trois espèces de geckonidés pour lesquels la connaissance était nettement insuffisante. On a confirmé la distinction entre *M. maculilabris* et *M. comorensis*, en montrant que *M. comorensis* a colonisé naturellement l'archipel des Comores, et encore, que les populations de cette espèce au Madagascar, ainsi que dans île de Mozambique, sont le résultat d'introductions humaines. Différentiation cryptique est encore décrite en *M. maculilabris* et *M striata*, malgré la distribution insulaire de cette dernière espèce qui est clairement très récente, n'existant pas de différentiation relativement aux populations continentales analysées.

Finalement et ayant pour base multiples marqueurs mitochondriaux et nucléaires, on a étudié la diversité, la structure, la génétique et l'histoire évolutive de trois groupes de lézards endémiques dans l'archipel des Seychelles. On a montré quelle différentiation cryptique et d'hauts niveaux de substruturaction génétique existent dans *Urocotyledon inexpectata*, une espèce apparemment plus abondante que ce qu'on avait antérieurement pensé ; on a raisonné sur la possibilité de la différentiation simultanée de chaque subespèce des espèces du genre *Phelsuma* aux Seychelles, mettant en évidence le possible rôle de déterminants écologiques dans les différents degrés de différentiation morphologique au dedans de chaque espèce et on a encore montré que hybridation et introgression massive entre *M. wrightii* et *M. sechellensis*, ce que explique la

paraphilie de ces espèces au niveau du DNA mitochondrial.

Malgré l'histoire évolutive unique de chaque groupe, quelques étalons communs sont passibles de se rendre évidents, tels que : **i)** l'existence d'une structuration génétique marquée, parmi les groupes d'îles du nord et du sud, indiquant une grande influence de la distance, dans la structuration et distribution de ces espèces ; **ii)** une plus grande proximité phylogénétique entre les populations de l'île de Frégate et celle des îles du sud ; **iii)** la présence d'une structuration additionnelle au dedans de chaque groupe géographique principal, et **iv)** l'âge pré-Pleistocénique de la principal différentiation, au-dedans de chaque groupe d'espèces. Les étalons observés peuvent maintenant être utilisés pour procéder à l'encadrement et à la formulation d'hypothèses biogéographiques pour l'archipel des Seychelles, à être testées dans d'autres taxa.

# CHAPTER 1

## GENERAL INTRODUCTION

## 1.1 Islands as Privileged Systems for the Study of Evolution

*"Although biological diversity has many causes,*
*the single most important factor*
*is the physical separation of populations."*

*(Dobzhansky, 1937; Mayr, 1942; Wagner, 1889)*

Although the paradigm where this prompt assertion was originally made has considerably changed (Bolnick and Fitzpatrick, 2007), it remains no less true, and it is not hard to appreciate why oceanic island settings are usually remarkable biodiversity hotspots, and such exciting places to study evolution. Not only do they provide the ideal conditions for the diversification of colonists, due to their inherent fragmented nature, as also, since many of them are volcanic archipelagos, initially "free" and "empty", they offer a wide range of niche opportunities to newcomers. Moreover, they do it in a replicated manner: each island and each new colonization event initiates a new and independent realization of evolution - an "*independent sample*" from the scientist's point of view.

These ideas are reflected in the central paradigm of island biogeography: that islands, being discrete, internally quantifiable, numerous, and varied entities, provide us with a suite of natural laboratories, from which the discerning natural scientist can make a selection that simplifies the complexity of the natural world, enabling theories of general importance to be developed and tested. To unveil the biogeographic affinities of island taxa, and how particular groups and lineages came to be distributed as they are, are necessary steps before the processes of evolution on islands can be tackled, and constitute the framework and general goals of this thesis, for selected reptile taxa across the Western Indian Ocean islands.

Remote island biotas differ from those of continents in a number of ways, being generally species poor and disharmonic (peculiar in taxonomic composition), yet rich in species found nowhere else, i.e., endemisms. Although these features are well known from more "popular" examples of "*unique creatures*", such as the Galapagos and Indian Ocean giant tortoises, the Mascarenes flightless Dodo, or the unusual Malagasy fauna, with their giant lemurs or minute chameleons and frogs, the global significance of island biodiversity in a general context is not so well appreciated. Current data (Whittaker and Fernández-Palacios, 2007) unequivocally demonstrate that islands, particularly if large and remote, contribute disproportionately for

biodiversity. Twenty of the 34 biodiversity hotspots defined by Conservation International are islands or have an important insular component (Mittermeier et al., 2005 and updates in http://www.biodiversityhotspots.org). Equally important, data are also clear in respect to attrition and extinction rates (Brooke et al., 2007; Courchamp et al., 2003), and many islands qualify today as "*biodiversity threatspots*" as well. The usually small population sizes and ranges of island organisms, and their unique characteristics resulting from prolonged evolution in isolation, make them particularly sensitive to anthropogenic changes (Frankham, 1998). Islands are invaluable but fragile arks of biodiversity.

### 1.2 The Western Indian Ocean Islands Biodiversity Hotspot

Madagascar and adjacent islands are one of the 34 "*hotspots*" defined by Conservation International in 2005 (Mittermeier et al., 2005). Scattered across the Western Indian Ocean, off the southeast coast of Africa, the "Indian Ocean Islands" hotspot, comprises several islands, including the Seychelles (and Aldabra), the Comoros, the Mascarenes and the Iles Esparses archipelagos, from which Madagascar stands out as the fourth largest island on Earth

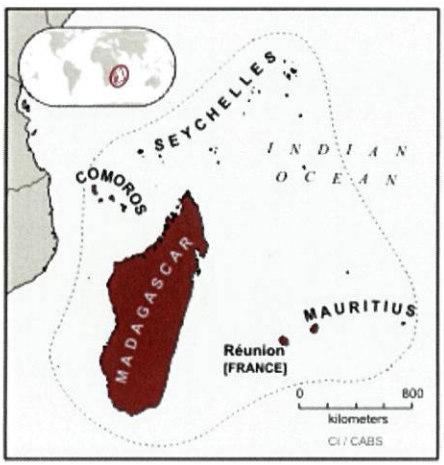



**Figure 1.1** The Indian Ocean Islands' biodiversity hotspot. Together these islands have an astounding total of eight plant families, four bird families and five primate families that live nowhere else on Earth. The Seychelles, Comoros and Mascarenes altogether support a considerable number of Critically Endangered species. Endemism values across different groups usually are above 90%. The Seychelles are also home to the only endemic family of Amphibians (the Sooglossidae) and the Aldabra giant tortoise, one of the region's most heralded endemic reptile (figure and data from www.biodiversityhotspots.org).

### 1.2.1 Geological Evolution of Western Indian Ocean Area: Gondwana Fragmentation and Islands Emergence

Geologically, the scenario is a complex one. When the supercontinent Pangea began to divide into the southern continental landmasses (Gondwana) and northern continental landmasses (Laurasia), Madagascar was tucked away, deep within Gondwana (Rakotosolofo et al., 1999; Reeves et al., 2002). The break-up of West and East Gondwana (starting 175 Mya, according to Schettino and Scotese, 2005) led to a true ocean floor forming between eastern-southern Africa and Madagascar in the Somali and Mozambique basins (Rabinowitz and Woods, 2006). Madagascar, together with Seychelles-India-Antarctica-Australia, migrated >1400 km SSE from its original site east of Tanzania-Kenya, first sliding along the Davie Fracture Zone, on a track roughly parallel to the coast of eastern Africa, and then moving eastwards, before spreading in the Somali basin ceased, at 120-116 Mya (Rabinowitz and Woods, 2006; Schettino and Scotese, 2005; Ségoufin et al., 2004, Figure 1.2). By 140 Mya, marine conditions were clearly prevalent along the entire west coast of Madagascar (Seward et al., 2004), and by 116 Mya Madagascar reached its current position relative to Africa (of 430 km distance, Ali and Aitchison, 2008).

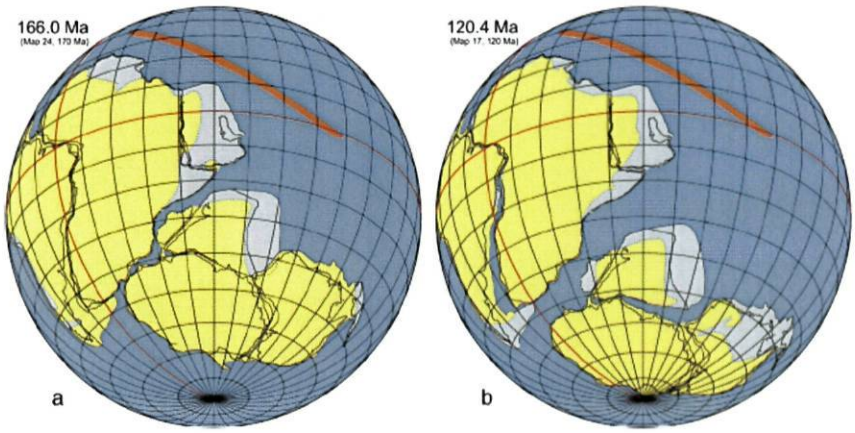



**Figure 1.2** Break-up and dispersal of Gondwana at 166.0 (a) and 120.4 Mya (b) (from Ali and Aitchison, 2008)

From this point onward, Madagascar and India remained in close contact; forming the Indo-Madagascar subcontinent, until the commencement of the northeasterly drift of the Seychelles-Indian block away from Madagascar's eastern margin. India's separation from Madagascar appears to have occurred over a 30 million years period, finishing around 85-90

Mya (Aitchison et al., 2007), and possibly beginning in the north of Madagascar and progressing to the south (Seward et al., 2004; Storey et al., 1995; Torsvik *et al.*, 1998). After that India continued its northwards drift until its ultimate collision with Asia in the earliest Eocene, 55 to 35 Mya (see Ali and Aitchison, 2008 for a discussion on the alternative models of India's arrival to Asia). Meanwhile, the Seychelles stood lagging behind, in between Madagascar and India, with the most approximate estimate of their complete isolation being at the Cretaceous/Tertiary boundary (KTB), 65 Mya (Plummer and Belle, 1995), associated with the Deccan volcanic events (Figure 1.3).

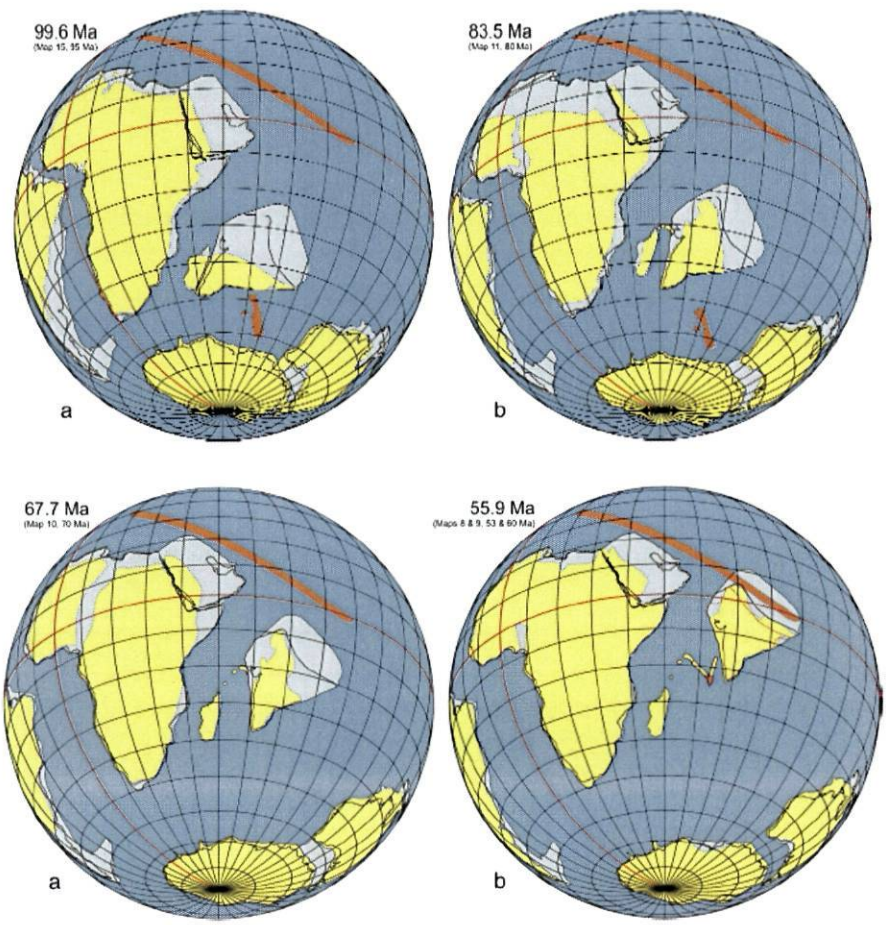



**Figure 1.3** Break-up and dispersal of Gondwana at 99.6 Mya (a, above), 83.5 Mya (b, above), 67.7 Mya (a, below) and 55.9 Mya (b, below) (from Ali and Aitchison, 2008).

After this, a new oceanic spreading ridge developed between the Seychelles, Mascarenes and India, forming what is today the Carlsberg ridge (McKenzie and Sclater, 1971; Norton and Sclater, 1979). The Seychelles/Mascarenes block remained thereafter an isolated micro-continent amid an ever-widening ocean as India drifted northwards. Where elevation was sufficient, such as perhaps sites of Deccan related volcanism; sedimentation was dominated by shelf carbonates, creating the present day plateaus and banks.

From 700 to 1400 km east of Madagascar, the Mascarenes (Reunion, Mauritius and Rodrigues) are three strikingly different islands, united only by their relative proximity and volcanic origin (Figure 1.4). Reunion is the largest and most southern and the nearest to Madagascar, followed by Mauritius and Rodrigues, the smallest, and by far the most isolated. According to potassium-argon and stratigraphic dating, the hotspot began to generate Mauritius some 10 Mya. The island remained volcanically active as it drifted off the main magma source, often with long periods of quiescence, until circa 25,000 years ago (Saddul, 1995). About 3 Mya the first eruptions forming Reunion broke the surface, and the island has grown (and eroded) actively since then (Montaggioni and Nativel, 1988). Its oldest known rocks are no more than 2.1 My old (McDougall, 1971). Off to the east, on a separate volcanic upwelling fracture zone, lies Rodrigues, with an estimated age similar to Mauritius, of 8-10 My (Anon., 1998; Giorgi and Borchellini, 1998).

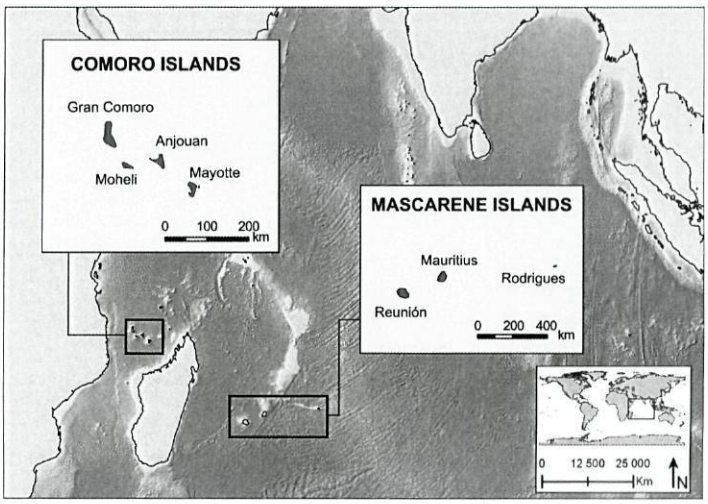



**Figure 1.4** The Mascarenes and Comoros archipelagos, Western Indian Ocean.

The Comoros (Figure 1.4), to the northwest of Madagascar, is a set of four major oceanic islands, formed by progressive hotspot volcanic activity in the last 15 My. They are Grand Comoro (Ngazidja), Anjouan (Ndzwani), Moheli (Mwali) and Mayotte (Maore). Distances range between 50 and 90 kilometres (km) between the islands and roughly 300 km to the East African Coast as well as to Madagascar. Grand Comoro is the largest of the islands with about 1,100 $km^2$, followed by Anjouan (425), Mayotte (375) and Moheli (210). The volcanic origin of the islands becomes most clearly visible on Grand Comoro, where Mt. Karthala, the archipelago's highest elevation, is still an active volcano. Relatively precise dating is possible only for exposed lavas, so results strongly depend on the quality of sampling. All estimates agree that Mayotte is the oldest and Grand Comoro the youngest, but aside from that age estimates still vary (Table 1).

**Table 1** - Summary of the estimates of geological age of the Comoro islands presented by several authors (in My). Age estimates usually refer to the oldest exposed lavas, while the estimated age of the volcanic origin of the island (*) should be considered as a better estimate of island sub-aerial emergence and availability for colonization (R. Duncan, *personal communication*)

| Grand Comore | Anjouan | Moheli | Mayotte | Source |
|---|---|---|---|---|
| | | | About 15 | Debouf et al.(2005) |
| | 5 | 3.9 | 8-15 | Battistini (1996) |
| | 3.9±0.3 | 5±0.4 | 7.7±1 | Nougier et al. ( 1986) |
| 0.13±0.002 | 3.5* | 2.81±0.08 | 5.4 1± 0.01 | Emerick and Duncan (1982) |
| 0.5 * | 11.5* | | 10-15* | Montaggioni and Nougier (1981) |
| 0.01±0.01 | 1.52±0.1 | 1.52±0.1 | 3.65±0.1 | Hajash and Armstrong (1972) |

Finally, the Seychelles (Figure 1.5) comprise 155 islands scattered across the Western Indian Ocean between 4-11ºS and 45-56º E. Traditionally divided into two main groups; the northern, granitic islands and the southern, coralline islands, they can in fact be differentiated into three geological categories: the granitic islands, the low coralline islands and the raised coralline islands.

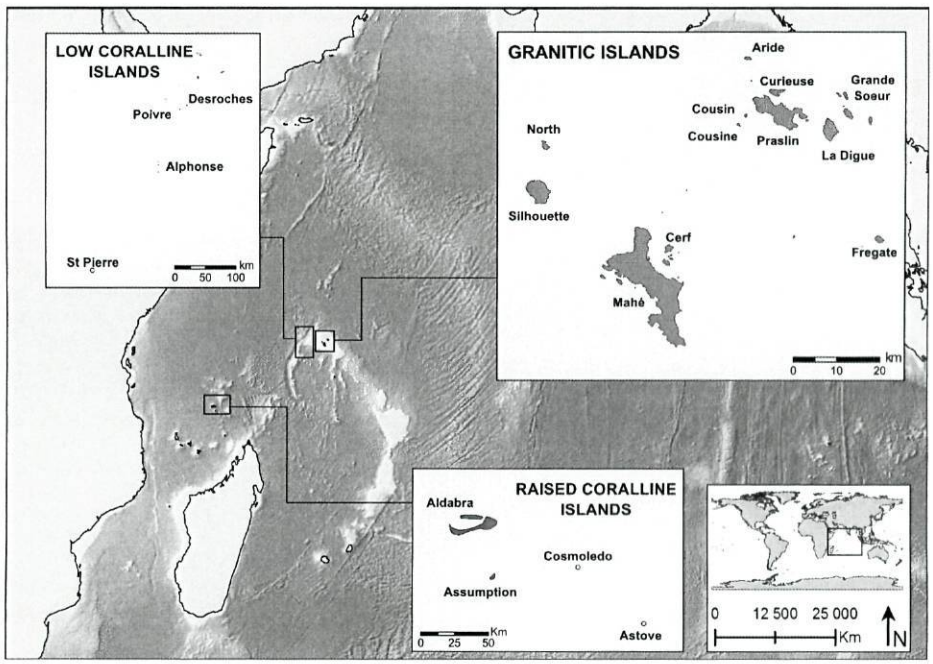



**Figure 1.5** The Seychelles Islands. Only main islands referred to along this thesis are labeled. For Cosmoledo, Astove and St. Pierre only locations are shown.

The granitic islands consist of a group of about 40, clustered together on the undersea shelf of granite that is the Seychelles Bank. These comprise the islands of North, Silhouette, Fregate, Mahé, Praslin, La Digue, Curieuse and several smaller islands encircling these (Figure 1.5). These islands are generally high, some very mountainous, reaching 914 m above sea level (Morne Seychellois, Mahé). Such height results in great habitat diversity and rainfall. The low coralline islands were formed very recently, probably less than 6,000 years ago, from marine sediments sometimes cemented by deposits of guano. Almost all are less than 3 m above sea level (Braithwaite, 1984). Finally, all islands of the Aldabra group (Aldabra, Cosmoledo and Astove atolls and Assumption island) are raised coralline islands. Like the low coralline islands,

they are oceanic in origin, formed by reef-building corals acting on submerged volcanic seamounts that may have formed some 20 Mya (Plummer, 1995) and have thus been submerged and emerged several times since their formation.

### 1.2.2 Sea Level Fluctuations and their Role on Indian Ocean Biota Evolution

New data on Indian Ocean sea-level changes support low-stands of up to 145 ± 5 m below present sea level (BPSL) at six episodes in the last 0.5 My, some of which persisted for up to 50,000 years at a time (Camoin et al., 2004; Colonna et al., 1996; Rohling et al., 1998; Siddall et al., 2003).

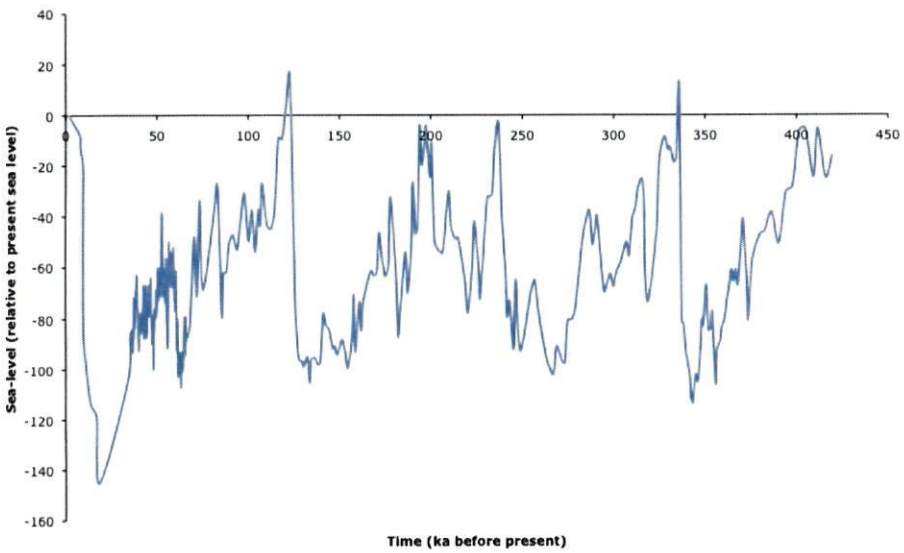



**Figure 1.6** Reconstruction of the sea level for the past 420,000 years. Data for the last 34,000 years come from the Mayotte reef (Comoros archipelago; Colonna et al., 1996), while those from 34,000 to 420,000 years come from the Red Sea (Siddall et al., 2003) (from Warren et al., 2010).

Geological records going back further suggest low-stands of 80–120 m BPSL at 0.64, 0.88, 1.04, 1.26 and 1.54 Mya, and 11 low-stand episodes of 50 m or more BPSL during the last 5 My (Miller et al., 2005). Although the topography of the Indian Ocean sea floor has been influenced by tectonic and volcanic activity, evidence suggests that bathymetry has remained largely constant for at least the last 5 My (Warren et al., 2010). Further back in the past, sea level mostly

oscillated around current zero through the Miocene, having had higher levels (>50m above present sea level; APSL) during the Oligocene and Eocene (30-55 Mya) (Miller et al., 2005).

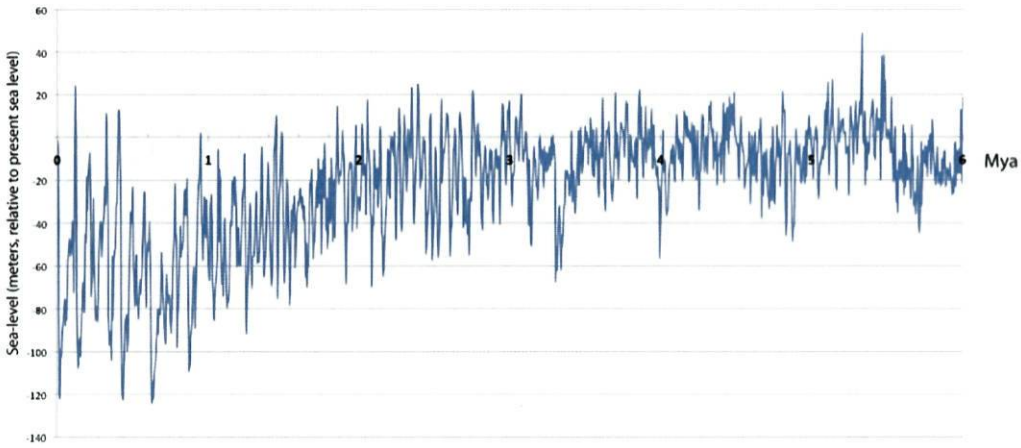



**Figure 1.7** Global sea level estimate derived from $\partial^{18}O$ for the last 6 Myr – from Miller et al. (2005) supplementary Table S1.

*The Mascarenes Plateau and the "Asiatic flavour" of Malagasy Region Biota*

On the basis of current bathymetry, at low stands of 10 m or more BPSL, a number of additional islands along the line of the Maldives, Chagos, and Mascarene Plateau would have been emergent, forming a stepping-stone chain between India and the Madagascar region. Additional islands between the Seychelles and Mascarenes would have further facilitated dispersal within the Malagasy region at these times, the largest being landmasses of 26,405 and 22,099 $km^2$, currently represented by the Saya de Malha and Nazareth submarine banks, respectively (based on low stands of 80 m BPSL, and yet larger during deeper low stands).




**Figure 1.8** Position and extent of the islands exposed by lower sea levels during the Pleistocene ice ages: a) current geography; b) geography at times when sea was 80 m BPSL; c) geography at times when sea was 135 m BPSL (from Warren et al., 2010). Although without direct evidence, and even accounting for higher Pre-Pliocene sea levels (Miller et al., 2005) there is every reason to hypothesize from the usual process of emergence, erosion and sinking that the banks along the Mascarenes plateau would have existed as sub-aerial emerging islands before the current Mascarenes (RE=Reunion; MU=Mauritius; RD=Rodrigues) emerged (Cheke and Hume, 2008). Saya de Malha was the first of the Mascarene islands to rise out of the Indian Ocean due to the Reunion hotspot (~40 Mya), followed by the Nazareth bank (~32 Mya), St. Brandon (also known as bank), Soudan bank (St. Geran) and finally by the presently emerged islands of Mauritius, Rodrigues and Reunion (O'Neill et al., 2005). Nazareth and St. Brandon were most probably still extant, and with some surviving endemic fauna, at the time Mauritius and Rodrigues emerged, though fading fast into atolls. Later, at Pleistocene sea level drops during glaciations, they submerged and re-emerged several times again as low islands, providing possible "stepping stones" for faunal colonization. (from Warren et al., 2010)

As well as the presence of additional islands, a number of currently existing islands would have been much larger in size. The granitic Seychelles, Maldives, Chagos archipelago, and St. Brandon, currently 218, 298, 63 and 1 $km^2$, respectively, would have been sizable regions of 41,615, 18,877, 13,379 and 8,973 $km^2$ (again based on low-stands of 80 m BPSL; see figure 1.8). The enlarged size of these islands is likely to have increased the frequency of arrival of waif dispersers, increasing the chances of populations becoming established. Similarly, it would have permitted populations of larger size to serve as the founding population for the colonization of neighboring islands and continents. Although the additional islands would have outsized all other islands in the Western Indian Ocean (except Madagascar) at low stands of around 40 m BPSL or more, low stands of only 10 m BPSL would have been sufficient to expose islands such as Saya de Malha. Consequently, within the last 500,000 years, frequent large reductions in the greatest inter-island oceanic crossing distance between India and the Madagascar region have occurred. Similar islands are likely to have also existed at earlier periods of low sea level between 5 and 34 Mya (Miller et al., 2005; Schatz, 1996). However, as ocean floor bathymetry beyond 5 Mya cannot currently be inferred with accuracy, the precise shapes and areas of these more ancient islands are speculative. Examining the situation further back in time, although the Saya de Malha and Nazareth banks are (like the granitic Seychelles) of continental-relict origin (Pilipenko, 1995; Plummer and Belle, 1995), it is clear from ocean drilling programs that they also have a major volcanic component, as do the Chagos–Maldives and Saint Brandon (Duncan, 1990; Duncan *et al.*, 1989). The age-progressive chain of volcanic rocks stretching across the Indian Ocean between the Mascarenes and India are generally believed to be the result of Indian and African plate movement over the Reunion hotspot (Bonneville et al., 1998; Duncan, 1990; Duncan et al., 1989; Sheth, 2005; Verzhbitsky, 2003). It is likely that many parts would have been sub-aerial at the time of formation, and would therefore have been available as stepping-stones for lineages dispersing between India and the Madagascar region at periods dating back to the separation of India and the granitic Seychelles at 65 Mya (Plummer and Belle, 1995; Rage, 1996). However, in the absence of any information about the rate of subsidence and erosion of such islands, their duration in the early Cenozoic is uncertain. The role of these submerged areas was recently explored and invoked (together with the direction of wind and Ocean currents), to explain the Asian affinities of a high proportion of Malagasy biota (Warren et al., 2010).

*Stepping Stones between Africa and Madagascar?*

The existence of "land bridges" between Africa and Madagascar, sub-aerial exposures of seamounts in the middle of the Mozambique Channel during the Cenozoic, along Davie Ridge (Figure 1.9), which would have facilitated dispersal between these two regions was long postulated, and supported (Godinot, 2006; Masters et al., 2006; McCall, 1997; Tattersall, 2006, 2008).



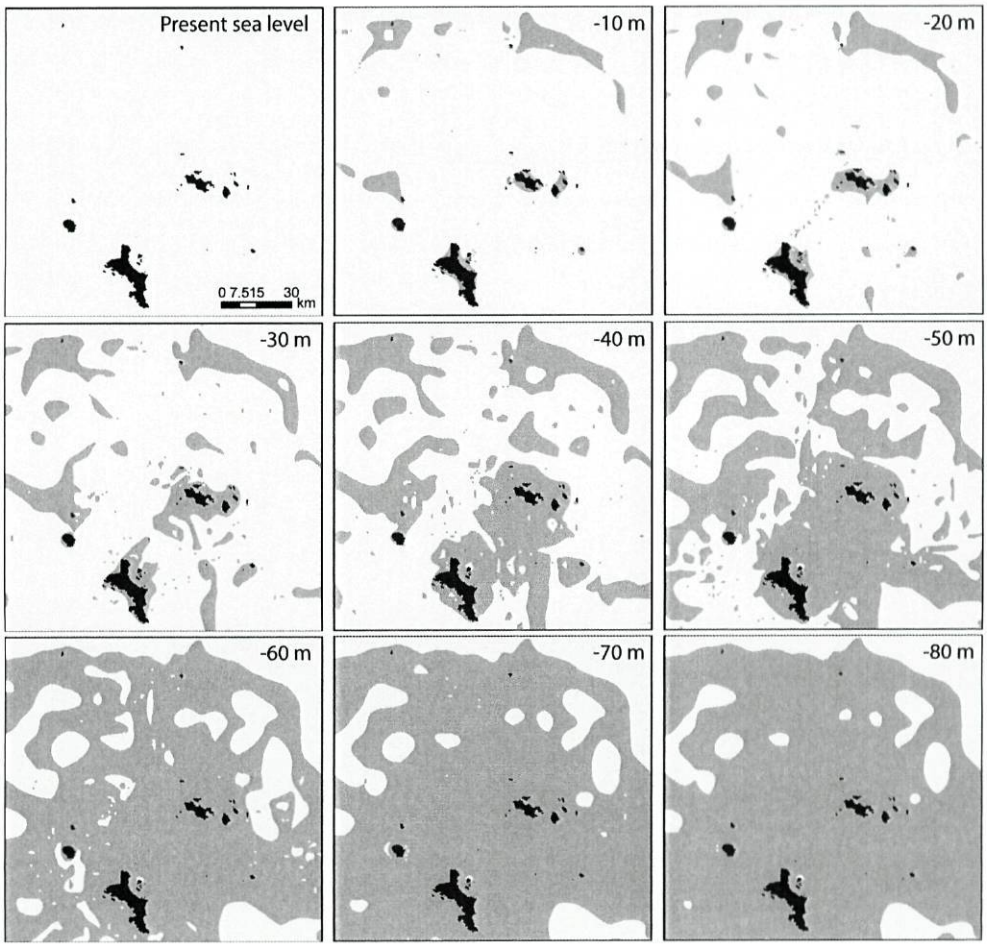

**Figure 1.9** Simplified bathymetric map of the southwest Indian Ocean region. Colors refer to depths. Note areas of lower depths along Davie Ridge (from Ali and Huber, 2010).

Nevertheless, their existence and extent have been sometimes questioned, and even if they existed they would certainly have been small and separated by several tens to hundreds of kilometers (Ali and Huber, 2010; Krause et al., 1997; Rabinowitz and Woods, 2006).

Furthermore, and although the Comoros have never been connected either between themselves or with Madagascar, potentially important stepping stones between Madagascar and Mayotte exist, like the "banc du Geyser" and the "banc du Leven" which both include significant areas less than 100 m below sea level (FTM map Antsiranana 1:1,000,000) and thus might have been exposed until the end of the last glaciation, and several times before.

*Sea Level Fluctuations in the Seychelles.*

Oscillations of the sea level throughout the last 5My (and even back further) must have played a major role in shaping diversity patterns across these islands: all the islands in the Seychelles plateau would have been united by low stands of roughly 60 m, but even much smaller ones greatly increased the size of each island and the possible connectivity between them. Above sea level stands during the Pleistocene (~11 and 17 m, according to Siddall et al. 2003) were much less dramatic than the low stands, with a high percentage of most granitic islands maintaining considerable habitat availability (Figure 1.10). It is thus most probable that instead of by simple directional colonization events, divergence and diversification within the granitic Seychelles have been mostly driven by vicariance, through cycles of allopatry and possible contact between populations.



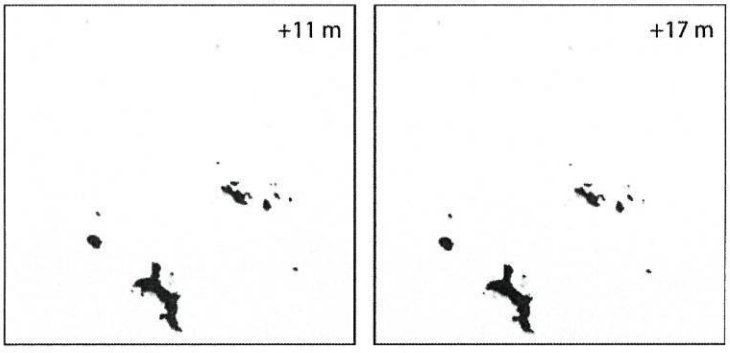



**Figure 1.10** The Seychelles plateau geography, at times of different bellow (-) and above (+) present sea levels, assuming constant ocean floor topography. Darker areas indicate emerged areas at different sea levels and light areas correspond to submerged areas. It is possible to sea that roughly below 60 m BPSL all the islands in the plateau could be connected and that APSL stands estimated during the Pleistocene apparently would not have much influence in most granitic islands areas relative to present.

Across the (lower) coralline islands, the impact of higher sea levels was obviously much higher. The last higher sea level period almost certainly led to the full submergence of Aldabra, which dates back to 125,000 years ago (118-140 Kya; Thomson and Walton, 1972). Remaining islands in the group, lower than Aldabra itself, were probably submerged during the last interglacial when sea level was roughly 10 m higher than present, re-emerging slightly later than Aldabra (Taylor et al., 1979).

### 1.2.3 The Ocean Currents

Ocean currents are another of the main factors to take into consideration when studying biogeography of island taxa. For a long time, Simpson's (1940) "sweepstakes hypothesis", according to which the ancestors of Madagascar's present day mammal stock rafted there from Africa, was severely questioned due to its inconsistency with local ocean currents, and the use of putative land bridges was favored (Masters *et al.*, 2006).

Using palaeo-geographic reconstructions and palaeo-oceanographic modeling, Ali and Huber (2010) recently showed that strong surface currents flowed eastwards from northeast Mozambique and Tanzania towards Madagascar during the Palaeogene (65.5 – 23.03 Mya), after which, as Madagascar advanced north towards the equatorial gyre, the regional currents system evolved into its modern configuration, i. e., it flows westwards from Madagascar to Africa. This may explain why no fully non-aquatic land mammals have colonized Madagascar since the arrival of rodents and carnivores during the early-Miocene epoch and provides a framework for understanding colonization patterns across the Western Indian Ocean region (Figure 1.10).

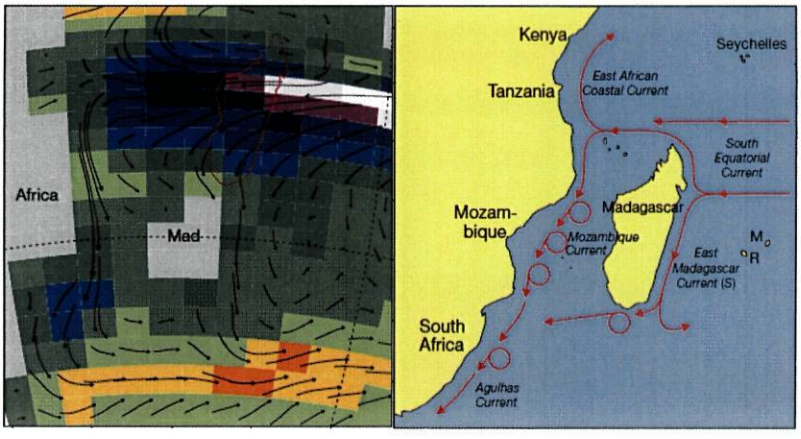



**Figure 1.11** Left, Eocene simulated Ocean currents (with current position of Madagascar outlined in red) showing the prevalent surface flows on an eastward direction (Africa to Madagascar); right, present-day surface-water circulation (from Ali and Huber, 2010).

## 1.3 The Origins of Western Indian Ocean Biota

### *1.3.1 Madagascar*

*"The biogeographic origins of the modern terrestrial and freshwater vertebrate fauna of Madagascar remain one of the greatest mysteries of natural history"*
(Krause et al., 1997 *in* Goodman and Benstead, 2003)

Malagasy biota shows a characteristic signature of evolution in isolation, both in the high levels of diversity within lineages and in the imbalance of lineages that are represented. Its peculiar composition inspired centuries of speculation relating to the mechanisms by which Madagascar's biota came to reside there: Gondwanan vicariance and/or Cenozoic dispersal. The debate on the origins of Malagasy fauna intermingled for a long time with the debate of the opposing schools of vicariance and dispersalist biogeography itself, and the understanding of plate tectonics (Wiley, 1988). Gillespie and Roderick (2002), in an analysis of terrestrial arthropod distributions, distinguish two types of island systems: "darwinian" and "fragment" islands. The former will never have been in contact with other biotic reservoirs and is presumed to have an abundant supply of empty ecological niches. On these islands, species numbers will increase at first via colonization, which, over time, will be followed by the formation of neo-endemics. According to this model, if isolation persists for vast periods of time, the ecological space will eventually be filled via speciation rather than immigration. In the case of fragment islands, it is posited that the ecological space will initially be filled due to contact with other landmasses, but that niches will open up, as the fragment island becomes more isolated, in a process called relaxation. From this point, speciation among the initial residents will then create paleo-endemics that over time can result in high levels of endemism. Gillespie and Roderick (2002) clearly placed Madagascar squarely within the later category.

Recent data however, making use of molecular tools and phylogenetic methods for the study of affinities of islands taxa and lineages' age estimations, show instead that Madagascar does not comfortably fit into either category, but instead shows patterns of neo-endemism that are as strong, if not considerably stronger, than patterns of paleo-endemism.

The overall summary of the emergent patterns from phylogenetic studies of Malagasy taxa (reviewed comprehensively by Yoder and Nowak, 2006) indicates that there are numerous endemic clades of Malagasy taxa whose closest sister group relationships are to African taxa and

that there appears to be an overwhelming indication of dispersal, with the majority of both crown and stem ages falling within the Cenozoic (<65 Mya). The inescapable inference seems to be that the living biota is predominantly comprised of neo-endemics that have evolved from transoceanic dispersers (mostly from Africa although several Asian groups also exist). Nevertheless, the number of studies that allow age estimation is paltry, relative to the number of endemic taxa, and conclusions have to be drawn with caution. Moreover, the fossil record of Madagascar places a severe handicap on temporal analysis, with terrestrial fossil exposures, especially for the Tertiary, virtually non-existent (Flynn and Wyss, 2003; Krause, 2003), and divergence time estimation, even making use of increasingly sophisticated phylogenetic and coalescent methods (Drummond et al., 2006; Thorne and Kishino, 2002; Yang and Rannala, 2006), usually cannot be made with high degrees of confidence, and should be viewed as rather gross approximations.

In the case of herpetofauna in particular, the distinction of Cenozoic dispersers and ancient vicars is fairly clear, and the predominant trend of thought is one of dispersal, typically from Africa (Vences, 2004), although a few exceptions exist (Noonan and Chippindale, 2006).

### *1.3.2 Mascarenes*

*"Of these two sorts of fowl, for ought we yet know, not any are to be found out of this island (...). A question may be demanded how they should be there, and not elsewhere, being so far from other land and can neither fly or swim; whether by mixture of kinds producing strange and monstruous forms, or the nature of the climate, air and earth in altering the first shapes in time and how (...)"*
Peter Mundi, 1638, commenting on the Dodo and the Red Hen from The Mascarenes.
*(in* Cheke and Hume, 2008*)*

The Mascarenes have in Madagascar their nearest land, so much nearer than any other, that one might consider any other source too improbable to be taken seriously, but with a deeper look into Mascarene biota it turns out that Madagascar's contribution, while important, is less than overwhelming, while the rise and fall of islands over time, along with ocean currents from the east, seem to have been very significant.

Some species in the Mascarenes, largely seabirds and waterbirds but also some bats and lizards are members of widespread species that have reached the islands recently, or whose members in different areas regularly mix. A few species are shared with Madagascar but more distantly related to forms outside the region. A substantial group of species is distinct but not greatly differentiated from related ones in Asia, Australasia, Madagascar, or, for seabirds, other

oceans. A final group consists of animals whose immediate relatives are far from obvious, or which have no close extant relatives at all - most of them now extinct (the origins of Mascarenes fauna is comprehensively reviewed in Cheke and Hume, 2008). The strong "*Asian flavour*" of the Mascarenes' fauna is particularly associated with the previously mentioned currently submerged banks and geological history of the region. Indeed, colonization and first differentiation within this nowadays submerged chain of islands was invoked from the emblematic (and extinct) Dodo and the Solitaire (Shapiro et al., 2002), and the same history is most probably true for many more groups (Cheke and Hume, 2008), including a few reptile taxa like *Cylindraspis* tortoises, *Leiolopisma* and *Gongylomorphus* skinks.

*1.3.3 Comoros*

After their formation, the Comoro islands were colonized mainly by fauna from Africa and Madagascar, but also eventually from Southeast Asia. The fauna of the Comoro islands comprises a diverse array of mammals, birds, reptiles, fishes, and invertebrates (Louette et al., 2004). Other classes are less abundant: amphibians, in particular, are represented by only two species, which, apparently, reached the archipelago by natural dispersal from Madagascar (Vences et al., 2003). There is a high degree of endemism within the Comoran fauna, but also a considerable number of apparently widespread and introduced species. Precise origins of most taxa remain unknown, but they are usually considered to have closer affinities to Madagascar. The reptile fauna of the Comoros has recently been subject to a few studies using molecular data and appears to result basically from three sources: ancient natural colonizations from Madagascar and Africa; more recent natural colonizations from Madagascar; and recent, human-aided dispersals. Multiple colonizations are common in most taxa, and oceanic dispersal, more than archipelago radiations or within-island divergence, seems to have led to the observed species diversity in the region. The emerging biogeographic pattern for the Comoros herpetofauna is that most endemic species have their closest relatives in Madagascar and not in East Africa. This is the case of chameleons, colubrids, geckonids, *Oplurus* iguanas, and skinks of the genera *Cryptoblepharus* and *Amphiglossus*. Groups with direct African affinities are reduced to the skink genus *Mabuya* and *Agamids* (Hawlitschek, 2008; Münchenberg et al., 2008; Rocha et al., 2006; 2009; Vidal et al., 2008)

*1.3.4 The Seychelles*

Seychelles' biota consists of a mixture of Afro-Malagasy- and Oriental-related forms: the ancient endemic groups seem to be largely derived from the Orient, while the most recent forms are mostly Ethiopian/Afro-Malagasy. A distinction has also to be made between the (northern) granitic Seychelles, and the central (Amirantes) and southern (Aldabra group) archipelagos; these last two usually display a much stronger African component.

Summerhayes (1931) showed that the primitive trees, which are the present rainforest dominants in the Seychelles, have oriental affinities. Endemic and indigenous flowering plants of the granitic islands are mostly related to Asian and Mascarene taxa, with only a small African component. Of the flora of the coralline islands though, the greatest proportion is Pan-tropical or Indo-Pacific in affinity (Procter, 1984; Stoddart and Fosberg, 1984). Christensen (1912) also reported a preponderance of ferns in the Seychelles with Oriental origins. The Oriental complexion of the granitic Seychelles insect fauna is so overwhelming that Holdhaus (1928) considered the Seychelles to be a sub-region of the Oriental, a point of view upheld by Scott (1933), and still highlighted in recent works (e.g. Dijkstra, 2007). Aldabra has a peculiar insect fauna, with 31% estimated to be of Ethiopian-Malagasy origin, 1% Oriental and 39% endemic. Many other groups of terrestrial invertebrates have predominantly oriental relationships (Gardiner, 1936), as the brachyuran decapod crustacean coral reef fauna (Garth, 1984) or the land snail fauna, that has greater affinities with the Indian one (Nevill, 1869). Recent species accounts and catalogs (Gerlach, 2006, 2008a; Gerlach and Haas, 2008; Gerlach and Matyot, 2006) have largely confirmed this trend for groups like Molluscs (for which 44% seem to be Gondwanan in origin and another 23% with Asian affinities - probably also Gondwanan), but are unveiling a growing amount of African and Madagascar-related species for groups that generally disperse well, such as Orthopteroidea, Lepidoptera or Diptera. The endemic Seychelles' spider fauna seem to have a mixed African and Oriental origin, with most species qualified has pan-tropical (Justin Gerlach, *personal communication*).

The terrestrial and freshwater vertebrate fauna of the Seychelles includes a high proportion of endemic taxa. In a general perspective, while the inter-island endemicity seems very low, reflecting the recent fragmentation of a widespread biota, the endemicity of the archipelago as a whole is very high. Again, those groups of ancient origins generally show affinities to India, while the more recent lineages have predominant affinities with Madagascar. Of the 117 terrestrial and freshwater vertebrate species recorded, only 24 are introduced

(Gerlach, 2007). According to Gerlach (2007), the 31 native seabirds and waterbirds are either cosmopolitan (45%), pantropical (20%), African (10%), Asian (3%), Indian Ocean (3%) or Western Indian Ocean (13%). Two are endemic subspecies but there are no endemic full species. In contrast, most of the land birds are endemics, closely related to other Western Indian Ocean species, but also with a strong Asian component (Benson, 1985). Although only about 5% of the species show direct Asian links, some 23% of the Western Indian Ocean bird species are probably descendant from Asian ancestors. It has been consistently proposed that the Seychelles acted as a stepping-stone in the colonization of Madagascar by Asian birds (Warren et al., 2005; Warren et al., 2010). Native mammals are restricted to six species of bats, with only two present on the granitic islands. All show affinities to Malagasy or African species. The freshwater fish of the Seychelles are primarily Indo-Pacific in distribution. Of the two endemic species, one belongs to an Indo-Pacific estuarine and marine genus and the other to a Malagasy freshwater genus. The amphibians are more diverse (one introduced and 11 endemic species) and show two different biogeographical patterns: the ancient endemic species (Caecilidae and Sooglosidae) as Gondwana relicts, whist the single Hyperoliid species is descendant from an ancestor rafting from Madagascar (Vences et al., 2003).

The reptiles show similar patterns: the endemic skink genera (*Pamelaescincus* and *Janetaescincus*) are basal to all remaining Afro-Malagasy "scincines", and possibly related to Indian and/or Sri Lankan groups (Brandley et al., 2005); the Seychelles' wolf snake is related to Ethiopian and Oriental natricines (Dowling, 1990; Vidal et al., 2008); and the endemic *Ailuronyx* genus and *Urocotyledon inexpectata* are basal respectively to Afro-Malagasy and Afro-Malagasy-Asian clades, without close relatives back almost to the origin of Gekkonidae sensu stricto, and thus with origins possibly going back to the Cretaceous (Aaron Bauer, *personal communication*). Remaining taxa are almost all closely related to other Western Indian Ocean ones, in great majority Malagasy and African.

In conclusion, although knowledge on the phylogenetic affinities of Western Indian Ocean islands taxa has certainly increased during recent years (see Cheke and Hume, 2008; Gerlach, 2007; Hawlitschek, 2008; Vences, 2004; Yoder and Nowak, 2006 for major reviews and most up-tp-date information), still many groups remain virtually unexplored and many of the hypothesized relationships untested.

## 1.4 Diversification of Western Indian Ocean Biota

As well as the problematic lack of knowledge regarding affinities of island taxa, knowledge of the intra-islands or archipelago phylogeographic patterns is even scarcer. Even in islands like Madagascar, internal patterns of diversification are still largely unknown, though a few hypotheses have been proposed. According to the "ecogeographic constraint" hypothesis, the sharp ecological distinction between eastern and western habitats would be the basis of barriers to gene flow, causing basal splits between eastern and western clades in the phylogeography of initially widespread species or species that expanded during different climatic conditions. Other model implies that mountain massifs, together with climate zonation act as barriers and lead to the frequently observed deep north-south breaks in the genealogies, with each clade spread over diverse ecological habitats in the west and east (Yoder and Heckman, 2006). Despite consistent observations across several groups, no obvious biogeographic barrier explains this primary division between north and south (Boumans et al., 2007). Wilmé et al. (2006) integrated these hypotheses in a mechanistic model where river catchments with sources at relatively low elevations, where forests became separated by larger intervening arid areas, constitute zones of isolation leading to speciation of locally endemic taxa, whereas watersheds with sources at higher elevations, served as zones of retreat and dispersion and hence contain proportionately lower levels of endemism. Nevertheless considerable controversy still exists on the relative merits of different factors in explaining Madagascar's remarkable diversity and endemism, and comprehensive phylogeographic studies to test it are still lacking (Vences et al., 2009), and largely dependent on the gathering of robust phylogenies for Malagasy taxa.

### *1.4.1 Patterns of Diversification within the Seychelles*

The recent history of the Seychellois faunas and floras must be intimately tied to the cycles of emergence and submergence associated with eustatic sea levels and glaciations' cycles described above. Its present fauna and flora are likely a non-random sample of the "original" Seychellois biota. Vast areas of lowland habitat must have disappeared along with many lowland adapted species. Large bodied species (like mammals and flightless birds, if ever present) with large home range requirements would have been subjected to higher extinction rates than small-bodied species. Swamp and pond-dwelling species would have been eliminated. It is probably no accident that the present endemic amphibian fauna consists of species that do not require

standing water for reproduction.

Nevertheless, diversification patterns within the Seychelles are virtually unknown, and very little is known about intra-archipelago structure of Seychellois taxa. Very few studies examined patterns of variation and diversification within the Seychelles, but historically, Mahé and surrounding islands plus Silhouette and North have been considered to form a biogeographic unit, with a separate Praslin - La Digue (and surrounding islands) group. Fregate is generally viewed as an isolated or intermediate unit (Cheke, 1984; Radtkey, 1996; Scott, 1933). This grouping has been detected in some faunal lists for insects (Scott, 1933), molluscs (Gerlach and van Bruggen, 1999) and also in (the few) studies of intraspecific variability within amphibians (Nussbaum and Wu, 1995) and reptiles (Gardner, 1987; Radtkey, 1996), but does not appear to stand for other groups (Gerlach, 1999; Nussbaum, 1984). The similarity between Mahé and Silhouette apparent in faunal lists could also be a consequence of these two islands, being the highest in the group, sharing most of their habitats, in particular extensive areas of mist forest, while lowland and palm forests predominantly cover Praslin, La Digue and Fregate. It will be most interesting to disentangle the influence of habitat versus shared evolutionary history in species distribution across the Seychelles.

Considering their ancient age, geographical (tropical) location and very particular bio-geological history, the Seychelles have a high potential to harbor highly genetically structured populations. To unveil this potential diversity is paramount, both from a general (alpha-taxonomic) perspective as well as from a conservation point of view (many of the Seychelles taxa are already considered Vulnerable, Endangered, or Critically endangered; Gerlach, 2008b). Furthermore, the understanding of the evolutionary processes that generated current diversity may not only supply us with remarkable systems within which to study those specific processes (because we're dealing with island populations), but also enable us to take those processes into consideration in future conservation efforts (Moritz, 2002).

To date, the use of molecular tools on the study of the diversity within the archipelago is almost completely lacking. As will be shown throughout this dissertation, they are crucial in unveiling cryptic differentiation and in understanding the islands' taxa evolutionary history.

## 1.5 Tools and Recent Advances on Phylogeography Inference Methodologies

To reconstruct historical biogeography, and in particular for the study of island colonizations, it is essential to integrate both topological and temporal information (Donoghue and Moore, 2003) in the study of the phylogenetic relationships among taxa. In recent years, the vast increase in capacity to generate large amounts of data, together with computational power, allowed the incredibly fast development of theoretical frameworks and methodologies. In molecular phylogenetics, Bayesian analysis was successfully introduced for tree inference and hypothesis testing (e.g. Drummond et al., 2006; Huelsenbeck and Ronquist, 2001), and new and faster algorithms facilitated the application of maximum likelihood and parsimony methods to large datasets as well (Guindon and Gascuel, 2003; Stamatakis, 2006; Varón et al., 2010; Zwickl, 2006). While the reliability of molecular phylogenies is in general beyond doubt, the use of DNA sequences to infer divergence times is much more controversial and complicated, and "local" or "relaxed" clock models and methods have been introduced in the last years to overcome the general inadequacy of most data to the "global" molecular clock hypothesis (Drummond et al., 2006; Sanderson, 2002; Thorne et al., 1998). Nevertheless, the accuracy of age/rate estimates in the phylogenetic framework is still discussible (Debruyne and Poinar, 2009; Emerson, 2007; Ho et al., 2005; Porter et al., 2005) and dependent on the availability of adequate (and enough) calibration tools and their correct application (Donoghue and Benton, 2007; Graur and Martin, 2004; Hedges and Kumar, 2004; Ho and Phillips, 2009; Ho et al., 2007; Hugall et al., 2007).

Using a population genetics framework, and primarily due to advances in the formulation and application of the coalescent (Wakeley, 2008), powerful methods became available to infer historical demographic parameters of populations (population sizes and size changes), as well as migration rates and splitting times, and to test hypotheses concerning these (Bahlo and Griffiths, 2000; Beerli and Felsenstein, 2001; Drummond et al., 2006; Heled and Drummond, 2008; Hey, 2010; Hey and Nielsen, 2004, 2007; Kuhner et al., 1998). In the context of the coalescent, the genealogy of a locus is a realization of a stochastic process, and estimating a single phylogeny for multiple unlinked loci is often a misleading exercise (Edwards, 2009). Instead of using estimated gene genealogies to directly infer the demographic history, coalescent methods generally treat these genealogies as a nuisance parameter to obtain estimates of biogeographically informative parameters (Hey and Machado, 2003). The use of statistical approaches based on coalescent models for parameter estimation and hypothesis testing has prompted a "statistical

phylogeography" framework (Knowles and Maddison, 2002): under this approach one hypothesis is identified as more probable relative to others when all available data are more likely under the corresponding model. Any competing model may be never "true" but nevertheless represent a useful approximation that should capture the essential features of a demographic history, and most useful if somewhat robust to violations of model assumptions (Anderson, 2007; Wakeley, 2004).

One of the insights from post-coalescent phylogeography was that the standard single-locus datasets that were most commonly collected are often insufficient and that multi-locus datasets could dramatically improve the performance of analytical methods for parameter estimation (Carling and Brumfield, 2007; Edwards and Beerli, 2000; Hickerson et al., 2006). This paradigmatic shift had its consequences for phylogenetics also, and the coalescent become relevant and necessary for species-level phylogeny inference at low levels of divergence. The tradition of equating an estimated gene tree with the history of species divergence has predominated in phylogenetics, and sequences of different loci are still typically combined (concatenated) and analyzed in very much the same format as a single gene, purportedly eliminating the problem of differing gene trees across independent loci. The lessons learned in phylogeography about the inherent benefits of explicitly considering the stochasticity of genetic processes led to the development of coalescent based approaches (Edwards et al., 2007; Heled and Drummond, 2008; Kubatko et al., 2009; Liu and Pearl, 2007; Oliver, 2008), with the focus changing to obtain data from multiple loci and multiple individuals per species or population, and using this data to obtain a direct estimate of the history of divergence - the species-tree - as opposed to focusing on the idiosyncrasies of individual gene trees (Belfiore et al., 2008; Brumfield et al., 2008; Carstens and Knowles, 2007; Kubatko et al., 2010).

The integration of phylogenetics and population genetics for investigating the connection between micro- and macro-evolutionary phenomena was already implicit in the original formulation (by Avise, 1987) of the phylogeography framework approximately 20 years ago. Since then the statistical rigor of the field has increased, largely due to the advances of coalescent theory and nowadays phylogeography is increasingly becoming the integrative and comparative multi-taxon endeavour that it was originally conceived to be (Hickerson et al., 2010). The growing framework of model-based phylogeography will certainly interface with numerous subfields to become one of the most integrative fields in all of ecology and evolutionary biology. A natural outcome is the application of this framework to comparative phylogeography

(Arbogast and Kenagy, 2001), i.e., a natural experiment where focal objects (co-distributed populations) have been independently submitted to the same "natural" evolutionary treatments (geological and/or climate-change scenarios). Methods are being developed that go beyond interpreting results from multiple single taxon analyses and actually try to integrate and infer demographic parameters across taxa given some models and/or depict potential drivers of evolution or demographic models congruence across co-distributed taxa (Hickerson et al., 2010). These methods are in their infancy but are a promising line of research.

## 1.6 Objectives and Organization of the Thesis

This work aimed to contribute to a better understanding of the distribution, phylogeographic patterns and evolutionary history of some groups of reptiles of Indian Ocean islands, and to use this knowledge to clarify the systematics of these groups and to distinguish natural from possibly anthropogenic-driven patterns. Several main objectives were defined for this work:

i) To obtain a robust estimate of the phylogenetic relationships for the genus *Phelsuma*, a group widely distributed across the Western Indian Ocean archipelagos, and use it to identify colonization events and to investigate on the monophyly or paraphyly of the several archipelagos' species assemblages and their degree of differentiation relative to their closest "mainland" forms.

ii) To use this knowledge, together with phenetic characteristics, to revise the systematics of this complex group and provide a classification system that better reflects the evolutionary history of the species.

iii) To decipher the inter-island relationships and the (native or introduced) status of populations from species whose distribution across Western Indian Ocean Islands is thought to have been influenced by human-mediated introductions (genus *Gehyra* and *Hemidactylus*), and

iv) To clarify the phylogenetic relationships between the species of the genus *Mabuya* (Squamata: Scincidae) inhabiting the Western Indian Ocean Islands (excluding Madagascar) and the distinction between different island (and mainland) populations.

v) To contribute to a better knowledge on the distribution, systematics and evolution of Seychelles endemic reptile groups, unveiling genetic diversity and structure across the archipelago for a few target species/species-groups, and to explore their evolutionary history and factors promoting and shaping their current diversity distribution.

This dissertation is organized in five chapters. After providing a *General Introduction* to this work in the present chapter, in *Chapter 2* we determined the phylogenetic relationships and colonization patterns of a major group of Western Indian Ocean Islands' lizards (day-geckos of the genus *Phelsuma*), based on data from multiple loci and an almost complete sampling of the genus (except two species). We further used this and morphological data to review the

intrageneric systematics of the group. These results are organized in three scientific papers, already published in international scientific periodicals with peer-review:

Paper I. Rocha S, Posada D, Carretero MA, Harris DJ (2007) Phylogenetic affinities of Comoroan and East African day geckos (genus *Phelsuma*): multiple natural colonisations, introductions and island radiations. *Molecular Phylogenetics and Evolution*, 43, 685-692.

Paper II. Rocha S, Vences M, Glaw F, Posada D, Harris, DJ (2009) Multigene phylogeny of Malagasy day geckos of the genus *Phelsuma. Molecular Phylogenetics and Evolution,* 52, 530-537.

Paper III. Rocha S, Rosler H, Gehring PS, Glaw F, Posada D, Harris DJ, Vences M (2010) Phylogenetic systematics of day geckos, genus *Phelsuma* based on molecular and morphological data (Squamata: Gekkonidae). *Zootaxa*, 2429, 1-28.

In ***Paper I*** we used mtDNA sequences, together with previously published data, to assess the phylogenetic position of Comoran and East African (Pemba Island) species of the genus and to obtain broad estimates of within-species diversity of these islands endemics. This analysis was extended in ***Paper II***, with a remarkably comprehensive sampling of the genus and the use of a multilocus dataset. We showed that the differentiation within this clade seems to have been relatively simultaneous and fast, in Madagascar, and that many of the previously hypothesized relationships were not correct. We unveiled several cases of cryptic differentiation and probable species complexes, and defined eight main clades. Overall, we identified three independent natural colonizations of the Comoros archipelago (plus two most probably human-mediated ones), one of them originating a remarkable Comoran radiation. The independent colonizations of Pemba and Aldabra archipelagos and the monophyly of both Mascarenes' and Seychelles' radiations were confirmed. We then reviewed the intrageneric systematics of this group, suggesting its division into 11 monophyletic species groups and provided an overview of 19 selected morphological, chromatic and behavioral characters for these groups (***Paper III***). After complementing the molecular data with new trees based on partial 16S rRNA gene sequences for the *P. madagascariensis-* and *P. lineata-groups* we further proposed to elevate *P. lineata*

*dorsivittata* and *P. quadriocellata parva* to full species rank as *P. dorsivittata* and *P. parva.*

In *Chapter 3* we explored the genetic diversity, variation and phylogenetic relationships across three groups of lizards with widespread distributions across different archipelagos of the Western Indian Ocean, and for which relationships between populations from different islands and their origin (natural or anthropogenically driven) were in the need of clarification. Results are presented in three scientific papers, all published in peer-reviewed international journals:

Paper IV. Rocha S, Ineich I, Harris DJ (2009) Cryptic variation and recent bipolar range expansion within the Stumped-Toed Gecko *Gehyra mutilata* (Wiegmann, 1834) across Indian and Pacific Ocean Islands. *Contributions to Zoology,* 78, 1-8.

Paper V. Rocha S, Carretero M, Harris DJ (2010) On the diversity, colonization patterns and status of *Hemidactylus* spp. (Reptilia: Gekkonidae) from the Western Indian Ocean Islands. *Herpetological Journal,* 20, 83-89.

Paper VI. Rocha S, Carretero M, Harris DJ (2010) Genetic diversity and phylogenetic relationships of *Mabuya spp* (Squamata: Scincidae) from Western Indian Ocean Islands. *Amphibia-Reptilia,* 31, 375-385.

In ***Paper IV*** we used mtDNA sequence data variation across Indian Ocean and Pacific Ocean islands populations of *Gehyra mutilata* to show that cryptic variation and strong geographic structure exist within this species and argued that the wide Indian and Pacific insular distribution of *Gehyra* is very recent, and, at least across the Indian Ocean islands, most probably the result of human-aided dispersal.

From a comprehensive analysis of the genetic variability of *Hemidactylus* species across Western Indian Ocean Islands (***Paper V***) we proposed that despite the four species widespread across the area, most of their range may actually be the result of very recent (possibly human-aided) dispersal events. We hypothesized that, most probably, all *Hemidactylus* species occurring in the Comoros and the granitic Seychelles archipelagos are not native and that instances of natural colonization seem to be only the ones of *H. mabouia* to Madagascar and from there to the coralline archipelago of Aldabra. We showed that Aldabra populations reveal a surprising degree

of genetic diversity and structure that strongly suggest their native status. Given the existence of highly differentiated lineages in South Africa and Mozambique, we proposed that *H. mercatorius* becomes the designation applied to insular (Gulf of Guinea islands, Comoros, Madagascar, Seychelles) populations of *H. mabouia*, with the status of eastern African populations remaining unchanged pending further studies. We further demonstrated cryptic variation in all species in their native range, with *H. platycephalus* and *H. mabouia* harbouring several highly divergent lineages, but warned that further taxonomic assignments should wait detailed assessments of distribution and molecular variation.

Western Indian Ocean islands (excluding Madagascar) are inhabited by at least five species of skinks of the genus *Mabuya*, for which diversity and inter-island phylogenetic relationships were still unclear, particularly concerning island populations of *M. maculilabris*, *M. comorensis* and *M. striata*. In ***Paper VI*** we used a comprehensive sampling across the Comoros archipelago and the East African coastal islands of Zanzibar and Pemba, together with previously published sequences, and reported cryptic diversity within *M. comorensis* and a West to East colonization of the Comoros archipelago. We argued that the Malagasy populations of this species result from human-mediated introductions from Moheli (Comoros), as well as *M. maculilabris casuarinae*, whose taxonomic status must be accordingly altered. Cryptic variation was further reported within *M. maculilabris*, which clearly harbors a higher number of lineages, and *M. striata*, although the Western Indian Ocean range of this last species is clearly very recent. We discussed the status of several continental and island populations and reported instances of paraphyly of some Afro-Malagasy species.

*Chapter 4* is devoted to the understanding of genetic diversity, structure and factors promoting diversification within the Seychelles archipelago, by focusing on the evolutionary history of three of their endemic clades. Based on multiple nuclear and mtDNA loci and model-based methods we described the genetic diversity and structure of three endemic lizard clades from the granitic Seychelles, exploring their patterns of diversification for congruency in space and/or time. Results are presented in three scientific papers, all submitted to international peer-reviewed journals:

Paper VII. Rocha S, Harris, D.J, Posada, D. One or more? Cryptic diversity within the endemic Prehensile-tailed gecko *Urocotyledon inexpectata* across the Seychelles Islands: patterns of phylogeographic structure and isolation at the multilocus level. *Submitted to Biological Journal of the Linnean Society.*

Paper VIII. Rocha S, Posada D, Harris DJ. The time frame of diversification of day-gecko genus *Phelsuma* on the Seychelles and its driving mechanisms. *Submitted to Systematic Biology.*

Paper IX. Rocha S, Perera A, Silva A, Posada D, Harris DJ. The evolutionary history of *Mabuya* skinks from the Seychelles islands: introgressive hybridization, morphological evolution and patterns of geographic structure. *Submitted to Molecular Ecology.*

In ***Paper VII*** we unveiled high levels of genetic diversity within *Urocotyledon inexpectata*, which was found to harbor two highly differentiated lineages, exclusively distributed across the northern and southern groups of islands. The main split between these lineages was dated back to the Miocene/Late Pliocene and gene flow between these groups of islands was apparently non-existent. Further structure within both groups was also depicted, possibly without gene flow also. We suggested the occurrence of at least two species, what warrants further investigation. The evolutionary history of the two endemic species of the genus *Phelsuma* from the Seychelles is explored in ***Paper VIII.*** Specifically, we thoroughly described genetic structure within both species and addressed the question of the asynchronous or synchronous divergence between their subspecies. Time estimates suggested a possibly synchronous divergence within each species, which led us to bring again to consideration the role of ecology, native vegetation distribution and differential selective pressures in these species (morphological) diversification across the archipelago. In ***Paper IX*** we explore phylogenetic relationships between populations of both species of the genus *Mabuya* endemic from the Seychelles. Paraphyly of *M. sechellensis* relative to *M. wrightii* had been observed at the mtDNA level (paper VI) and was largely confirmed, but only for mtDNA markers. We argue that hybridization between these species leading to massive mtDNA introgression better explain the results and that analyses of morphological variation seem also to reflect this reticulation pattern. Gene flow and divergence time estimates reflected some putative shared patterns across these three groups, namely: a

Mio-Pliocene divergence between both species, a north-south marked phylogeographic structure; a close relationship between populations from Fregate island and further differentiation and apparent isolation both within the northern (*Urocotyledon* and *Phelsuma*) and the southern (*Urocotyledon* and *Mabuya*) islands populations.

Finally, in Chapter 5 a *General Discussion* of the most relevant results obtained in this work is presented. Also, the major conclusions of this dissertation are exposed and future research pathways are discussed.

## 1.7 References


Aitchison, J.C., Ali, J.R., Davis, A.M., 2007. When and where did India and Asia collide? Journal of Geophysical Research 112, B05423.

Ali, J.R., Aitchison, J.C., 2008. Gondwana to Asia: Plate tectonics, paleogeography and the biological connectivity of the Indian sub-continent from the Middle Jurassic through latest Eocene (166-35†Ma). Earth-Science Reviews 88, 145-166.

Ali, J.R., Huber, M., 2010. Mammalian biodiversity on Madagascar controlled by ocean currents. Nature 463, 653-656.

Anderson, D.R., 2007. Model Based Inference in the Life Sciences: A Primer on Evidence. Springer, New York.

Anon., 1998. Ile Rodrigues. Carte Géologique au 1:25,000. In: hydrogéologique, S. (Ed.). Geolab, Paris.

Arbogast, B.S., Kenagy, G.J., 2001. Comparative phylogeography as an integrative approach to historical biogeography. Journal of Biogeography 28, 819-825.

Avise, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigel, J.E., Reeb, C.A., Saunders, N.C., 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annual Review in Ecology and Systematics 18, 489-522.

Bahlo, M., Griffiths, R.C., 2000. Inference from Gene Trees in a Subdivided Population. Theoretical Population Biology 57, 79-95.

Battistini, R., 1996. Paleogéographie et variété des milieux naturels à Madagascar et dans les îles voisines: quelques données de base pour l'étude biogéographique de la 'région malgache'. In: Lourenço, W.R. (Ed.), Actes du colloque international biogéographie de Madagascar. Société de Biogéographie – Muséum – ORSTOM, Paris, pp. 1-17.

Beerli, P., Felsenstein, J., 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proceedings of the National Academy of Sciences of the United States of America 98.

Belfiore, N.M., Liu, L., Moritz, C., 2008. Multilocus phylogenetics of a rapid radiation in the genus *Thomomys* (Rodentia: Geomyidae). Systematic Biology 57, 294-310.

Benson, C.W., 1985. Origins of Seychelles land birds. In: Stoddart, D.R. (Ed.), Biogeography and ecology of the Seychelles Islands. Dr. W. Junk, The Hague, pp. 469-486.

Bolnick, D.I., Fitzpatrick, B.M., 2007. Sympatric Speciation: Models and Empirical Evidence. Annual Review of Ecology, Evolution, and Systematics 38, 459-487.

Bonneville, A., Barriot, J.P., Bayer, R., 1998. Evidence from Geoid data of a hot spot origin for the southern Mascarene Plateau and Mascarene islands (Indian Ocean). Journal of Geophysical Research 95, 4199-4212.

Boumans, L., Vieites, D.R., Glaw, F., Vences, M., 2007. Geographical patterns of deep mitochondrial differentiation in widespread Malagasy reptiles. Molecular Phylogenetics and Evolution 45, 822-839.

Braithwaite, C.J.R., 1984. Scientfic studies in the Seychelles. In: Stoddart, D.R., Junk, W. (Eds.), Biogeography of the Seychelles Islands, Netherlands: The Hague, pp. 1-16.

Brandley, M.C., Schmitz, A., Reeder, T.W., 2005. Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. Systematic Biology 54, 373-390.

Brooke, M.D., Hilton, G.M., Martins, T.L.F., 2007. Prioritizing the world's islands for vertebrate irradication programmes. Animal Conservation 10, 380-390

Brumfield, R.T., Liu, L., Lum, D.E., Edwards, S.V., 2008. Comparison of species tree methods for reconstructing the phylogeny of bearded manakins (Aves: Pipridae, *Manacus*) from multilocus sequence data. Systematic Biology 57, 719-731.

Camoin, G.F., Montaggioni, L.F., Braithwaite, C.J.R., 2004. Late glacial to post glacial sea levels in the Western Indian Ocean. Marine Geology 206, 119-146.

Carling, M.D., Brumfield, R.T., 2007. Gene sampling strategies for multi-locus opulation estimates of genetic diversity (theta). PLos One 2, e160.

Carstens, B.C., Knowles, L.L., 2007. Estimating species phylogeny from gene-tree probabilities despite incomplete lineage sorting: an example from *Melanoplus* grasshoppers. Systematic Biology 56, 400-411.

Cheke, A.S., 1984. Lizards of the Seychelles. In: Stoddart, D.R. (Ed.), Biogeography and ecology of the Seychelles Islands. Dr. W. Junk, The Hague, pp. 245–258.

Cheke, A.S., Hume, J.P., 2008. Lost Land of the Dodo. An Ecological History of Mauritius, Réunion & Rodrigues. A. & C. Black and New Haven Conn.: Yale University Press, London.

Christensen, C., 1912. On the ferns of the Seychelles and the Aldabra Group. Transactions of the Linnean Society of London 1, 409-425.

Colonna, M.J., Casanova, J., Dullo, W.C., Camoin, G., 1996. Sea-level changes and $\partial$18O record for the past 34,000 yr from Mayotte reef, Indian Ocean. Quaternary Research 46, 335-339.

Courchamp, F., Chapuis, J.L., Pascal, M., 2003. Mammal invaders on islands: impact, control and control impact. Biological Reviews 78, 347-383.

Debouf, D., Bachelery, P., Sigmarsson, O., 2005. Importance of extensional tectonics in Comorean archipelago magmatism: the case of Mayotte Island (Mozambique Channel, Indian Ocean). Geophysical Research Abstracts 7, 05538, SRef-ID: 1607-7962/gra/EGU1605-A-05538. European Geosciences Union.

Debruyne, R., Poinar, H.N., 2009. Time Dependency of Molecular Rates in Ancient DNA Data Sets, A Sampling Artifact? Systematic Biology 58, 348-360.

Dijkstra, K.-D.B., 2007. Gone with the wind: westward dispersal across the Indian Ocean and island speciation in Hemicordulia dragonflies (Odonata: Corduliidae). Zootaxa 1493, 27-48.

Dobzhansky, T., 1937. Genetics and the origin of species. Columbia University Press, New York.

Donoghue, M.J., Moore, B.R., 2003. Toward an Integrative Historical Biogeography. Integrative and Comparative Biology 43, 261-270.

Donoghue, P.C.J., Benton, M.J., 2007. Rocks and clocks: calibrating the Tree of Life using fossils and molecules. Trends in Ecology and Evolution 22, 424-431.

Dowling, H., 1990. Taxonomic status and relationships of the genus Lycognathophis. Herpetologica 46, 60-66.

Drummond, A.J., Ho, S.Y., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol 4, e88.

Duncan, R.A., 1990. The volcanic record of the Réunion hotspot. In: Duncan, R.A., Backman, J., Peterson, L.C., Baker, P.A., Baxter, A.N., Boersma, A.W., Cullen, J.L., Droxler, A.W., Fisk, M.R., Greenough, J.D., Hargreaves, R.B., Hempel, P., Hobart, M.A., Hurley, M.T., Johnson, D.A., Macdonald, A.H., Mikkelsen, N., Okada, H., Rio, D., Robinson, S.G., Schneider, D., Swart, P.K., Tatsumi, Y., Vandamme, D., Vilks, G., Vincent, E. (Eds.), Proceedings of the Ocean Drilling Program, Scientific Results, 115. Ocean Drilling Program, College Station, TX, USA, pp. 3-10.

Duncan, R.A., Backman, J., Peterson, L., 1989. Reunion hotspot activity through Tertiary time: initial results from the Ocean Drilling Program, Leg 115. Journal of Volcanology and Geothermal Research 36, 193-198.

Edwards, S.V., 2009. Is a new and general theory of molecular systematics emerging? Evolution 63, 1-19.

Edwards, S.V., Beerli, P., 2000. Perspective: gene divergence, population divergence and the variance in coalescence time in phylogeographic studies. Evolution 54, 1839-1854.

Edwards, S.V., Liu, L., Pearl, D.K., 2007. High-resolution species trees without concatenation. Proceedings of the National Academy of Sciences of the United States of America 104, 5936-5941.

Emerick, C.M., Duncan, R.A., 1982. Age progressive volcanism in the Comores Archipelago, western Indian Ocean and Implications for Somali plate tectonics. Earth and Planetary Science Letters 60.

Emerson, B.C., 2007. Alarm bells for the molecular clock? No support for Ho et al.'s model of time-dependent molecular rate estimates. Systematic Biology 56, 337-345.

Flynn, J.J., Wyss, A.R., 2003. Mesozoic terrestrial vertebrate faunas: the early history of Madagascar's vertebrate diversity. In: Goodman, S.M., Benstead, J.P. (Eds.), The Natural History of Madagascar. The University of Chicago Press, pp. 34-40.

Gardiner, J.S., 1936. Concluding remarks on the distribution of the land and marine fauna, with a list of the water temperature observations. Transactions of the Linnean Society of London 19, 447-464.

Gardner, A.S., 1987. The systematics of the *Phelsuma madagascariensis* species group of day geckos (Reptilia: Gekkonidae) in the Seychelles. Zoological Journal of the Linnean Society 91, 93-105.

Garth, J.S., 1984. Brachyuran decapod crustaceans of coral reef communities of the Seychelles and Amirante islands. In: Stoddart, D.R. (Ed.), Biogeography and ecology of the Seychelles Islands. Dr. W. Junk, The Hague, pp. 103-122.

Gerlach, J., 1999. Snails of the genus *Pachnodus* (Mollusca; Gastropoda; Enidae): their origins and evolution. Journal of Biogeography 26, 251-255.

Gerlach, J., 2006. Terrestrial and freshwater Mollusca of the Seychelles islands. Backhuys Publishers, Leiden, The Netherlands.

Gerlach, J., 2007. Terrestrial and freshwater vertebrates of the Seychelles islands. Backhuys Publishers, Leiden, The Netherlands.

Gerlach, J., 2008a. Diptera of the Seychelles Islands. Backhuys Publishers, Leiden, The Netherlands.

Gerlach, J., 2008b. Setting Conservation Priorities – A Key Biodiversity Areas Analysis for the Seychelles Islands. The Open Conservation Biology Journal 2, 44-53.

Gerlach, J., Haas, F., 2008. Orthopteroidea of the Seychelles Islands. Orthopteroidea Leiden, The Netherlands.

Gerlach, J., Matyot, P., 2006. Lepidoptera of the Seychelles Islands. Backhuys Publishers, Leiden, The Netherlands.

Gerlach, J., van Bruggen, A.C., 1999. The Streptaxidae (Mollusca; Pulmonata; Gastropoda) of the Seychelles islands, western Indian Ocean. Zool. Verh. Leiden 328, 1-60.

Gillespie, R.G., Roderick, G.K., 2002. Arthropods on islands: colonization, speciation, and conservation. Annual Review on Entomology 47, 595–632.

Giorgi, L., Borchellini, S., 1998. Carte géologique de l'Ile Rodrigues au 1:25.000. Le schema hydrogeologique. La notice explicative. Ministère Délégué à la Cooperation et de la Francophonie & Geolab, Paris.

Godinot, M., 2006. Lemuriform origins as viewed from the fossil record. Folia Primatologica 77, 446-464.

Goodman, S.M., Benstead, J.P., 2003. The Natural History of Madagascar. The University of Chicago Press.

Graur, D., Martin, W., 2004. Reading the entrails of chickens: molecular timescales of evolution and the illusion of precision. Trends in Genetics 20, 80-86.

Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52, 696-704.

Hajash, A., Armstrong, R.L., 1972. Paleomagnetic and radiometric evidence for the ages of the Comoro islands, west central Indian Ocean. Earth and Planetary Science Letters 16, 231-236.

Hawlitschek, O., 2008. Systematics and distribution of the herpetofauna of the Comoro islands. University of Munich.

Hedges, S.B., Kumar, S., 2004. Precision of molecular time estimates. Trends in Genetics 20, 242-247.

Heled, J., Drummond, A.J., 2008. Bayesian inference of population size history from multiple loci. BMC Evolutionary Biology 8, 289.

Hey, J., 2010. Isolation with Migration Models for more than Two Populations. Molecular Biology and Evolution 27, 905-920.

Hey, J., Machado, C.A., 2003. The study of structured populations--new hope for a difficult and divided science. Nature Review in Genetics 4, 535-543.

Hey, J., Nielsen, R., 2004. Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura* and *D. persimilis*. Genetics 167.

Hey, J., Nielsen, R., 2007. Integration within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. Proceedings of the National Academy of Sciences of the United States of America 104, 2785-2790.

Hickerson, M.J., Carstens, B.C., Cavender-Bares, J., Crandall, K.A., Graham, C.H., Johnson, J.B., Rissler, L., Victoriano, P.F., Yoder, A.D., 2010. Phylogeography's past, present, and future: 10 years after. Molecular Phylogenetics and Evolution 54, 291-301.

Hickerson, M.J., Dolman, G., Moritz, C., 2006. Comparative phylogeographic summary statistics for testing simultaneous vicariance. Molecular Ecology 15, 209-223.

Ho, S.Y.W., Phillips, M.J., 2009. Accounting for Calibration Uncertainty in Phylogenetic Estimation of Evolutionary Divergence Times. Systematic Biology 58, 367-380.

Ho, S.Y.W., Phillips, M.J., Cooper, A., Drummond, A.J., 2005. Time Dependency of Molecular Rate Estimates and Systematic Overestimation of Recent Divergence Times. Molecular Biology and Evolution 22, 1561-1568.

Ho, S.Y.W., Shapiro, B., Phillips, M.J., Cooper, A., Drummond, A.J., 2007. Evidence for time dependency of molecular rate estimates. Systematic Biology 56, 515-522.

Holdhaus, K., 1928. Die geographishe verbreitung der Insekten. In: Schroder, C. (Ed.), Handbuch der Entomologie, pp. 592-1057.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754-755.

Hugall, A.F., Foster, R., Lee, M.S.Y., 2007. Calibration Choice, Rate Smoothing, and the Pattern of Tetrapod Diversification According to the Long Nuclear Gene RAG-1. Systematic Biology 56, 543-563.

Knowles, L.L., Maddison, W.P., 2002. Statistical phylogeography. Molecular Ecology 11, 2623-2635.

Krause, D.W., 2003. Late Cretaceous vertebrates of Madagascar: a window into Gondwanan biogeography at the end of the age of dinosaurs. In: Goodman, S.M., Benstead, J.P. (Eds.), The Natural History of Madagascar. The University of Chicago Press, pp. 40-47.

Krause, D.W., Hartman, J.H., Wells, N.A., 1997. Late Cretaceous vertebrates from Madagascar: Implications for biotic change in deep time. In: Goodman, S.D., Patterson, B.D. (Eds.), Natural Change and Human Impact in Madagascar. Smithsonian Institution Press, Washington, D.C.

Kubatko, L.S., Carstens, B.C., Knowles, L.L., 2009. STEM: species tree estimation using maximum likelihood for gene trees under coalescence. Bioinformatics 25, 971-973.

Kubatko, L.S., Gibbs, H.L., Bloomquist, E.W., 2010. Infering species-level phylogenies using multilocus data for a recent radiation of Sistrurus rattlesnakes. Systematic Biology In press.

Kuhner, M.K., Yamato, J., Felsenstein, J., 1998. Maximum likelihood estimation of population growth rates based on the coalescent. Genetics 149, 429-434.

Liu, L., Pearl, D.K., 2007. Species trees from gene trees: reconstructing Bayesian posterior distributions of a species phylogeny using estimated gene tree distributions. Systematic Biology 56, 504-514.

Louette, M., Meirte, D., Jocqué, R., 2004. La faune terrestre de'l archipel des Comores. MRAC, Ternvuren.

Masters, J.C., de Wit, M.J., Asher, R.J., 2006. Reconciling the origins of Africa, India and Madagascar with vertebrate dispersal scenarios. Folia Primatologica 77, 399-418.

Mayr, E., 1942. Systematics and the origin of species. Columbia University Press, New York.

McCall, R., 1997. Implications of recent geological investigations of the Mozambique Channel for the mammalian colonization of Madagascar. Proceedings of the Royal Society of London Series- B 264, 663-665.

McDougall, I., 1971. The geochronology and evolution of the young volcanic island of R eunion, Indian Ocean. Geochim. Cosmochim. Acta 35, 261-288.

McKenzie, D.P., Sclater, J.G., 1971. The evolution of the Indian Ocean since the late Cretaceous. Geophys. J. R.A.S. 25, 437-528.

Miller, K.G., Kominz, M.A., Browning, J.V., Wright, J.D., Mountain, G.S., Katz, M.E., Sugarman, P.J., Cramer, B.S., Christie-Blick, N., Pekar, S.F., 2005. The Phanerozoic record of global sea-level change. Science 310, 1293-1298.

Mittermeier, R.A., Robles Gil, P., Hoffman, J., Pilgrim, J., Brooks, T., Mittermeier, C.G., Lamoreaux, J., da Fonseca, G.A.B., 2005. Hotspots Revisited: Earth's Biologically Richest and Most Endangered Terrestrial Ecoregions. University of Chicago Press, Chicago.

Montaggioni, L., Nativel, P., 1988. La Réunion, Ile Maurice, Géologie et aperçus biologiques. Masson, Paris.

Montaggioni, L., Nougier, J., 1981. Les enclaves de roches dé tritiques dans les volcans d'Anjouan (Archipel des Comores): origine et interprétation dans le cadre de l'é volution du canal de Mozambique. Bull. Soc. géol. France 23, 595–601.

Moritz, C., 2002. Strategies to protect biological diversity and the evolutionary processes that sustain it. Systematic Biology 51, 238-254.

Münchenberg, T., Wollenberg, K.C., Glaw, F., Vences, M., 2008. Molecular phylogeny and geographic variation of Malagasy iguanas (*Oplurus* and *Chalarodon*). Amphibia-Reptilia 29, 319-327.

Nevill, G., 1869. Aditional notes on the land-shells of the Seychelles islands. Proceedings of the Zoological Society of London 4, 61-66.

Noonan, B.P., Chippindale, P.T., 2006. Vicariant Origin of Malagasy Reptiles Supports Late Cretaceous Antarctic Land Bridge. The American Naturalist 168, 730-741.

Norton, I.O., Sclater, J.G., 1979. A model for the evolution of the Indian Ocean and the break-up of Gondwanaland. Journal of Geophysical Research 84, 6803-6830.

Nougier, J., Cantagrel, J.M., Karche, J.P., 1986. The Comores Archipelago in the western Indian Ocean: volcanology, geochronology, and geodynamic setting. Journal of African Earth Sciences 5, 135-145.

Nussbaum, R.A., 1984. The amphibians of the Seychelles. In: Stoddart, D.R. (Ed.), Biogeography invertebrate and vertebrate taxonomy, ecology and and ecology of the Seychelles Islands. Dr. W. Junk, The Hague, pp. 378-415.

Nussbaum, R.A., Wu, S.H., 1995. Distribution, variation, and systematics of the Seychelles treefrog, *Tachycnemis seychellensis* (Amphibia: Anura: Hyperoliidae). Journal of Zoology 236, 383-406.

O'Neill, C., M¸ller, D., Steinberger, B., 2005. On the uncertainties in hot spot reconstructions and the significance of moving hot spot reference frames. Geochemistry Geophysics Geosystems 6, Q04003.

Oliver, J.C., 2008. AUGIST: inferring species trees while accommodating gene tree uncertainty. Bioinformatics 24, 2932-2933.

Pilipenko, A.I., 1995. Structure of aseismic ridges along the Mascarene–Australia geotraverse (Indian Ocean). Geotectonics 28, 494-504.

Plummer, P.S., 1995. Planet Aldabra. In: Amin, M., Willetts, D., Skerett, A. (Eds.), Aldabra World Heritage Site. Camerapix Publishers International, Kenya: Nairobi, pp. 49-70.

Plummer, P.S., Belle, E.R., 1995. Mesozoic tectono-stratigraphic evolution of the Seychelles microcontinent. Sedimentary Geology 96, 73-91.

Porter, M.L., PÈrez-Losada, M., Crandall, K.A., 2005. Model-based multi-locus estimation of decapod phylogeny and divergence times. Molecular Phylogenetics and Evolution 37, 355-369.

Procter, J., 1984. Vegetation of the granitic islands of the Seychelles. In: Stoddart, D.R. (Ed.), Biogeography and ecology of the Seychelles Islands. Dr. W. Junk, The Hague, pp. 193-207.

Rabinowitz, P.D., Woods, S., 2006. The Africa-Madagascar connection and mammalian migrations. Journal of African Earth Sciences 44, 270-276.

Radtkey, R.R., 1996. Adaptive radiation of day-geckos (*Phelsuma*) in the Seychelles archipelago: a phylogenetic analysis. Evolution 50, 604-623.

Rage, J.-C., 1996. Le peuplement animal de Madagascar: une composante venue de Laurasia est-elle envisageable? In: Lourenço, W.R. (Ed.), Biogéographie de Madagascar. ORSTOM, Paris, pp. 27-35.

Rakotosolofo, N.A., Torsvik, T.H., Ashwal, L.D., Eide, E.A., De Wit, M.J., 1999. The Karoo Supergroup revisited and Madagascar-Africa fits. Journal of African Earth Sciences 29, 135-151.

Reeves, C.V., Sahu, B.K., de Wit, M., 2002. A re-examination of the paleo-position of Africa's eastern neighbours in Gondwana. Journal of African Earth Sciences 34, 101-108.

Rocha, S., Carretero, M.A., Vences, M., Glaw, F., Harris, D.J., 2006. Deciphering patterns of transoceanic dispersal: the evolutionary origin and biogeography of coastal lizards *Cryptoblepharus* in the Western Indian Ocean region. Journal of Biogeography 33, 13-22.

Rocha, S., Vences, M., Glaw, F., Posada, D., Harris, D.J., 2009. Multigene phylogeny of Malagasy day geckos of the genus Phelsuma. Mol Phylogenet Evol 52, 530-537.

Rohling, E.J., Fenton, M., Jorissen, F.J., Bertrand, P., Ganssen, G., Caulet, J.P., 1998. Magnitudes of sea-level lowstands of the past 500,000 years. Nature 394, 162-165.

Saddul, P., 1995. Mauritius - a geomorphological analysis. Mahatma Ghandi Institute, Moka, Mauritius.

Sanderson, M.J., 2002. Estimating Absolute Rates of Molecular Evolution and Divergence Times: A Penalized Likelihood Approach. Mol Biol Evol 19, 101-109.

Schatz, G.E., 1996. Malagasy / Indo-Australo-Malesian phytogeographic connections. In: Lourenço, W.R. (Ed.), Biogéographie de Madagascar. ORSTOM, Paris, pp. 73-88.

Schettino, A., Scotese, C.R., 2005. Apparent polar wander paths for the major continents (200 Ma to the present day): a palaeomagnetic reference frame for global plate tectonic reconstructions. Geophysics Journal International 163, 727-759.

Scott, H., 1933. General conclusions regarding the insect fauna of the Seychelles and adjacent islands. Transactions of the Linnean Society of London 2nd series (Zoology) 19, 307-391.

Ségoufin, J., Munschy, M., Bouysse, P., Mendel, G., Grikurov, G., Leitchenkov, G., Subrahmanyan, C., Chand, S., Pubellier, M., Patriat, P., 2004. Map of the Indian Ocean (1:20,000,000), sheet 2, structural map. In: Edition, C. (Ed.), Paris.

Seward, D., Grujic, D., Schreurs, G., 2004. An insight into the breakup of Gondwana: identifying events through low-temperature thermochronology from the basement rocks of Madagascar. Tectonics 23, TC3007.

Shapiro, B., Sibthorpe, D., Rambaut, A., Austin, J., Wragg, G.M., Bininda-Emonds, O.R.P., Lee, P.L.M., Cooper, A., 2002. Flight of the Dodo. Science 295, 1683-.

Sheth, H.C., 2005. From Deccan to Réunion: no trace of a mantle plume. In: Foulger, G.R., Natland, J.H., Presnall, D.C., Anderson, D.L. (Eds.), Plates, Plumes, and Paradigms. Geol. Soc. Am. Spec. Pap., pp. 447-501.

Siddall, M., Rohling, E.J., Almogi-Labin, A., Hemleben, C., Meischner, D., Schmelzer, I., Smeed, D.A., 2003. Sea-level fluctuations during the last glacial cycle. Nature 423, 853-858.

Simpson, G.G., 1940. Mammals and land bridges. Journal of the Washington Academy of Sciences 30, 137-163.

Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688-2690.

Stoddart, D.R., Fosberg, F.R., 1984. Vegetation and floristics of the western Indian Ocean coral islands. In: Stoddart, D.R. (Ed.), Biogeography and ecology of the Seychelles Islands. Dr. W. Junk, The Hague, pp. 221-238.

Storey, M., Mahoney, J.J., Saunders, A.D., Duncan, R.A., Kelley, S.P., Coffin, M.F., 1995. Timing of Hot Spot--Related Volcanism and the Breakup of Madagascar and India. Science 267, 852-855.

Summerhayes, V.S., 1931. An enumeration of the angiosperms of the Seychelles archipelago. Transactions of the Linnean Society of London 19, 261-299.

Tattersall, I., 2006. Historical biogeography of the strepsirhine primates of Madagascar. Folia Primatologica 77, 477-487.

Tattersall, I., 2008. Vicariance vs. dispersal in the origin of the Malagasy Mammal Fauna. In: Fleagle, J.G., Gilbert, C.G. (Eds.), Elwyn Simons: A Search for Origins. Springer, New York, pp. 397-408.

Taylor, J.D., Braithwaite, C.J.R., Peake, J.F., Arnold, E.N., 1979. Terrestrial faunas and habitats of Aldabra during the late Pleistocene. Philosophical Transactions of the Royal Society of London B 286, 47-66.

Thomson, J., Walton, A., 1972. Redetermination of the chronology of the Aldabra Atoll by Th/U dating. Nature 240, 145-146.

Thorne, J., Kishino, H., Painter, I., 1998. Estimating the rate of evolution of the rate of molecular evolution. Molecular Biology and Evolution 15, 1647-1657.

Thorne, J.L., Kishino, H., 2002. Divergence time and evolutionary rate estimation with multilocus data. Systematic Biology 51, 689-702.

Torsvik, T.H., Tucker, R.D., Ashwal, L.D., Eide, E.A., Rakotosolofo, N.A., 1998. Late Cretaceous magmatism in Madagascar:

paleomagnetic evidence for a stationary Marion hot spot. Earth and Planetary Science Letters 164, 221-232.

Varón, A., Vinh, L.S., Wheeler, W.C., 2010. POY version 4: phylogenetic analysis using dynamic homologies. Cladistics 26, in press.

Vences, M., 2004. Origin of Madagascar's extant fauna: a perspective from amphibians, reptiles and other non-flying vertebrates. Italian Journal of Zoology 2, 217-228.

Vences, M., Vieites, D.R., Glaw, F., Brinkmann, H., Kosuch, J., Veith, M., Meyer, A., 2003. Multiple overseas dispersal in amphibians. Proceedings of the Royal Society London Series-B 270, 2435-2442.

Vences, M., Wollenberg, K.C., Vieites, D.R., Lees, D.C., 2009. Madagascar as a model region of species diversification. Trends in Ecology and Evolution 24, 456-465.

Verzhbitsky, E.V., 2003. Geothermal regime and genesis of the Ninety-East and Chagos-Laccadive ridges. Journal of Geodynamics 35, 289-302.

Vidal, N., Branch, W.R., Pauwels, O.S.G., Hedges, S.B., Broadley, D.G., Wink, M., Cruaud, C., Joger, U., Nagy, Z.T., 2008. Dissecting the major African snake radiation: a molecular phylogeny of the Lamprophiidae Fitzinger (Serpentes, Caenophidia). Zootaxa 1945, 51-66.

Wagner, M., 1889. Die Entstehung der Arten durch raumliche Sonderung. Benno Schwalbe, Basel, Switzerland.

Wakeley, J., 2004. Recent trends in population genetics: More data! More math! Simple models? Journal of Heredity 95, 397-405.

Wakeley, J., 2008. Coalescent Theory. Roberts and Company Publishers, Greenwood Village, CO.

Warren, B.H., Bermingham, E., Prys-Jones, R.P., Thebaud, C., 2005. Tracking island colonization history and phenotypic shifts in Indian Ocean bulbuls Hypsipetes: Pycnonotidae). Biological Journal of the Linnean Society 85, 271-287.

Warren, B.H., Dominique, S., Bruggemann, J.H., Robert, P.P.-J., Christophe, T., 2010. Why does the biota of the Madagascar region have such a strong Asiatic flavour? Cladistics 26, 526-538.

Whittaker, R.J., Fernández-Palacios, J.M., 2007. Island Biogeography: Ecology, Evolution and Conservation. Oxford University Press, Oxford.

Wiley, E.O., 1988. Vicariance biogeography. Annual Review on Ecology and Systematics 19, 513-542.

Wilmé, L., Goodman, S.M., Ganzhorn, J.U., 2006. Biogeographic Evolution of Madagascar's Microendemic Biota. Science 312, 1063-1065.

Yang, Z., Rannala, B., 2006. Bayesian estimation of species divergence times under a molecular clock using multiple fossil calibrations with soft bounds. Molecular Biology and Evolution 23, 212-226.

Yoder, A.D., Heckman, K., 2006. Mouse lemur phylogeography revises a model of ecogeographic constraint in Madagascar. In: Freagle, J., Lehman, S.M. (Eds.), Primate Biogeography: Progress and Prospects. Kluwer, pp. 255-268.

Yoder, A.D., Nowak, M.D., 2006. Has Vicariance or Dispersal Been the Predominant Biogeographic Force in Madagascar? Only Time Will Tell. Annual Review of Ecology, Evolution, and Systematics 37, 405-431.

Zwickl, D.J., 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. The University of Texas at Austin.

# CHAPTER 2

## PHYLOGENETIC RELATIONSHIPS, COLONIZATION PATTERNS AND SYSTEMATICS OF *Phelsuma* DAY-GECKOS

Available online at www.sciencedirect.com
ScienceDirect
Molecular Phylogenetics and Evolution 43 (2007) 685–692
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Short communication

# Phylogenetic affinities of Comoroan and East African day geckos (genus *Phelsuma*): Multiple natural colonisations, introductions and island radiations

Sara Rocha [a,b,c,*], David Posada [c], Miguel A. Carretero [a], D. James Harris [a,b]

[a] *CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, 4485-661 Vairão, Portugal*
[b] *Departamento de Zoologia e Antropologia, Faculdade de Ciências, Universidade do Porto, Praça Gomes Teixeira 4099-002, Portugal*
[c] *Departamento de Bioquímica, Genética e Inmunología, Facultad de Biología, Universidad de Vigo, Vigo 36310, Spain*

Received 25 April 2006; revised 13 July 2006; accepted 18 July 2006
Available online 26 July 2006

## 1. Introduction

The day-geckos of the genus *Phelsuma* (Gray, 1825) are amongst the most prominent reptiles from the Malagasy region. The whole genus consists of about 42 species, most of them inhabiting exclusively Madagascar. It is generally accepted that *Phelsuma* originated in Madagascar, and that from there they dispersed to several other Indian Ocean islands. In fact, they can be found on essentially every Indian Ocean archipelago: the Comoros, the Mascarenes, the Seychelles, Aldabra and even the Andamans. Having colonised these archipelagos they often radiated, producing several sets of endemic species. One of the species, *P. dubia*, mostly distributed on the western Malagasy coast, can also be found on mainland Africa, in coastal Kenya and Tanzania and in the islands of Zanzibar and Pemba (Loveridge, 1942). The island of Pemba also contains an additional endemic species, *P. parkeri* (Loveridge, 1941).

The Comoro islands, a set of four major volcanic islands located north-west of Madagascar, are inhabited by four endemic species (Fig. 1): *P. v-nigra* (with subspecies *v-nigra*, *anjouanensis*, *comoraegrandensis* and *pasteuri*), *P. nigristriata*, *P. robertmertensi* and *P. comorensis*. Additionally, *P. dubia* and *P. laticauda*, which are also found on Madagascar (and on the East African coast in the case of *P. dubia*), can be found on the Comoros (Glaw and Vences, 1994). This species abundance, as opposed to what happens for example in the Seychelles, inhabited by only two species of *Phelsuma*, is presumably due to the proximity of the Comoro Islands to Madagascar, and reflects once again the propensity of these geckos to long distance over-water dispersal.

In the absence of a thorough phylogenetic analysis, Glaw et al. (1999), in the only, so far, comprehensive study of this genus, defined nine Malagasy species groups, based on phenetic characters: *P. guttata*-group, *P. madagascariensis*-group, *P. lineata*-group, *P. mutabilis*-group, *P. laticauda*-group, *P. klemmeri*-group, *P. dubia*-group, *P. modesta*-group and *P. barbouri*-group.

Previous works by Radtkey (1996) and Austin et al. (2004) showed that both the Seychelles and the Mascarenes sets of species constitute endemic archipelago radiations, single monophyletic units that diversified as a result of both historical and ecological factors. Austin et al. (2004) also provided molecular support for at least one of the phenetic groups previously proposed, supporting close phylogenetic relationships between *P. astriatta*, *P. abbotti* and *P. madagascariensis* (*P. madagascariensis*-group). The other resolved clade in their analysis comprises *P. lineata*, *P. laticauda*, *P. quadriocellata* and *P. serraticauda*, species previously proposed to be included in two groups, *P. lineata*- and *P. laticauda*-group. However, none of these works included Comoroan species of *Phelsuma*. Thus, with the exception of the relationships within the Mascarene *Phelsuma*, the phylogenetic relationships between the extant members of the genus remain, so far, mostly unknown. *P. parkeri*, the endemic species from Pemba Island (off Tanzania) has never been included in any of the previous analyses.

Here, we used mitochondrial DNA sequence data (12S rRNA and cytochrome *b* gene fragments) from Comoroan and African (*P. parkeri*) species, together with previously published sequences from Austin et al. (2004), to assess

* Corresponding author. Fax: +351252661780.
*E-mail address:* sara.rocha@mail.icav.up.pt (S. Rocha).

1055-7903/$ - see front matter © 2007 Published by Elsevier Inc.
doi:10.1016/j.ympev.2006.07.010

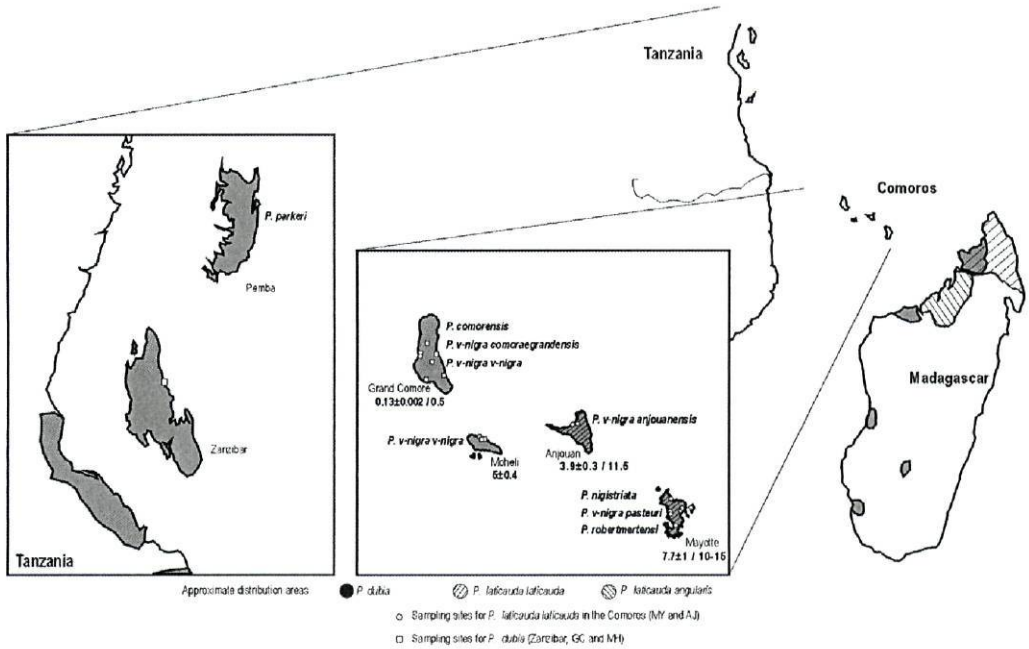



Fig. 1. Distribution of Comoroan and East African *Phelsuma* species sequenced in this study. The approximate distribution of the more widespread species (Glaw and Vences, 1994; Spawls et al., 2001) is indicated, respectively, by grey (*P. dubia*) and striped (*P. laticauda*) areas. Island ages estimates (age of the oldest exposed lavas/estimated age of the volcanic origin of the islands) follow Montaggioni and Nougier (1981); Emerick and Duncan (1982) and Nougier et al. (1986). Small white circles and squares represent, respectively, location of samples of *P. laticauda* and *P. dubia* in the Comoros and Zanzibar.

their position within the genus *Phelsuma* and to obtain broad estimates of within-species divergence of these island endemics. Furthermore, for the widespread species, *P. dubia* and *P. laticauda*, we used cytochrome *b* sequences to analyse the relationships between Malagasy, Comoroan and African haplotypes, in order to determine if these latter localities were the results of natural colonisations or anthropogenic introductions. Likelihood and Bayesian methods, and available information about island ages were also used to estimate divergence times and to test for congruency between previous divergence time estimates of main clades made by Austin et al. (2004) and others obtained using other islands ages as calibration points, and to test for congruence between obtained values using different calibration schemes.

## 2. Materials and methods

Geographic origins of samples used in this study are listed in Table 1. Fifty-eight *Phelsuma* tissue samples were collected from the Comoros archipelago and the East African islands of Zanzibar and Pemba. All the species from these islands are represented, with the exception of *P. nigristriata*, an endemic species from Mayotte Island. Total genomic DNA was extracted from muscle tissue (autotomized tails) preserved in pure ethanol using standard methods (Sambrook et al., 1989). Segments of two mitochondrial genes were amplified (12s rRNA, ~370 bp and cytochrome *b*, 714 bp) using universal primers 12Sa and 12Sb (Kocher et al., 1989) and CBL14753 (Austin et al., 2004) and CBH15579 (5′-TGG GAT TGA TCG TAG GAT GGC GTA-3′, Salvador Carranza, personal communication), respectively. Amplification of the 12S rRNA gene fragment followed Harris et al. (1998) and that of cytochrome *b* (cyt *b*) followed Austin et al. (2004), except that 30 s were used for annealing with temperatures varying between 44 and 53 °C depending on the species. Amplification products were directly purified using a standard enzymatic procedure, except for amplification products of cytochrome *b* from *P. comorensis*, for which unspecific bands of around 300 bp were always present and thus bands of the expected cyt *b* size were cut from the gel and then purified with a gel band purification kit (Amersham, Biosciences). Amplified fragments were sequenced on a 310 Applied Biosystem DNA sequencing apparatus (12S fragments) and on a 3730xl DNA Analyser (cyt *b*) and sequences aligned using the ClustalW (Thompson et al., 1994) application from BioEdit (Hall, 1999) with default parameters, against published sequences of other *Phelsuma* species (Austin et al., 2004). *Lygodactylus* sp., *Rhoptropella ocellata* and *Rhoptropus boultoni* (also from Austin et al., 2004) were used as outgroup.

For the cytochrome *b* gene fragment alignment was unambiguous, while the 12S rRNA gene fragment was more difficult to reliably align between species due to highly variable loop regions in the secondary structure. Thus,

Table 1
Specimens used in this study, respective locations (see Carretero et al., 2005) and accession numbers. First two letters of individual's code correspond to island of origin (AJ = Anjouan, GC = Grand Comoro, MH = Moheli, MY = Mayotte, PB = Pemba and Z = Zanzibar)

| Species | Individual | Location | Accession Number 12S/Cytb |
|---|---|---|---|
| *Phelsuma comorensis* | GC22 | Foret de La Guille | DQ911636/DQ911694 |
| *Phelsuma comorensis* | GC24 | Foret de La Guille | DQ911637/DQ911695 |
| *Phelsuma comorensis* | GC23 | Foret de La Guille | DQ911638/DQ911696 |
| *Phelsuma robertmertensi* | MY73 | Passamenti | DQ911639/DQ911697 |
| *Phelsuma robertmertensi* | MY84 | Longoni | DQ911640/DQ911698 |
| *Phelsuma robertmertensi* | MY85 | Longoni | DQ911641/DQ911699 |
| *Phelsuma robertmertensi* | MY23 | M'zouazia | DQ911642/DQ911700 |
| *Phelsuma robertmertensi* | MY24 | M'zouazia | DQ911643/DQ911701 |
| *Phelsuma dubia* | GC9 | Moroni | DQ911644/DQ911702 |
| *Phelsuma dubia* | GC32 | Itsoundzou | DQ911645/DQ911703 |
| *Phelsuma dubia* | GC44 | Foumbouni | DQ911646/DQ911704 |
| *Phelsuma dubia* | MH5 | Fomboni | DQ911647/DQ911705 |
| *Phelsuma dubia* | MH33 | Mbouerani | DQ911648/DQ911706 |
| *Phelsuma dubia* | Z33 | Kiwengwa, Zanzibar (Tanzania) | DQ911649/DQ911707 |
| *Phelsuma dubia* | GC38 | Mouadzazi | DQ911650/DQ911708 |
| *Phelsuma dubia* | GC39 | Mouadzazi | DQ911651/DQ911709 |
| *Phelsuma dubia* | GC50 | Konbani | DQ911652/DQ911710 |
| *Phelsuma dubia* | GC53 | Mvouni | DQ911653/DQ911711 |
| *Phelsuma dubia* | MH6 | Fomboni | DQ911654/DQ911712 |
| *Phelsuma dubia* | MH34 | Mbouerani | DQ911655/DQ911713 |
| *Phelsuma dubia* | MH29 | Fomboni | DQ911656/DQ911714 |
| *Phelsuma dubia* | MH36 | Mbatsé | DQ911657/DQ911715 |
| *Phelsuma dubia* | MH38 | Mbatsé | DQ911658/DQ911716 |
| *Phelsuma dubia* | GC62 | Belvedere (Karthala) | DQ911659/DQ911717 |
| *Phelsuma dubia* | GC63 | Belvedere (Karthala) | DQ911660/DQ911718 |
| *Phelsuma dubia* | GC a | Moroni, Pension Laizam | DQ911661/DQ911719 |
| *Phelsuma laticauda* | MY44 | Chiroungoui | DQ911662/DQ911720 |
| *Phelsuma laticauda* | MY6 | Mamoutzu | DQ911663/DQ911721 |
| *Phelsuma laticauda* | AJ35 | Moutsamoudou | DQ911664/DQ911722 |
| *Phelsuma laticauda* | MY7 | Mamoutzu | DQ911665/DQ911723 |
| *Phelsuma laticauda* | MY8 | Mamoutzu | DQ911666/DQ911724 |
| *Phelsuma laticauda* | MY50 | Ouangani | DQ911667/DQ911725 |
| *Phelsuma laticauda* | MY56 | Sada road | DQ911668/DQ911726 |
| *Phelsuma laticauda* | AJ10 | Bazimini | DQ911669/DQ911727 |
| *Phelsuma laticauda* | AJ11 | Bazimini | DQ911670/DQ911728 |
| *Phelsuma laticauda* | MY74 | Passamenti | DQ911671/DQ911729 |
| *Phelsuma laticauda* | MY86 | Longoni | DQ911672/DQ911730 |
| *Phelsuma laticauda* | MY87 | Dzaouzi (near airport) | DQ911673/DQ911731 |
| *Phelsuma laticauda* | (Platic)* | Madagascar | AY221280/AY221386 |
| *Phelsuma v-nigra v-nigra* | MH10 | Badjo, mountain | DQ911674/DQ911732 |
| *Phelsuma v-nigra v-nigra* | MH19 | Gnombéni | DQ911675/DQ911733 |
| *Phelsuma v-nigra v-nigra* | MH22 | Ouanani | DQ911676/DQ911734 |
| *Phelsuma v-nigra anjouanensis* | AJ34 | Houngouni | DQ911677/DQ911735 |
| *Phelsuma v-nigra anjouanensis* | AJ19 | Foret de Moya | DQ911678/DQ911736 |
| *Phelsuma v-nigra comoraegrandensis* | GC10 | Moroni | DQ911679/DQ911737 |
| *Phelsuma v-nigra comoraegrandensis* | GC11 | Moroni | DQ911680/DQ911738 |
| *Phelsuma v-nigra comoraegrandensis* | GC12 | Moroni | DQ911681/DQ911739 |
| *Phelsuma v-nigra comoraegrandensis* | GC31 | Itzanzéni | DQ911682/DQ911740 |
| *Phelsuma v-nigra comoraegrandensis* | GC49 | Bandanadji | DQ911683/DQ911741 |
| *Phelsuma v-nigra comoraegrandensis* | GC51 | Konbani | DQ911684/DQ911742 |
| *Phelsuma v-nigra comoraegrandensis* | GC54 | Mvouni | DQ911685/DQ911743 |
| *Phelsuma v-nigra comoraegrandensis* | GC55 | Mvouni | DQ911686/DQ911745 |
| *Phelsuma v-nigra comoraegrandensis* | GC56 | Mvouni | DQ911687/DQ911744 |
| *Phelsuma v-nigra comoraegrandensis* | GC65 | Belvedere (Karthala) | DQ911688/DQ911746 |
| *Phelsuma v-nigra comoraegrandensis* | GC66 | Belvedere (Karthala) | DQ911689/DQ911747 |
| *Phelsuma v-nigra comoraegrandensis* | GC 21 | Mouadja | DQ911690/DQ911748 |
| *Phesuma v-nigra pasteuri* | MY65 | Koualé | DQ911691/DQ911749 |
| *Phelsuma parkeri* | PB15 | Jondeni, Pemba (Tanzania) | DQ911692/DQ911750 |
| *Phelsuma parkeri* | PB17 | Jondeni, Pemba (Tanzania) | DQ911693/DQ911751 |
| *Phelsuma standingi* | * | Madagascar | AY221281/AY221387 |
| *Phelsuma astriatta* | * | Mahé, Seychelles | AY221273/AY221379 |
| *Phelsuma abbotti* | * | Aldabra attol | AY221285/AY221391 |

(*continued on next page*)

Table 1 (*continued*)

| Species | Individual | Location | Accession Number 12S/Cytb |
|---|---|---|---|
| *Phelsuma madagascariensis kochi* | * | Madagascar | AY221276/AY221382 |
| *Phelsuma madagascariensis grandis* | * | Madagascar | AY221274/AY221380 |
| *Phelsuma madagascariensis grandis* | * | Madagascar | AY221275/AY221381 |
| *Phelsuma andamanensis* | * | Windoor, Andamanes | AY221277/AY221383 |
| *Phelsuma mutabilis* | * | Madagascar | AY221272/AY221378 |
| *Phelsuma guentheri* | * | Mascarenes | AY221311/AY221442 |
| *Phelsuma borbonica borbonica* | * | Mascarenes | AY221289/AY221395 |
| *Phelsuma cepediana* | * | Mascarenes | AY221294/AY221400 |
| *Phelsuma guimbeaui* | * | Mascarenes | AY221330/AY221461 |
| *Phelsuma rosagularis* | * | Mascarenes | AY221327/AY221458 |
| *Phelsuma ornata ornata* | * | Mascarenes | AY221323/AY221454 |
| *Phelsuma serraticauda* | * | Madagascar | AY221278/AY221384 |
| *Phelsuma quadriocellata* | * | Madagascar | AY221282/AY221388 |
| *Phelsuma lineata* | * | Madagascar | AY221279/AY221385 |
| *Rhoptropella ocellata* | * | SW Africa | AY221271/AY221377 |
| *Rhoptropus boultoni* | * | Twyfelfontein, Namibia | AY221269/AY221375 |
| *Lygodactylus sp.* | * | Southern Africa | AY221270/AY221376 |

* Sequences from Austin et al. (2004).

GBlocks v0.91b (Castresana, 2000) was used to eliminate poorly aligned positions and extremely variable regions, with the following parameters: IS = 25/FS = 30/CP = 2/BL2 = 5/allowed gap position = with half; resulting in a 341 bp alignment. The Akaike Information Criterion (AIC) in Modeltest3.06 (Posada and Crandall, 1998; Posada and Buckley, 2004) was used to select an appropriate model of nucleotide substitution given the model likelihoods estimated in PAUP* (Swofford, 2000). Sequence datasets were analyzed both separately (each gene) and combined (1055 bp). For phylogeny estimation sequences from both mitochondrial genes were concatenated and collapsed into haplotypes using Collapse1.2 (a program by D. Posada, available at http://darwin.uvigo.es) with gaps treated as a fifth state. Generated datasets were used to reconstruct phylogenies using Maximum Likelihood (ML) and Bayesian (BMCMC) methods, as implemented in PHYML (Guindon and Gascuel, 2003) and MrBayes (Huelsenbeck and Ronquist, 2001) v3.1, respectively. Confidence in the ML resulting relationships was assessed using the nonparametric bootstrap technique (1000 bootstrap replicates). For the BMCMC, a combined analysis was performed in which each mitochondrial gene had independent substitution parameters and branch lengths, but the same underlying topology. Two runs with four independent chains (with default heating values) were implemented, and checked for convergence. Both in ML and BMCMC analyses model parameters values were treated as unknown and estimated during the runs. For the BMCMC, each Markov chain started with a random tree and was run for $11 \times 10^6$ generations, sampling every 1000 generations. Burnin values were always of 1001 trees, long after the log-likelihood of each run reached stability. The posterior probabilities (pP) for individual clades obtained from each analysis were checked for congruence and both analysis combined and summarized in a 50% majority-rule consensus.

Clock-like evolution of mtDNA sequences was tested by comparing likelihood scores of ML trees unconstrained and under the molecular clock. In each case, the molecular clock was rejected, and therefore divergence times were estimated using the Bayesian method of Thorne and Kishino (2002) implemented in the multidivtime package (http://statgen.ncsu.edu/thorne/multidivtime.html). This program can accommodate multiple calibration points and multiple genes, which are expected to provide improved estimates of both divergence time and rates of evolution. In this method, branch lengths are estimated without assuming a molecular clock and then times and rates are estimated by minimizing the discrepancies in branch lengths and the rate changes over branches. This method also has the added advantage of allowing the specified calibrations as upper or lower bounds on node ages. As prior ages we adopted 5 million years for the ingroup root depth (rttm, SD = 5 Myr), following Austin et al. (2004), and 0.06 substitution per site per million year for the rate at the root node (rtrate), also with a standard deviation of 0.06 (rtratesd). The rtrate and rtratesd parameters were estimated as suggested in the multidivtime manual (Rutshmann, 2005). The Brownian rate parameter was set to the default value. The MCMC analysis involved an initial burn-in of 100,000 cycles, after which the chain was sampled 10,000 times, every 100th cycle. The R8s (version 1.7) computer program (Sanderson, 1997, 2002) was also used to obtain rough estimates of divergence times, using the same calibration schemes. This program implements several methods for estimating absolute rates of molecular evolution, ranging from standard maximum likelihood methods to more experimental semiparametric and nonparametric methods that relax the stringency of the clock assumptions using smoothing methods. One of the advantages of this program is that it provides a cross-validation test that allows the user to explore the fidelity with which any of these methods explains the branch length variation, by removing each terminal branch in turn, estimating the remaining parameters of the model without the branch, predicting the expected numbers of substitution on the pruned branch and reporting the performance of this

predictions as a cross validation score. This allows the user to select the method that best explains the branch length variation (Sanderson, 2002).

Calibration times for the divergence time analysis were accommodated differently in each analysis. Island ages represent maximum ages for colonisation, i.e. upper calibration limits, the maximum age for monophyletic group inhabiting that respective island. Multidivtime allows the specification of calibration points as upper and lower limits on node ages, but it needs at least one upper and one lower limit to provide reasonable age estimates. Thus, in the absence of any appropriate lower age limit, we decided to use each island age and island age –0.5 million years as upper and lower bounds on age node priors. When using r8s to estimate node ages we introduced island ages as fixed calibration points.

For the widespread species *P. laticauda* and *P. dubia*, and because the observed level of divergence was low, haplotype networks were constructed using the program TCS v1.21 (Clement et al., 2000).

## 3. Results and discussion

### 3.1. Phylogenetic analysis

According to the AIC; the General Time Reversible model (GTR, e.g. Yang, 1994) was the best fitting model, with a gamma distribution (+G) for rate heterogeneity for 12S, and with both G and a proportion of invariable sites (+I) for Cyt *b*.

Both ML and BMCMC analyses of the combined dataset yielded highly similar estimates of the phylogenetic relationships (Fig. 2a). Although deeper relationships could not be resolved, having very low bootstrap support values (not shown), the phylogenetic affinities of the majority of the Comoroan and African species with remaining *Phelsuma*

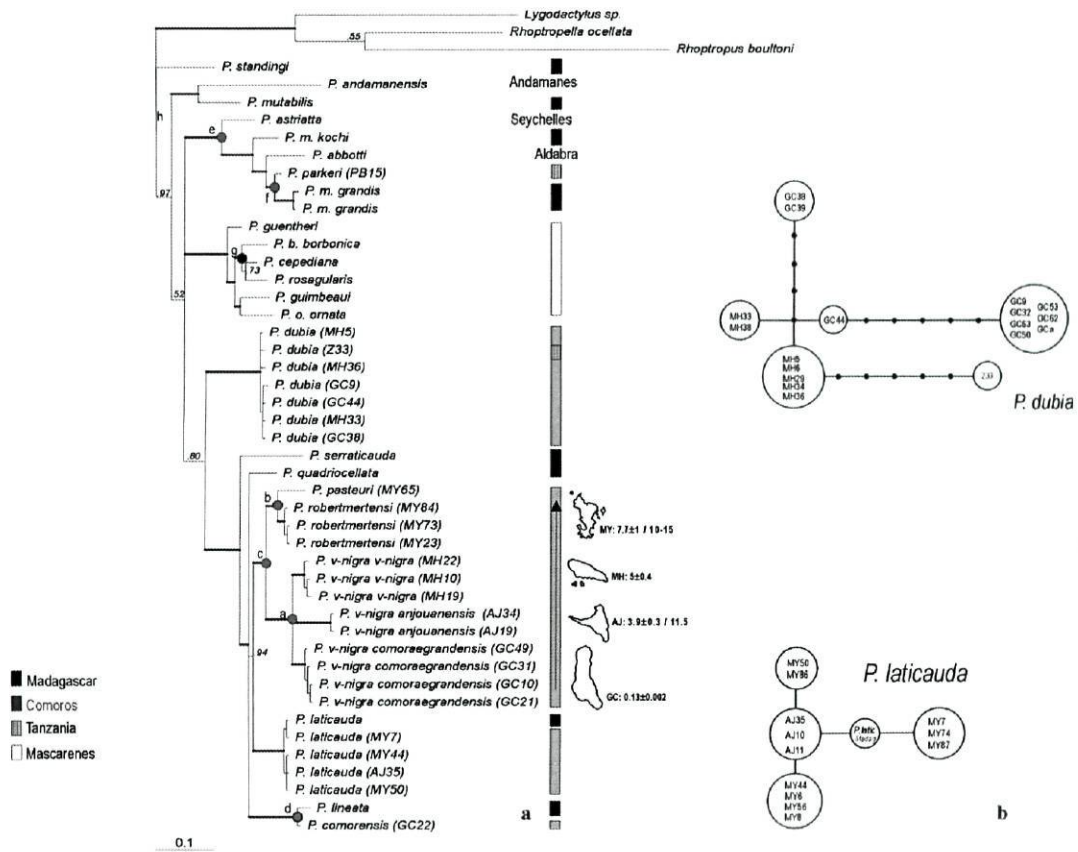



Fig. 2. (a) Phylogenetic relationships of the studied *Phelsuma* species inferred from Bayesian analysis of 1055 bp of mtDNA data. Branches in bold indicate well supported nodes with Bayesian Posterior probabilities (pP) of 1. Other important pP values are signalled in the figure (in italics). The Maximum Likelihood analysis (not shown) resulted in a similar tree topology with all the supported groups being the same. The black dot indicates the main calibration node (2.1 Myr) and grey nodes (numbered) indicate nodes for which age estimates are given, both from multidivtime and r8s software packages. Results of age estimates can be found on Table 2. (b) Haplotype networks for *Phelsuma laticauda* and *Phelsuma dubia* based on 714 bp of cytochrome *b*. Circle size is proportional to the number of individuals exhibiting the haplotype and individual code is given inside each circle (sample locations given in Table 1 and shown in Fig. 1). Small black dots represent missing/not sampled haplotypes.

included in the analysis could be estimated with high confidence. According to these analyses, *Phelsuma parkeri*, the endemic species from Pemba Island (Tanzania) clearly belongs to the *P. madagascariensis*-group (with *P. astriatta*; *P. madagascariensis* and *P. abbotti*), being consistently placed within *P. madagascariensis*, more closely related to *P. madagascariensis grandis*, from north Madagascar from which it differs by 6.77%, compared to the 12.7% sequence divergence between *P. madagascariensis grandis* and *P. madagascariensis kochi* (12S and Cyt *b*, uncorrected *p*-distances). This is the second species, together with *P. abbotti*, to be placed within *P. madagascariensis*. With four subspecies with allopatric distributions, *P. madagascariensis* is one of the most widely distributed species in Madagascar, and again here, evidence of its paraphyly further supports the treatment of at least *P. m. grandis* and *P. m. kochi* as separate species. The status of other extant subspecies remains unknown pending further molecular studies. *Phelsuma parkeri* thus represents another example within this genus of an ancient and natural transmarine dispersal and colonisation, in this case from Madagascar to Pemba Island.

The Comoroan endemic species *P. v-nigra* and *P. robertmertensi,* interestingly, do form a single clade, with very high support values. This clade is further subdivided in two groups also strongly supported: *P. (v-nigra) pasteuri* with *P. robertmertensi* (with a 1.0 posterior probability and 0.95 bootstrap value (not shown)), and the remaining subspecies of *P. v-nigra*. The nucleotide sequence divergence between *P. (v-nigra) pasteuri* and the other *P. v-nigra* subspecies ranges from 12.4 to 14.3% (uncorr. *p*-dist.), while the distances between *P. v-nigra v-nigra*, *P. v-nigra anjouanensis* and *P. v-nigra comoraegrandensis* vary between 6.5 and 7.8%. The observations above indicate that *P. v-nigra* is also paraphyletic and that *P. (v-nigra) pasteuri* should be treated as a full species, as already proposed by Meirte (2004). *Phelsuma robertmertensi* is thus the most closely related species to *P. pasteuri*, with 9.7–9.9% sequence divergence. With both species being endemic to the island of Mayotte, if this phylogeny estimate continues to be supported with the addition of the remaining species of this genus, it may represent a within-island speciation phenomenon. Within the Comoros, so far, these are the only reptile taxa exhibiting this pattern, since neither *Hemidactylus* (Rocha et al., 2005) nor *Cryptoblepharus* skinks (Rocha et al., 2006) present such a structure. Mayotte has a complex topography, with several volcanoes and also a complex geological history with at least three different active volcanic phases recognized. Lava sample ages span a range of around 5 Myr, with the most recent parts of the island dating from at least $1.53 \pm 0.1$ Myr. *Phelsuma robertmertensi* is found in forested coastal localities while *P. pasteuri* inhabits inland forest and plantations (Carretero et al., 2005), but their distribution ranges are not well known. Without a better knowledge regarding their ecological preferences, if they occur or not in sympatry and if recent volcanic phenomena on Mayotte could have caused isolation of populations, any hypothesis about their speciation mechanism is preliminary. As to *P. v-nigra* subspecies, the branching pattern is not resolved, preventing the reconstruction of the patterns of island colonisation. Taking into account the similar levels of sequence divergence between the subspecies, the three islands might have been colonised by a common ancestor at roughly the same time. Whether these Comoroan species are a monophyletic unit or two, or the identity of their respective sister species, is still not clear, and will only be known when all *Phelsuma* species are included in a comprehensive phylogeny of the genus.

*Phelsuma comorensis*, the endemic species from Grand Comoro, is clearly the sister taxa of *P. lineata*, which comprises five subspecies, three of them with very restricted distributions, while the others, *P. lineata lineata* and *P. lineata elanthana*, are widespread in the eastern Malagasy coast. Nucleotide sequence divergence between *P. comorensis* and the *P. lineata* specimen analyzed is only 3.6%, a very low value, compared to the degree of divergence between other species or even subspecies, which implies that a more recent colonisation and speciation event gave rise to this Grand Comoro endemism.

Concerning *P. dubia* and *P. laticauda*, both present in Madagascar and the Comoros, and in the case of *P. dubia,* also in the East African coast, the levels of differentiation between individuals from different locations/islands is minimum, without any clear geographic structure. Such a pattern most probably reflects very recent human-aided dispersals, rather than natural colonisations (Fig. 2b). Nevertheless, within *P. dubia* the level of differentiation is higher than within *P. laticauda*, where all the haplotypes are just one mutation step away from the others (cytochrome *b* data). *P. laticauda laticauda* (the same subspecies that occurs on Comoros) has a very restricted distribution in the humid regions of northern Madagascar, while *P. dubia* occurs along the western coast of Madagascar, possibly with disjunct populations (Glaw and Vences, 1994). Therefore, it is not surprising that, despite having originated from recent introductions, the Comoroan populations of *P. dubia* exhibit more genetic variation than *P. laticauda*, simply reflecting the diversity within the "source" populations. Both species are the ones with the largest distribution areas outside Madagascar, and *P. laticauda* has been introduced even onto the Hawaii islands. It would be interesting to further examine African populations of *P. dubia* to accurately understand their origin. Certainly, the fact that both species inhabit a wide variety of habitats, including human buildings, with palms and banana trees as their preferred vegetation (Carretero et al., 2005), influences the extent to which they can be subject to human-aided dispersals, making them some of the most prone species to this kind of phenomena within the genus.

### 3.2. Divergence time estimates

For the relaxed Bayesian analyses performed in multidivtime, as mentioned above, we allowed some variation in island colonisation age; specifying each calibration as an

Table 2
Posterior estimates of divergence ages (±standard deviation) and 95% credibility intervals for some of the nodes inferred from the concatenated dataset using the Bayesian relaxed molecular clock implemented in multidivtime and the Langley–Fitch method implemented in r8s

| Clade, node id | Calibration | | | | | |
|---|---|---|---|---|---|---|
| | 2.1–1.6 Myr on node g *(2.1 on node g for LF)* | | 2.1–1.6 Myr on node g 0.5–0 Myr on node d *(2.1 on node g and 0.5 on node d for LF)* | | 0.5–0 Myr on node d *(0.5 on node d for LF)* | |
| | Age ± SD | 95 % CI | Age ± SD | 95 % CI | Age ± SD | 95 % CI |
| Bayesian relaxed clock (MULTIDIVTIME) *Likelihood LF (r8s) (estimated ages only)* | | | | | | |
| *P. v-nigra*, **a** | 1.12 ± 0.53 *1.26* | [0.38–2.39] | 1.10 ± 0.46 *1.23* | [0.35–2.1] | 0.32 ± 0.28 *1.02* | [0.05–1.04] |
| *P. robertmertensi* + *P. pasteuri*, **b** | 1.05 ± 0.54 *1.99* | [0.3–2.33] | 0.95 ± 0.5 *1.94* | [0.28–2.08] | 0.29 ± 0.26 *1.94* | [0.05–1] |
| *P. robertmertensi* + *P. pasteuri* + *P. v-nigra*, **c** | 1.64 ± 0.68 *2.98* | [0.65–3.29] | 1.49 ± 0.59 *2.90* | [0.61–2.96] | 0.47 ± 0.39 *2.39* | [0.06–0.41] |
| *P. lineata* + *P. comorensis*, **d** *(calibration)* | 0.48 ± 0.36 *0.62* | [0.03–1.4] | 0.27 ± 0.13 — | [0.02–0.48] | 0.13 ± 0.1 — | [0.06–0.4] |
| *P. astriatta*, **e** | 3.18 ± 1.05 *4.96* | [1.62–5.62] | 3.02 ± 0.95 *4.84* | [1.57–5.31] | 0.97 ± 0.73 *3.99* | [0.21–2.80] |
| *P. parkeri*, **f** | 1.03 ± 0.53 *1.40* | [0.28–2.36] | 0.98 ± 0.50 *1.37* | [0.26–2.18] | 0.31 ± 0.27 *1.13* | [0.04–0.99] |
| *P. borbonica*, **g** *(calibration)* | 1.81 ± 0.14 — | [1.61–2.08] | 1.8 ± 0.14 — | [1.61–2.08] | 0.45 ± 0.4 *1.68* | [0.07–1.34] |
| Ingroup root, **h** | 4.95 ± 1.43 *6.95* | [2.91–8.44] | 4.68 ± 1.28 *6.7* | [2.82–7.77] | 1.55 ± 1.10 *5.59* | [0.35–4.29] |

interval, with island age as the upper limit and island age—0.5 Myr as the lower limit. Thus, we used the same calibration point of Austin et al. (2004), i.e., 2.1 Myr of the oldest rocks of Reunion under the assumption that the ancestor of *P. borbonica* colonised it soon after island formation, and estimates for remaining island colonisations (Comoros) were obtained. Additionally we used the estimated age of the volcanic origin of Grand Comoro (0.5 Myr) as a calibration point on node d (*P. lineata* + *P. comorensis*, Fig. 2), assuming that *P. comorensis* colonised Grand Comoro also soon after its emergence. Node ages were estimated using either each calibration point alone or both together. The cross-validation test implemented in R8s indicated that branch length variation was explained with the highest fidelity by the Langley–Fitch method (LF) (Langley and Fitch, 1973, 1974), which uses maximum likelihood to reconstruct divergence times under the assumption of a molecular clock. As a result of that, the LF method was run with the TN algorithm (which is the best and fastest for use with LF—Sanderson, 2004). Ages of some relevant nodes obtained under the different methods and calibration schemes are given on Table 2.

Our absolute age estimates do vary considerably depending on the calibration point and the method used, but this variation is higher for deeper nodes than for recent ones, where this variability is considerably reduced. However, relative ages are somewhat consistent across methods and calibrations. Number and phylogenetic distribution of calibrations are known to severely affect estimates, and using only younger calibrations may result in severe underestimation of older node ages (Porter et al., 2005). The lack of consistency in our estimates is most likely due to the lack of more calibrations points, and therefore results must be taken cautiously. Nevertheless, these estimates are roughly in agreement with previous studies that imply that diversification of main *Phelsuma* clades should have happened around 5 Myr ago (Austin et al., 2004). The time estimates for the nodes leading to the Comoroan species are all very young, in comparison to island ages (Table 2), with the single exception of the node leading to *P. comorensis*, which is dated to around 0.5 Myr (when not set as a calibration), i.e., soon after the island emergence. All the estimates of the age of the *P. v-nigra* clade were at most 1.26 Myr, and the splitting of *P. robertmertensi* and *P. pasteuri* does not seem to be older than 2 Myr. Even if we consider this set of three species as a real monophyletic group, age estimates are not older than 3 Myr, much later than islands emergence: minimum age estimates for Mayotte (*P. pasteuri* and *P. robertmertensi*) are around 7 Myr old and for Anjouan and Moheli of around 3.9 and 5 Myr old, respectively. Also, the colonisation of Pemba by the ancestor of *P. parkeri* seems to have happened around 1 Myr ago.

## 4. Conclusions

The Comoro islands are inhabited by at least five endemic species: *P. robertmertensi*, *P. pasteuri*, *P. v-nigra*, *P. comorensis* and *P. nigristriata*. While *Phelsuma* from the Mascarenes and the Seychelles form apparently monophyletic groups, at least two, and possibly three colonisation events from Madagascar explain the origin of the studied Comoroan endemic species, with the phylogenetic position of *P. nigristriata* still unknown. *Phelsuma dubia* and *P. laticauda* populations lack any kind of geographic structure, reflecting patterns more probably related with multiple recent and/or anthropogenic movements. *Phelsuma parkeri*,

the endemic species from Pemba Island (Tanzania) has its closest relatives within *P. madagascariensis*, a widespread and subspecies-rich group from Madagascar, representing another independent transmarine colonisation. All these colonisation events are relatively young when compared to islands age, with the possible exception of the ancestor of *P. comorensis* that appears to have colonised Grand Comoro soon after its emergence. Age estimates confirm previous results about the age of diversification of main clades within *Phelsuma*, but these estimates have to be taken carefully until a more complete phylogeny is available for this genus.

## Acknowledgments

This project was supported by grants from Fundação para a Ciê ncia e Tecnologia (FCT) POCTI/ BSE/46647/ 2002, SFRH/BPD/5702/2001 (to D.J.H.), SFRH/BPD/3596/ 2000 (to M.A.C.) and SFRH/ BD/ 17541/ 2004 (to S.R.). Part of this work was accomplished during a long-term visit of S.R. at the University of Vigo (Spain). Thanks to Salvador Carranza, to Paloma Morán for providing the laboratory facilities, to Nieves Santamaría and colleagues at the University of Vigo for help and advice during experimental work.

## References

Austin, J.J., Arnold, E.N., Jones, C.G., 2004. Reconstructing an island radiation using ancient and recent DNA: the extinct and living day geckos (*Phelsuma*) of the Mascarene islands. Mol. Phylogenet. Evol. 31, 102–109.

Carretero, M.A., Harris, D.J., Rocha, S., 2005. Recent observations of reptiles in the Comoro islands (Western Indian Ocean). Herpetol. Bull. 91, 19–28.

Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. 17, 540–552.

Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9, 1650–1667.

Emerick, C.M., Duncan, R.A., 1982. Age progressive volcanism in the Comores Archipelago, western Indian Ocean and Implications for Somali plate tectonics. Earth Planet Sci. Lett. 60, 415–428.

Glaw, F., Vences, M., 1994. A Fieldguide to the Amphibians and Reptiles of Madagascar, Second Ed. Vences and Glaw Verlags GbR, Kö ln. 480 pp.

Glaw, F., Vences, M., Ziegler, T., 1999. Bemerkungen zu *Phelsuma dubia* (Boettger, 1881): Wiederentdeckung des Holotypus, Verwandtschaftsbeziehungen und Daten zur Fortpflanzung. Salamandra 35, 267–278.

Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.

Hall, T.A., 1999. BioEdit: a user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids Symp. Ser. 41, 95–98.

Harris, D.J., Arnold, E.N., Thomas, R.H., 1998. Relationships of lacertid lizards (Reptilia: Lacertidae) estimated from mitochondrial DNA sequences and morphology. Proc. R. Soc. Lond. B 265, 1936–1948.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C., 1989. Dynamics of mitochondrial DNA evolution in mammals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA 86, 6196–6200.

Langley, C.H., Fitch, W., 1973. The constancy of evolution: a statistical analysis of a and b haemoglobins, cytochrome *c*, and fibrinopeptide A. In: Morton, N.E. (Ed.), Genetic Structure of Populations. University of Hawaii Press, Honolulu, pp. 246–262.

Langley, C.H., Fitch, W., 1974. An estimation of the constancy of the rate of molecular evolution. J. Mol. Evol. 3, 161–177.

Loveridge, A., 1941. New geckos (*Phelsuma* and *Lygodactylus*), snake (*Leptotyphlops*), and frog (*Phrynobatrachus*) from Pemba Island. East Africa Proc. Biol. Soc. Wash. 54, 175–178.

Loveridge, A., 1942. Revision of the Afro-oriental geckos of the genus *Phelsuma*. Bull. Mus. Comparat. Zool., Cambridge Mass. 89, 338–343.

Meirte, D., 2004. Les Reptiles. La faune terrestre de l'Archipel des Comores (ed. by M. Louette, D. Meirte and R. Jocque'), pp. 209–210. Studies in Afrotropical Zoology, 293, MRAC, Tervuren.

Montaggioni, L., Nougier, J., 1981. Les enclaves de roches dé tritiques dans les volcans d'Anjouan (Archipel des Comores): origine et interpré tation dans le cadre de l'é volution du canal de Mozambique. Bull. Soc. géol. France 23, 595–601.

Nougier, J., Cantagrel, J.M., Karche, J.P., 1986. The Comores Archipelago in the western Indian Ocean: volcanology, geochronology, and geodynamic setting. J. Afr. Earth Sci. 5, 135–145.

Posada, D., Buckley, T.R., 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over Likelihood ratio tests. Syst. Biol. 53, 793–808.

Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Porter, M., Pérez-Losada, M., Crandall, K.A., 2005. Model based multilocus estimation of decapod phylogeny and divergence times. Mol. Phylogenet. Evol. 37, 355–369.

Radtkey, R.R., 1996. Adaptive radiation of Day-Geckos (*Phelsuma*) in the Seychelles archipelago: a phylogenetic analysis. Evolution 50, 604–623.

Rocha, S., Carretero, M.A., Harris, D.J., 2005. Diversity and phylogenetic relationships of *Hemidactylus* geckos from the Comoro islands. Mol. Phylogenet. Evol. 35, 292–299.

Rocha, S., Carretero, M.A., Vences, M., Glaw, F., Harris, D.J., 2006. Deciphering patterns of transoceanic dispersal: the evolutionary origin and biogeography of coastal lizards (*Cryptoblepharus*) in the Western Indian Ocean region. J. Biogeogr. 33, 13–22.

Rutshmann, F., 2005. Bayesian Molecular dating using PAML/multidivtime. A step-by-step manual. University of Zurich, Switzerland. Available at http://www.plant.ch.

Sambrook, J., Fritsh, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual, second ed. Cold Spring Harbor Press, New York.

Sanderson, M.J., 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. Mol. Biol. Evol. 14, 1218–1231.

Sanderson, M.J., 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. Mol. Biol. Evol. 19, 101–109.

Sanderson, M.J., 2004. r8s, version 1.70 User's manual (available at http://ginger.ucdavis.edu/r8s/).

Spawls, S., Howell, K., Drewes, R.C., Ash, J., 2001. Field Guide to the Reptiles of East Africa: All the Reptiles of Kenya, Tanzania, Uganda, Rwanda and Burundi. Princeton University, Princeton, USA.

Swofford, D.L., 2000. PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods). Sinauer Associates, Sunderland, Massachusetts.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

Thorne, J., Kishino, H., 2002. Divergence time and evolutionary rate estimation with multilocus data. Syst. Biol. 51, 689–702.

Yang, Z., 1994. Estimating the pattern of nucleotide substitution. J. Mol. Evol. 39, 105–111.

Molecular Phylogenetics and Evolution 52 (2009) 530–537

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Short Communication

# Multigene phylogeny of Malagasy day geckos of the genus *Phelsuma*

S. Rocha [a,b,c,*], M. Vences [d], F. Glaw [e], D. Posada [c], D.J. Harris [a,b]

[a] CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Instituto de Ciências Agrárias de Vairão, Rua Padre Armando Quintas, 4485-661 Vairão, Portugal
[b] Departamento de Zoologia e Antropologia, Faculdade de Ciências, Universidade do Porto, Praça Gomes Teixeira 4099-002, Portugal
[c] Departamento de Bioquímica, Genética e Inmunología, Facultad de Biología, Universidad de Vigo, Vigo 36310, Spain
[d] Technical University of Braunschweig, Zoological Institute, Spielmannstr. 8, 38106 Braunschweig, Germany
[e] Zoologische Staatssammlung München, Münchhausenstr. 21, 81247 München, Germany

ARTICLE INFO

*Article history:*
Received 19 November 2008
Revised 18 March 2009
Accepted 29 March 2009
Available online 9 April 2009

## 1. Introduction

Geckos of the genus *Phelsuma* are amongst the most conspicuous lizards of the Malagasy region. The genus contains around 43 extant species, of which ca. 29 occur in Madagascar, and 25 of these are endemic to this island. *Rhoptropella ocellata*, endemic to a small region of Namibia and South Africa is, together with *Lygodactylus* sp., the closest relative of *Phelsuma*, which is considered a monophyletic genus (Austin et al., 2004). With Madagascar as their main centre of diversity, *Phelsuma* are also present on almost all neighbouring islands and locally at the East African coast (Fig. 1). The Comoros harbour seven species (five of them endemic), the Mascarenes have seven endemic extant species (two extinct species are known: *P. gigas* and *P. edwardnewtoni*), with one subspecies endemic to Agalega island (1100 km north of Mauritius), one (non-endemic) species in two islands of the Aldabra archipelago, two endemic species in the granitic islands of the Seychelles, one endemic species in the island of Pemba off the East African coast of Tanzania, and one species in the Andaman islands, off Myanmar, in the Bay of Bengal. Within Madagascar, *Phelsuma* species are found in a variety of habitats spanning the island, mainly in primary forest regions, either dry southern seasonal forests (e.g. *P. breviceps*), scrublands (e.g. *P. mutabilis*, *P. modesta*, *P. hielscheri*), western coastal forests (e.g. *P. abbotti*), low and mid-altitude humid forests (*P. madagascariensis*, *P. guttata*, *P. lineata* ssp., *P. quadriocellata* ssp.) and even in some high-altitude regions (*P. barbouri*, *P. malamakibo*).

Despite extensive work on *Phelsuma* taxonomy, ecology, biogeography and ethology, even its alpha-taxonomy is not fully understood. Many taxa, especially subspecies, are based only on chromatic characters and not well defined. In several cases color transitions/polymorphisms can be observed and thus some species may represent artificial taxa based on local color morphs. Previous studies, based on phenetic characters (Loveridge, 1942; Mertens, 1962; Glaw et al., 1999; Van Heygen, 2004), have led to recognition of eight species groups of Malagasy species. Close relationships between some of the groups were postulated (e.g. *P. guttata*- and *P. madagascariensis*-group and the Mascarene Islands species with the *P. modesta*-group) but some species were not assigned to any particular phenetic group (e.g. *P. vanheygeni*).

The main potential difficulty with *Phelsuma* taxonomy is thus its reliance on highly variable coloration characters, which has led to the description of a large number of species and subspecies. Taxon sampling has always been incomplete in previous molecular studies of *Phelsuma* (Radtkey, 1996; Austin et al., 2004; Sound et al., 2006; Rocha et al., 2007; Raxworthy et al., 2007; Harmon et al., 2008), making it difficult to evaluate intrageneric relationships. Additionally, the validity of many taxa is yet to be assessed using molecular markers.

Here, we present the most comprehensive molecular phylogenetic analysis of *Phelsuma* to date, based on a near-complete taxon sampling of all but two species, as well as most subspecies, and on a multilocus dataset including both fast-evolving mitochondrial genes and more moderate-to-slow-evolving nuclear DNA markers.

## 2. Materials and methods

### 2.1. Biological material, DNA isolation and sequencing

Tissue samples (tail tips) of 104 specimens of *Phelsuma* and five additional taxa (outgroup) were obtained during fieldwork in Madagascar, Seychelles, the Comoros and Tanzania in the period 2000–2006 and preserved in 70–100% ethanol. Additional samples were obtained from breeders. All recognized *Phelsuma* species, with the exception of *P. masohoala* and *P. guimbeaui*,

* Corresponding author. Address: CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Instituto de Ciências Agrárias de Vairão, Rua Padre Armando Quintas, 4485-661 Vairão, Portugal. Fax: +351 252 661 780.
*E-mail address:* sara.rocha@mail.icav.up.pt (S. Rocha).

1055-7903/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2009.03.032

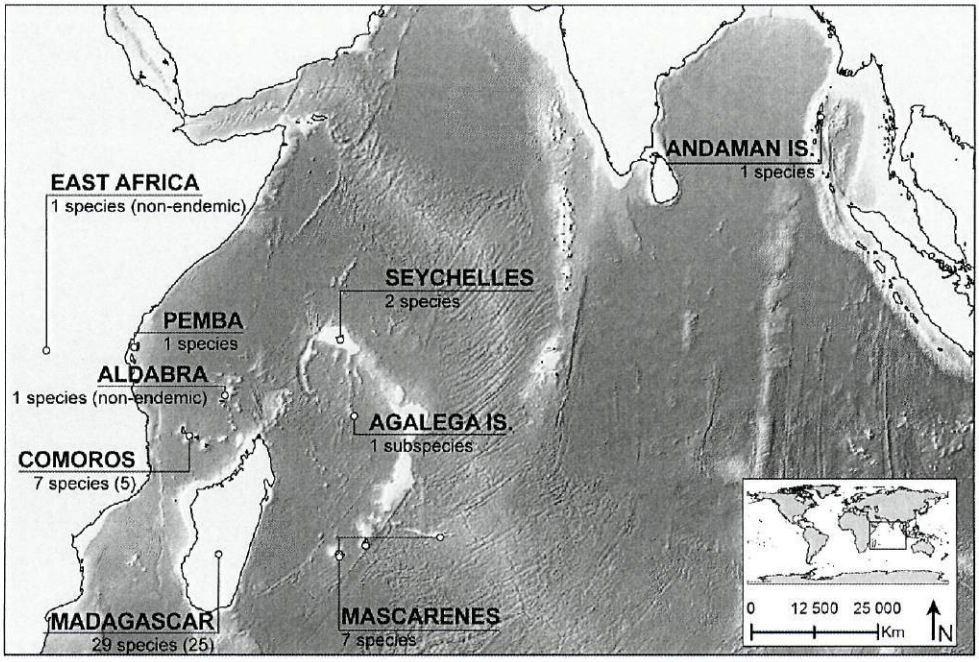



**Fig. 1.** Distribution of *Phelsuma* in the Indian Ocean Region and number of species per island/archipelago. The number of endemic species is given in parenthesis, when different from the total. Lighter (non-delimitated) areas correspond to areas of lower sea-level.

and most recognized subspecies were represented (Table 1). We refrained from including partial sequences of *P. guimbeaui* available from Genbank (cytochrome *b*, 16S rRNA and *C-mos*) to avoid large amounts of missing data in our data set, and because this species clearly belongs into the well-studied Mascarene clade (Austin et al., 2004). For species with ample distributions, an effort was made to include samples from widespread locations to obtain preliminary information on intraspecific variation. DNA extraction followed standard salt or phenol–chloroform protocols (Kocher et al., 1989). Fragments of two mitochondrial (16s rRNA and cytochrome *b*) and three nuclear (*C-mos*, Rag-1 and Rag-2) genes were amplified via PCR. Primers used were 16sA-L and 16sB-H for 16srRNA (Palumbi et al., 1991); forward CBL14753 (Austin et al., 2004) and CBL14841 (Austin et al., 2004, modified from Kocher et al., 1989) and reverse CB3H (Palumbi et al., 1991) and CBH15579 (Rocha et al., 2007) for cytochrome *b*; G73 and G74 (Saint et al., 1998) for *C-mos*; L2408 and H2920 for Rag-1 (Vidal and Hedges, 2004) and Lung35F, Lung320R, Lung460R and 31FNVenk (Hoegg et al., 2004) for Rag-2. Amplified fragments were of 545, 714, 344, 473 and 796 bp, respectively, for 16srRNA, Cyt-*b*, *C-mos*, Rag-1 and Rag-2 fragments. All PCR amplifications were conducted in 25 μl reactions using standard conditions. Annealing temperatures varied between 50 °C and 52 °C for 16srRNA, 42 and 53 °C for Cyt-*b*, 46 and 56 °C for *C-mos*, 52 and 57 °C for Rag-1 and 53 and 57 °C for Rag-2 and magnesium concentration varied between 1 and 4 mM. Amplification products were directly purified using a standard enzyme procedure or were sometimes cut from the gel and then purified using a gel band purification kit (Amersham Biosciences). Amplified fragments were sequenced on an ABI 3730xl automated capillary DNA sequencer. Sequences obtained for this study have been deposited in GenBank under the Accession Nos. FJ829886–FJ830328.

### 2.2. Sequence alignment and phylogenetic analyses

Cytochrome *b*, Rag-1 and Rag-2 gene sequences were aligned manually using BioEdit (Hall, 1999). Within *C-mos*, indels were present and thus nucleotide sequences were translated to amino acid sequences to aid the alignment. The 16srRNA nucleotide dataset contained highly variable regions and many indels, and therefore ProAlign version 0.5a3 (Löytynoja and Milinkovitch, 2003) was used. Hidden Markov Model parameters *delta* and *epsilon* were estimated from the data, while remaining parameters were set to their default values. All the columns with posterior probabilities lower than 90 were removed, for a final alignment of 384 bp. All protein-coding genes were translated to amino acids to check for stop codons that could indicate the presence of pseudogenes.

The concatenated data were filtered to remove redundant sequences, resulting in a data set with 84 sequences. The appropriate model of nucleotide substitution for each gene was determined using PAUP* 4.0b10 (Swofford, 2002) and Modeltest 3.06 (Posada and Crandall, 1998) under the AIC criterion (Akaike, 1974). Models were determined also for each codon position of the protein coding genes. $\chi^2$ tests of sequence composition and likelihood mapping analysis (Strimmer and von Haesler, 1997), which allow an *a priori* examination of conflict versus support signal in molecular sequence data, were performed using Tree-Puzzle 5.0 (Schmidt et al., 2002).

Bayesian phylogenetic analyses were conducted using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001). These began with a random starting tree and were run for 11,000,000 generations (20 million in the case of Cyt-*b*), and sampled every 1000 generations. Multiple runs were always performed. Convergence was checked using AWTY (Wilgenbusch et al., 2004), which provides plots of clade posterior probabilities (PPs) across post-burnin generations (cumulative function). Following Brown and Lemmon (2007), we

**Table 1**
Specimens used in this study, respective locations and Accession Nos.

| Species | Individual | Location | GenBank Accession Nos. | | | | |
|---|---|---|---|---|---|---|---|
| | | | 16s | Cyt-*b* | Rag-1 | Rag-2 | C-*mos* |
| *P. abbotti abbotti* | AD1 | Picard, Aldabra, 09° 24′ 03.8″ S, 46° 12′ 21.6″ E | FJ829886 | FJ829974 | FJ830147 | FJ830238 | FJ830055 |
| *P. abbotti abbotti* | AD36 | Grand Terre, Aldabra | FJ829887 | FJ829975 | FJ830148 | FJ830239 | FJ830056 |
| *P. abbotti chekei* | FGZC 553 | Ankarana, Madagascar | FJ829888 | FJ829976 | FJ830149 | FJ830240 | FJ830057 |
| *P. abbotti chekei* | FGZC 795 | Tsingy de Bemaraha, Madagascar, 18° 42′ 31″ S, 44° 43′ 08″ E | FJ829889 | FJ829977 | FJ830150 | FJ830241 | FJ830058 |
| *P. abbotti chekei* | FGZC 947 | Tsingy de Bemaraha, Madagascar, 18° 47′ 03″ S, 44° 46′ 46″ E; 177 m | FJ829890 | FJ829978 | FJ830151 | FJ830242 | FJ830059 |
| *P. abbotti sumptio* | AP3 | Assumption, 09° 44′ 03.0″ S, 46° 30′ 01.2″ E | FJ829891 | FJ829979 | FJ830152 | FJ830243 | FJ830060 |
| *P. abbotti sumptio* | AP5 | Assumption, 09° 44′ 03.0″ S, 46° 30′ 01.2″ E | FJ829892 | FJ829980 | FJ830153 | FJ830244 | FJ830061 |
| *P. andamanense* | P-111 | Andamans | FJ829893 | FJ829981 | FJ830154 | FJ830245 | FJ830062 |
| *P. andamanense* | Pandamane | Andamans | FJ829894 | FJ829982 | FJ830155 | FJ830246 | FJ830063 |
| *P. antanosy* | FGZC 2647 | Ste. Luce, Madagascar, 24° 46,79′ S, 47° 10,28′ E | FJ829895 | FJ829983 | FJ830156 | FJ830247 | FJ830064 |
| *P. astriata astriata* | AT6 | Astove, 10° 04′ 12.7″ S, 47° 44′ 20.4″ E | FJ829896 | FJ829984 | FJ830157 | FJ830248 | FJ830065 |
| *P. astriata astriata* | AT15 | Astove, 10° 04′ 12.7″ S, 47° 44′ 20.4″ E | FJ829897 | FJ829985 | FJ830158 | FJ830249 | FJ830066 |
| *P. astriata semicarinata* | APH1 | Alphonse, 07° 00′ 31.9″ S, 52° 43′ 50.4″ E | FJ829898 | FJ829986 | FJ830159 | FJ830250 | FJ830067 |
| *P. astriata semicarinata* | APH10 | Alphonse, 07° 00′ 31.9″ S, 52° 43∗ 50.4″ E | FJ829899 | FJ829987 | FJ830160 | FJ830251 | FJ830068 |
| *P. barbouri* | FG/MV 2002-59 | Antoetra, Madagascar | FJ829900 | FJ829988 | FJ830161 | FJ830252 | FJ830069 |
| *P. berghofi* | ZCMV 518 | Manombo Camp, Madagascar, 23° 01.699′ S, 47° 43.892′ E | FJ829901 | FJ829989 | FJ830162 | FJ830253 | FJ830070 |
| *P. borbonica mater* | P-24 | Basse Vallee, Reunion | DQ270574 | FJ829990 | FJ830163 | FJ830254 | FJ830071 |
| *P. breviceps* | 2000-564 | Arboretum, Toliara, Madagascar | FJ829902 | FJ829991 | FJ830164 | FJ830255 | FJ830072 |
| *P. cepediana* | PcepD | No loc. data | FJ829903 | FJ829992 | FJ830165 | FJ830256 | FJ830073 |
| *P. comorensis* | GC22 | Grand Comore, Comoros | FJ829904 | DQ911694 | FJ830166 | FJ830257 | FJ830074 |
| *P. dubia* | Z33 | Zanzibar, Tanzania | FJ829905 | DQ911707 | FJ830167 | FJ830258 | FJ830075 |
| *P. dubia* | MH6 | Moheli, Comoros | FJ829906 | DQ911712 | FJ830168 | FJ830259 | FJ830076 |
| *P. dubia* | FG/MV 2002-2201 | Ambanja, Madagascar | FJ829907 | FJ829993 | FJ830169 | FJ830260 | FJ830077 |
| *P. ravenala* | PdubMan | Mananjary, Madagascar | FJ829908 | FJ829994 | FJ830170 | FJ830261 | FJ830078 |
| *P. dubia* | FGZC 961 | Antsalova, Madagascar | FJ829909 | FJ829995 | FJ830171 | FJ830262 | FJ830079 |
| *P. dubia* | FGZC 962 | Antsalova, Madagascar | FJ829910 | FJ829996 | FJ830172 | FJ830263 | FJ830080 |
| *P. flavigularis* | Pflav | No loc. data | FJ829911 | FJ829997 | FJ830173 | FJ830264 | FJ830081 |
| *P. flavigularis* | Pflav2 | No loc. data | FJ829912 | FJ829998 | FJ830174 | FJ830265 | FJ830082 |
| *P. grandis* | FG/MV 2002-2246 | Antsiranana, Madagascar | FJ829913 | FJ829999 | FJ830175 | FJ830266 | FJ830083 |
| *P. guentheri* | P-112 | No loc. data | FJ829914 | FJ830000 | FJ830176 | FJ830267 | FJ830084 |
| *P. guttata* | ZCMV 2172 | Nosy Mangabe, Madagascar | FJ829915 | FJ830001 | FJ830177 | FJ830268 | FJ830085 |
| *P. hielscheri* | FGZC 358 | Isalo, Madagascar, 22° 35′ 09″ S, 45° 23′ 57″ E, 812 m | FJ829916 | FJ830002 | FJ830178 | FJ830269 | FJ830086 |
| *P. inexpectata* | Pinexpect | No loc. data | FJ829917 | FJ830003 | FJ830179 | FJ830270 | FJ830087 |
| *P. kely* | ZSM 607/2003 | ca. 65 km south of Toamasina, Madagascar | FJ829918 | FJ830004 | FJ830180 | FJ830271 | FJ830088 |
| *P. klemmeri* | Pklemm1 | Ampasindava Peninsula, Madagascar | FJ829919 | FJ830005 | FJ830181 | FJ830272 | FJ830089 |
| *P. klemmeri* | Pklemm2 | Ampasindava Peninsula, Madagascar | FJ829920 | FJ830006 | FJ830182 | FJ830273 | FJ830090 |
| *P. kochi* | FGZC 680 | Tsingy de Bemaraha, Madagascar; 18° 42′ 31″ S, 44° 43′ 08″ E, 146 m | FJ829921 | FJ830007 | FJ830183 | FJ830274 | FJ830091 |
| *P. kochi* | FG/MV 2002-797 | Manongarivo, Madagascar | FJ829922 | FJ830008 | FJ830184 | FJ830275 | FJ830092 |
| *P. kochi* | MV 2001-388 | Ankarafantsika/Ampijoroa, Madagascar | FJ829923 | FJ830009 | FJ830185 | FJ830276 | FJ830093 |
| *P. laticauda laticauda* | FG/MV 2002-2202 | Ambanja, Madagascar | FJ829924 | FJ830010 | FJ830186 | FJ830277 | FJ830094 |
| *P. laticauda laticauda* | FGZC 2705 | Antalaha, Madagascar | FJ829925 | FJ830011 | FJ830187 | FJ830278 | FJ830095 |
| *P. laticauda laticauda* | MY 44 | Mayotte, Comoros | FJ829926 | DQ911720 | FJ830188 | FJ830279 | FJ830096 |
| *P. laticauda laticauda* | Platic JG | Farquhars, Seychelles | FJ829927 | FJ830012 | FJ830189 | FJ830280 | FJ830097 |
| *P. lineata dorsivittata* | FG/MV 2002-937 | Montagne d Ambre, Madagascar | FJ829928 | FJ830013 | FJ830190 | FJ830281 | FJ830098 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *P. lineata dorsivittata* | FGZC 488 | Montagne d'Ambre, Madagascar, 12° 31′ 37″ S, 49° 10′ 19″ E, 1050 m | FJ829929 | FJ830014 | FJ830191 | FJ830282 | FJ830099 |
| *P. lineata dorsivittata* | PlindB1 | Vohemar/Iharana, Madagascar | FJ829930 | FJ830015 | FJ830192 | FJ830283 | FJ830100 |
| *P. lineata dorsivittata* | PlindB2 | Vohemar/Iharana, Madagascar | FJ829931 | FJ830016 | FJ830193 | FJ830284 | FJ830101 |
| *P. lineata dorsivittata* | ZCMV 2029 | Marojejy, Camp Simpona, Madagascar, 14° 26.199′ S, 49° 44.601′ E | FJ829932 | FJ830017 | FJ830194 | FJ830285 | FJ830102 |
| *P. lineata lineata* | FG/MV 2002-299 | Ranomafana, Madagascar | FJ829933 | FJ830018 | FJ830195 | FJ830286 | FJ830103 |
| *P. lineata lineata* | FGZC 2634 | Ste. Luce, Madagascar, 24° 46.79′ S, 47° 10.28′ E | FJ829934 | FJ830019 | FJ830196 | FJ830287 | FJ830104 |
| *P. lineata lineata* | ZCMV 113 | Ambohitsara, Madagascar; 21° 21.431′ S, 47° 48.941′ E | FJ829935 | FJ830020 | FJ830197 | FJ830288 | FJ830105 |
| *P. lineata lineata* | ZCMV 813 | Besariaka, Madagascar | FJ829936 | FJ830021 | FJ830198 | FJ830289 | FJ830106 |
| *P. madagascariensis* | FG/MV 2002-500 | Ambohitsara, Madagascar | FJ829937 | FJ830022 | FJ830199 | FJ830290 | FJ830107 |
| *P. malamakibo* | FGZC 2556 | Andohahela, Madagascar; 24° 32.642′ S, 46° 42.847′ E | FJ829938 | FJ830023 | FJ830200 | FJ830291 | FJ830108 |
| *P. modesta modesta* | FGZC 56 | Ambovombe, Madagascar | FJ829939 | FJ830024 | FJ830201 | FJ830292 | FJ830109 |
| *P. modesta isakae* | FGZC 310 | Ilasombe, Madagascar | FJ829940 | FJ830025 | FJ830202 | FJ830293 | FJ830110 |
| *P. (modesta) leiogaster* | FG/MV 2002-1490 | Toliara, Madagascar | FJ829941 | FJ830026 | FJ830203 | FJ830294 | FJ830111 |
| *P. mutabilis* | FG/MV 2002-1494 | Toliara, Madagascar | FJ829942 | FJ830027 | FJ830204 | FJ830295 | FJ830112 |
| *P. mutabilis* | FGZC 350 | Ejeda, Madagascar | FJ829943 | FJ830028 | FJ830205 | FJ830296 | FJ830113 |
| *P. mutabilis* | FGZC 971 | Antsalova, Madagascar | FJ829944 | FJ830029 | FJ830206 | FJ830297 | FJ830114 |
| *P.* sp. *aff. mutabilis* | FGZC 881 | Tsingy de Bemaraha, Madagascar; 18° 47′ 03″ S, 44° 46′ 46″ E, 177 m | FJ829945 | FJ830030 | FJ830207 | FJ830298 | FJ830115 |
| *P. nigristriata* | 2000/870 | Mayotte, Comoros | FJ829946 | FJ830031 | FJ830208 | FJ830299 | FJ830116 |
| *P. nigristriata* | Pnigris | Mayotte, Comoros | FJ829947 | FJ830032 | FJ830209 | FJ830300 | FJ830117 |
| *P. ornata* | PornF1 | No loc. data | FJ829948 | FJ830033 | FJ830210 | FJ830301 | FJ830118 |
| *P. parkeri* | PB15 | Pemba, Tanzania | FJ829949 | DQ911750 | FJ830211 | FJ830302 | FJ830119 |
| *P. pasteuri* | MY65 | Mayotte, Comoros | FJ829950 | DQ911749 | FJ830212 | FJ830303 | FJ830120 |
| *P. pronki* | FGZC 2703 | Moramanga region, Madagascar | FJ829951 | FJ830034 | FJ830213 | FJ830304 | FJ830121 |
| *P. pusilla* | ZCMV 2174 | Nosy Mangabe, Madagascar | FJ829952 | FJ830035 | FJ830214 | FJ830305 | FJ830122 |
| *P. quadriocellata cf. bimaculata* | ZCMV 3211 | Nosy Boraha, Madagascar | FJ829953 | FJ830036 | FJ830215 | FJ830306 | FJ830123 |
| *P. quadriocellata parva* | ZCMV 375 | Ifanadiana-Tolongoina, Madagascar, 21° 21.215′ S, 47° 36.467′ E | FJ829954 | FJ830037 | FJ830216 | FJ830307 | FJ830124 |
| *P. quadriocellata parva* | FGZC 2640 | Ste. Luce, Madagascar, 24° 46.50′ S, 47° 09.05′ E | FJ829955 | FJ830038 | FJ830217 | FJ830308 | FJ830125 |
| *P. quadriocellata quadriocellata* | ZCMV 380 | Ranomafana, Madagascar, 21° 15.699′ S, 47° 27.571′ E | FJ829956 | FJ830039 | FJ830218 | FJ830309 | FJ830126 |
| *P. quadriocellata quadriocellata* | MV 2001-1060 | Ambohimanarivo, Madagascar | FJ829957 | FJ830040 | FJ830219 | FJ830310 | FJ830127 |
| *P. robertmertensi* | MY 73 | Mayotte, Comoros | FJ829958 | DQ911697 | FJ830220 | FJ830311 | FJ830128 |
| *P. rosagularis* | P-25 | Gorges de la Riviere Noire, Mauritius | DQ270549 | FJ830041 | FJ830221 | FJ830312 | FJ830129 |
| *P. seippi* | Pseippi | No loc. data | FJ829959 | FJ830042 | FJ830222 | FJ830313 | FJ830130 |
| *P. serraticauda* | Pserratica | No loc. data | FJ829960 | FJ830043 | FJ830223 | FJ830314 | FJ830131 |
| *P. standingi* | FGMV 2002-1562 | Ifaty, Madagascar | FJ829961 | FJ830044 | FJ830224 | FJ830315 | FJ830132 |
| *P. standingi* | FGMV 2002-2058 | Ifaty, Madagascar | FJ829962 | FJ830045 | FJ830225 | FJ830316 | FJ830133 |
| *P. sundbergi sundbergi* | PV3 | Poivre, Seychelles | FJ829963 | FJ830046 | FJ830226 | FJ830317 | FJ830134 |
| *P. sundbergi longinsulae* | MA1 | Mahé, Seychelles | FJ829964 | FJ830047 | FJ830227 | FJ830318 | FJ830135 |
| *P. sundbergi longinsulae* | CM5 | Cosmoledo, 09° 42′ 35.9″S, 47° 30′ 28.4″E | FJ829965 | FJ830048 | FJ830228 | FJ830319 | FJ830136 |
| *P. vanheygeni* | Pvanheig1 | Madagascar | FJ829966 | FJ830049 | FJ830229 | FJ830320 | FJ830137 |
| *P. v-nigra v-nigra* | MH 10 | Moheli, Comoros | FJ829967 | DQ911732 | FJ830230 | FJ830321 | FJ830138 |
| *P. v-nigra anjouanensis* | AJ 19 | Anjouan, Comoros | FJ829968 | DQ911736 | FJ830231 | FJ830322 | FJ830139 |
| *P. v-nigra comoraegrandensis* | GC 66 | Grand Comore, Comoros | FJ829969 | DQ911747 | FJ830232 | FJ830323 | FJ830140 |
| *Rhoptropella ocellatta* | AMB5687 | Namibia | FJ829970 | FJ830050 | FJ830233 | FJ830324 | FJ830141 |
| *Lygodactylus luteopicturatus* | TZ2 | Dar es Salaam, Tanzania | FJ829971 | FJ830051 | FJ830234 | FJ830325 | FJ830142 |
| *Ailuronyx* sp. | 2MA62 | Mahé, Seychelles | FJ829972 | n/a | FJ830235 | FJ830326 | FJ830143 |
| *Ailuronyx seychellensis* | PL17 | Praslin, Seychelles | n/a | FJ830052 | n/a | n/a | n/a |
| *Ebenavia inunguis* | GC70 | Grand Comore, Comoros | FJ829973 | FJ830053 | FJ830236 | FJ830327 | FJ830144 |
| *Gehyra mutilata* | GmReu1 | Reunion, Mascarenes | FJ613444 | FJ830054 | FJ830237 | FJ830328 | FJ830145 |

used Bayes Factors (BF) to identify the appropriate partitioning strategy for the coding genes. Because in all cases BFs clearly (>150, "very strong"; Kass and Raftery, 1995) favoured partitioning by codon position with rate variation, partitions by gene and codon position were used in the analysis of the concatenated dataset (hereinafter called *combined* dataset), that ran for 50,000,000 generations. Cumulative plots of PPs and their comparison across runs were also performed to assess convergence. Separate analyses of (1) the concatenated mtDNA fragments (*mitochondrial* dataset) and (2) the concatenated nuclear fragments (*nuclear* dataset) were also performed, and run for 30 and 25 million generations, respectively. Substitution-model parameters were always unlinked across partitions. Topology and branch lengths were linked across all partitions, but each partition was allowed to have its own rate (*prset* = variable).

Maximum likelihood (ML) analyses were performed on individual genes and on the *combined* dataset using GARLI (Zwickl, 2006) under the models specified by the AIC in Modeltest with 5000 to 1,000,000 generations after better scoring topology (*genthreshfortopoterm*) as a termination condition (five different analyses for each dataset). As no significant differences in the topology were observed with increasing numbers of generations, bootstrap support was calculated using 5000 *genthresfortopterm* for each replicate. Several independent GARLI runs, each starting with a different random tree topology, were performed. Because distant outgroup taxa can influence the relationships within the ingroup taxa, and correctly rooting rapid radiations may be difficult (Shavit et al., 2007), we also conducted ML searches without the outgroup taxa to ensure that incorrect rooting was not breaking otherwise supported relationships within the ingroup. We used SH-tests (Shimodaira and Hasegawa, 1999) as implemented in PAUP*, to test the fit of individual gene trees to the different partitions. SH-tests were implemented with the RELL approximation with 1000 bootstrap replicates.

## 3. Results and discussion

### 3.1. Alignment, gene composition, gene variability and phylogenetic analysis

After removing ambiguous positions in the 16srRNA gene dataset, 384 positions were used for phylogenetic inference. In the cytochrome *b*, C-*mos*, Rag-1 and Rag-2 datasets no positions were excluded. The analysed fragments had very different levels of variation. After excluding the outgroup taxa, 16s had 133 variable (34.6%) and 102 parsimony informative sites (PI: 26.6%), while Cyt-*b* had 431 variable (60.4%) and 425 PI sites (57.9%). Rag-1 was the slowest evolving gene, with 60 variable and 33 PI sites (12.7% and 6.9%, respectively). C-*mos* and Rag-2 showed intermediate values of variation: there were 53 (15.4%) variable and 34 (9.9%) PI sites at C-*mos* and 129 (16.2%) variable and 78 (9.9%) PI positions at Rag-2. Despite the overall low variation in the nuclear genes, they still exhibited considerable phylogenetic signal – 67.2%, 66.3% and 65.4% resolved quartet topologies respectively for "C-*mos*", "Rag-1" and "Rag-2" – as revealed by the four-cluster likelihood mapping analysis. Moreover, combining all the genes increases the phylogenetic signal up to 95.7% (sum of quartets falling in "signal" regions in the triangular plot of probability vectors, in the *combined* dataset). A number of heterozygous positions were observed at the nuclear genes (36 at C-*mos*, 33 at Rag-1 and 42 at Rag-2), and coded as ambiguities.

Analysis with and without the outgroup (the latter not shown) recovered the same ingroup relationships. Judging from the similar results compared across runs, GARLI was unlikely to have been trapped in local optima. For all individual and combined dataset estimates, ML and Bayesian topologies and support values were largely concordant (Supplementary Figs. 1–3). While the SH tests (Table 2) rejected the null hypothesis of congruence among most of the individual gene fragments used, no individual dataset rejected the *combined* topology. Fig. 2 represents the tree obtained from the *combined* dataset. We consider this tree as our best estimate of the phylogeny of *Phelsuma* (*mitochondrial* and *nuclear* phylogenies are also presented in Supplementary Fig. 2).

Highly supported clades (PP $\geqslant$ 99) in the combined analysis are depicted in different colors, and the same individuals are also identically color-coded in the mtDNA, nuclear and individual trees. The *mitochondrial* and *combined* datasets highly supported the same groups. Most of these were also recovered using the combined nuclear dataset, the few incongruent clades from the nuclear analysis being poorly supported (Suppl. Fig. 2).

### 3.2. Phylogenetic relationships among species of Phelsuma

Our analysis recovered eight clades whose differentiation appears to have been relatively simultaneous (clades J, K, N, P, Q, B, D and E, using node labeling from Fig. 2). Most of the clades reflect previously hypothesized relationships but several novel relationships were recovered:

(1) The recently described *P. vanheygeni* is the sister-taxon to the Seychelles radiation, and related to members of the previously designated *P. guttata* and *P. madagascariensis* species groups (which all compose clade E);
(2) *Phelsuma antanosy*, not previously included in any published molecular phylogeny, is closely related to *P. quadriocellata*, and together with *P. lineata*, *P. kely*, *P. pusilla* and *P. comorensis* is part of a strongly supported clade (J);
(3) *Phelsuma laticauda* and *P. serraticauda* (and *P. antanosy*) are not closely related, and the previous *P. laticauda* species group (Glaw et al., 1999) does not represent a clade. Instead,

**Table 2**
Shimodaira-Hasegawa test *P*-values from the tests of gene trees obtained from analysis of different data partitions. Each value reflects the fit of different individual or combined gene trees (lines) to different data partitions (columns). *P*-values below 0.05 are indicated by an asterisk.

| | DATA | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 16s | Cyt-*b* | mtDNA | Rag-1 | Rag-2 | C-*mos* | nucDNA | Combined |
| *TREES* | | | | | | | | |
| 16s | (best) | * | * | * | * | * | * | * |
| Cyt-*b* | 0.322 | (best) | 0.819 | * | 0.050 | * | * | 0.632 |
| mtDNA | 0.579 | 0.802 | (best) | * | * | * | * | 0.629 |
| Rag-1 | * | * | * | (best) | * | * | * | ** |
| Rag-2 | * | * | * | * | (best) | * | 0.237 | * |
| C-*mos* | * | * | * | * | * | (best) | * | * |
| nucDNA | * | * | * | 0.221 | 0.509 | 0.290 | (best) | * |
| Combined | 0.449 | 0.644 | 0.773 | 0.064 | 0.107 | 0.072 | 0.209 | (best) |

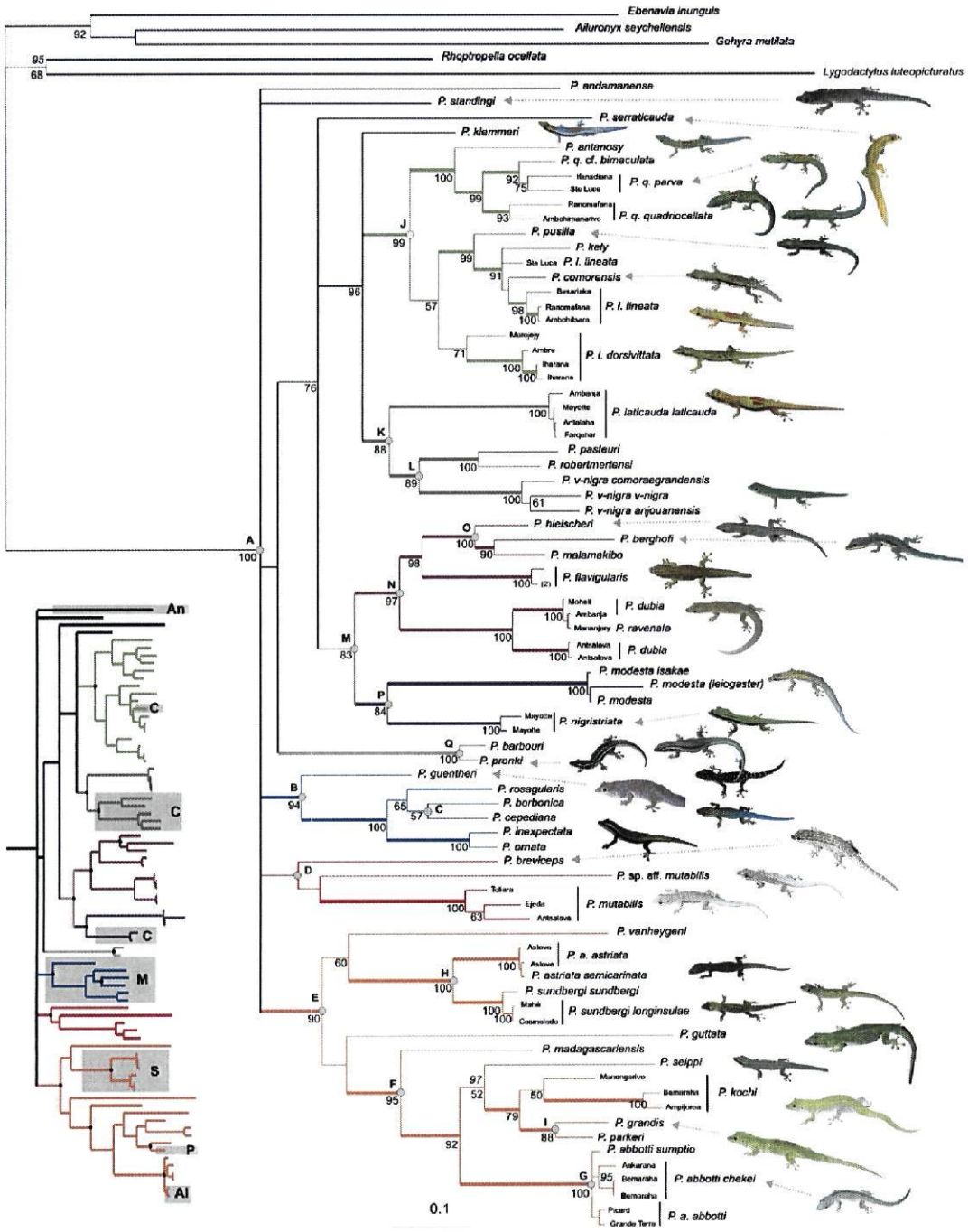



**Fig. 2.** Bayesian 50% majority-rule consensus phylogenetic tree of the combined data. Groups supported by PP ⩾ 99 are color coded (and discussed in detail in the text: blue lineage, Mascarenes group; red lineage, *P. mutabilis* group; grey lineage, *P. barbouri* and *P. pronki*; orange lineage, *P. guttata*- and *P. madagascariensis*-group; purple, *P. dubia* and *P. modesta* species group; yellow, *P. laticauda* species group and green lineage, *P. lineata* species group). Branches supported by PP ⩾ 99 are highlighted in bold. Bootstrap support values from ML analyses above 50 are given below corresponding branch. Relevant PP values above 95 are given above respective branches (italics). Node labels (A–M) given correspond to major clades discussed in the text. Inset figure in lower left corner reproduces schematically the same phylogenetic tree and indicates *Phelsuma* lineages not occurring on Madagascar: Al, Aldabra; An, Andamans; C, Comoros; M, Mascarenes; P, Pemba; S, Seychelles.

*P. serraticauda* represents an early diverging *Phelsuma* lineage and *P. laticauda* seems to be related to a group containing the endemic Comoran *P. v-nigra*. The separate position of *P. serraticauda* in our tree requires further study as it is in apparent conflict with the placement of this species in a clade with *P. laticauda*, *P. lineata* and *P. quadriocellata* in Austin et al. (2004), Raxworthy et al. (2007) and Harmon et al. (2008);

(4) *Phelsuma klemmeri* is not closely related to *P. pronki* and *P. barbouri* (which do form a supported clade, Q), and;

(5) *Phelsuma leiogaster* (sensu Glaw and Vences, 1994) is nested within *P. modesta*, confirming that these two taxa, which so far have not been included together in a molecular phylogeny, are probably conspecific (Nussbaum et al., 2000). They are closely related to *P. nigristriata* from the Comoros and not to the Mascarenes clade.

Beside these main novelties, our phylogeny contributes to clarifying *Phelsuma* systematics in various further aspects. Close relationships between *P. lineata*, *P. kely*, *P. quadriocellata* and *P. pusilla* (clade J) have been long hypothesized (e.g. Glaw et al. 1999) and were found for *P. lineata* and *P. quadriocellata* (the other taxa not included) in previous studies (Austin et al., 2004; Raxworthy et al., 2007; Harmon et al., 2008). The four species' close relationship is now also confirmed by our results, which also highlight that the species-level taxonomy in this clade needs to be revised. Indeed, a species status is probably warranted for several subspecies (Boumans et al., 2007).

Clade N, containing *Phelsuma dubia*, *P. flavigularis*, *P. malamakibo*, *P. berghofi* and *P. hielscheri*, largely corroborates previous classifications (Glaw et al., 1999; Van Heygen, 2004). The placement of *P. hielscheri* close to *P. lineata* and *P. laticauda* in the analysis of Raxworthy et al. (2007) is likely due to a confusion of samples, and/or use of a very short DNA fragment for *P. hielscheri* (193 bp). Our analysis further indicates a strong differentiation of western *P. dubia* specimens (Antsalova), rendering this species paraphyletic with respect to the recently described *P. ravenala* (Raxworthy et al., 2007) and indicating that the taxonomy of these species needs revision.

Clade Q, containing *Phelsuma barbouri* and *P. pronki*, was strongly supported by the mitochondrial and nuclear data sets, contradicting their most recent classification (Van Heygen, 2004).

Clade D contains three species from south-western (*P. breviceps*) and western (*P. mutabilis*, *P.* sp. aff. *mutabilis*) Madagascar. The close relationships between *P. breviceps* and *P. mutabilis* had previously been hypothesized based on morphometric and scale features (Loveridge, 1942). Our results support their distinctiveness, and suggest that *P.* sp. aff. *mutabilis* might be a distinctive undescribed species that occurs sympatrically with *P. mutabilis* in the Tsingy de Bemaraha area in western Madagascar (Glaw and coworkers, unpublished observations).

The placement of the previous *P. madagascariensis* subspecies (clade E) supports the proposal of Raxworthy et al. (2007) to elevate these taxa to species rank. The monophyly of the Seychelles species is here confirmed, with *P. vanheygeni* as the sister taxon of this clade. Lerner (2004) excluded *P. vanheygeni* from the *P. guttata* and *P. madagascariensis* species groups (and others) due to its egg-gluing behaviour, but our molecular data imply multiple independent evolution (and/or loss) of egg-gluing behaviour: species of *P. dubia* and *P. modesta* groups (except *P. nigristriata*), *P. barbouri* and *P. vanheygeni* are egg gluers.

Our analysis confirms previously recognized island species/lineage affinities and also the monophyly of both Mascarenes and Seychelles radiation, as well as the monophyly of the Comoran radiation comprising *P. pasteuri*, *P. robertmertensi* and *P. v-nigra*. *Phelsuma nigristriata* is most closely related to *P. modesta* from Madagascar; thus, the Comoros underwent more independent colonisations (three) than any other archipelago. *Phelsuma andamanense* seems to be an ancient lineage, lacking clear relationships to any other extant lineage.

The complex geological island setting where *Phelsuma* evolved certainly provided numerous and unique opportunities for isolation and diversification. The phylogeny proposed here should lead to a better understanding of causes, patterns and dynamics of diversification within this genus and in Madagascar. This newly generated phylogeny is also a valuable framework for further research on *Phelsuma* relationships at a phylogeographic level.

## Acknowledgments

Financial support to this work was obtained from the Portuguese Fundação para a Ciência e Tecnologia, through a PhD grant to S.R. (SFRH/BD/17541/ 2004) and research Grants POCTI/BSE/46647/2002 and PTDC/BIA-BDE/65745/2006. Laboratory work was conducted at the University of Vigo (Spain), where we would like to thank Paloma Morán, Pilar Alvariño, Nieves Santamaria and Graciela Sotelo for help and advice during experimental work. We thank Centro de Investigación Príncipe Felipe (Valencia), for allowing the use of Computational Resources at the Bioinformatics Department. Fieldwork of MV and FG in Madagascar was made possible by collaboration agreements of the author's institutions with the Université d'Antananarivo (Département de Biologie Animale) and the Association Nationale pour la Gestion des Aires Protegées, and supported by the Volkswagen Foundation and the Deutsche Forschungsgemeinschaft. For assistance in the field, we are grateful to Miguel A. Carretero, Franco Andreone, Parfait Bora, Kathrin Glaw, Roger-Daniel Randrianiaina and David R. Vieites. We also thank Aaron Bauer for very useful remarks on *Phelsuma* relationships and Anthony Cheke for insightful discussions on *Phelsuma* and Indian Ocean Island biogeography. Thanks to Pedro Tarroso for Fig. 1. Finally we are very grateful to Hans-Peter Berghof, Achim Lerner, Patrick Schönecker and Peter Sound for providing crucial tissue samples.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2009.03.032.

## References

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Aut. Control 19, 716–723.

Austin, J.J., Arnold, E.N., Jones, C.G., 2004. Reconstructing an island radiation using ancient and recent DNA: the extinct and living day geckos (*Phelsuma*) of the Mascarene islands. Mol. Phylogenet. Evol. 31, 102–109.

Boumans, L., Vieites, D.R., Glaw, F., Vences, M., 2007. Geographical patterns of deep mitochondrial differentiation in widespread Malagasy reptiles. Mol. Phylogenet. Evol. 45, 822–839.

Brown, J.M., Lemmon, A.R., 2007. The importance of data partitioning and the utility of Bayes factors in Bayesian phylogenetics. Syst. Biol. 56, 643–655.

Glaw, F., Vences, M., 1994. A Fieldguide to the Amphibians and Reptiles of Madagascar. M. Vences and F. Glaw GbR, Koln.

Glaw, F., Vences, M., Ziegler, T., 1999. Bemerkungen zu *Phelsuma dubia* (Boettger, 1881): Wiederentdeckung des Holotypus, Verwandtschaftsbeziehungen und Daten zur Fortpflanzung. Salamandra 35, 267–278.

Hall, T.A., 1999. BioEdit: a user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Harmon, L., Melville, J., Larson, A., Losos, J.B., 2008. The role of geography and ecological opportunity in the diversification of day geckos (*Phelsuma*). Syst. Biol. 57, 562–573.

Hoegg, S., Vences, M., Brinkmann, H., Meyer, A., 2004. Phylogeny and comparative substitution rates of frogs inferred from sequences of three nuclear genes. Mol. Biol. Evol. 21, 1188–1200.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Kass, R.E., Raftery, A.E., 1995. Bayes factors. J. Am. Stat. Assoc. 90, 773–795.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C., 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA 86, 6196–6200.

Lerner, A., 2004. A new taxonomically isolated species of the genus *Phelsuma* Gray, 1825 from the Ampasindava peninsula, Madagascar. Phelsuma 12, 91–98.

Löytynoja, A., Milinkovitch, M., 2003. A hidden Markov model for progressive alignment. Bioinformatics 19, 1505–1513.

Loveridge, A., 1942. Revision of the Afro-Oriental geckos of the genus *Phelsuma*. Bull. Mus. Comp. Zool. Harvard 89, 439–482.

Mertens, R., 1962. Die Arten und Unterarten der Geckonengattung *Phelsuma*. Senckenb. Biol. 43, 81–127.

Nussbaum, R.A., Raxworthy, C.J., Raselimanana, A.P., Ramanamanjato, J.B., 2000. New species of Day Gecko, *Phelsuma* Gray (Reptilia: Squamata: Gekkonidae), from the Réserve Naturelle Intégrale d'Andohahela, southern Madagascar. Copeia 2000, 763–770.

Palumbi, S.R., Martin, A., Romano, S., McMillan, W.O., Stice, L., Grabowski, G., 1991. The simple fool's guide to PCR, Version 2.0. Privately Published, Hawaii.

Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Radtkey, R., 1996. Adaptive radiation of day-geckos (*Phelsuma*) in the Seychelles Archipelago: a phylogenetic analysis. Evolution 50, 604–623.

Raxworthy, C.J., Ingram, C.M., Rabibisoa, N., Pearson, R.G., 2007. Applications of ecological niche modeling for species delimitation: a review and empirical evaluation using day geckos (*Phelsuma*) from Madagascar. Syst. Biol. 56, 907–923.

Rocha, S., Posada, D., Carretero, M.A., Harris, D.J., 2007. Phylogenetic affinities of Comoroan and East African day geckos (genus *Phelsuma*): multiple natural colonisations, introductions and island radiations. Mol. Phylogenet. Evol. 43, 685–692.

Saint, K.M., Austin, C.C., Donnellan, S.C., Hutchinson, M.N., 1998. *cmos*, a nuclear marker useful for squamate phylogenetic analysis. Mol. Phylogenet. Evol. 10, 259–263.

Schmidt, H.A., Strimmer, K., Vingron, M., von Haeseler, A., 2002. TREEPUZZLE: maximum likelihood phylogenetic analysis using quartets and parallel computing. Bioinformatics 18, 502–504.

Shavit, L., Penny, D., Hendy, M.D., Holland, B.R., 2007. The problem of rooting rapid radiations. Mol. Biol. Evol. 24, 2400–2411.

Shimodaira, H., Hasegawa, M., 1999. Multiple comparison of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16, 1114–1116.

Sound, P., Kosuch, J., Vences, M., Seitz, A., Veith, M., 2006. Preliminary molecular relationships of Comoroan day geckos (Phelsuma). In: Vences, M., Köhler, J., Ziegler, T., Böhme, W. (Eds.), Herpetologia Bonnensis II. Proceedings of the 13th Congress of the Societas Europaea Herpetologica. Bonn, Zoologisches Forschungsmuseum A. Koenig and Societas Europaea Herpetologica, pp. 175-179.

Strimmer, K., von Haesler, A., 1997. Likelihood-mapping: a simple method to visualize phylogenetic content of a sequence alignment. Proc. Natl. Acad. Sci. USA 94, 6815–6819.

Swofford, D.L., 2002. PAUP*. Phylogenetic Analysis Using Parsimony (* and other Methods). Version 4. Sinauer Associates, Sunderland, MA.

Van Heygen, E., 2004. Remarks on the *Phelsuma barbouri*- and *Phelsuma klemmeri*-phenetic groups. Phelsuma 12, 153–154.

Vidal, N., Hedges, S.B., 2004. Molecular evidence for a terrestrial origin of snakes. Proc. R. Soc. Lond. B 271, 226–229.

Wilgenbusch, J.C., Warren, D.L., Swofford, D.L., 2004. AWTY: a system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference. Available from: <http://www.ceb.csit.fsu.edu/awty>.

Zwickl, D.J., 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation, The University of Texas, Austin. Available from: <http://www.bio.utexas.edu/faculty/antisense/garli/Garli.html>.

**Appendix A**

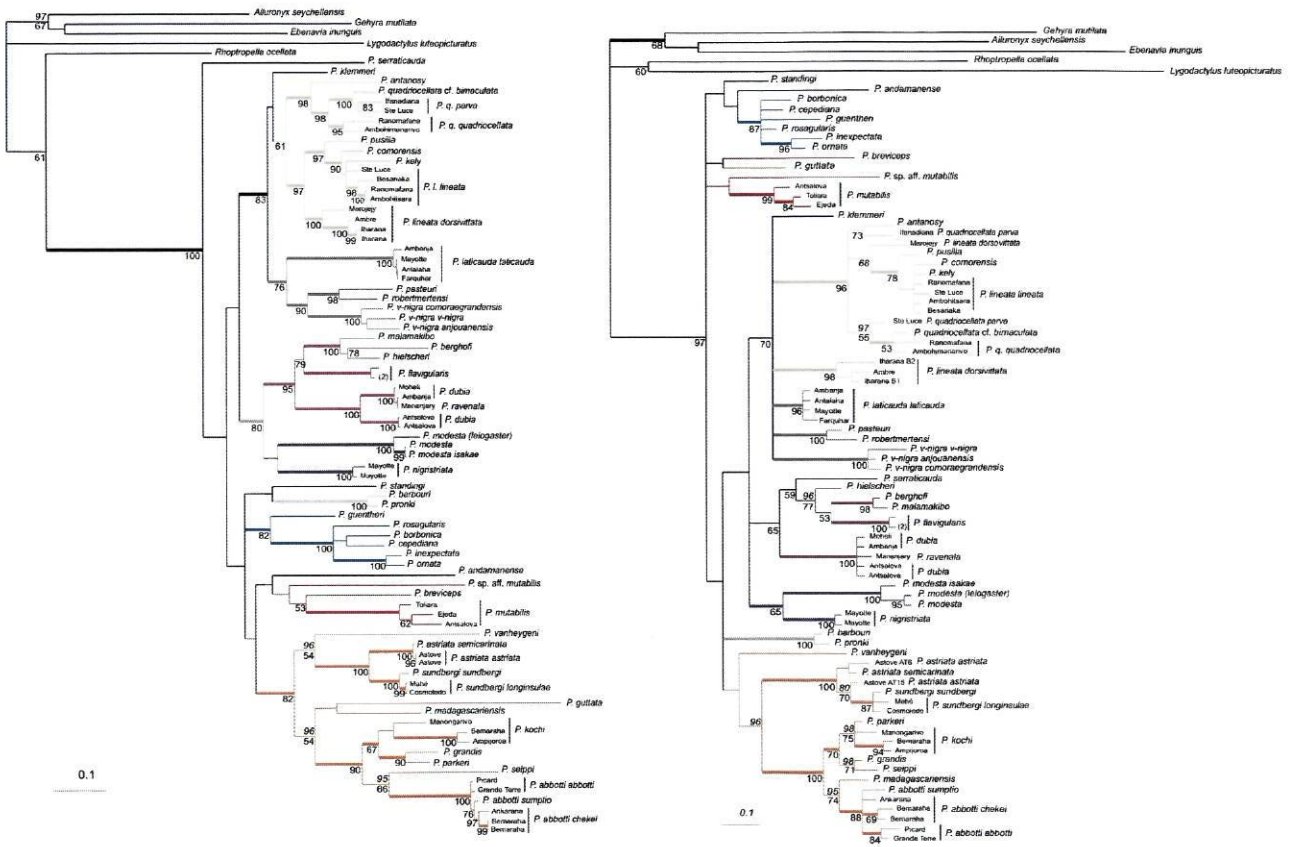
(S. Rocha et al., 2009. *Molecular Phylogenetics and Evolution* 52, 530-537)

**Supplementary Figure 1:** Bayesian 50% majority-rule consensus trees for the combined dataset of mitochondrial genes (left) and nuclear genes (right). Color-coding distinguishes groups supported by PP ≥ 99 in the combined phylogeny (Figure 2). Branches supported by PP ≥ 99 are highlighted in bold and ML bootstraps support above 50 is given below corresponding branch. Relevant PP values above 95 are given above respective branches (italics).

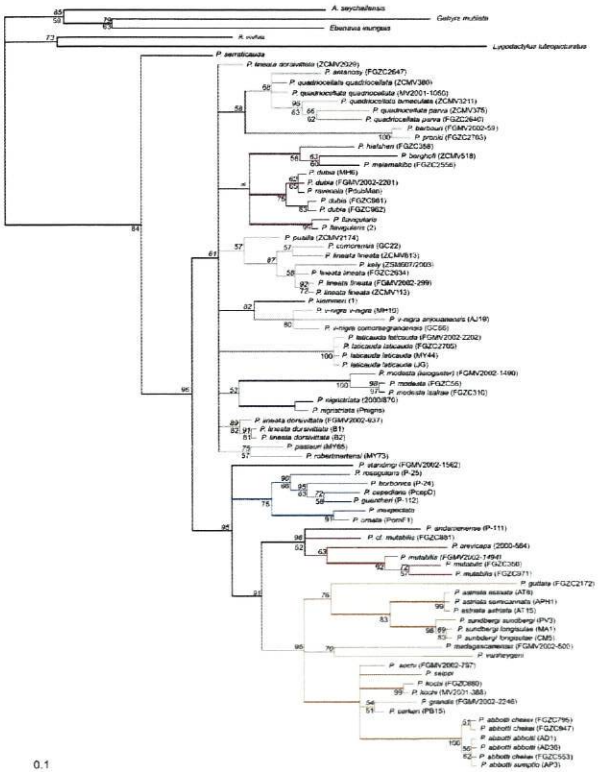
**Supplementary Figure 2:** Bayesian 50% majority-rule consensus trees for individual mitochondrial genes: 16s rRNA and Cyt-*b*. Color-coding distinguishes groups supported by PP ≥ 99 in the combined phylogeny (Figure 2). Branches supported by PP ≥ 99 are highlighted in bold and ML bootstraps support above 50 is given below corresponding branch. Relevant PP values above 95 are given above respective branches (italics).

**Supplementary Figure 3:** Bayesian 50% majority-rule consensus trees for individual nuclear genes: Rag-1, Rag-2 and C-mos. Color-coding distinguishes groups supported by PP ≥ 99 in the combined phylogeny (Figure 2). Branches supported by PP ≥ 99 are highlighted in bold and ML bootstraps support above 50 is given below corresponding branch. Relevant PP values above 95 are given above respective branches (italics).

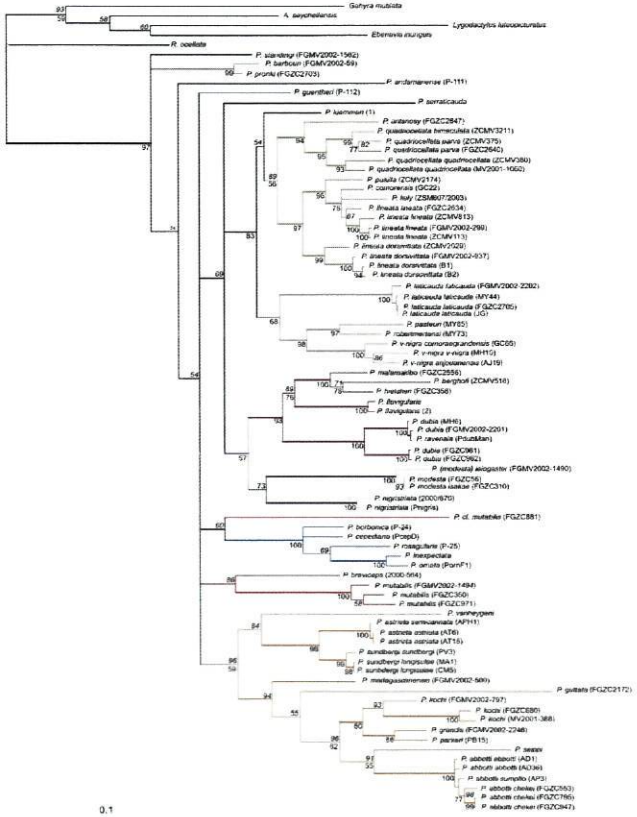
## Supplementary Figure 1



## Supplementary Figure 2

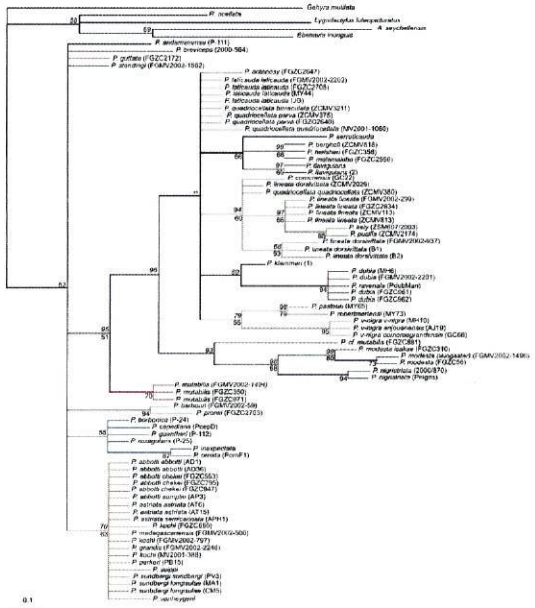



Gene: 16s

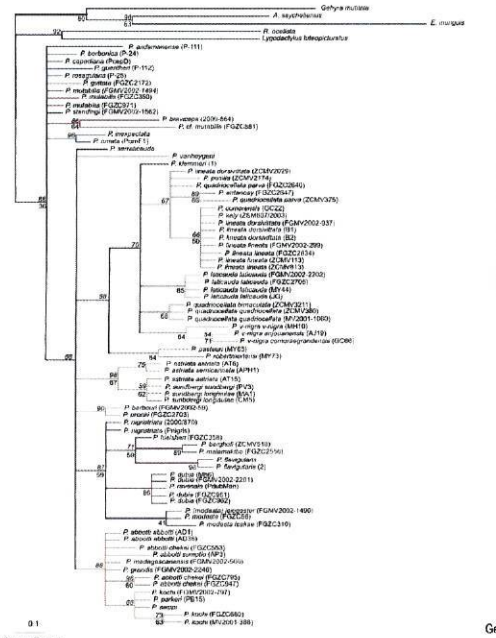



Gene: Cyt-*b*

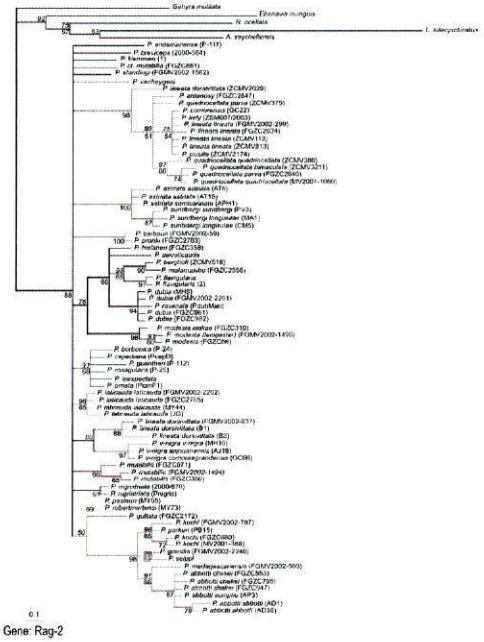
## Supplementary Figure 3



Gene: C-mos



Gene: Rag-1



Gene: Rag-2

*Zootaxa* 2429: 1–28 (2010)
www.mapress.com/zootaxa/
Copyright © 2010 · Magnolia Press

**Article**

ISSN 1175-5326 (print edition)
**ZOOTAXA**
ISSN 1175-5334 (online edition)

# Phylogenetic systematics of day geckos, genus *Phelsuma*, based on molecular and morphological data (Squamata: Gekkonidae)

SARA ROCHA[1,2,3], HERBERT RÖSLER[4], PHILIP-SEBASTIAN GEHRING[5], FRANK GLAW[6], DAVID POSADA[3], D. JAMES HARRIS[1,2], & MIGUEL VENCES[5,7]

[1]*CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, 4485-661 Vairão, Portugal*
[2]*Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre FC4, 4169-007 Porto, Portugal*
[3]*Departamento de Bioquímica, Genética e Inmunología, Facultad de Biología, Universidad de Vigo, Vigo 36310, Spain*
[4]*Senckenberg Naturhistorische Sammlungen Dresden, Museum für Tierkunde, Sektion Herpetologie, Königsbrücker Landstr. 159, 01109 Dresden, Germany*
[5]*Technical University of Braunschweig, Zoological Institute, Spielmannstr. 8, 38106 Braunschweig, Germany*
[6]*Zoologische Staatssammlung München, Münchhausenstr. 21, 81247 München, Germany*
[7]*Corresponding author. E-mail: m.vences@tu-bs.de*

## Table of contents

Abstract ..... 2
Introduction ..... 2
Material and methods ..... 3
Results and discussion ..... 4
*Phelsuma barbouri* group ..... 9
*Phelsuma dubia* group ..... 11
*Phelsuma mutabilis* group ..... 13
*Phelsuma cepediana* group ..... 13
*Phelsuma astriata* group ..... 14
*Phelsuma madagascariensis* group ..... 15
*Phelsuma lineata* group ..... 19
*Phelsuma laticauda* group ..... 23
*Phelsuma klemmeri* group ..... 23
*Phelsuma andamanense* group ..... 24
*Phelsuma standingi* group ..... 24
Species not assigned to any species group ..... 24
Conclusion ..... 25
Acknowledgements ..... 25
References ..... 26

*Accepted by A. Bauer: 1 Mar. 2010; published: 19 Apr. 2010*

## Abstract

We review the intrageneric systematics of day geckos, genus *Phelsuma* (Squamata: Sauria: Gekkonidae) in the light of a recently published multigene phylogeny, and provide an overview of 19 selected chromatic, morphological and behavioural characters for all currently recognized species and subspecies in the genus. To replace previous attempts of dividing the genus into phenetic species assemblages, we propose the following monophyletic species groups and summarize the morphological characters that partly support this grouping: *Phelsuma barbouri* group (*P. barbouri, P. pronki*); *P. dubia* group (*P. berghofi, P. dubia, P. flavigularis, P. hielscheri, P. malamakibo, P. modesta, P. nigristriata, P. ravenala*); *P. mutabilis* group (*P. borai, P. breviceps, P. mutabilis*); *P. cepediana* group (*P. borbonica, P. cepediana, P. guentheri, P. guimbeaui, P. inexpectata, P. ornata, P. rosagularis* as well as the extinct species *P. edwardnewtonii* and *P. gigas*); *P. astriata* group (*P. astriata, P. sundbergi*); *P. madagascariensis* group (*P. abbotti, P. grandis, P. guttata, P. kochi, P. madagascariensis, P. parkeri, P. seippi* and probably *P. masohoala*); *P. lineata* group (*P. antanosy, P. comorensis, P. kely, P. lineata, P. pusilla, P. quadriocellata* and probably *P. hoeschi*); *P. laticauda* group (*P. laticauda, P. pasteuri, P. robertmertensi, P. v-nigra*); *P. klemmeri* group (*P. klemmeri*); *P. andamanense* group (*P. andamanense*) and *P. standingi* group (*P. standingi*). Due to their equivocal placements based on different molecular markers, we refrain from assigning *P. serraticauda* and *P. vanheygeni* to any of these species groups at this time. We complement the molecular data with new trees based on partial sequences of the 16S rRNA gene for the *P. madagascariensis*- and *P. lineata* groups and propose to elevate *P. lineata dorsivittata* and *P. quadriocellata parva* to full species rank as *P. dorsivittata* and *P. parva*.

**Key words:** Squamata: Gekkonidae: *Phelsuma*, *Phelsuma parva*, *Phelsuma dorsivittata*; Andamans; Comoros, Madagascar, Mascarenes, Seychelles, species groups

## Introduction

Day geckos, genus *Phelsuma* Gray, are one of the most prominent groups of lizards of the Malagasy region. They are naturally distributed on most islands and in the western Indian Ocean, and have additionally been widely introduced, both in remote locations like Hawaii (Lever 2003), Florida (Krysko *et al.* 2003), and Moorea, French Polinesia (Ota & Ineich 2006) as well as in closer archipelagos like the Mascarenes (Checke & Hume 2008). Most of the circa 44 species of *Phelsuma* occur on Madagascar, but these geckos have also naturally colonized the Comoros, Mascarenes, Seychelles, Andamans, Zanzibar and Pemba, as well as several other small islands and a few localities on the East African mainland. *Phelsuma* are colourful lizards of great morphological homogeneity, with round pupils (except *P. guentheri*) and undivided toe lamellae. A putative synapomorphy of *Phelsuma* might be the contact between the prefrontal and postorbitofrontal bones, a feature otherwise only present among pygopodids (see Daza & Bauer 2010). Besides body size, few morphological characters are known that can be used to distinguish species, or define species groups within *Phelsuma*, and their taxonomy is largely based on colour that ranges from bright green, with red spots and markings in most species, to dull grey or brownish in a few others.

Despite the extensive published works on *Phelsuma* taxonomy, ecology, biogeography and ethology (Loveridge 1941, 1942; Mertens 1954, 1963a, 1963b, 1964, 1966; Cheke 1975, 1981, 1982; Meier 1982; Gardner 1986, 1987; Losos 1986; Radtkey 1996; Meier & Böhme 1991, 1996; Krüger 1996c; Ikeuchi *et al.* 2005; Van Heygen 2004) there are still many uncertainties, even regarding the alpha-taxonomy of these lizards. Many taxa, especially subspecies, have been described only based on chromatic characters, and many are poorly defined. In several cases colour transitions/polymorphisms can be observed and thus some subspecies and even species may represent artificial taxa based on local colour morphs (Glaw and Vences 1994). Loveridge (1942) made the first important attempt to sort out relationships within these day-geckos, in the form of a phenetic key, and Mertens (1962) in a review of *Phelsuma* designated informal species groups within the genus based on the same characters used by Loveridge. This classification has since been revised and updated (Glaw & Vences 1994; Glaw *et al.* 1999; Van Heygen 2004). The latter author defined nine Malagasy species groups, based on phenetic characters: *P. barbouri* group (*P. barbouri*), *P. guttata* group (*P. abbotti, P. guttata, P. masohoala, P. seippi*), *P. madagascariensis* group (*P. madagascariensis* [including *P. grandis* and *P. kochi* as subspecies], *P. standingi*), *P. lineata* group (*P. kely, P. lineata, P. pusilla, P. quadriocellata*), *P. mutabilis* group (*P. breviceps* and *P. mutabilis*), *P. laticauda* group (*P. antanosy, P.*

*laticauda*, *P. serraticauda*), *P. klemmeri* group (*P. klemmeri*, *P. pronki*), *P. dubia* group (*P. berghofi*, *P. dubia*, *P. flavigularis*), and *P. modesta* group (*P. modesta*). Close relationships between some of the groups were postulated (*P. guttata*- and *P. madagascariensis* groups for example and also of the species from the Mascarene Islands with the *P. modesta* group), but three species (*P. hielscheri*, *P. malamakibo*, *P. vanheygeni*) were not assigned to any group.

Several molecular studies have contributed to a better understanding of *Phelsuma* relationships (Radtkey 1996; Austin *et al.* 2004; Sound *et al.* 2006; Raxworthy *et al.* 2007; Harmon *et al.* 2008; Rocha *et al.* 2007, 2009). *Rhoptropella ocellata* from south-western Africa and/or the diurnal dwarf geckos of the genera *Lygodactylus*, *Microscalabotes* and *Vanzoia* (e.g., Puente *et al.* 2009) appear to be the closest relatives of *Phelsuma* (e.g., Austin *et al.* 2004). It seems clear now that the Seychellois and Mascarene radiations of *Phelsuma* are monophyletic while the Comoros have been colonized multiple times independently by these lizards. Most probably, Madagascar was the origin of the diversification of *Phelsuma* after the initial colonization of this island by rafting ancestors from Africa. Apparently most or all of the other islands and island groups have subsequently been colonized by overseas dispersal from Madagascar. In a recent study that included all but two described species of *Phelsuma* (Rocha *et al.* 2009), a multi-gene data set of mitochondrial as well as nuclear DNA sequences was analyzed, and shed light on various novel aspects of the evolutionary history of day geckos. Although basal relationships in the genus were not well defined, a number of strongly supported clades were identified that, in numerous cases, suggested surprising relationships among species that were not previously thought to be closely related based on morphology. Furthermore, several studies (Boumans *et al.* 2007; Raxworthy *et al.* 2007, Rocha *et al.* 2009) indicated the existence of problematic species complexes, with morphologically apparently poorly differentiated forms of high genetic divergence, and with a topology that rendered several well-known taxa paraphyletic.

In this paper we wish to contribute to *Phelsuma* systematics by (1) discussing the major clades identified by molecular analysis in the light of a survey of morphological characters, including the definition of formal species groups for all species in the genus based on their phylogenetic relationships, and to characterize these groups for relevant morphological characters, (2) discussing several taxa of controversial phylogenetic placement due to either conflict between data sets or confusion of samples or sequences in previous works, and (3) presenting new mitochondrial data for selected species and species complexes and exploring their diversity and relationships as well as some alpha-taxonomic consequences.

In fact, given the current knowledge on the evolutionary relationships within this genus, fostered by the latest molecular phylogenetic studies, a reorganization of the species within the genus and its division into species groups is crucial, and will greatly contribute to make this clade more manageable, and more efficiently direct future research within this genus, either in ecology, conservation, taxonomy or phylogeography of specific groups. It is now evident that despite the plasticity of many characters, some can be useful in defining particular clades and should not be overlooked. Although some members of this genus are more common in anthropogenic habitats than in pristine forest (e.g., D'Cruze *et al.* 2009), others are threatened by habitat degradation and subject to additional pressure from collection for international pet trade, with all species currently receiving protection on CITES appendices (Bauer 2003). A reclassification that better reflects the evolutionary history of the species, as proposed herein, will therefore also be a valuable tool for conservation purposes.

## Material and methods

Tissue samples (tail tips) of a large number of specimens of *Phelsuma* and outgroups were obtained during fieldwork in Madagascar, Seychelles, the Comoros and Tanzania in the period between 2000 and 2008 and preserved in 70% to 100% ethanol. Most or all collections from Madagascar are furthermore backed up by voucher specimens, deposited in the Zoologische Staatssammlung München, Zoological Museum Amsterdam, and Université d'Antananarivo, Département de Biologie Animale. A multigene phylogeny based on 104 specimens of all but two extant recognized *Phelsuma* species (with the exceptions of *P. masohoala* and *P. guimbeaui*) and most recognized subspecies was obtained, and published elsewhere (Rocha *et al.* 2009),

and is the basis for the figures and the discussion of species groups in the genus herein. This estimate of phylogeny was based on partial DNA sequences of two mitochondrial genes (cytochrome *b*, 714 nucleotides; 16S rRNA, 384 bp after exclusion of hypervariable regions) and three nuclear genes (Rag1, 473 bp; Rag2, 796 bp, C-*mos*, 384 bp). For details on laboratory protocols and primers used to obtain these sequences, and methods to analyse them, see Rocha *et al.* (2009). In brief, analyses were partitioned Bayesian inference using MrBayes ver. 3.1.2 (Huelsenbeck & Ronquist 2001), and maximum likelihood using GARLI (Zwickl 2006), after determining the appropriate substitution models using PAUP* ver. 4.0b10 (Swofford 2002) and Modeltest 3.06 (Posada & Crandall 1998) under the AIC criterion (Akaike 1974). We here compare the published estimate of phylogeny based on the concatenated nuclear and mitochondrial sequences (Rocha *et al.* 2009; excerpts of the tree shown here), with trees based on separate analyses of the concatenated mitochondrial and concatenated nuclear genes, respectively. In the discussion we refer to Bayesian posterior probabilities, transformed into percent, abbreviated as PP, and bootstrap values in percent, abbreviated as BS.

In addition we performed new analyses based on samples from more specimens of several species of the *Phelsuma lineata*- and *P. madagascariensis* groups. From these specimens, we extracted DNA using standard salt or phenol-chloroform protocols (Sambrook *et al.* 1999; Taggart *et al.* 1992), and amplified a fragment of the 16S rRNA gene with primers 16sar-L and 16sbr-H (Palumbi *et al.* 1991), using standard PCR protocols. Amplified fragments were sequenced on a 3730xl DNA analyser. The obtained sequences were combined with those from Rocha *et al.* (2009), Boumans *et al.* (2007), and Sound *et al.* (2006). New sequences obtained for this study have been deposited in GenBank under the accession numbers GU734090-GU734103. Alignments were performed with MAFFT version 6 (Katoh & Toh 2008) under the G-INS-I strategy and Bayesian analyses were performed using MrBayes ver. 3.1.2 (Huelsenbeck & Ronquist 2001), under the most appropriate model of evolution, determined using jModeltest (Posada 2008; Guindon & Gascuel 2003) under the AIC (Akaike 1974) criterion. In Bayesian analysis MCMC 3 heated and 1 cold chains were run for 5 million generations with default heating values. Two runs were performed for each dataset and convergence and congruence across runs assessed using "cumulative" and "compare" functions in AWTY (Nylander *et al.* 2008).

Based on an extensive survey of literature (Berghof & Trautmann 2009; Böhme & Meier 1981; Börner 1972; Börner & Minuth 1984; Boulenger 1885; Cheke 1975, 1981; Glaw *et al.* 2009; Hallmann *et al.* 2008; Krüger 1993, 1996a, 1996b, 1996c; Lerner 2004; Loveridge 1942; Meier 1977, 1981, 1982, 1983, 1984, 1986, 1987, 1989, 1993; Mertens 1962, 1963a, 1963b, 1964, 1966, 1970, 1973; Nussbaum *et al.* 2000; Raxworthy *et al.* 2007; Raxworthy & Nussbaum 1993, 1994; Rendahl 1939; Rösler 1998, 2001; Rösler *et al.* 2000; Schönecker 2008; Schönecker *et al.* 2004; Seipp 1990; Vinson & Vinson 1969) and own measurements we also compiled a database of phenotypic characters of *Phelsuma* species (including mainly external morphology and colouration, but also one behavioural character, egg-gluing). Although the few phenotypic characters available for these geckos all appear to be affected by homoplasy (see below), several of them turned out to be uniform in the clades recovered by molecular analysis and are therefore here presented (Tables 1–2) in order to allow a phenotypic characterization of the species groups we propose. We also examined type specimens from the collections of the Senckenberg Museum Frankfurt (SMF), Senckenberg Naturhistorische Sammlung Dresden (SNSD, Zoologisches Forschungsmuseum Alexander Koenig, Bonn (ZFMK), and the Zoologische Staatssammlung München (ZSM). We see the combination of literature data with original scale counts in Tables 1–2 as only a preliminary step towards a comprehensive revision of the genus, or of particular species groups, which should be accompanied by original counts and measurements carried out by the same person and that should be restricted to specimens with clear locality data and if possible associated to molecular data of the same individuals.

## Results and discussion

The general phylogeny among species groups of *Phelsuma* as proposed in the following is schematically represented in Fig. 1, based on the tree of Rocha *et al.* (2009). This figure also summarizes the main phenotypic characters of each species group, and the species assigned to each group.

**TABLE 1.** Body size, colour characters and egg-laying behaviour of recognized *Phelsuma* species and subspecies. Abbreviations: TL, maximum total length; SVL, maximum snout-vent length (both in mm). Colour is coded as follows: green, predominant colour green or blue; grey, predominant colour grey or brown (not green); interm., intermediate or mixed colour. Pattern: strong, contrast of main pattern compared with ground colour strong respectively weak, or intermediate. Stripes: tendency of dorsal or dorsolateral stripes present, absent, or intermediate; elongated patches on head, neck and lower back not considered as stripes. Lateral stripes: dark lateral/sublateral stripes present, absent or weak. *Phelsuma* can either glue their eggs to the substrate (+) or not (-). Missing data are represented by question marks (?).

| | TL | SVL | Colour | Pattern | Stripes | Lateral stripes | Egg gluer |
|---|---|---|---|---|---|---|---|
| *Pheluma barbouri* group | | | | | | | |
| *Phelsuma barbouri* Loveridge, 1942 | 150 | 64 | grey | strong | present | present | + |
| *Phelsuma pronki* Seipp, 1994 | 112 | 50 | grey | strong | present | present | - |
| *Phelsuma dubia* group | | | | | | | |
| *Phelsuma berghofi* Krüger, 1996 | 122 | 58 | interm. | weak | absent | absent | + |
| *Phelsuma dubia* (Boettger, 1881) | 155 | 68 | interm. | weak | absent | absent | + |
| *Phelsuma flavigularis* Mertens, 1962 | 170 | 66 | green | weak | absent | absent | + |
| *Phelsuma hielscheri* Rösler, Obst & Seipp, 2001 | 161 | 73.4 | interm. | strong | absent | absent | + |
| *Phelsuma malamakibo* Nussbaum, Raxworthy, Raselimanana & Ramanamanjato, 2000 | 123 | 61 | green | weak | absent | present | + |
| *Phelsuma modesta modesta* Mertens, 1970 | 96 | 46 | interm. | strong | absent | weak | + |
| *Phelsuma modesta isakae* Meier, 1993 | 120 | 57 | interm. | weak | absent | weak | + |
| *Phelsuma modesta leiogaster* Mertens, 1973 | 130 | 48 | grey | interm. | absent | weak | + |
| *Phelsuma nigristriata* Meier, 1984 | 110 | 46 | green | weak | absent | present | - |
| *Phelsuma ravenala* Raxworthy, Ingram, Rabibisoa & Pearson, 2007 | 129 | 61 | green | weak | absent | absent | + |
| *Phelsuma mutabilis* group | | | | | | | |
| *Phelsuma breviceps* Boettger, 1894 | 120 | 48 | grey | strong | absent | absent | - |
| *Phelsuma mutabilis* (Grandidier, 1869) | 102 | 50 | grey | strong | absent | absent | - |
| *Phelsuma borai* Glaw, Köhler, Vences 2009 | 88 | 43 | grey | strong | absent | absent | ? |
| *Phelsuma cepediana* group | | | | | | | |
| *Phelsuma borbonica borbonica* Mertens, 1966 | 160 | 69 | interm. | interm. | absent | absent | + |
| *Phelsuma borbonica agalegae* Cheke, 1975 | 170 | 71 | interm. | strong | absent | absent | + |
| *Phelsuma edwardnewtonii* Vinson & Vinson, 1969 | 223 | 108 | green | strong | absent | absent | ? |
| *Phelsuma cepediana* (Milbert, 1812) | 133 | 58 | green | strong | interm. | present | + |
| *Phelsuma guentheri* Boulenger, 1885 | >260 | 133 | grey | interm. | absent | absent | + |
| *Phelsuma guimbeaui* Mertens, 1963 | 150 | 62 | green | interm. | interm. | absent | + |
| *Phelsuma inexpectata* Mertens, 1966 | 130 | 58 | green | strong | absent | absent | + |
| *Phelsuma ornata* Gray, 1825 | 130 | 52 | green | strong | interm. | absent | + |
| *Phelsuma rosagularis* Vinson & Vinson, 1969 | 170 | 69 | interm. | interm. | absent | absent | + |
| *Phelsuma astriata* group | | | | | | | |
| *Phelsuma astriata astriata* Tornier, 1901 | 130 | 60 | green | weak | interm. | absent | - |
| *Phelsuma astriata semicarinata* Cheke, 1981 | 120 | 56 | green | weak | interm. | absent | - |
| *Phelsuma sundbergi sundbergi* Rendahl, 1939 | 220 | 93.5 | green | weak | absent | absent | - |
| *Phelsuma sundbergi ladiguensis* Böhme & Meier, 1981 | 170 | 80.5 | green | weak | absent | absent | - |

continued next page

**TABLE 1**. (continued)

| | TL | SVL | Colour | Pattern | Stripes | Lateral stripes | Egg-gluer |
|---|---|---|---|---|---|---|---|
| *Phelsuma sundbergi longinsulae* Rendahl, 1939 | 150 | 65 | green | weak | absent | weak | - |
| | | | | | | | |
| *Phelsuma madagascariensis* group | | | | | | | |
| *Phelsuma abbotti abbotti* Stejneger, 1893 | 110 | 56 | interm. | strong | interm. | absent | - |
| *Phelsuma abbotti chekei* Börner & Minuth, 1984 | 160 | 60 | interm. | strong | interm. | absent | - |
| *Phelsuma abbotti sumptio* Cheke, 1981 | 160 | 71 | green | weak | interm. | absent | - |
| *Phelsuma guttata* Kaudern, 1922 | 140 | 52 | green | weak | absent | absent | - |
| *Phelsuma grandis* Gray, 1870 | 300 | 102.4 | green | weak | absent | absent | - |
| *Phelsuma kochi* Mertens, 1954 | 254 | 95 | green | weak | absent | absent | - |
| *Phelsuma madagascariensis madagascariensis* (Gray, 1831) | 240 | 120 | green | weak | absent | absent | - |
| *Phelsuma madagascariensis boehmei* Meier, 1982 | 220 | 99 | green | weak | absent | absent | - |
| *Phelsuma masohoala* Raxworthy & Nussbaum, 1994 | 120 | 47 | grey | strong | absent | weak | - |
| *Phelsuma parkeri* Loveridge, 1941 | 165 | 65 | green | weak | absent | absent | - |
| *Phelsuma seippi* Meier, 1987 | 150 | 55 | green | weak | interm. | absent | - |
| | | | | | | | |
| *Phelsuma lineata* group | | | | | | | |
| *Phelsuma antanosy* Raxworthy & Nussbaum, 1993 | 130 | 48 | green | weak | absent | weak | - |
| *Phelsuma comorensis* Boettger, 1913 | 120 | 50 | green | weak | absent | present | - |
| *Phelsuma dorsivittata* Mertens, 1964 | 130 | 57 | green | weak | absent | present | - |
| *Phelsuma hoeschi* Berghof & Trautmann 2009 | 85 | 36,6 | green | strong | present | present | - |
| *Phelsuma kely* Schönecker, Bach & Glaw, 2004 | 71 | 33 | grey | weak | absent | weak | - |
| *Phelsuma lineata lineata* Gray, 1842 | 145 | 64 | green | interm. | absent | present | - |
| *Phelsuma lineata bombetokensis* Mertens, 1964 | 110 | 46 | green | interm. | absent | present | - |
| *Phelsuma lineata elanthana* Krüger, 1996 | 125 | 60 | green | weak | absent | present | - |
| *Phelsuma lineata punctulata* Mertens, 1970 | 115 | 55 | interm. | interm. | absent | present | ? |
| *Phelsuma parva* Meier, 1983 | 85 | 36 | green | weak | absent | weak | - |
| *Phelsuma pusilla pusilla* Mertens, 1964 | 85 | 37 | green | weak | absent | present | - |
| *Phelsuma pusilla hallmanni* Meier, 1989 | 100 | 42 | green | weak | absent | present | - |
| *Phelsuma quadriocellata quadriocellata* (Peters, 1883) | 110 | 63 | green | weak | absent | absent | - |
| *Phelsuma quadriocellata bimaculata* Kaudern, 1922 | 90 | 52 | green | weak | absent | absent | - |
| *Phelsuma quadriocellata lepida* Krüger, 1993 | 127 | 61 | green | interm. | absent | weak | - |
| | | | | | | | |
| *Phelsuma laticauda* group | | | | | | | |
| *Phelsuma laticauda laticauda* (Boettger, 1880) | 130 | 58 | green | interm. | absent | weak | - |
| *Phelsuma laticauda angularis* Mertens, 1964 | 120 | 52 | green | interm. | absent | weak | - |
| *Phelsuma pasteuri* Meier, 1984 | 115 | 53 | green | weak | absent | absent | - |
| *Phelsuma robertmertensi* Meier, 1980 | 110 | 50 | interm. | weak | present | weak | - |
| *Phelsuma v-nigra v-nigra* Boettger, 1913 | 110 | 46 | green | weak | absent | absent | - |
| *Phelsuma v-nigra anjouanensis* Meier, 1986 | 110 | 53 | green | weak | absent | absent | - |
| *Phelsuma v-nigra comoraegrandensis* Meier, 1986 | 100 | 48 | green | weak | absent | weak | - |
| | | | | | | | |
| *Phelsuma klemmeri* group | | | | | | | |

continued next page

**TABLE 1.** (continued)

| | TL | SVL | Colour | Pattern | Stripes | Lateral stripes | Egg gluer |
|---|---|---|---|---|---|---|---|
| *Phelsuma klemmeri* Seipp, 1990 | 100 | 43 | green | weak | present | present | - |
| *Phelsuma andamanense* group | | | | | | | |
| *Phelsuma andamanense* Blyth, 1860 | 140 | 56 | green | weak | absent | absent | - |
| *Phelsuma standingi* group | | | | | | | |
| *Phelsuma standingi* Methuen & Hewitt, 1913 | 280 | 135 | grey | strong | absent | absent | - |
| Unassigned species | | | | | | | |
| *Phelsuma serraticauda* Mertens, 1963 | 160 | 60 | green | weak | absent | absent | - |
| *Phelsuma vanheygeni* Lerner, 2004 | 80 | 35 | green | weak | absent | absent | + |

**TABLE 2.** Selected scale characters in species and subspecies of day geckos. Abbreviations: SL, number of supralabials (min - max); IL, number of infralabials (min-max); N, number of nasalia (defined as scales in contact with nostril); NC, contact of nostril only with first supralabial (0) or with rostral and first supralabial (1); DL, dorsal and lateral scales keeled (k) or smooth (s); V, ventrals keeled (k) or smooth (s); DST, dorsal scales on tail keeled (k) or smooth (s); SC, subcaudalia keeled (k) or smooth (s); SC broad, subcaudalia broadened (+) or not broadened (-); VSS, vertebral region with smaller scales (+) or without such scales (-); PFP, number of preanofemoral pores in males; Midbody scales, number of scales around midbody (minimum-maximum). Missing data are represented by question marks (?).

| | SL (min-max) | IL (min-max) | N | NC | DL | V | DST | SC | SC broad | VSS | PFP | Mid-body scales |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Pheluma barbouri* group | | | | | | | | | | | | |
| *Phelsuma barbouri* | 6–9 | 6 | 3 | 0 | s | s | s | s | - | - | 26–27 | 92–108 |
| *Phelsuma pronki* | 6–7 | 6–7 | 3 | 0 | s | s | s | s | + | - | 31 | 82–93 |
| *Phelsuma dubia* group | | | | | | | | | | | | |
| *Phelsuma berghofi* | 9–10 | 8–9 | 3 | 0 | k | k | k | k | - | - | 22–28 | 66–68 |
| *Phelsuma dubia* | 9–12 | 8–10 | 2–3 | 0 | k | s | k | s | - | + | 19–30 | 68–89 |
| *Phelsuma flavigularis* | 10–11 | 8–9 | 3 | 0 | k | k | k | k | - | + | 22–29 | 60–72 |
| *Phelsuma hielscheri* | 9–11 | 8–10 | 3 | 0 | k | k | s | s | - | - | 25–30 | 86–98 |
| *Phelsuma malamakibo* | 8–11 | 6–9 | 3 | 0 | s | s | s | s | - | + | ? | 69–79 |
| *Phelsuma modesta modesta* | 8–9 | 7–8 | 3 | 0 | k | s | s | s | + | - | 16–24 | 82–87 |
| *Phelsuma modesta isakae* | 8–9 | 6–8 | 3 | 0 | k | s | s | s | + | - | 16–20 | 88–95 |
| *Phelsuma modesta leiogaster* | 8–9 | 7–8 | 3 | 0 | k | s | s | s | + | - | 16–20 | 84 |
| *Phelsuma nigristriata* | 7 | 6 | 3 | 0 | k | s | s | s | + | - | 23 | 77–80 |
| *Phelsuma ravenala* | 9–11 | 9–11 | 3–4 | 0 | s | s | k | s | - | - | 27–29 | 64–76 |
| *Phelsuma mutabilis* group | | | | | | | | | | | | |
| *Phelsuma breviceps* | 6–7 | 5–6 | 2 | 0 | s | s | s | s | + | - | 27–31 | 95–119 |
| *Phelsuma mutabilis* | 6–8 | 5–6 | 2–3 | 0 | s | s | s | s | + | - | 27–32 | 91–98 |
| *Phelsuma borai* | 9–10 | 6–7 | 3 | 0 | s | s | s | s | + | - | 29 | 96 |
| *Phelsuma cepediana* group | | | | | | | | | | | | |
| *Phelsuma borbonica borbonica* | 8–11 | 6–10 | 3 | 1 | s | s | s | s | - | - | 22–23 | 99–114 |

continued next page

**TABLE 2.** (continued)

| | SL (min-max) | IL (min-max) | N | NC | DL | V | DST | SC | SC broad | VSS | PFP | Mid-body scales |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Phelsuma borbonica agalegae* | 7–9 | 6–8 | 3 | 1 | s | s | s | s | + | - | 11–16 | 98–115 |
| *Phelsuma cepediana* | 7–11 | 6–10 | 3 | 1 | k | s | s | s | - | - | 14–25 | 87–104 |
| *Phelsuma edwardnewtonii* | 7–10 | 6–8 | 3 | 1 | s | s | s | s | + | + | 11–14 | 107 |
| *Phelsuma guentheri* | 10–14 | 6–12 | 3 | 1 | k | s | s | s | + | - | 43–50 | 144–162 |
| *Phelsuma guimbeaui* | 6–10 | 6–11 | 3 | 1 | s | s | s | s | - | - | 21–28 | 89–98 |
| *Phelsuma inexpectata* | 8–12 | 7–10 | 3 | 1 | k | s | k | s | - | - | 19–22 | 100–103 |
| *Phelsuma ornata* | 7–10 | 5–9 | 3 | 1 | k | s | s | s | + | - | 17–23 | 91–105 |
| *Phelsuma rosagularis* | 7–9 | 5–8 | 3 | 0 | s | s | s | s | + | - | 25–26 | 96 |
| | | | | | | | | | | | | |
| *Phelsuma astriata* group | | | | | | | | | | | | |
| *Phelsuma astriata astriata* | 8–11 | 7–8 | 3 | 0 | k | k | k | k | - | + | 26–30 | 88–96 |
| *Phelsuma astriata semicarinata* | 9–10 | 7–9 | 3 | 0 | k | k | k | s | + | + | ? | 90–95 |
| *Phelsuma sundbergi sundbergi* | 8–11 | 6–9 | 3 | 0 | k | s | k | s | + | + | 27–36 | 87–99 |
| *Phelsuma sundbergi ladiguensis* | 8–10 | 7–9 | 3 | 0 | k | s | s | s | + | + | 21–32 | 85–100 |
| *Phelsuma sundbergii longinsulae* | 8–11 | 7–9 | 3 | 0 | k | s | s | s | + | + | 28–35 | 78–97 |
| | | | | | | | | | | | | |
| *Phelsuma madagascariensis* group | | | | | | | | | | | | |
| *Phelsuma abbotti abbotti* | 8–10 | 6–7 | 3 | 0 | k | s | s | s | + | + | 30–35 | 78–80 |
| *Phelsuma abbotti chekei* | 6–9 | 7–9 | 3 | 0 | k | s | k | s | + | + | 33–38 | 68–82 |
| *Phelsuma abbotti sumptio* | 8–10 | 6–7 | 3 | 0 | k | s | k | s | + | + | ? | 79 |
| *Phelsuma guttata* | 8–11 | 6–9 | 3 | 0 | k | s | s | s | - | + | 28 | 92–98 |
| *Phelsuma grandis* | 9–11 | 7–9 | 3 | 0 | k | s | k | s | + | + | 41–44 | 67–77 |
| *Phelsuma kochi* | 7–8 | 7 | 3 | 0 | k | s | k | s | + | + | 42–48 | 78–95 |
| *Phelsuma m. madagascariensis* | 6–10 | 6–9 | 3–4 | 0 | k | s | k | s | + | + | 35–43 | 73–88 |
| *Phelsuma m. boehmei* | 7–8 | 7–8 | 3 | 0 | k | s | s | s | + | + | 40 | 95–101 |
| *Phelsuma masohoala* | 8–9 | 7 | 3 | 0 | k | s | k | s | + | + | ? | 76 |
| *Phelsuma parkeri* | 8–10 | 6–9 | 3 | 0 | k | s | s | s | + | + | 32–38 | 76 |
| *Phelsuma seippi* | 9 | 6–7 | 3 | 0 | k | s | s | s | + | + | 36 | 105–110 |
| | | | | | | | | | | | | |
| *Phelsuma lineata* group | | | | | | | | | | | | |
| *Phelsuma antanosy* | 8–11 | 7–8 | 3 | 0 | k | k | k | k | - | - | 22–29 | 78–98 |
| *Phelsuma comorensis* | 7–9 | 6–8 | 3 | 0 | k | s | k | s | - | - | 25–31 | 78–89 |
| *Phelsuma dorsivittata* | 8–9 | 6–9 | 3 | 0 | k | k | k | k | - | - | 25–29 | 76 |
| *Phelsuma hoeschi* | 8–9 | 7 | 3 | 0 | k | k | k | k | - | - | 29 | 86 |
| *Phelsuma kely* | 8–10 | 7–9 | 3–4 | 0 | k | k | k | k | - | - | 22–29 | 80–86 |
| *Phelsuma l. lineata* | 7–10 | 6–9 | 3 | 0 | k | k | k | k | - | - | 20–34 | 74 |
| *Phelsuma l. bombetokensis* | 7–9 | 6–8 | 2–3 | 0 | k | k | k | k | - | - | 21–26 | 63–78 |
| *Phelsuma l. elanthana* | 6–10 | 6–8 | ? | 0 | k | k | s | k | - | - | 24–30 | 73–80 |
| *Phelsuma l. punctulata* | 7–8 | 6–8 | 3 | 0 | k | k | k | s | - | - | 26 | 66 |
| *Phelsuma p. pusilla* | 7–9 | 6–8 | 3 | 0 | k | k | k | k | - | - | 21–25 | 62–80 |
| *Phelsuma p. hallmanni* | 9 | 8 | 3 | 0 | s | k | k | k | - | - | 28–30 | 82 |

continued next page

**TABLE 2.** (continued)

| | SL (min-max) | IL (min-max) | N | NC | DL | V | DST | SC | SC broad | VSS | PFP | Mid-body scales |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Phelsuma parva* | 7–10 | 7–8 | 3 | 0 | k | k | k | k | - | - | 20–24 | 67–71 |
| *Phelsuma q. quadriocellata* | 7–10 | 6–9 | 3 | 0 | k | s | k | k | - | - | 24–30 | 68–77 |
| *Phelsuma q. bimaculata* | 8–11 | 6–9 | 3 | 0 | k | k | k | k | - | - | 24–30 | 65–71 |
| *Phelsuma q. lepida* | 8–10 | 7–8 | 3 | 0 | k | k | k | k | - | - | 26–30 | 71 |
| *Phelsuma laticauda* group | | | | | | | | | | | | |
| *Phelsuma l. laticauda* | 6–9 | 6–9 | 3 | 0 | k | s | k | s | + | + | 20–28 | 72–81 |
| *Phelsuma l. angularis* | 7–9 | 6–7 | 3 | 0 | k | s | k | s | + | + | 25 | 84 |
| *Phelsuma pasteuri* | 8 | 8 | 3 | 0 | k | s | k | s | - | + | 25–31 | 80–87 |
| *Phelsuma robertmertensi* | 8–9 | 7–8 | 3 | 0 | s | s | s | s | - | + | 26–30 | 75–86 |
| *Phelsuma v-nigra v-nigra* | 7–8 | 6–8 | 3 | 0 | s | ? | s | s | - | - | 20–28 | 97–104 |
| *Phelsuma v-nigra anjouanensis* | 7–8 | 7 | 3 | 0 | s | s | s | s | - | - | 20–25 | 101 |
| *Phelsuma v-nigra comoraegrandensis* | 6–8 | 7 | 3 | 0 | s | s | s | s | - | - | 25 | 91–97 |
| *Phelsuma klemmeri* group | | | | | | | | | | | | |
| *Phelsuma klemmeri* | 9–10 | 7 | 3 | 0 | s | s | s | s | + | + | 31 | 75–80 |
| *Phelsuma andamanense* group | | | | | | | | | | | | |
| *Phelsuma andamanense* | 8–11 | 6–10 | 3 | 0 | s | s | s | s | + | - | 24–32 | 80–90 |
| *Phelsuma standingi* group | | | | | | | | | | | | |
| *Phelsuma standingi* | 9–12 | 7–8 | 3 | 0 | s | s | s | s | - | - | 43 | 112–122 |
| Unassigned species | | | | | | | | | | | | |
| *Phelsuma serraticauda* | 9–10 | 8–9 | 3 | 0 | k | k | k | k | - | - | 18–21 | 88–96 |
| *Phelsuma vanheygeni* | 8–10 | 5–9 | 3–4 | 1 | s | s | s | s | + | - | 26–28 | 80–82 |

### ***Phelsuma barbouri* group**

(contains *P. barbouri* and *P. pronki*)

These two species were recovered as sister taxa with strong support by both the mitochondrial and nuclear sequences, and the combined analysis (clade Q in Rocha *et al.* 2009; not shown here). They were previously thought to be closely related (Glaw *et al.* 1999) but later *P. pronki* was reclassified within the *P. klemmeri* group based on being a non-egg-gluer (Van Heygen 2004), contrary to *P. barbouri*. The molecular data suggests instead that *P. klemmeri* does not belong to any of the previously identified groups; although it may be more related to the *P. lineata* and *P. laticauda* species groups (see below). *Phelsuma barbouri* and *P. pronki* individuals exhibit a genetic distance of only 5.1% (uncorrected p-distance, cytochrome *b*), which is low compared to other interspecific differentiation values within this genus and points to their relatively recent differentiation.

*Phelsuma barbouri* and *P. pronki* share a rather depressed body and head shape, and a rather similar and distinct colour pattern without clear predominance of green, but differ in the number of midbody scales (higher in *P. barbouri*), number of ventral scales and egg-gluing behaviour (Tables 1–2).

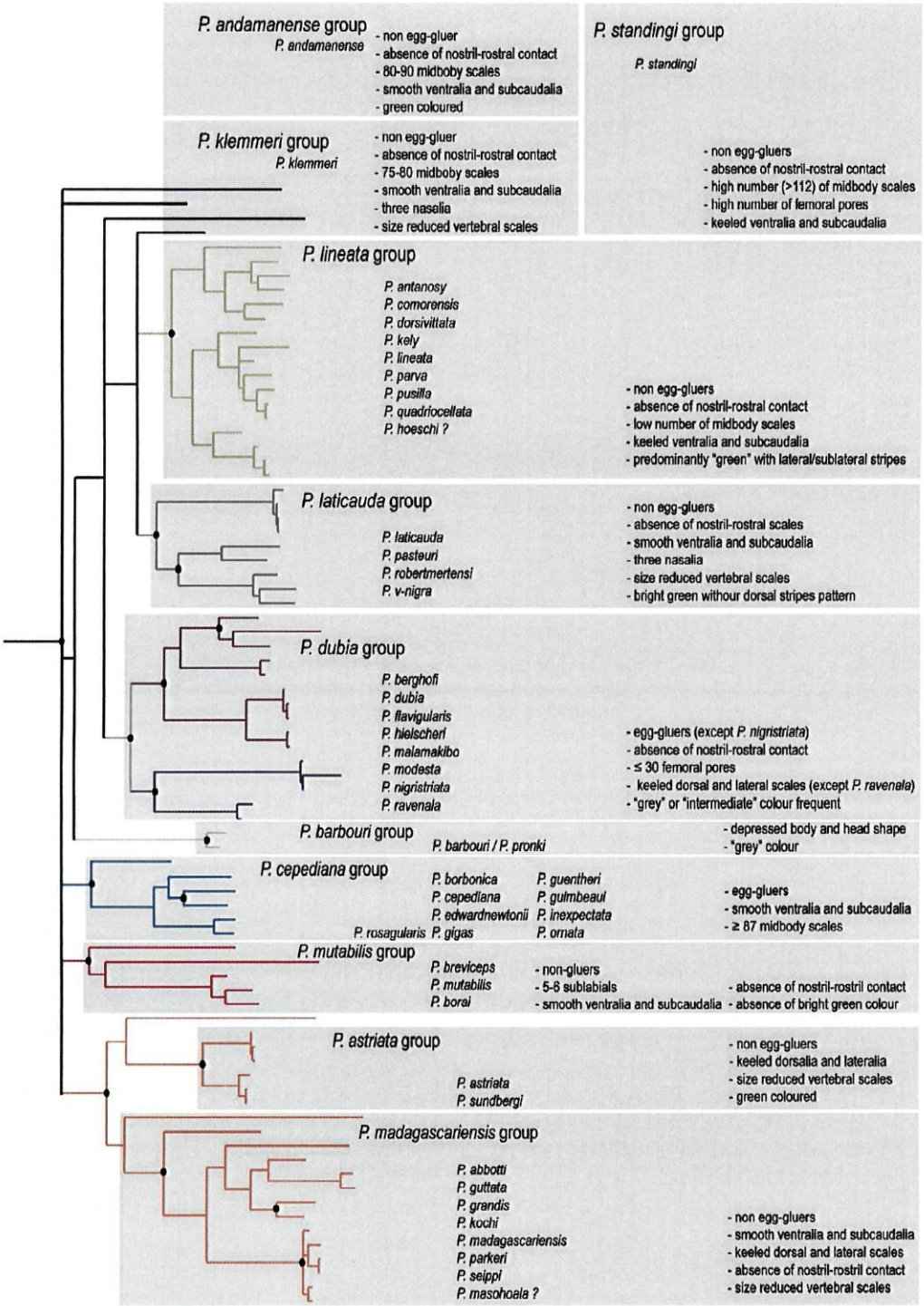



**FIGURE 1.** Schematic cladogram summarizing phylogenetic relationships of species groups proposed herein, species contained in each group, and characters useful for their phenotypic characterization. The tree is adapted from Rocha *et al.* (2009) and represents the BI 50% majority-rule consensus tree of combined mitochondrial and nuclear data. Species groups containing more that one species are colour coded.

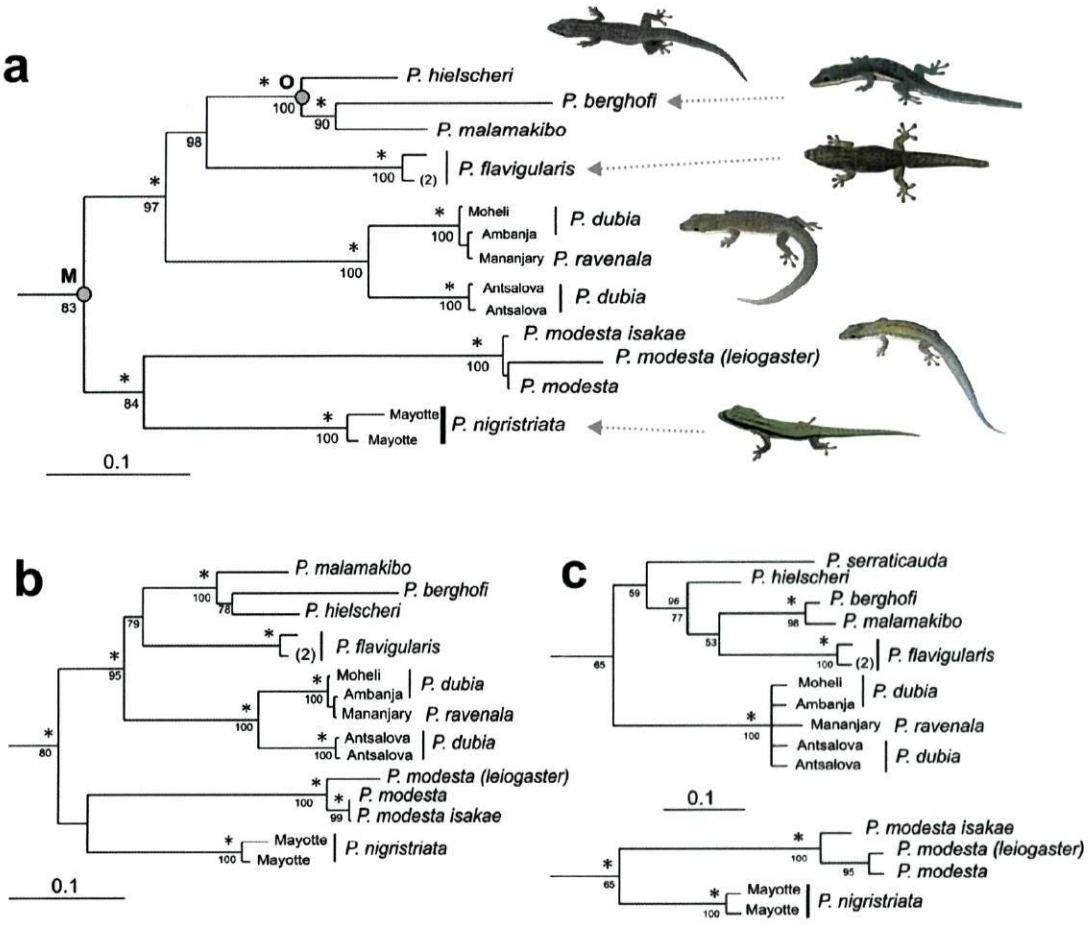



**FIGURE 2.** Molecular phylogeny of the *Phelsuma dubia* group as calculated from the multi-gene dataset of Rocha *et al.* (2009). Shown are the respective clades from the Bayesian 50% majority-rule consensus trees based on (a) the combined dataset of mitochondrial and nuclear genes, (b) the mitochondrial genes only, and (c) the nuclear genes only. Asterisks mark nodes supported by Bayesian posterior probabilities of 99% or higher. ML bootstraps supports above 50 are given below the corresponding branch. Relevant PP values above 95 are given above respective branches (italics). Clade numbers M and O are as in Rocha *et al.* (2009) and discussed in the text.

*P. barbouri* is the most ground-dwelling species within the genus, found on and around the exposed rocky outcrops and boulders of unforested habitat areas of Madagascar's central mountain chain. In contrast, *P. pronki* is an arboreal species in the mid-altitude rainforests bordering Madagascar's central high plateau. The divergence between these two species is likely a recent one, and may have been driven by ecological specialization along the sharp ecotone between these habitat types. The distribution of *P. pronki* is very poorly known (Glaw & Vences 2007), and the species is probably rare and/or localized. Additional surveys are needed to understand if these two taxa are geographically isolated or have a contact or hybrid zone.

### *Phelsuma dubia* group

(contains *P. berghofi, P. dubia, P. flavigularis, P. hielscheri, P. malamakibo, P. modesta, P. nigristriata* and *P. ravenala*)

Evidence for a close relationship between *P. dubia*, *P. flavigularis*, *P. malamakibo*, *P. berghofi* and *P.*

*hielscheri* and of these with *P. modesta* and *P. nigristriata* is apparent from the molecular phylogenies (Fig. 2; clade M), corroborating morphological data. This group is strongly supported by the combined phylogeny and mtDNA data and also recovered (though not strongly supported – 89 PP, including also *P. serraticauda*) by the nuclear phylogeny.

Within this clade, *P. hielscheri*, *P. berghofi* and *P. malamakibo* (clade O) plus *P. flavigularis*, form a generally strongly supported clade (Fig. 2), but the position of this latter species respective to others differs between the mitochondrial and combined phylogenies (where P. *flavigularis* is basal to remaining three species) and the nuclear data (which instead recovers *P. flavigularis* as sister to *P. berghofi* and *P. malamakibo*, with *P. hiescheri* basal to these three). The placement of this group of species (*P. malamakibo, P. berghofi, P. hielscheri* and *P. flavigularis*) with the *P. dubia / P. ravenala* clade is strongly supported by mtDNA data and, although weakly, also by the nuclear data.

The placement of *P. hielscheri* (and *P. malamakibo*) in the *P. dubia* group is congruent with the morphological data (Table 1–2; see also Van Heygen 2004) but contrasts with recent results from Raxworthy *et al.* (2007) in which their individual of *P. hielscheri* was more closely related to *P. lineata* and *P. laticauda*. Specimens of *P. hielscheri* have previously been considered as *P. dubia* (see Glaw *et al.* 1999), and the differences between the two species only recently noted (Rösler *et al.* 2001). In our analysis, even when it is not placed in a monophyletic group with *P. malamakibo* and *P. berghofi*, *P. hielscheri* is always recovered as included in the *P. dubia* group, and never close to the *P. lineata* or *P. laticauda* species groups as in the analysis of Raxworthy *et al.* (2007). A reanalysis of the data of these authors as available from Genbank revealed that they had only a very short partial sequence of cytochrome *b* available for this species (193 bp; EF434870); which, when aligned with our data, clustered deep inside the *Phelsuma lineata* group. Since our samples come from a well-identified specimen of *P. hielscheri* and the various markers we sequenced are congruent in placing it into the *P. dubia* group, we conclude that the deviant placement of the species in the phylogeny of Raxworthy *et al.* (2007) is likely erroneous.

The mitochondrial genes reveal additional deep differentiation within *P. dubia* (although not apparent in the nuclear phylogeny, possibly due to incomplete lineage sorting). *P. dubia* has a patchy distribution along the northwestern coast of Madagascar, possibly with disjunct populations (Glaw & Vences 1994). We find a deep divergence between the individual from the northern isolate (Ambanja) and the southern one (Antsalova) (12.2% uncorrected p-distance in the cytochrome *b* marker). The included specimen from Moheli, Comoros, is very closely related to specimens from northwestern Madagascar (Ambanja), as are specimens from Zanzibar, Tanzania: see Rocha *et al.* 2007). Recently, Raxworthy *et al.* (2007) described *P. ravenala* from the eastern coast of Madagascar as a new species from the *P. dubia* group. Although genetic differentiation relative to *P. dubia* was minimal (~0.3%, 12S rRNA uncorrected p-distance; 0.4–0.7% cytochrome *b* distance according to our data), allopatric distribution, morphological differentiation and better fit of distribution models when considering it a different species were invoked to argue for its specific status. Our *P. dubia* individuals from the Antsalova region in western Madagascar exhibit a much higher degree of differentiation relative to remaining *P. dubia* (14% uncorrected p-distance at cytochrome *b*) and might also be allopatrically distributed. This indicates that the taxonomy in the *P. dubia/ravenala* complex of species merits further investigation; *P. dubia* is at the moment paraphyletic with respect to *P. ravenala*, and possibly contains yet further undescribed species, with the western Antsalova population being one candidate. Also, the validity of *P. ravenala* is in need of confirmation, especially regarding its morphological differentiation (in number of midbody scales, colour and number of preanofemoral pores; see Raxworthy *et al.* 2007 and Tables 1–2).

The second major clade in the *P. dubia* group is composed of *P. modesta* and the Comoran *P. nigristriata*. The sister group relationship of these two species is supported by nuclear as well as mitochondrial data. The placement of this clade with the *P. dubia* group is strongly supported by the combined and mitochondrial data sets but unresolved with nuclear data alone. Our results are partly in agreement with the opinion of Nussbaum *et al.* (2000) who considered the subspecies of *Phelsuma modesta* as invalid forms representing colour variations. The level of differentiation between two of the subspecies (*P. modesta modesta* from Tolagnaro and *P. m. isakae* from near the type locality Isaka) is very low (0.1% uncorrected p-distance in cytochrome *b*) and the blue head colouration of males, the only character to distinguish *P. m. isakae* from *P. m. modesta* is

also seen in specimens from the Tolagnaro region, usually assigned to *P. m. modesta*. On the other hand, *P. modesta leiogaster* presents a higher level of differentiation (6%), but the topology recovered differs between mitochondrial and nuclear genes.

Species in the *Phelsuma dubia* group share egg-gluing behaviour (except for *P. nigristriata*), absence of nostril-rostral contact, a relatively low number of preanofemoral pores in males (30 or less), and keeled dorsal and lateral scales (except for *P. ravenala*). Most species have a weak expression of green colour, and in some species of the group the ventrals and/or subcaudals are keeled (Tables 1–2).

### ***Phelsuma mutabilis* group**

(contains *P. borai* [named *P.* sp. aff. *mutabilis* in Rocha *et al.* 2009], *P. breviceps,* and *P. mutabilis*)

The three species in this group inhabit southwestern (*P. breviceps, P. mutabilis*) and western Madagascar (*P. mutabilis, P. borai*). Close relationships between *P. breviceps* and *P. mutabilis* had already been hypothesized based on morphometric and scale features (Loveridge 1942); in fact, the two species have been synonymized and resurrected several times (Loveridge 1942; Blanc 1972; Kluge 1991; Raxworthy & Nussbaum 1994). The mitochondrial and combined molecular data recover this group as monophyletic, although the nuclear data alone fail to support this grouping (Fig. 3). Furthermore, the combined analysis recovers a monophyletic *P. mutabilis* sister to the very divergent *P. borai.*

Genetic distances within this clade are high: average uncorrected p-distance between *P. breviceps* and *P. mutabilis* for cytochrome *b* is of 18.9%, with the distance of *P. borai* to *P. breviceps* (22.1%) and to *P. mutabilis* (23.5%) being even higher. Cytochrome *b* p-distances within *P. mutabilis* reach 8% (see also Glaw *et al.* 2009).

Species in the *P. mutabilis* group share, among other character states, the absence of bright green colour, non-gluing egg laying behaviour, a relatively low number of sublabials (5–6), smooth ventrals and subcaudals, and absence of nostril-rostral contact (Tables 1-2).

The combined mitochondrial data (Fig. 3b) indicate possible affinities of the *P. mutabilis* group with *P. andamanense.* However, this grouping received less than 50% bootstrap and very low Bayesian support and warrants further analysis.

### ***Phelsuma cepediana* group**

(contains *P. borbonica, P. cepediana, P. edwardnewtonii, P. gigas, P. guentheri, P. guimbeaui, P. inexpectata, P. ornata,* and *P. rosagularis*)

The lineage containing all species from the Mascarene Islands was previously studied in detail by Austin *et al.* (2004) with a considerably better sampling including the two extinct species *P. edwardnewtonii* and *P. gigas.* Corroborating previous results, we also consistently recovered it as a strongly supported monophyletic clade (clade B in Fig. 4). Clearly recovered by both the mitochondrial and combined data, *P. guentheri* is basal to the remaining species, and appears to have differentiated from these at an early stage of the Mascarene radiation although the two extinct species, not included in our study, might have been even more basal (Austin *et al.* 2004).

The extant species in the *P. cepediana* group are quite variable in many morphological and chromatic characters but share egg-gluing behaviour, smooth ventralia and subcaudalia, a contact between rostral and nostril (apparently all except *P. rosagularis*), and a high number of midbody scales (87 or more) (Tables 1–2). *Phelsuma guentheri* differs from all extant species in the *P. cepediana* group (including *P. edwardnewtonii*), and all other *Phelsuma* species, by the ability to change the pupil to a vertical slit. This and its associated "crepuscular" activity, as in the closely related (and extinct) *P. gigas*, are most probably of secondary origin (Austin 2004).

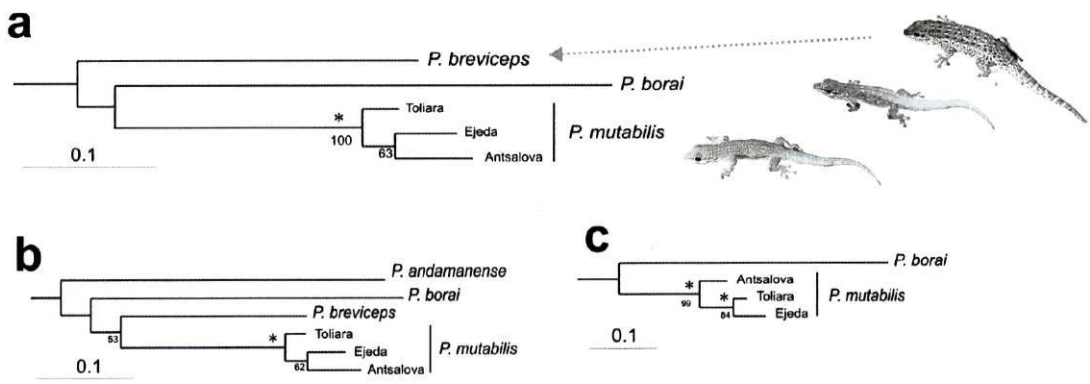



**FIGURE 3.** Molecular phylogeny of the *Phelsuma mutabilis* group as calculated from the multi-gene dataset of Rocha *et al.* (2009). Shown are the respective clades from the Bayesian 50% majority-rule consensus trees based on (a) the combined dataset of mitochondrial and nuclear genes, (b) the mitochondrial genes only, and (c) the nuclear genes only. See caption to Fig. 2 for further explanations.

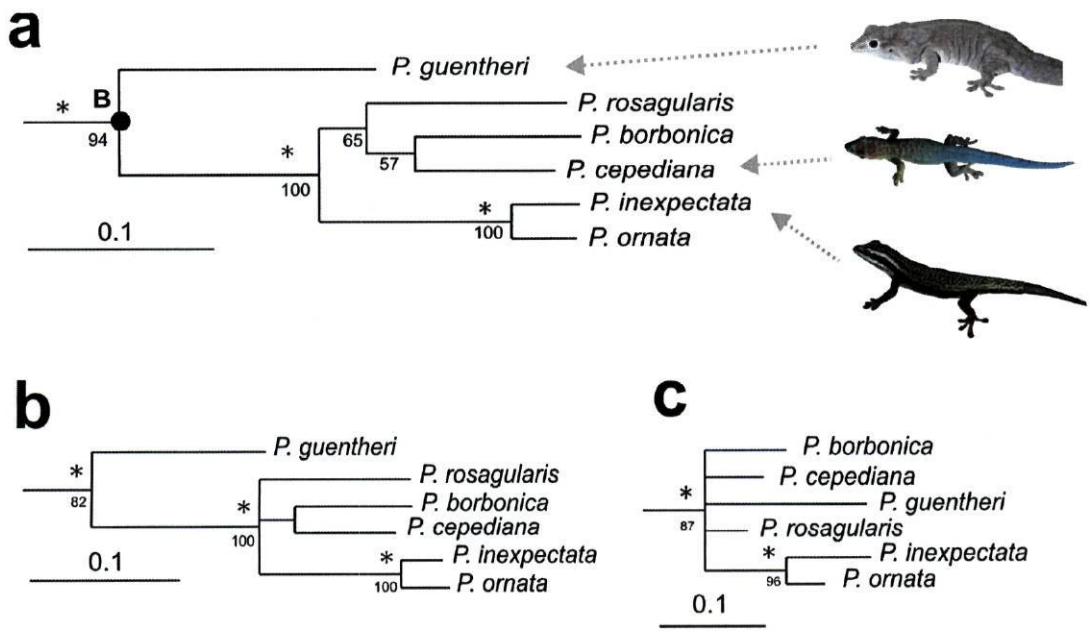



**FIGURE 4.** Molecular phylogeny of the *Phelsuma cepediana* group as calculated from the multi-gene dataset of Rocha *et al.* (2009). Shown are the respective clades from the Bayesian 50% majority-rule consensus trees based on (a) the combined dataset of mitochondrial and nuclear genes, (b) the mitochondrial genes only, and (c) the nuclear genes only. See caption to Fig. 2 for further explanations.

***Phelsuma astriata* group**
(contains *P. astriata* and *P. sundbergi*)

These two species form a small endemic radiation on the Seychelles islands and various subspecies have been described for both. Variation within this Seychellois group was studied by Cheke (1982), Gardner (1984, 1986, 1987), and more recently, by Radtkey (1996). The available molecular data, nuclear and mitochondrial, is unambiguous in supporting monophyly of this group (Fig. 5: clade H), which furthermore was recovered in

single-gene analyses of all genes except C-*mos*, where differentiation relative to other species in clade E (see Fig. 5) is very low. The mitochondrial and combined analyses indicate that, possibly, *Phelsuma vanheygeni* is the sister species of the Seychellois clade and may be in the future included in the *P. astriata* group (see section on this species below).

Species in the *Phelsuma astriata* group are all green coloured with a weak dorsal pattern and no or only weak dorsal or lateral lines, keeled dorsalia and lateralia, size-reduced vertebral scales, and are non-gluers, but otherwise appear to be quite variable in morphological characters (Tables 1–2).

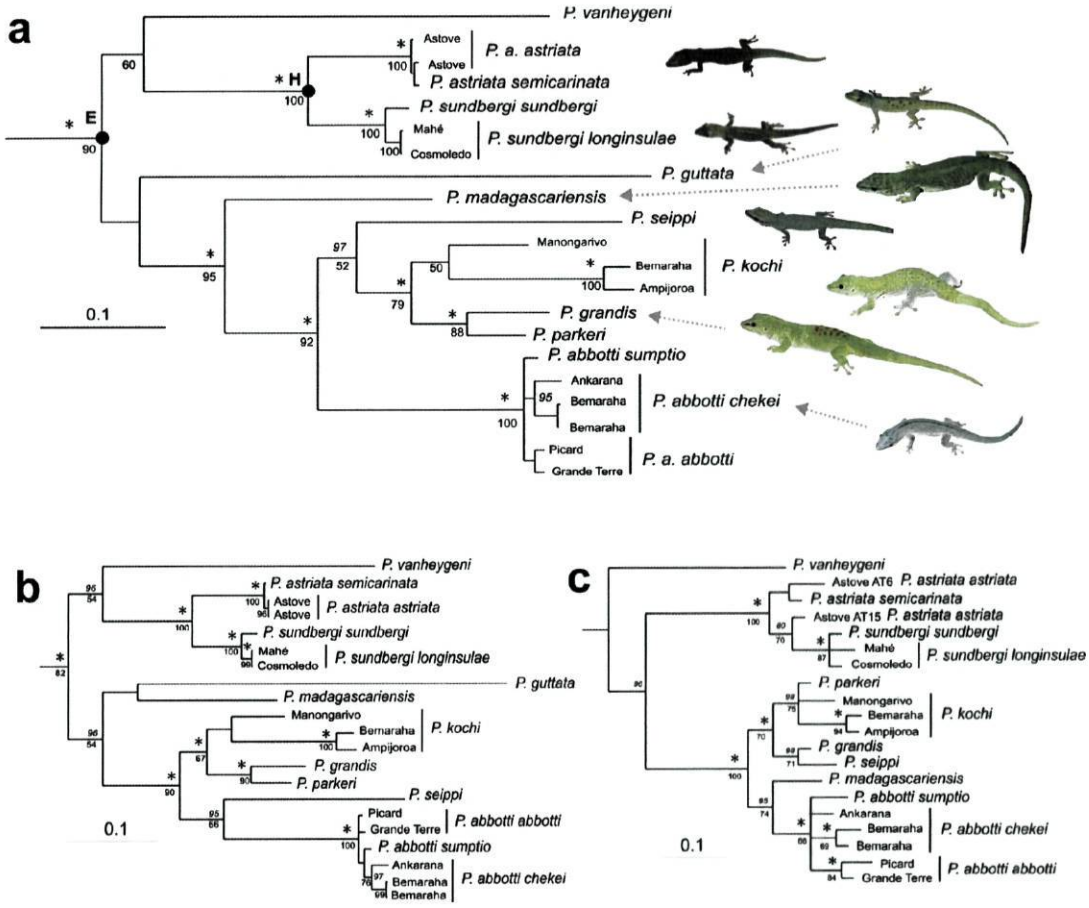



**FIGURE 5.** Molecular phylogeny of the *Phelsuma astriata* group (clade H) the *P. madagascariensis* group, and *P. vanheygeni* as calculated from the multi-gene dataset of Rocha *et al.* (2009). Shown are the respective clades from the Bayesian 50% majority-rule consensus trees based on (a) the combined dataset of mitochondrial and nuclear genes, (b) the mitochondrial genes only, and (c) the nuclear genes only. See caption to Fig. 2 for further explanations.

### ***Phelsuma madagascariensis*** **group**

(contains *Phelsuma abbotti, P. grandis, P. guttata, P. kochi, P. madagascariensis, P. parkeri, P. seippi* and probably *P. masohoala*)

Relationships within the *P. madagascariensis* group

This group is in a well-supported clade (Fig. 5; clade E) with the Seychellean *P. astriata* group, and with *P. vanheygeni.* The basal topology of clade E is uncertain; the position of both *P. vanheygeni* and *P. guttata* is unresolved, mainly because of conflicts between the mitochondrial and nuclear datasets. In the mitochondrial,

as well as in the combined data, *P. vanheygeni* is sister taxon to the *P. astriata* group and *P. guttata* is placed in the *P. madagascariensis* group. The nuclear data, however, place *P. vanheygeni* basal in clade E, and *P. guttata* completely outside of it, albeit both placements with less than 50% PP/BS support. Because of its morphological affinities to the *P. madagascariensis* group we nevertheless include *P. guttata* in this group, but we emphasize that further study on its phylogenetic position is needed.

Within the *P. madagascariensis* group, the *P. guttata* species group as previously defined (*P. guttata, P. abbotti, P. masohoala,* and *P. seippi*; Glaw & Vences 1994; Glaw *et al.* 1999; Van Heygen 2004) does not form a monophyletic group; instead, *P. guttata* is placed basal in the group, and *P. seippi* and *P. abbotti* also do not appear to be sister species. Two members of this group originated by (long) distance colonization in a northwestwards direction: *P. abbotti abbotti* (Aldabra) and *P. parkeri* (Pemba). Both have their closest relatives in the northwestern/northern Malagasy coast, the most likely area in fact from which colonizations to occur from, considering surface sea-currents in the region (Ali & Huber 2010).

One of the few nominal *Phelsuma* species for which no DNA sequences are available until now is *P. masohoala.* As discussed by Meier & Böhme (1996) it is possible that *P. masohoala* is related to *P. abbotti* and thus belongs to the *P. madagascariensis* group where we provisionally include it.

Relationships among *P. madagascariensis, P. grandis,* and *P. kochi*

In our molecular analysis, *Phelsuma grandis, P. kochi,* and *P. madagascariensis* which were long considered subspecies of a single species (*P. madagascariensis*) did not form a clade, although the mitochondrial and nuclear data suggested different relationships among these taxa. This confirms the results of Raxworthy *et al.* (2007) who recently proposed to elevate them to species level, based on data from a molecular analysis and from environmental niche modelling. Neither Raxworthy *et al.* (2007) nor ourselves had sequences of *Phelsuma madagascariensis boehmei* Meier, 1982, and the status of this taxon thus remains unclarified.

We detected deep genetic divergences among *P. grandis, P. kochi,* and *P. madagascariensis,* reaching up to 14% (uncorrected p-distances from cytochrome *b*). Within *P. grandis* and *P. kochi* distances of up to 1.1% and 9% were, respectively, observed for cytochrome *b*. These surprisingly high distances prompted us to undertake a wider survey of variability in the *P. madagascariensis* group, based on mitochondrial 16S rDNA sequences. The three taxa, *P. grandis, P. kochi,* and *P. madagascariensis* are large geckos that are thought to be allopatrically distributed mainly in coastal Madagascar: *P. madagascariensis* in the east, *P. grandis* in the north, and *P. kochi* in the west (Meier & Böhme 1991), with contiguous distributions and contact zones between them. The new data (Figs. 5 and 6) add various new perspectives to this picture:

First, we detected a second deep lineage that we here assign to *P. kochi* in a preliminary way: a specimen (identified as *P. kochi*) from Manongarivo in the Sambirano region, far northwestern Madagascar, differed strongly from specimens from the west (Tsingy de Bemaraha and Ankarafantsika). For 16S alone, it did not even form a monophyletic group with the remaining *P. kochi* (Fig. 6), and although it did in the combined analysis (Fig. 5), support was not significant (PP<50%; BS=50%).

Second, also samples of *P. madagascariensis* were deeply differentiated, with an eastern-southeastern clade (from Ambohitsara and Nosy Boraha) and a north-eastern clade (from Ankalampo), and with a differentiation between these of 7.5% uncorrected p-distance in the 16S gene (Fig. 6).

Differentiation within *P. abbotti*

A last aspect obvious from the multi-gene phylogeny (Fig. 5) and the 16S data (Fig. 6) is the low differentiation among the various subspecies of *Phelsuma abbotti.* In Madagascar, the subspecies *P. a. chekei* shows a slight differentiation between specimens from the north (Ankarana and Montagne des Francais) and west (Tsingy de Bemaraha). *Phelsuma a. abbotti* and *P. a. sumptio* from Aldabra and Assumption islands show some differentiation but certainly have colonized these islands very recently, as expected.

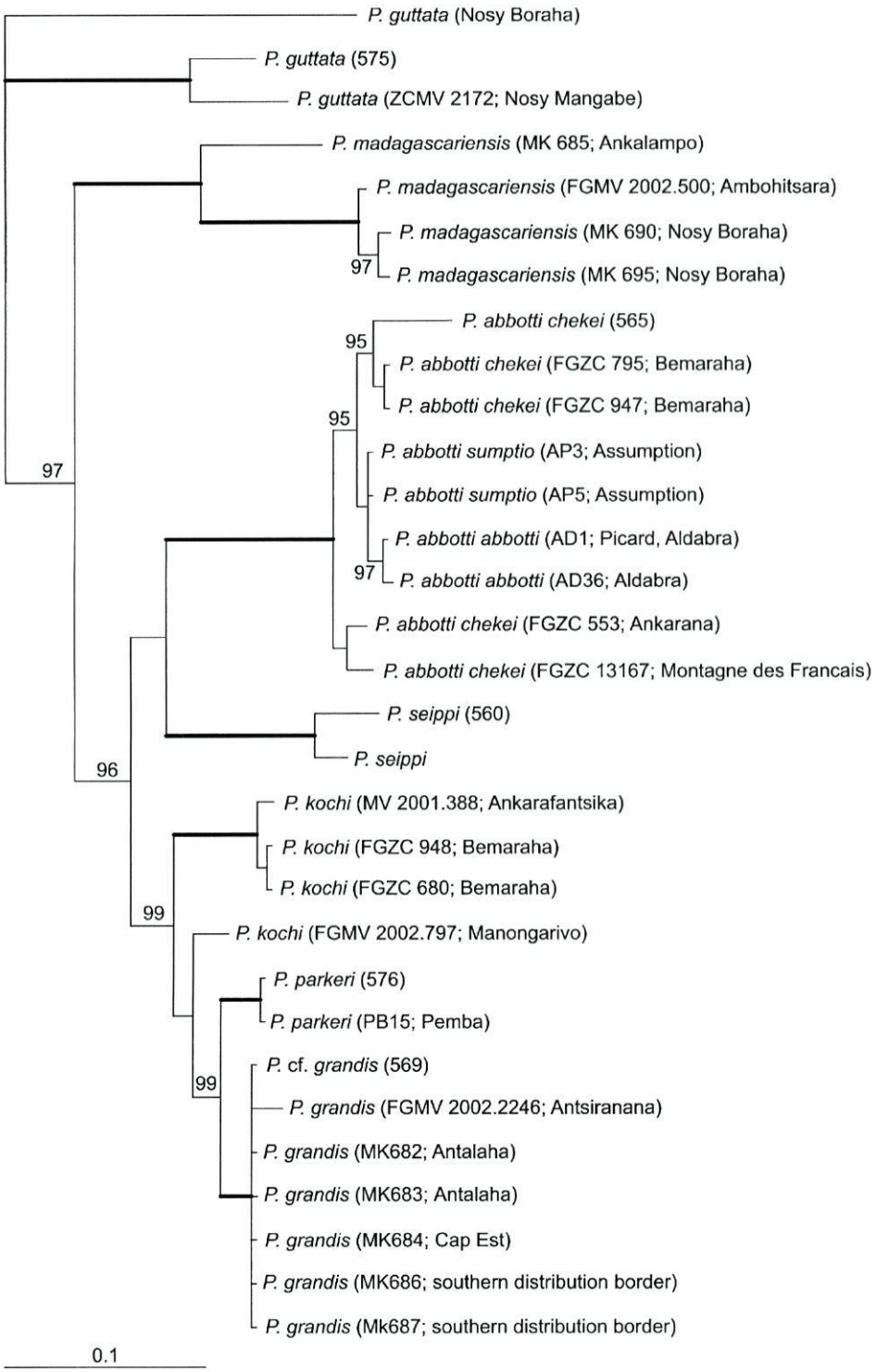



**FIGURE 6.** BI tree of available sequences of a fragment of the 16S rRNA gene in specimens of the *Phelsuma madagascariensis* group. Branches in bold are supported by PP values of 100; other relevant PP values above 50 are given as numbers.

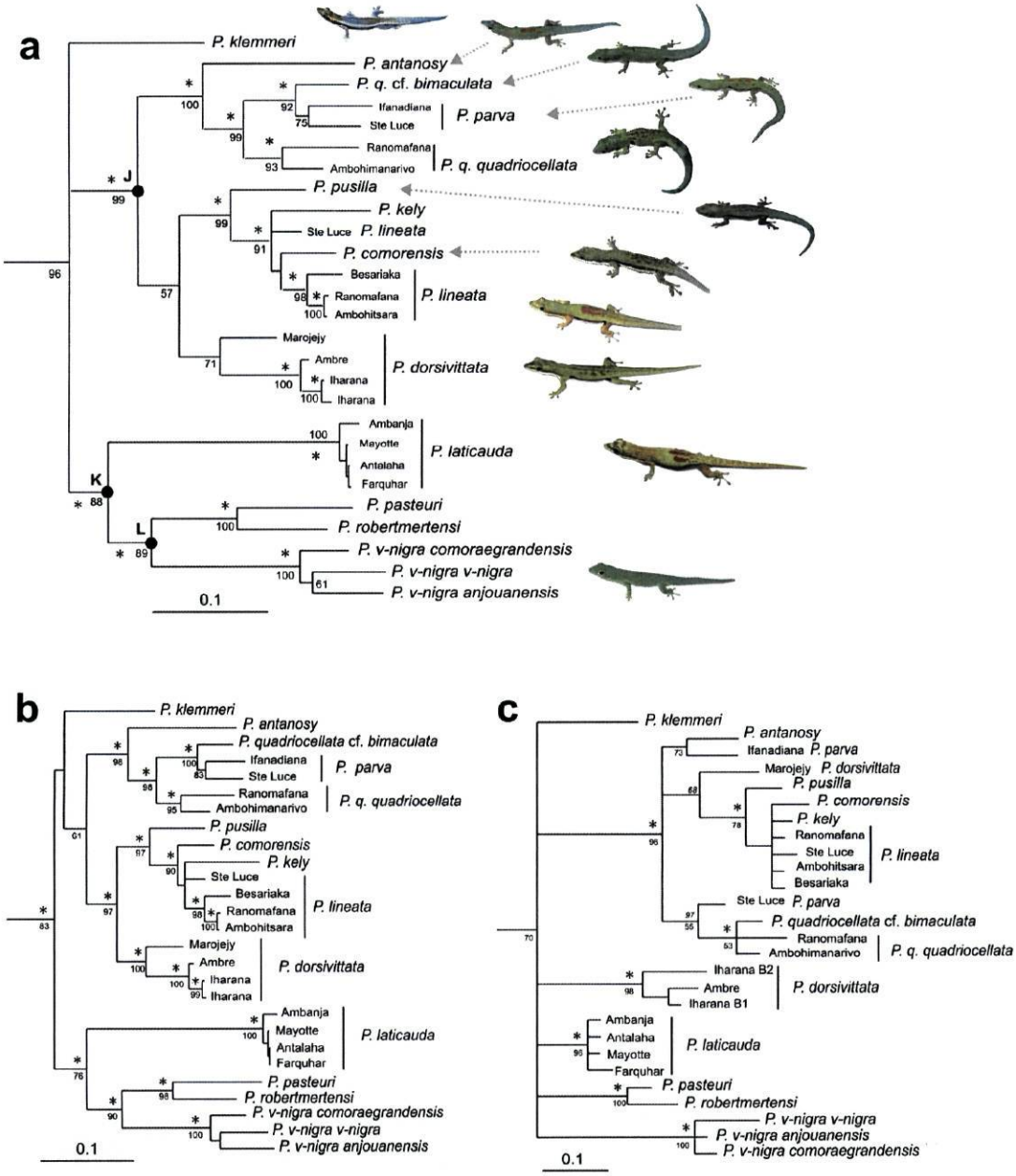



**FIGURE 7.** Molecular phylogeny of the *Phelsuma lineata* group (clade J), the *P. laticauda* group (clade K) and *P. klemmeri* as calculated from the multi-gene dataset of Rocha *et al.* (2009). Shown are the respective clades from the Bayesian 50% majority-rule consensus trees based on (a) the combined dataset of mitochondrial and nuclear genes, (b) the mitochondrial genes only, and (c) the nuclear genes only. See caption to Fig. 2 for further explanations.

Phenotypic characterization of the *P. madagascariensis* group

Species in the *Phelsuma madagascariensis* group are characterized by non-gluing egg laying behaviour, smooth ventral and subcaudal scales, keeled dorsal and lateral scales absence of nostril-rostral contact, and size-reduced vertebral scales, whereas other characters are variable among species in the group, possibly in part related to the size differences (Tables 1–2). For example, the number of preanofemoral pores is higher in

the larger species (*P. grandis, P. kochi, P. madagascariensis, P. parkeri*). Several characters such as non-gluing behaviour, and absence of rostral-nostril contact are shared by the *P. madagascariensis* group and the *P. astriata* group, in accordance with their placement in a monophyletic group by the molecular data, but *P. vanheygeni* which also belongs into this clade is an egg-gluer (see below).

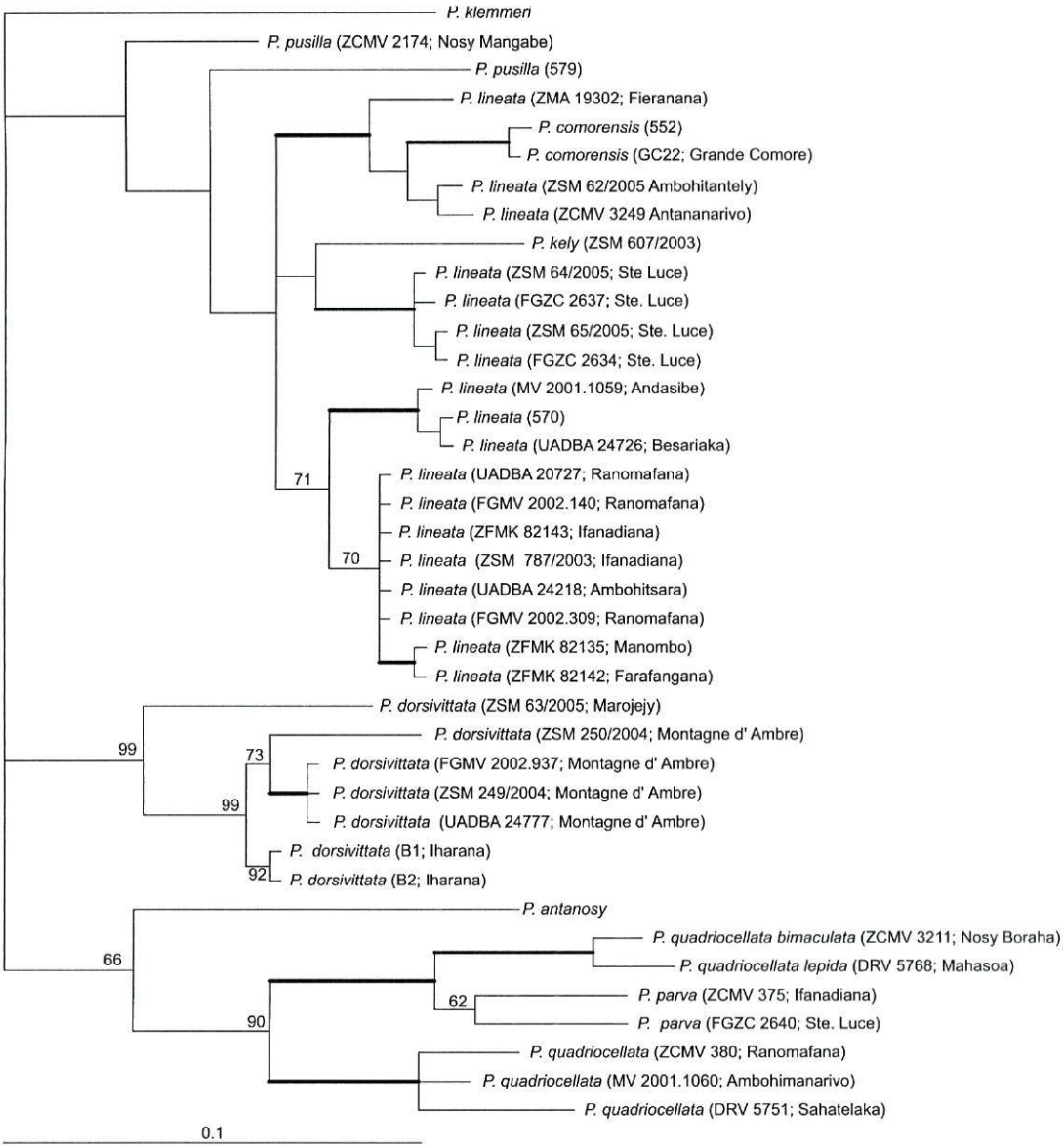



**FIGURE 8.** BI tree of available sequences of a fragment of the 16S rRNA gene in specimens of the *Phelsuma lineata* group. Branches in bold are supported by PP values of 100; other relevant PP values above 50 are given as numbers.

### *Phelsuma lineata* group

(contains *P. antanosy, P. comorensis, P. dorsivittata, P. kely, P. lineata, P. parva, P. pusilla* and, *P. quadriocellata,* and probably, *P. hoeschi*)

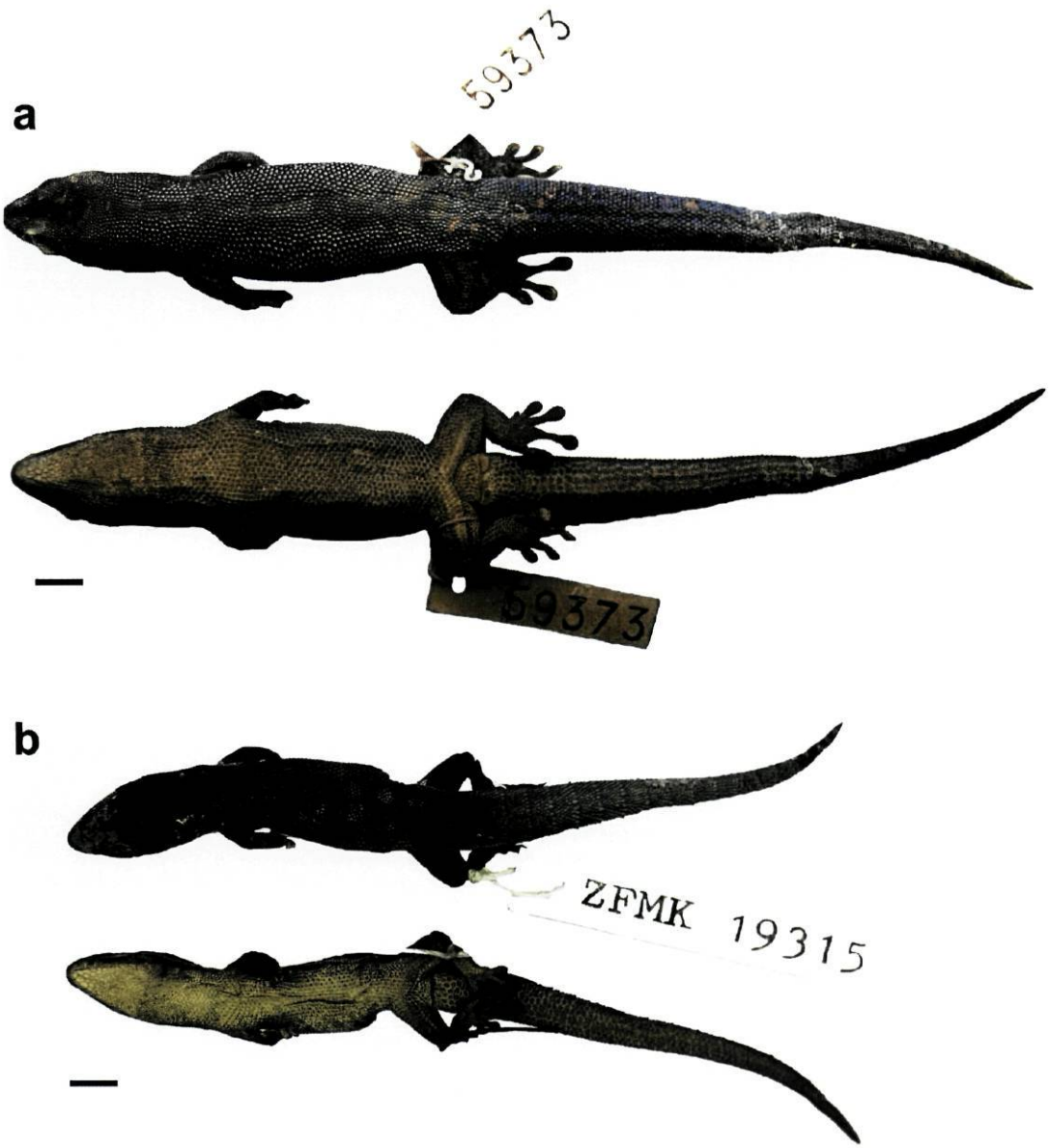



**FIGURE 9.** Holotypes of (a) *Phelsuma dorsivittata* (SMF 59373) and (b) *Phelsuma parva* (ZFMK 19315) in dorsal and ventral view, as photographed in August 2009. Scale bars represent 10 mm.

Definition and content of the group

Molecular data strongly corroborate the previously suggested (Glaw & Vences 1994) evolutionary relationships between *P. lineata*, *P. quadriocellata* and *P. pusilla*. Other species belonging to this well supported group are *P. kely*, the Comoran *P. comorensis,* and *P. antanosy* (Fig. 7a; clade J) Two subgroups can further be defined: (1) all *P. quadriocellata* subspecies (including *P. quadriocellata bimaculata*, sometimes regarded as a distinct species – Glaw & Vences, 1994) and *P. antanosy* and (2) *P. lineata*, *P. pusilla*, *P. kely* and *P. comorensis*. Although nuclear genes reveal unresolved or paraphyletic relationships between these (Figure 7c), for the faster evolving mtDNA markers these groups already reached reciprocal monophyly and are recovered with strong support (Fig. 7b).

*Phelsuma antanosy* was previously thought to be more closely related to either the species from the

Mascarenes (Raxworthy & Nussbaum 1994), i.e., the *P. cepediana* group as defined here, or to the *P. laticauda* group (Glaw & Vences 1994) but in the molecular analysis it is clearly nested within the *P. lineata* group, sister to *P. quadriocellata*.

Although no molecular data are yet available, we here assign the recently described (Berghof & Trautmann 2009) *Phelsuma hoeschi* to the *P. lineata* group based on its dark lateral stripe and its similarities to *P. pusilla* and *P. kely* in scale characters, as also emphasized in the original description (see also Tables 1–2).

Loveridge (1942) hypothesized about the dark spots of *P. quadriocellata* being derived from the dark lateral band of *P. lineata*. It is worth mentioning that *P. l. bombetokensis*, a geographically isolated taxon from dry forest in north-western Madagascar (still unstudied from a molecular perspective), has an intermediate pattern in this respect.

Variation within the *P. lineata* complex and recognition of *P. dorsivittata* at species level

*Phelsuma lineata*, being paraphyletic in respect to *P. kely*, *P. comorensis* and *P. pusilla* (if *lineata* is considered as including *dorsivittata* as subspecies), exhibits consistent deep differentiation (12.8% uncorrected p-distances for cytochrome *b*) between *P. lineata dorsivittata* from the far north of Madagascar (including a specimen of uncertain status from Marojejy in north-eastern Madagascar) and the remaining individuals. Genetic distances within remaining *P. lineata* individuals for cytochrome *b* reach 7.7% uncorrected p-distances. Also, p-distances reach as much as 8.8% within *P. lineata dorsivittata*. The expanded analysis based on 16S sequences only, but including many more specimens (those sequenced by Boumans *et al.* 2007, plus 16 others) largely confirms these conclusions (Fig. 8). *Phelsuma kely* and *P. comorensis* are deeply nested within the *P. lineata* clade. A taxonomic consequence from this fact could be to accept a paraphyletic species *P. lineata* or, given the known colour differences between populations (Krüger 1996c), its partitioning into various species. The following deeply differentiated clades can be distinguished: (1) *P. lineata* populations from the highlands (Antananarivo, Ambohitantely, Fierenana), which would correspond to *P. lineata elanthana* according to Krüger (1996c), (2) populations from the south east (Ste. Luce), (3) populations from the northern central east and southern central east (Andasibe, Besariaka, Ranomafana, Ifanadiana, Ambohitsara, Farafangana), and (4) as the most divergent lineage, the populations from the north, currently corresponding to *P. l. dorsivittata*. However, *P. lineata* sensu lato is almost continuously distributed over most of Madagascar's rainforests, with probable contact and possibly hybrid zones, especially between population groups from the highlands and the northern central east. Furthermore, the nuclear genes provide no evidence for separating lineages 2 versus 3 (Fig. 7c; highland specimens of lineage 1 not included). The available evidence is therefore insufficient for conclusive statements on the taxonomic status of the lineages 1–3. On the contrary, lineage 4 from the northern localities Montagne d'Ambre, Iharana (= Vohemar) and Marojejy is consistently recovered by mitochondrial and nuclear markers (Fig. 7b–c) and is grouped basal to the clade containing *P. lineata* lineages 1–3, *P. pusilla*, *P. kely* and *P. comorensis* (Fig. 7a). We see this as sufficient support for a status of the northern populations as separate species, *Phelsuma dorsivittata*.

Typical specimens of *Phelsuma dorsivittata* have several red spots on the back, which can often fuse to larger markings. Usually a red vertebral stripe is present on the anterior back. A rather indistinct dark lateral spot is present behind the forelimb, and can be also present in front of the hindlimbs, forming the anterior and posterior part of a dark lateral line (distinctly recognizable in the holotype). However, the anterior spot is not isolated and not bordered by blue colour in life. In *Phelsuma dorsivittata* 8–9 supralabialia (min-max) and 6–9 infralabialia (min-max) are present. Selected morphological characters of the male holotype (SMF 59373, Fig. 9) of *Phelsuma dorsivittata* are as follows: SVL 55.3 mm; TL 124.4; 9/9 supralabials on the left and right side of the head; 8/7 infralabials; 1 internasal; nostril in contact with rostral and first supralabial; 28 preanofemoral pores; mental triangle-shaped; dorsals, ventrals, and subcaudals keeled. For a more detailed description of the holotype see Mertens (1964). The holotype, upon examination in 2009 and based on the original description, shows the colour pattern as described above (distinct anterior dark marking on the flanks, red dorsal markings forming a midventral line on the anterior part of the back. However, a specimen from Marojejy (the same as included in our molecular trees) as well as another specimen from the type locality Montagne d'Ambre (see

Glaw & Vences 2007: p. 403 and their fig 1f) show a rather uniform dorsum with no or few red markings which do not form a longitudinal line. Whether this variation is related to the substantial molecular variation observed among these populations and among specimens from Montagne d'Ambre requires further study.

Variation within the *P. quadriocellata* complex and recognition of *P. parva* at species level

Within *P. quadriocellata,* uncorrected p-distances for cytochrome *b* reach 13.4% and three main clades seem to be distinguishable by the mtDNA markers (Figs. 7–8), although nuclear markers do not provide resolution (and show evidence of ancestral polymorphism) at this level. One of these likely corresponds to typical *P. quadriocellata* and appears to be distributed over much of the mid-altitude localities in the northern central east and southern central east, from Sahatelaka (near Lake Alaotra) to Ranomafana. A second clade was found in the lowlands of the southern central east and south east (Ifanadiana and Ste. Luce). The specimens from these two sites are distinctly smaller and have a different pattern of red dorsal markings than those from nearby Ranomafana, and thus corresponded to the current subspecies *P. quadriocellata parva.* Specimens from lower elevations in the northern central east (Nosy Boraha and Mahasoa) clustered together (uncorrected p-distance for the 16S gene 2.2%) (Fig. 8) and correspond morphologically to two subspecies: specimens from Mahasoa possibly to *P. q. lepida* and specimens from Nosy Boraha possibly to *P. q. bimaculata.*

Given that especially *quadriocellata* and *parva* occur in close geographic proximity (Ranomafana vs. Ifanadiana) but each of them is genetically and morphologically constant over wide areas of eastern Madagascar (*parva*: Ste Luce in the southern central east to Ifanadiana in the southern central east; *quadriocellata*: Ranomafana in the southern central east to Sahatelaka in the northern central east; Fig. 8), we hypothesize that these two taxa are distinct at the species level, and consider *Phelsuma parva* as full species. Average cytochrome *b* distances between *P. q. quadriocellata* and *P. parva* are high (11.9%), and average distances within *P. parva* and *P. q. quadriocellata* are high as well, 7.3% and 5.6% respectively, for cytochrome *b*.

*Phelsuma parva* is one of the smallest species within the genus *Phelsuma*; its total length does not reach more than 90 mm (Meier 1983; Glaw & Vences 2007). The body has a slender shape with a bright green colouration. It has distinct red spots on the back and a dark spot behind the forelimb that is variable in size, more or less round and often surrounded by a blue ring. *Phelsuma parva* is characterised by 7–10 supralabialia (min-max), 7–8 infralabialia (min-max). Selected morphological characters of the male holotype (ZFMK 19315, Fig. 9) of *Phelsuma parva* are as follows: SVL 36.2 mm; TL 80.6; 7/7 supralabials; 7/7 infralabials; 1 internasal; nostril in contact with rostral and first supralabial; 22 preanofemoral pores; mental triangle-shaped; dorsals, ventrals, and subcaudals keeled. For a more detailed description of the holotype see Meier (1983). All specimens collected by us and included in our molecular trees agree in size and colour closely with the holotype.

Specimens assigned to the newly defined species *Phelsuma parva* do not form a monophyletic group in the analysis based on nuclear DNA sequences (Fig. 7c). However, the same would also be true for *P. quadriocellata* sensu lato, if *parva* was seen as subspecies of such an inclusive species. Moreover, the fact that rather well-defined species such as *P. antanosy, P. comorensis, P. pusilla,* and *P. kely* cluster among individuals of *P. parva* and *P. lineata* in the nuclear gene tree indicates that these placements are probably due to incomplete lineage sorting in these complexes of species.

The recognition of *P. parva* at species level also rises questions about the status of individuals here assigned to *P. q. bimaculata* and *P. q. lepida* which in the mitochondrial analysis form a clade with *P. parva* rather than *P. q. quadriocellata* (Fig. 7). These forms have distinct differences in colour patterns from each other, from *P. q. quadriocellata,* and from *P. parva.* We have little doubts that they are to be assigned to one or possibly even two separate species. However, the nomenclatural situation is convoluted because (1) a clear definition of the taxon *bimaculata* based on a re-examination of type material is missing, and (2) our analysis does not include topotypical samples of *lepida* (which was described from near Andapa in the North-East), so that the identity of this taxon requires confirmation too. We therefore here refrain from taxonomic conclusions and postpone these to forthcoming studies.

Phenotypic characterization of the *P. lineata* group

The *Phelsuma lineata* group is characterized by a predominant green colour with usually a pattern of lateral/sublateral stripes, typically with keeled ventral and subcaudal scales (except *P. lineata punctulata P. lineata elanthana* and *P. comorensis*), absence of rostral-nostril contact, a relatively low number of midbody scales (below 98, except for *P. antanosy*) and absence of size-reduced vertebral scales (Tables 1–2). The *P. lineata* group shares with the *P. laticauda* group (see below), which is its sister group (Fig. 7), the non-gluing behaviour and absence of nostril-rostral contact, but species in the *P. laticauda* group have smooth ventral and subcaudal scales, and several of them have size-reduced vertebral scales.

The *P. lineata* group as defined here is one of the genetically most diverse clades within *Phelsuma*, and will probably turn out to be the most species-rich clade after a thorough taxonomic study. We had no *P. lineata punctulata* or *P. lineata bombetokensis* available but considering their isolate distribution (in central North and North-West Madagascar, respectively) it is possible that these taxa will again turn out to be genetically differentiated from the remaining.

***Phelsuma laticauda* group**

(contains *P. laticauda, P. pasteuri, P. robertmertensi* and *P. v-nigra*)

None of the members of *P. laticauda* group as defined by Glaw *et al.* (1999) (*P. serraticauda* and *P. antanosy*) is actually closely related to *P. laticauda*, according to our analysis: *P. antanosy* is in fact more closely related to *P. quadriocellata* and *P. serraticauda* cannot be clearly allocated to any species group (see below).

Instead, *P. laticauda* is recovered as the sister species to a clade containing the Comoran species *P. robertmertensi, P. pasteuri* and *P. v-nigra* (Fig. 7; 100 PP in the mtDNA and combined analysis). Nuclear markers *per se* do not provide enough resolution to recover these groups relationship, however, the monophyly of these three Comoroan species is strongly supported by both mtDNA and combined phylogenies (100 PP; clade L in Fig. 7a). *Phelsuma pasteuri* and *P. robertmertensi* which are both endemic to the oldest island of the Comoros archipelago (Mayotte) form a monophyletic group, strongly corroborated by all gene phylogenies, confirming previous results (Rocha *et al.* 2007) and supporting the specific status of *P. pasteuri*. The *P. laticauda* group species (clade K in Fig. 7a) are characterized by being non-gluers with smooth ventral and sub-caudal scales, three nasalia and without nostril-rostral contact. They are mostly bright green coloured and have no lateral or dorsal pattern of stripes (Tables 1–2).

***Phelsuma klemmeri* group**

(contains *P. klemmeri*)

Affinities of this species to the *P. lineata* and *P. laticauda* groups are apparent and well-supported from our analysis (Fig. 7), but mitochondrial as well as nuclear markers place *P. klemmeri* at a basal position within the clade containing these two species groups. Because its colouration is very characteristic and strongly differs from all other *Phelsuma*, the isolated position of this species is best reflected by placing it in a separate species group. Phenotypically, several characters of *P. klemmeri* are in agreement with its affinities: non-gluing behaviour and absence of nostril-rostral contact are shared with the *P. lineata* and *P. laticauda* groups, and presence of smooth ventral and subcaudal scales, number of nasalia (3) as well as of size-reduced vertebral scales are similar to the *P. laticauda* group (Tables 1–2).

***Phelsuma andamanense* group**

(contains *P. andamanense*)

A single species of *Phelsuma* is known from the geographically most disparate archipelago colonized by this

genus, the Andaman Islands, in the Bay of Bengal, with a surface of over 6,000 km$^2$ and extensive tropical rainforest surface. Phylogenetic relationships of this species remain unresolved (isolated in the basal polytomy of the genus, Rocha *et al.* 2009), although possible affinities to the *P. mutabilis* group were detected in some analyses. The basal phylogenetic position of *P. andamanense* indicates that it has colonized the Andamans relatively early. It is intriguing that despite this early colonization, apparently no radiation has occurred on this archipelago, unlike what has happened on the Mascarenes, Seychelles, and Comoros.

*P. andamanense* is a green-coloured non-gluer with smooth ventral and subcaudal scales and without nostril-rostral contact or size-reduced vertebral scales. Except for the colour these characters agree with those observed in the *P. mutabilis* group (Tables 1–2).

### *Phelsuma standingi* group

(contains *P. standingi*)

*Phelsuma standingi*, a species for which Glaw & Vences (1994) hypothesized relationships to *P. madagascariensis* occupies instead a basal position arising from the basal polytomy of the tree as presented in Rocha *et al.* (2009). This is a large species occurring in the very arid spiny forest of south-western Madagascar, and seems to have no close living relatives.

This species stands out by a high number of preanofemoral pores and of midbody scales. By being a non-gluer without nostril-rostral contact it resembles the other Malagasy large sized day geckos in the *P. madagascariensis* group but differs in the absence of size-reduced vertebral scales and not broadened subcaudalia (Tables 1–2). Again, the isolated position of this species is best reflected by assigning it to its own species group.

### Species not assigned to any species group

The relationships of two species (*Phelsuma serraticauda* and *P. vanheygeni*) must at present be considered to be largely unresolved, and their assignment to any of the existing or new species groups is pending further analyses:

*Phelsuma serraticauda* was hypothesized to belong to the *P. laticauda* group by Glaw *et al.* (1999), and such a relationship was in fact recovered by the molecular analyses of Austin *et al.* (2004), Harmon *et al.* (2008) and Raxworthy *et al.* (2007), although never with strong support. In our study (trees in Rocha *et al.* 2009, and herein), on the contrary, the position of this species remained unresolved by mitochondrial genes and it was placed in the *P. dubia* group by nuclear genes (unsupported) (Fig. 2). The incongruence between analyses might be due to the poorer taxon sampling and shorter DNA sequences in previous studies. We exclude possible sequencing errors as source for the controversial position of our sample because the sequences obtained by us from this individual are nearly identical to the ones previously available from a different individual (from Austin *et al.* 2004: identical in C-*mos* and 2 bp difference in cytochrome *b*). To clarify the phylogenetic position of *P. serraticauda* it is possibly necessary to sequence additional markers. Morphologically, *P. serraticauda* shares most morphological characters with species of the *P. laticauda* group but differs in the absence (vs. presence) of keeled ventral and subcaudal scales (Tables 1–2). From species in the *P. dubia* group it differs by the same character plus by its non-gluing (vs. gluing) behaviour. *Phelsuma serraticauda* is also unique in having a broad, flattened tail.

*Phelsuma vanheygeni* was excluded by Lerner (2004) from possibly belonging to the *P. madagascariensis* species group (as defined here) due to its egg-gluing reproduction behaviour. However, our data clearly indicate its relationships with the *P. astriata* and *P. madagascariensis* groups (clade E in Fig. 5) and also imply multiple independent evolution (gain or/and loss) of egg-gluing behaviour (species of *P. dubia*- and *P. modesta* groups except *P. nigristriata*, as well as *P. barbouri* and *P. vanheygeni* are egg-gluers). At present it is uncertain if the species is basal in clade E (and thus merits inclusion in its own species group), or basal to the

*P. astriata*- group (and thus could be included as the only Malagasy species in this otherwise Seychellois species group).

## Conclusion

Summarizing, we here propose to divide *Phelsuma* into 11 species groups whose constitution aims to reflect evolutionary relationships within the genus. As exemplified by Hedges *et al.* (2008) for the extremely species rich group of frogs previously all considered to belong to a single genus (*Eleutherodactylus*) and today summarized in the unranked taxon Terrarana, higher taxa that contain too many lower taxa (e.g., species) can hinder further research simply because of their large size. Even if *Phelsuma* is not one of the largest genera in the Gekkonidae, with currently at least 46 species (after revalidation of *P. dorsivittata* and *P. parva* herein) it is large enough to be considered as a unit useful to break up into groups that are monophyletic and biogeographically meaningful, thereby facilitating further research. Guayasamin *et al.* (2009) propose various very useful criteria to define such new taxa, for example congruence between analysis methods and datasets to minimize the probability of further change of the classification scheme, and morphological diagnosability of the new taxonomic groups. In the case of *Phelsuma*, the genus is well supported by molecular data as monophyletic (Austin *et al.* 2004; Rocha *et al.* 2009), and it is usually easy to recognize a species as belonging to this genus, mainly by their vivid green-colouration, diurnal habits, round pupil and undivided toe lamellae. On the contrary, the morphological distinctness and diagnosability of the lineages identified within *Phelsuma* is rather low. We therefore see no need for partitioning *Phelsuma* into various subgenera and certainly not into various genera, and we consider the use of informal species groups as most appropriate to classify the species diversity within *Phelsuma*. Given that the independent mitochondrial and nuclear markers supported most of the species groups proposed here, we are confident that this classification scheme will be rather stable.

At the level of alpha taxonomy, we here propose the elevation to species-rank of *P. dorsivittata* and *P. parva*. Two species are not assigned to any species group and the assignations of *P. masohoala* and *P. hoeschi* remain provisional pending further molecular studies. Several species may yet harbor considerable cryptic diversity, but fine-scale molecular and morphological analysis should precede any further species-status elevation. The new molecular data and classification here proposed should promote and ease further work on *Phelsuma* taxonomy and biogeography, as manageable units of work can now be considered for proper taxonomic and phylogeographic assessment.

## Acknowledgements

This study has been financially supported by the Portuguese Fundação para a Ciência e Tecnologia, through a Ph. D grant to SR (SFRH/ BD/ 17541/ 2004) and research grants POCTI/ BSE/46647/2002 and PTDC/BIA-BDE/65745/2006. Laboratory work was conducted at the University of Vigo and the Technical University of Braunschweig, where we would like to thank Angelica Crottini, Gabriele Keunecke, Meike Kondermann, Paloma Morán, Pilar Alvariño, Nieves Santamaria, Eva Saxinger, and Graciela Sotelo. Fieldwork of MV and FG in Madagascar was made possible by collaboration agreements of the author's institutions with the Université d'Antananarivo (Département de Biologie Animale) and the Association Nationale pour la Gestion des Aires Protegées, and supported by the Volkswagen Foundation and the Deutsche Forschungsgemeinschaft. The work of PSG was supported by a project of the evolutionary biology funding initiative of the Volkswagen Foundation. For assistance in the field, we are grateful to Franco Andreone, Parfait Bora, Kathrin Glaw, Jim and Carol Patton, Roger-Daniel Randrianiaina and David R. Vieites. Finally we are very grateful to Hans-Peter Berghof, Achim Lerner, Patrick Schönecker and Peter Sound for providing crucial tissue samples.

## References


Akaike, H. (1974) A new look at the statistical model identification. *IEEE Transactions on automatic Control*, 19, 716–723.

Ali, J.R. & Huber, M. (2010) Mammalian biodiversity on Madagascar controlled by ocean currents. *Nature*, 653–656.

Austin, J.J., Arnold, E.N. & Jones, C.G. (2004) Reconstructing an island radiation using ancient and recent DNA: the extinct and living day geckos (*Phelsuma*) of the Mascarene islands. *Molecular Phylogenetics and Evolution*, 31, 102–109.

Bauer, A.M. (2003) Gekkonidae, Geckos. Pp. 973–977 In: *The Natural History of Madagascar*. Goodman, S.M. and J.P. Benstead (Eds.). The University of Chicago Press, Chicago, Illinois, USA.

Berghof, H.P. & Trautmann, G. (2009) Eine neue Art der Gattung *Phelsuma* Gray, 1825 (Sauria: Gekkonidae) von der Ostküste Madagaskars. *Sauria*, 31, 5–14.

Blanc, C.P. (1972) Les Reptiles de Madagascar et des Iles Voisines. In: Battisttini, R. & Richard-Vindard, G. (Eds.), *Biogeography and Ecology in Madagascar*. W Junk, The Hague, pp. 501–614.

Böhme, W. & Meier, H. (1981) Eine neue Form der *madagascariensis*-Gruppe der Gattung *Phelsuma* von den Seychellen (Reptilia: Sauria: Gekkonidae). *Salamandra*, 17, 12–19.

Börner, A.R. (1972) Revision der Gekkonidengattung *Phelsuma* Gray 1825. *Saurologica* [published privately], 1, 1-145.

Börner, A.R. & Minuth, W. (1984) On the taxonomy of the Indian Ocean lizards of the *Phelsuma madagascariensis* group (Reptilia: Gekkonidae). *Journal of the Bombay Natural History Society*, 81, 243–281.

Boulenger, G.A. (1885) *Catalogue of the lizards in the British Museum (Natural History)*. Volume I. Geckonidae, Eublepharidae, Uroplatidae, Pygopodidae, Agamidae. Trustees of the British Museum, London, I-XII, 436 pp.

Boumans, L., Vieites, D.R., Glaw, F. & Vences, M. (2007) Geographical patterns of deep mitochondrial differentiation in widespread Malagasy reptiles. *Molecular Phylogenetics and Evolution*, 45, 822–839.

Cheke, A.S. (1975) An undescribed gecko from Agalega, *Phelsuma agalega* sp. nov. *Bulletin of the Mauritius Institute*, 8, 33–48.

Cheke, A.S. (1981) What the book does not tell you about day geckos: Some comments on Oostveen's "*Phelsuma's*", on new species and on recent research. *Herplite*, 6, 4–10.

Cheke, A.S. (1982) *Phelsuma* Gray 1825 in the Seychelles and neighboring islands: A re-appraisal of their taxonomy and description of two new forms. *Senckenbergiana biologica*, 62, 181–198.

Checke, A.S. Hume, J. (2008) *Lost land of the Dodo: The ecological history of Mauritius, Réunion and Rodrigues*. A&C Black.

Daza, J. D. & Bauer, A. M. (2010 / Anatomical Record, 293, 402–413) The circumorbital bones of the Gekkota (Reptilia: Squamata). *Anatomical Record*.

D'Cruze, N., Sabel, J., Dawson, J. & Kumar, S. (2009) The influence of habitat type and structure on the abundance of *Phelsuma madagascariensis grandis* (Gekkonidae) in northern Madagascar. *Herpetological Conservation and Biology*, 4, 55–61.

Gardner, A.S. (1984) *The evolutionary ecology and population systematics of day geckos* (Phelsuma) *in the Seychelles*. Unpublished Ph.D Thesis, University of Aberdeen.

Gardner, A.S. (1986) Morphological evolution in the day gecko *Phelsuma sundbergi* in the Seychelles: A multivariate study. *Biological Journal of the Linnean Society*, 29, 223–244.

Gardner, A.S. (1987) The systematics of the *Phelsuma madagascariensis* species group of day geckos (Reptilia: Gekkonidae) in the Seychelles. *Zoological Journal of the Linnean Society*, 91, 93–105.

Glaw, F., Köhler. J. & Vences, M. (2009) A new species of cryptically coloured day gecko (*Phelsuma*) from the Tsingy de Bemaraha National Park in western Madagascar. *Zootaxa*, 2195, 61–68.

Glaw, F. & Vences, M. (1994) *A Fieldguide to the Amphibians and Reptiles of Madagascar*. Second Edition. Vences and Glaw Verlag, Köln, 480 pp.

Glaw, F. & Vences, M. (2007) *A Field Guide to the Amphibians and Reptiles of Madagascar*. Third Edition. Vences and Glaw Verlag, Köln, 496 pp.

Glaw, F., Vences, M. & Ziegler, T. (1999) Bemerkungen zu *Phelsuma dubia* (Boettger, 1881): Wiederentdeckung des Holotypus, Verwandtschaftsbeziehungen und Daten zur Fortpflanzung. *Salamandra*, 35, 267–278.

Guayasamin, J. M., Castroviejo-Fisher, S., Trueb, L., Ayarzagüena, J., Rada, M. & Vilá, C. (2009) Phylogenetic systematics of Glassfrogs (Amphibia: Centrolenidae) and their sister taxon *Allophryne ruthveni*. *Zootaxa*, 2100, 1–97.

Guindon S. & Gascuel O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, 52, 696–704.

Hallmann, G., Krüger, J. & Trautmann, G. (2008, eds.) Faszinierende Taggeckos. Die Gattung *Phelsuma*. Second Edition. Natur und Tier-Verlag, Münster.

Harmon, L., Melville, J., Larson, A. & Losos, J.B. (2008) The role of geography and ecological opportunity in the diversification of day geckos (*Phelsuma*). *Systematic Biology*, 57, 562–573.

Hedges, S. B., Duellman, W. E. & Heinicke, M. P. (2008) New World direct-developing frogs (Anura: Terrarana):

Molecular phylogeny, classification, biogeography, and conservation. *Zootaxa*, 1737, 1–182.
Huelsenbeck, J.P., Ronquist, F. (2001) MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17, 754–755.
Ikeuchi, I., Mori, A. & Hasegawa, M. (2005) Natural history of *Phelsuma madagascariensis kochi* from a dry forest in Madagascar. *Amphibia-Reptilia* 26, 475–483.
Katoh, K. & Toh, H. (2008) Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics*, 9, 286–298.
Kluge, A.R. (1991) Checklist of gekkonid lizards. *Smithsonian Herpetological Information Service,* 85, 1–35.
Krüger, J. (1993) Beschreibung einer neuen Unterart von *Phelsuma quadriocellata* aus dem Nord-Osten Madagaskars. *Salamandra*, 29, 133–139.
Krüger, J. (1996a) Untersuchungen zur Validität einiger Taxa der Gattung *Phelsuma*. *Herpetofauna*, 18 (102), 5–11.
Krüger, J. (1996b) Beschreibung einer neuen Art aus der Gattung *Phelsuma* aus dem Süd-Osten Madagaskars. *Herpetofauna*, 18 (105), 14–18.
Krüger, J. (1996c) Zur Nomenklatur der *lineata*-Gruppe in der Gattung *Phelsuma* (Reptilia: Sauria: Gekkonidae). *Sauria*, 18 (3), 37–42.
Krysko, K. L., Nichole, A. N., Sheehy, C. M. (2003) The Madagascar giant gecko, *Phelsuma madagascariensis grandis* Gray 1870 (Sauria: Gekkonidae): A new established species in Florida. *Florida Scientist*, 66, 222–225.
Lerner, A. (2004) A new taxonomically isolated species of the genus *Phelsuma* Gray, 1825 from the Ampasindava Peninsula, Madagascar. *Phelsuma*, 12, 91–98.
Lever, C. (2003) *Naturalized Reptiles and Amphibians of the World.* Oxford University Press, Oxford.
Losos, J. (1986) Island biogeography of day geckos (*Phelsuma*) in the Indian Ocean. *Oecologia*, 68, 338–343.
Loveridge, A. (1941) New geckos (*Phelsuma* & *Lygodactylus*), snake (*Leptotyphlops*), and frog (*Phrynobatrachus*) from Pemba Island, East Africa. *Proceedings of the Biological Society of Washington*, 54, 175–176.
Loveridge, A. (1942) Revision of the Afro-Oriental geckos of the genus *Phelsuma*. *Bulletin of the Museum Comparative Zoology, Harvard*, 89, 439–482.
Meier, H. (1977) Beobachtungen an *Phelsuma standingi* (Reptilia: Sauria: Gekkonidae). *Salamandra*, 13, 1–12.
Meier, H. (1981) Zur Taxonomie und Ökologie der Gattung *Phelsuma* (Reptilia: Sauria: Gekkonidae) auf den Komoren, mit Beschreibung einer neuen Art. *Bonner Zoologische Beiträge*, 31 [1980], 323–331.
Meier, H. (1982) Ergebnisse zur Taxonomie und Ökologie einiger Arten und Unterarten der Gattung *Phelsuma* auf Madagaskar, gesammelt in den Jahren 1972 bis 1981, mit Beschreibung einer neuen Form (Reptilia: Sauria: Gekkonidae). *Salamandra*, 18, 168–190.
Meier, H. (1983) Neue Ergebnisse über *Phelsuma lineata pusilla* Mertens, 1964, *Phelsuma bimaculata* Kaudern, 1922 und *Phelsuma quadriocellata* (Peters, 1883), mit Beschreibung von zwei neuen Unterarten (Sauria: Gekkonidae). *Salamandra*, 19, 108–122.
Meier, H. (1984) Zwei neue Formen der Gattung *Phelsuma* von den Komoren (Sauria: Gekkonidae). *Salamandra*, 20, 32–38.
Meier, H. (1986) Der Formenkreis von *Phelsuma v-nigra* (Boettger, 1913) (Sauria: Gekkonidae) auf den Komoren: Beschreibung von zwei neuen Unterarten. *Salamandra*, 22, 11–20.
Meier, H. (1987) Vorläufige Beschreibung einer neuen Art der Gattung *Phelsuma* von Madagaskar (Sauria: Gekkonidae). *Salamandra*, 23, 204–211.
Meier, H. (1989) Eine neue Form aus der *lineata*-Gruppe der Gattung *Phelsuma* auf Madagaskar. Salamandra, 25, 230–236.
Meier, H. (1993) Neues über einige Taxa der Gattung *Phelsuma* auf Madagaskar, mit Beschreibung zweier neuer Formen. Salamandra, 29, 119–132.
Meier, H. & Böhme, W. (1991) Zur Arealkunde von *Phelsuma madagascariensis* (Gray, 1831) anhand der Museumssammlungen A. Koenig und Senckenberg, mit Bemerkungen zur Variabilität von *P. m. kochi* Mertens, 1954. *Salamandra*, 27, 143–151.
Meier, H. & Böhme, W. (1996) Zum taxonomischen Status des Formenkreises von *Phelsuma abbotti* Stejneger, 1893, mit Bemerkungen über *P. masohoala* Raxworthy & Nussbaum, 1994. *Salamandra*, 32, 85–98.
Mertens, R. (1954) Studien über die Reptilienfauna Madagaskars II. Ein neue Rasse von *Phelsuma madagascariensis*. *Senckenbergiana biologica,* 35, 13–16.
Mertens, R. (1962) Studien über die Reptilienfauna Madagaskars. III. Die Arten und Unterarten der Geckonengattung *Phelsuma*. *Senckenbergiana biologica*, 43, 81–127.
Mertens, R. (1963a) Studien über die Reptilienfauna Madagascars IV. Zwei neue Arten der Geckonengattung *Phelsuma*. *Senckenbergiana biologica*,.44, 349–356.
Mertens, R. (1963b) The geckos of the genus *Phelsuma* on Mauritius and adjacent islands. Bulletin of the Mauritius Institute, 5, 299–304.
Mertens, R. (1964) Studien über die Reptilienfauna Madagaskars V. Fünf neue Rassen der Geckonengattung *Phelsuma*. *Senckenbergiana biologica,* 45, 99–112.
Mertens, R. (1966) Die nichtmadegassischen Arten und Unterarten der Geckonengattung *Phelsuma*. *Senckenbergiana*

*biologica*, 47, 85–110.
Mertens, R. (1970) Neues über einige Taxa der Geckonengattung *Phelsuma*. *Senckenbergiana biologica*, 51, 1–13.
Mertens, R. (1973) Studien über die Reptilienfauna Madagaskars. VII. Eine neue Unterart des Taggeckos *Phelsuma lineata*. *Senckenbergiana biologica*, 54, 299–301.
Nussbaum, R.A., Raxworthy, C.J., Raselimanana, A.P. & Ramanamanjato, J.B. (2000) New species of Day Gecko, *Phelsuma* Gray (Reptilia: Squamata: Gekkonidae), from the Réserve Naturelle Intégrale d'Andohahela, southern Madagascar. *Copeia*, 2000, 763–770.
Nylander, J.A., Wilgenbusch, J.C., Warren, D.L., Swofford, D.L. (2008) Awty (are we there yet?): A system for graphical exploration of mcmc convergence in Bayesian phylogenetics. *Bioinformatics*, 24, 581–583.
Ota, H., Ineich, I. (2006) Colonization of the gold dust day gecko, *Phelsuma laticauda* (Reptilia: Gekkonidae), in Moorea Archipelago, French Polynesia. *Current Herpetology*, 25, 97–99.
Palumbi, S.R., Martin, A., Romano, S., McMillan, W.O., Stice, L. & Grabowski, G. (1991) *The Simple Fool's Guide to PCR, Version 2.0*. Privately published, Hawaii.
Posada, D. (2008) jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution*, 25, 1253–1256.
Posada, D. & Crandall, K.A. (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics*, 14, 817–818.
Puente, M., Glaw, F., Vieites, D. R. & Vences, M. (2009) Review of the systematics, morphology and distribution of Malagasy dwarf geckos, genera *Lygodactylus* and *Microscalabotes* (Squamata: Gekkonidae). *Zootaxa*, 2103, 1–76.
Radtkey, R. (1996) Adaptive radiation of day-geckos (*Phelsuma*) in the Seychelles Archipelago: A phylogenetic analysis. *Evolution*, 50, 604–623.
Raxworthy, C.J. & Nussbaum, R.A. (1993) A new Madagascan *Phelsuma*, with a review of *Phelsuma trilineata* and comments on *Phelsuma cepediana* in Madagascar (Squamata: Gekkonidae). *Herpetologica*, 49, 342–349.
Raxworthy, C. J. & Nussbaum, R.A. (1994) A partial systematic revision of the day geckos, *Phelsuma* Gray, of Madagascar (Reptilia: Squamata: Gekkonidae). *Zoological Journal of the Linnean Society*, 112, 321–335.
Raxworthy, C.J., Ingram, C.M., Rabibisoa, N. & Pearson, R.G. (2007) Applications of ecological niche modelling for species delimitation: A review and empirical evaluation using day geckos (*Phelsuma*) from Madagascar. *Systematic Biology*, 56, 907–923.
Rendahl, H. (1939) Zur Herpetofauna der Seychellen. I. Reptilien. *Zoologische Jahrbücher, Abteilung für Systematik, Ökologie und Geographie der Tiere*, 72, 157–328.
Rocha S., Posada D., Carretero M.A., Harris D.J., 2007. Phylogenetic affinities of Comoroan and East African day geckos (genus *Phelsuma*): multiple natural colonisations, introductions and island radiations. *Molecular Phylogenetics and Evolution*, 43, 685–692.
Rocha S., Vences M., Glaw F., Posada D., Harris D.J., 2009. Multigene phylogeny of Malagasy day geckos of the genus *Phelsuma*. *Molecular Phylogenetics and Evolution*, 52, 530–537.
Rösler, H. (1998) Zur Taxonomie und Verbreitung von *Phelsuma madagascariensis* (Gray, 1831) in Nordost-Madagaskar (Sauria: Gekkonidae). *Gekkota*, 1, 21–32.
Rösler, H. (2001) Zur Rumpfbeschuppung der Gattung *Phelsuma* Gray, 1825: zoogeographische Aspekte (Sauria: Gekkonidae). *Gekkota*, 3, 47–73.
Rösler, H., Obst, F.J. & Seipp, R. (2001) Eine neue Taggecko-Art von Westmadagaskar: *Phelsuma hielscheri* sp.n. (Reptilia: Sauria: Gekkonidae). *Zoologische Abhandlungen Staatliches Museum für Tierkunde in Dresden*, 51, 51–60.
Sambrook, J., Fritsh, E.F. & Maniatis, T. (1999) *Molecular Cloning: A Laboratory Manual, second ed.* Cold Spring Harbor Press, New York.
Schönecker, P. (2008) *Geckos of Madagascar, the Seychelles, Comoros and Mascarene Islands*. Terralog. Edition Chimaira, Frankfurt a. M., 144 pp.
Schönecker, P., Bach, S. & Glaw, F. (2004) Eine neue Taggecko-Art der Gattung *Phelsuma* aus Ost-Madagaskar (Reptilia: Squamata: Gekkonidae). *Salamandra*, 40, 105–112.
Seipp, R. (1990) Eine neue Art der Gattung *Phelsuma* Gray, 1825 von Madagaskar (Reptilia: Sauria: Gekkonidae). *Senckenbergiana biologica*, 71, 11–14.
Sound, P., Kosuch, J., Vences, M., Seitz, A. & Veith, M. (2006) Preliminary molecular relationships of Comoran day geckos (*Phelsuma*). *Proceedings of the 13th Congress Societas Europaea Herpetologica*, 175–179
Swofford, D.L. (2002) PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, MA.
Taggart, J.B., Hynes, R.A., Prodoul, P.A., Ferguson, A. (1992) A simplified protocol for routine total DNA isolation from salmonid fishes. *Journal of Fish Biology*, 40, 963–965.
Van Heygen, E. (2004) Remarks on the *Phelsuma barbouri*- and *Phelsuma klemmeri*-phenetic groups. *Phelsuma*, 12, 153–154.
Vinson, J. & Vinson, J.M. (1969) The saurian fauna of the Mascarene Islands. *Mauritius Institute Bulletin*, 4, 203–320.
Zwickl, D.J. (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D.dissertation, The University of Texas at Austin. Available from http://www.bio.utexas.edu/faculty/antisense/garli/Garli.html

# CHAPTER 3

## PHYLOGENETIC RELATIONSHIPS, GENETIC DIVERSITY AND ISLANDS POPULATION STATUS OF THREE WIDESPREAD WESTERN INDIAN OCEAN GROUPS: *Gehyra*, *Hemidactylus* AND *Mabuya spp.*

Contributions to Zoology, 78 (1) 1-8 (2009)

# Cryptic variation and recent bipolar range expansion within the Stumped-Toed Gecko *Gehyra mutilata* across Indian and Pacific Ocean islands

Sara Rocha[1, 2, 3, 5], Ivan Ineich[4, 6], D. James Harris[1, 2, 7]

[1] *CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, 4485-661 Vairão, Portugal*

[2] *Departamento de Zoologia e Antropologia, Faculdade de Ciências, Universidade do Porto, Praça Gomes Teixeira, 4099-002, Portugal*

[3] *Departamento de Bioquímica, Genética e Inmunología, Facultad de Biología, Universidad de Vigo, Vigo 36310, Spain*

[4] *Muséum national d'Histoire naturelle, Département de Systématique et Evolution, Section Reptiles, CP n° 30 - 25 Rue Cuvier, 75005 Paris, France*

[5] *E-mail: sara.rocha@mail.icav.up.pt*

[6] *E-mail: ineich@mnhn.fr*

[7] *E-mail: james@mail.icav.up.pt*

Key words: 16s rRNA, Asia, biogeography, *Gehyra insulensis*, *Gehyra mutilata*, Indian Ocean, introductions, island colonisations, Oceania

## Abstract

With an impressively wide distribution, *Gehyra mutilata* is present on almost all Indian and Pacific Ocean islands and in large regions of Southeast Asia and Indonesia. Mitochondrial sequence data (~500 bp) from individuals covering large parts of its (mainly insular) distribution reveals deep cryptic variation and strong geographic structure, with two well differentiated lineages. Molecular data also reveals that the wide Indian and Pacific insular distribution of *Gehyra* is very recent and, at least across the Indian Ocean islands, most probably the result of human-aided dispersal, as no variation within this lineage was found. Further research is needed to determine geographic patterns of variation across Southeast Asia, the level of genetic variation, and possible mechanisms of speciation. If recognized as distinct taxa, the binomen *Gehyra insulensis* should be resurrected, and applied to the 'Pacific lineage'.

## Contents

Introduction ..... 1
Material and methods ..... 2
Results ..... 3
Discussion ..... 3
Acknowledgements ..... 5
References ..... 5
Appendix ..... 8

## Introduction

Geckos of the genus *Gehyra* (Gray, 1834) are some of the most prominent and widespread geckos within the Australasian and Pacific regions. *Gehyra mutilata* (Wiegmann, 1834) is widespread throughout the Pacific basin, large regions of Southeast Asia, Indonesia and the Indian Ocean. It also occurs in Mexico and it is known to have been introduced in California (USA) and in the French Guiana (Ineich and de Massary, 1997). It occurs in both natural forests and disturbed garden or urban areas and it is the least studied widespread gecko in the world, with little information available on genetic or morphological variation across its distribution.

Fisher (1997) examined allozyme variation within this species across some Southeast Asian (Thailand, Philippines) and Pacific (from Belau to Hawaii) localities and found that 1) samples across the Pacific showed no genetic variation for the loci surveyed, and 2) there were fixed differences at three loci between a group consisting of the Pacific and two southern Asian populations (the 'southern mainland type', sensu Fisher, 1997) and a second group of four southern Asian populations (the 'northern mainland type', sensu Fisher, 1997). Both groups were present in the Philippines and in Thailand, although sympatry was not found. Fisher (1997) interpreted his results as indicating that the source of the Pacific populations was probably southern Asia and pointed to a recent, possibly human-mediated, range expansion, but the lack of variation at these markers prevented assessment of alternative dispersal hypotheses. Indian Ocean populations were not studied, despite the fact that they are considered to

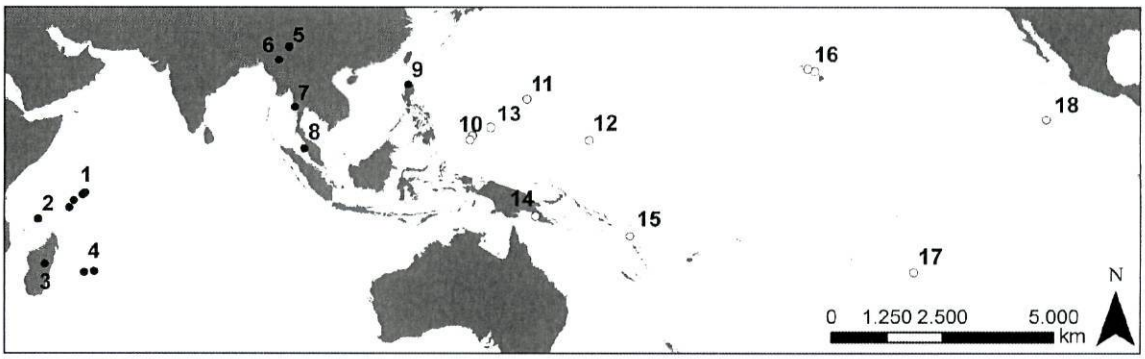



*Fig. 1.* Sampling localities: Seychelles (1); Aldabra (2); Madagascar (3); Mascarenes (4); Myanmar (5-7); Malaysia (8); Luzon, Philippines (9); Palau (10); Mariana (11); Caroline (12, 13); Papua New Guinea (14); Vanuatu (15); Hawaii (16); French Polynesia (17) and Clipperton Atoll (18) (see Appendix 1 for exact location and details on origin of the samples). Localities harbouring different mtDNA lineages (see results) are colour coded (black versus white dots).

have been introduced throughout this region (Cheke, 1984). It has been assumed (de Massary, 1992; I. Ineich quoted in Glaw and Vences, 1994) that *Gehyra* populations of Indonesia and from the western Indian Ocean islands are distinct from those of Oceania, and that *G. insulensis* should be resurrected and used as the proper designation for some or all Pacific populations. Faster evolving mtDNA data could provide increased resolution regarding genetic variation of *G. mutilata* across the Pacific and could help in assessing the source and mechanisms related to the origin of Indian Ocean islands populations.

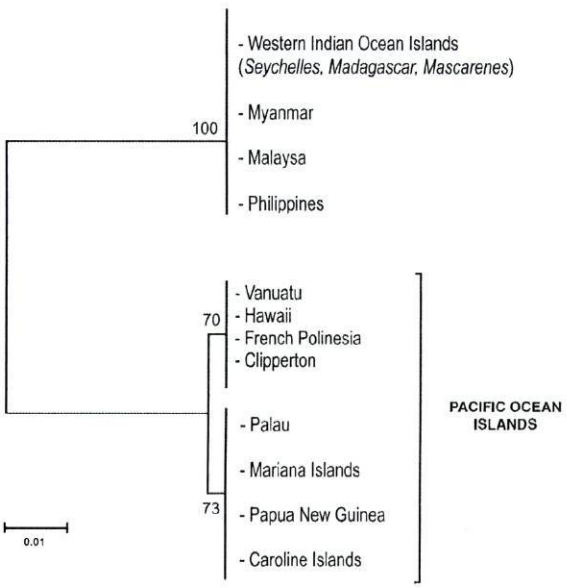



*Fig. 2.* ME tree for the 517bp data matrix (complete gap deletion option) with bootstrap values shown alongside the branches. The two haplotypes from the Pacific Ocean clade and the two main clades are separated by two and 53 mutational steps, respectively.

It is important to assess the mechanisms explaining the presence of this species in oceanic islands; introduced reptiles can have varied negative impacts on native species, including predation, competition for food, basking sites and other resources, hybridisation and spread of diseases and parasites, and they may alter the habitat of native species or disrupt ecosystem dynamics. All these processes are especially dangerous when they occur on islands, where the number of endemic species is generally higher and ecosystems are more vulnerable to introductions (Shine *et al.*, 2000). Furthermore, it is on islands that introductions are more frequent and that invasive species have a higher probability of successful establishment (Kraus, 2003). Indeed, several examples exist of strong negative impact of introduced reptiles in island systems (Cogger *et al.*, 1983; Powell *et al.*, 1990; Petren and Case, 1996; Cole *et al.*, 2005).

Our main goal was to assess the origins and genetic variation of Indian Ocean populations of *Gehyra mutilata*. By comparing them to other southern Asian and Pacific populations, we could assess their putative distinctiveness and better understand previously hypothesised dispersal mechanisms (Fisher, 1997).

## Material and methods

Specimens were collected during fieldtrips across the Indian Ocean and Pacific Islands and additional sam-

ples obtained from museums (see Fig. 1 and Appendix 1 for locality details).

Genomic DNA was extracted following a standard high-salt protocol (Sambrook *et al.*, 1989) and part of the 16s rRNA gene (517 bp) was amplified and sequenced using primers 16sA-L and 16sB-H (Palumbi *et al.*, 1991). Amplification conditions are described in Harris *et al.* (1998). Amplified fragments were directly purified with ExoSap-IT (Amersham-Pharmacia Biotech) and sequenced on an ABI 3730xl automated capillary DNA sequencer and aligned with available *Gehyra* sequences from GenBank. Generated sequences are deposited in GenBank under accession numbers FJ613425-FJ613473 and the full alignment is available on the TreeBASE website (www.treebase.org/treebase; study accession number = S2324). As the pattern of genetic variation was clear, evolutionary history was inferred using the Minimum Evolution method (Rzhetsky and Nei, 1992), with the evolutionary distances computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004) and presented in units of number of base substitutions per site. One thousand bootstrap replicates (Felsenstein, 1985) were performed to estimate nodal support. The ME tree was searched for using the Close Neighbor Interchange (CNI) algorithm (Nei and Kumar, 2000) at a search level of 2. The Neighbor Joining algorithm (Saitou and Nei, 1987) was used to generate the initial tree. Due to the shorter size of some of the sequences (Genbank sequences DQ857338/Madagascar; DQ270549 and AY517559/Mauritius), positions containing gaps and missing data were eliminated from the dataset ('complete gap deletion' option), leaving a total of 318 positions in the final dataset, from which the ME tree topology was inferred. Phylogenetic analyses were conducted in MEGA4 (Tamura *et al.*, 2007). Additionally, a haplotype median-joining network (Bandelt *et al.*, 1999) was obtained using NETWORK4.5 (Fluxus Engineering, Suffolk, UK), in which the shorter sequences were excluded, indels of more than 1bp were coded as single events, and the full fragment length (517 bp) was considered.

## Results

From a total of 53 individuals analysed from the Indian Ocean islands (24), Southeast Asia (14), East (6) and West (9) Pacific Islands, sequencing of the 16s rRNA partial fragment revealed three haplotypes, belonging to two strongly differentiated lineages, and with a remarkable geographic structure. Southern Asian (Myanmar, Malaysia and Luzon, Philippines) and Indian Ocean islands individuals exhibited the same haplotype, with around 10% sequence divergence from the 'Pacific lineage', which harbours two closely related haplotypes (Fig. 2).

There were 55 variable positions, 53 of them diagnostic for the two clades, including four indels of variable (1-10 bp) size. In every case alignment was unambiguous and no 'gappy' region was excluded from the analysis. GenBank sequences from Madagascar and Mauritius had considerable 5' or 3' missing data regions, thus restricting the comparable fragment size including all sequences to 334 bp. As they were identical to all other Indian Ocean islands samples for the available sequence fragment (in total 418 bp for DQ857338/Madagascar; 495 bp for DQ270549/Mauritius; and 499bp for AY517559/Mauritius), they were excluded from the haplotype network construction, so that all the differences within the full length of the sequenced fragment could be represented.

## Discussion

As had been hypothesized based on allozyme data (Fisher, 1997), there are two cryptic lineages within *G. mutilata*. Levels of differentiation at the 16s rRNA marker (~10%) are higher or equivalent to differentiation observed between known distinct species of geckos (Jesus *et al.*, 2005; Rocha *et al.*, 2005; Weiss and Hedges, 2007).

Our data show that Western Indian Ocean populations (Seychelles, Mascarenes, Madagascar) belong to the same lineage as individuals analysed from the Philippines (Luzon Island) and mainland Asia (Myanmar and Malaysia).

Fisher (1997) observed the coexistence of two lineages in the Philippines (and Thailand) that possibly correspond to the ones that are now evident at the mtDNA level, with the Negros Island population belonging to the 'southern mainland type', also widespread across the Pacific, and the other two populations (Luzon and Caminguin) belonging to the same lineage as the two northern Thailand populations (the 'northern mainland type'). The Philippine material included in our study is from Luzon Island, which is also the type locality of *G. mutilata* (Manila), and belongs to the same clade of the remaining Southern Asia and Indian Ocean individuals. On the basis of these data,

we argue that they should all be referred to as *Gehyra mutilata* sensu stricto with *Hemidactylus peronii* Duméril and Bibron, 1836 (type locality Mauritius), *Gecko pardus* Tytler, 1865 (type localities Rangoon, Moulmein and Port Blair) and *Peropus packardii* Cope, 1869 (type locality Penang) as synonyms. Indonesian populations (on which the names *Hemidactylus platurus* Bleeker, 1859 and *Gehyra beebei* Annandale, 1913 are based) were not included in our study and their status cannot yet be assessed. If future work leads to the recognition of the Oceanian population as a separate species, priority shall be given to the name *Dactyloperus (= Gehyra) insulensis* Girard, 1857 (type locality Sandwich Islands). We suggest however, that a formal nomenclatural change be delayed pending further studies on the effective isolation of the two lineages and the geographic structure of genetic variation.

Remarkably, little or no variation was found within the 'Indian Ocean and Southern Asia' and 'Pacific' lineages, respectively, indicating a recent and fast spread of the species throughout these areas, probably from sources located in Southern Asia. The hypothesis of a recent, human-mediated, dispersal of *G. mutilata* across Indian Ocean islands is supported by the observation that records of *G. mutilata* are either recent (for Madagascar, the Mascarenes and the Seychelles - Glaw and Vences, 1994; Gerlach, 2007; Cheke and Hume, 2008) or absent (for the Comoros - Carretero *et al.*, 2005) across this region.

Despite the near-complete absence of variation, the two haplotypes constituting the 'Pacific lineage' exhibit some geographic structure, being exclusive of either Western populations (comprising Palau, New Guinea and Micronesia – Mariana and Caroline) or Eastern Pacific populations (Polynesia, Vanuatu and Clipperton). This may indicate that two colonisation routes existed, although a single colonisation followed by limited gene flow would also explain the data. Interestingly, the 'two routes' pattern would fit Fisher's (1997) 'non-European' human-mediated dispersal hypothesis involving two - Australoid and proto-Polynesian - Pacific colonisations. However, the Melanesian populations from Vanuatu, which share the Eastern and not the Western Pacific haplotype, do not fit the picture. The only specimen from Vanuatu used in this study was recently collected on Torres Islands, in northern Vanuatu, where the species was locally abundant, thus possibly reflecting a recent introduction. Previous records from Vanuatu are very scarce (only one record from a single specimen from Efate, collected in 1924 or 1925 - Medway and Marshall, 1975; Cranbrook and Pickering, 1981) and it is possible that both Western and Eastern lineages now occur on that archipelago, the Eastern one being very recently introduced. It would be interesting to compare Fijian and Solomon Islands populations with Vanuatu to test conflicting biogeographical hypotheses for that area (Bauer, 1988).

Clipperton Atoll populations belong to the Eastern Pacific lineage and were generally considered as being recently introduced from Mexico (Lorvelec and Pascal, 2006). However, a natural occurrence on the atoll cannot be excluded. Likewise, the origin of Mexican populations is still under debate (Ineich and Blanc, 1987).

It is well known that *G. mutilata* is an effective island colonist possessing several morphological and ecological adaptations that facilitate successful inter-island travel (see Ineich and Blanc, 1987). It even recently recolonized Krakatau Island in Indonesia, which was completely covered by lava after an historical volcanic eruption (O'Shea and Cook, 2006) and is also present in southern Japan (Ota and Yamashita, 1985). Genetic uniformity of both lineages, across the Indian Ocean on the one hand and the Pacific on the other, is classically interpreted as proof of their recent spread through human agency (see *e.g.*, Bruna *et al.*, 1996). However, given that human colonisation of Pacific islands is also extremely recent (Belwood, 1997; Blust, 1995; Kirch, 1997), the hypothesis that this species was already present on the islands before man cannot be rejected with present data, *i.e.*, colonisation can be natural, and 'pre-human', but still recent, and thus without time for organisms to exhibit geographical patterns of genetic variation that would allow the trace of the colonisation routes. Across most of the Pacific Islands, *Gehyra mutilata* occupies a wide range of habitats, and not only the ones related to man. It is usually not very abundant but geographically widespread and there are no clear gaps in its distribution in French Polynesia, as would be expected for a very recent 'anthropogenic' species (Ineich and Blanc, 1988).

Use of faster evolving genetic markers and further sampling along south-eastern territories between Myanmar and Indonesia/Papua New Guinea and comparison with proposed human migration routes should clarify this question and may possibly shed light on the causes of speciation within this apparent species complex.

The fact that *G. mutilata* is clearly a 'newcomer' across the major oceanic island areas is relevant for consideration of its effect on island ecosystems. As al-

ready discussed, the impact of alien species on islands can be considerable. The impact of *G. mutilata* on other Indian Ocean gecko populations is unknown. Across the Pacific this species is less abundant than many other introduced house geckos (*e.g.*, several species of the genus *Hemidactylus* Gray, 1825) but it is nowadays widespread and abundant across the Seychelles, with established populations on both coralline and granitic islands. Bringsøe (2006; see also Rösler, 2007) hypothesised that nocturnal activity of *G. mutilata* (that he considered as recently introduced) had a significant impact on, and led to nocturnal activity of some, Seychelles day geckos such as *Phelsuma sundbergi* Rendahl, 1939. We do not agree with that hypothesis and rather think that well known behavioural plasticity of island reptiles (Ineich and Blanc, 1987) better explains this recent shift of *P. sundbergi* to exploit an easily obtained and abundant food resource located around artificial lights. Across Indian Ocean islands this species seems to be rapidly spreading: earliest *G. mutilata* records date back from 1885 (1905 for the first precise locality record - Victoria, Mahé Island - Gerlach, 2007), but this species is nowadays present on almost all islands of the archipelago, including the outer ones (Gerlach, 2007). Not exclusive to urban areas, *G. mutilata* in the Seychelles can be also found in more pristine vegetation and montane areas, and it has even been observed co-existing with the native gecko *Urocotyledon inexpectata* Stejneger, 1893 at the very specific habitat/nesting sites of that species: rocks covered with empty wasp nests in which the eggs are laid (S. Rocha, *pers. obs.*, 2007). Interspecific competition may have an enormous impact on endemic gecko populations (see Cole *et al.*, 2005 and references therein), such as *Hemidactylus mercatorius* (sensu Vences *et al.*, 2004) on Aldabra (S. Rocha, *pers. obs.*, 2007) or *Urocotyledon inexpectata* in the granitic Seychelles, and should be the target of specific monitoring.

Concerning Pacific island populations, we have found that they clearly avoid artificial lights, contrary to Indian Ocean *G. mutilata*. It is also noteworthy that they avoid humid forest habitats were they are rarely observed. Instead, they are more abundant in littoral dry forests (*e.g.*, the Tuamotu atolls in French Polynesia), even when isolated and far from human habitations, but also occur in and around houses. They shelter by day under loose bark on dead trees, and lay two adhesive eggs (sometimes in communal laying sites) in the same microhabitat. They occupy more xeric habitats than *G. oceanica* (Lesson, 1839), a more common Pacific island congener, thus explaining its colonization success on the American continent, even in Mediterranean climates such as in California, and on some remote and dry areas like Clipperton Atoll in Eastern Pacific.

Competition with other species in the Pacific islands apparently does not occur, or at least not at a significant level. The impact of *G. mutilata* on native species, if really recently introduced, seems limited. Perhaps this can be considered as evidence of its slightly 'older' arrival in the Pacific, and establishment of equilibrium with other sympatric species. The rarity of *G. mutilata* on Pacific islands compared to the initial explosive demography of *H. frenatus* Duméril and Bibron, 1836 (Case and Bolger, 1991), suggests that its current impact on other local species is not significant and that the species does not constitute a major threat to other native species.

## Acknowledgements

SR and JH are grateful to SBS of Seychelles for permits for fieldwork and tissue collection and to the Ministry of Environment and Natural Resources (especially Dr. Denis Matatiken and Wilna Accouche) for logistic support during fieldwork. We thank Miguel Carretero, Raquel Vasconcelos and Ana Perera for help during fieldwork and Justin Gerlach from NPTS for support and information. This research was supported by grants from Fundação para a Ciência e Tecnologia (FCT) SFRH/BD/1745/2004 (to SR) and PTDC/BIA-BDE/65745/2006. II collected samples on Clipperton Atoll through French Clipperton Expedition and on Torres Islands through PPF/MNHN. II's Torres Islands field trip was made possible through funding from MNHN (Programme 'Etat et structure de la biodiversité actuelle et fossile'), the Delegation of the European Commission in Vanuatu, and CNRS. Thanks to Dr Philippe Bouchet, the Environment Unit of the Vanuatu Ministry of Lands, Mr Titus Noel and Mr Rufino Pineda for their help during Torres (Vanuatu) field trip of one of us (II). We thank Addison H. Wynn and George R. Zug (USNM, Washington), Ronald Crombie, Carla Kishinami (BPBM, Hawaii), Grégory Déso and Jean-Michel Probst (La Réunion) and Jens Vindum at the California Academy of Sciences (CAS) for providing tissue samples, and Dr. Glenn Shea, Dr. Aaron M. Bauer and two anonymous reviewers for constructive comments.

## References

Annandale N. 1913. Some new and interesting Batrachia and lizards from India, Ceylon and Borneo. *Records of the Indian Museum* 9: 301-307.

Bandelt HJ, Forster P, Rohl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16: 37-45.

Bauer AM. 1988. Hypothesis: A geological basis for some herpetological disjunctions in the southwest Pacific, with special reference to Vanuatu. *Herpetological Journal* 1: 259-263.

Belwood P. 1997. The Prehistory of the Indo-Malaysian Archipelago. 2nd ed. Honolulu: University of Hawaii Press.

Bleeker P. 1859. Hemidactylus platurus. *Natuurkundig Tijdschrift voor Nederlandsch Indie* 16: 30-31.

Blust R. 1995. The prehistory of the Austranesian speaking peoples: a view from language. *Journal of World Prehistory* 9: 453-510.

Bringsøe H. 2006. Beobachtungen zur Nachtaktivität von *Phelsuma sundbergi* s.l. in der Nähe von künstlicher Beleuchtung auf den Seychellen. *Der Taggecko* 57: 20-23.

Bruna EM, Fisher RN, Case TJ. 1996. Morphological and genetic evolution appear decoupled in Pacific skinks (Squamata: Scincidae: *Emoia*). *Proceedings of the Royal Society of London - B* 263: 681-688.

Carretero MA, Harris DJ, Rocha S. 2005. Recent observations of reptiles in the Comoro Islands (Western Indian Ocean). *Herpetological Bulletin* 91: 19-28.

Case TJ, Bolger DT. 1991. The role of introduced species in shaping the distribution and abundance of island reptiles. *Evolutionary Biology* 5: 272-290.

Cheke AS. 1984. Lizards of the Seychelles. In: Stoddart, DR. *Biogeography and ecology of the Seychelles*. The Hague: DR W. Junk Publishers, 331-360.

Cheke A, Hume J. 2008. *Lost land of the Dodo*. London: T & AD Poyser.

Cogger HG, Sadlier RA, Cameron EE. 1983. The terrestrial reptiles of Australia's island territories. *Australian National Parks and Wildlife Service Special Publication* 11: 1-80.

Cole NC, Jones CG, Harris S. 2005. The need for enemy-free space: the impact of an invasive gecko on island endemics. *Biological Conservation* 125: 467-474.

Cope ED. 1869. Observations on reptiles of the Old World. *Proceedings of the Academy of Natural Sciences of Philadelphia* 20: 316-323.

Cranbrook E, Pickering R. 1981. A Checklist and Preliminary Key to the Lizards of Vanuatu. *Naika* 4: 3-6.

De Massary JC. 1992. *Comparaison morphométrique et méristique entre les populations de (Reptilia, Gekkonidae) de l'Océan Pacifique et de l'Océan Indien. Mémoire de Maîtrise*. Paris: Université de Paris VI.

Duméril AMC, Bibron G. 1836. *Erpétologie Générale ou Histoire naturelle complète des Reptiles, 3*. Paris: Librairie Encyclopédique de Roret.

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39: 783-791.

Fisher RN. 1997. Dispersal and evolution of the Pacific basin gekkonid lizards *Gehyra oceanica* and *Gehyra mutilata*. *Evolution* 51: 906-921.

Gerlach J. 2007. *Terrestrial and Freshwater vertebrates of the Seychelles islands*. Leiden: Backhuys Publishers.

Girard C. 1857. Descriptions of some new reptiles, collected by the United States Exploring Expedition, under the command of Capt. Charles Wilkes, U.S.N. Part 4: Including the species of Saurians, exotic to North America. *Proceedings of the Academy of Natural Sciences of Philadelphia* 9: 195-199.

Glaw F, Vences M. 1994. *A Fieldguide to the Amphibians and Reptiles of Madagascar*. Second Edition, including Mammals and Freshwater Fish. Cologne: Vences & Glaw.

Gray JE. 1825. A synopsis of the genera of reptiles and amphibia. *Annals of Philosophy*, London, Ser. 2, 10: 193-217.

Gray JE. 1834. Characters of two new genera of reptile (*Geomyda* and *Gehyra*). *Proceedings of the Zoological Society of London 1834 (1834)*: 99-101.

Harris DJ, Arnold EN, Thomas RH. 1998. Relationships of the lacertid lizards (Reptilia: Lacertidae) estimated from mitochondrial DNA sequences and morphology. *Proceedings of the Royal Society of London – B* 265: 1939-1948.

Ineich I, Blanc CP. 1987. Le peuplement herpétologique de Polynésie française. Adaptations et aléas. *Bulletin de la Société zoologique de France* 112: 381-400.

Ineich I, Blanc CP. 1988. Distribution des Reptiles terrestres en Polynésie orientale. *Atoll Research Bulletin* 318: 1-75.

Ineich I, de Massary JC. 1997. Distribution of *Gehyra mutilata*. *Herpetological Review* 28: 95.

Jesus J, Brehm A, Harris DJ. 2005. Phylogenetic relationships of *Hemidactylus* geckos from the Gulf of Guinea islands: patterns of natural colonizations and anthropogenic introductions estimated from mitochondrial and nuclear DNA sequences. *Molecular Phylogenetics and Evolution* 34: 480-485.

Kirch PV. 1997. *The Lapita peoples*. Cambridge, Mass.: Blackwell.

Kraus F. 2003. Invasion pathways for terrestrial vertebrates. In: Carlton J, Ruiz G, Mack R. eds. *Invasive Species: Vectors and Management Strategies*. Washington, DC.: Island Press, 68-92.

Lesson RP. 1830. Description de quelques reptiles nouveaux ou peu connus. In: Duperrey MLI. Voyage Autour du Monde executé pendant les annees 1822, 1823, 1824 et 1825. *Zoology* 2. Paris: Arthur Bertrand, 1-65.

Lorvelec O, Pascal M. 2006. Les vertébrés de Clipperton soumis à un siècle et demi de bouleversements écologiques. *Revue d'Ecologie (Terre Vie)* 61: 135-158.

Medway L, Marshall AG. 1975. Terrestrial vertebrates of the New Hebrides: origin and distribution. *Philosophical Transactions of the Royal Society of London, series B* 272: 423-465.

Nei M, Kumar S. 2000. *Molecular Evolution and Phylogenetics*. New York: Oxford University Press.

O'Shea MT, Cook S. 2006. Geographic Distribution - Lacertilia - *Gehyra mutilata* (Mutilated Gecko). Indonesia: Krakatau Islands. *Herpetological Review* 37: 360-361.

Ota H, Yamashita A. 1985. New record of the gekkonid lizard *Gehyra mutilata* (Wiegmann) from Hateruma-jima Island, Okinawa, Southern Japan. *Biological Magazine Okinawa* 23: 33-34.

Palumbi S, Martin A, Romano S, Mcmillan W, Stick L, Grabowski G. 1991. *The simple fools guide to PCR*. Version 2, University of Hawaii Press, Honolulu, Hawaii.

Petren K, Case TJ. 1996. An experimental demonstration of exploitation competition in an ongoing invasion. *Ecology* 77: 118-132.

Powell R, Smith DD, Parmerlee JS, Taylor CV, Jolley ML. 1990. Range expansion by an introduced anole: *Anolis porcatus* in the Dominican Republic. *Amphibia-Reptilia* 11: 421-425.

Rendahl H. 1939. Zur Herpetologie der Seychellen. I. Reptilien. *Zoologische Jahrbucher, abteilung für Systematik, Geographie und Biologie der Tiere*. 72: 255-328.

Rocha S, Carretero MA, Harris DJ. 2005. Diversity and phylogenetic relationships of *Hemidactylus* geckos from the Comoro islands. *Molecular Phylogenetics and Evolution* 35: 292-299.

Rösler H. 2007. Bemerkungen zur Nachaktivität von Taggeckos bei Kunstlicht. *Der Taggecko* 58: 4-5.

Rzhetsky A, Nei M. 1992. A simple method for estimating and testing minimum evolution trees. *Molecular Biology and Evolution* 9: 945-967.

Saitou N, Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 406-425.

Sambrook J, Fritsch EF, Maniatis T. 1989. *Molecular Cloning: a Laboratory Manual*, 2nd edn. New York: Cold Spring Harbor Press.

Shine S, Williams N, Gündling L. 2000. *A Guide to Designing Legal Institutional Frameworks on Alien Invasive Species*. IUCN, Gland, Switzerland, Cambridge and Bonn.

Stejneger L. 1893. On some collections of reptiles and batrachians from East Africa and the adjacent islands, recently received from Dr. W. L. Abbott and Mr. William Astor Chanler, with descriptions of new species. *Proceedings of the U.S. National Museum* 16: 711-741.

Tamura K, Nei M, Kumar S. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences* 101: 11030-11035.

Tamura K, Dudley J, Nei M, Kumar S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24, 1596-1599.

Tytler RC. 1865. Observations on a few species of geckos alive in the possession of the author. *Journal of the Asiatic Society of Bengal* 33: 535-548.

Vences M, Wanke S, Vieites DR, Branch WR, Glaw F, Meyer A. 2004. Natural colonization or introduction? Phylogeographical relationships and morphological differentiation of house geckos (*Hemidactylus*) from Madagascar. *Biological Journal of the Linnean Society* 83: 115-130.

Weiss AJ, Hedges SB. 2007. Molecular phylogeny and biogeography of the Antillean geckos *Phyllodactylus wirshingi*, *Tarentola americana*, and *Hemidactylus haitianus* (Reptilia, Squamata). *Molecular Phylogenetics and Evolution* 45: 409-16.

Wiegmann AFA. 1834. Amphibien, pp 433-522, plates LII-LXI In: Meyen FJF. *Reise um die Erde ausgeführt auf dem Königlich Preussischen Seehandlungs-Schiffe Prinzess Louise, comandiert von Capitain W. Wendt, in den Jahren 1830, 1831 und 1832. von Dr. F.J.F. Meyen*. Dritter Theil. Zoologischer Bericht. Berlin: Sanderschen Buchhandlung (C.W. Eichhoff). Pp viii, 522, 61 plates.

Received: 19 November 2008
Accepted: 29 December 2008
Published online: 31 March 2009
Editor: J.W. Arntzen

## Appendix

Specimens used in this study and respective locations. Sequences obtained from GenBank are identified by the accession number. Museum acronyms are BPBM: Bernice P. Bishop Museum, Hawaii, USA; CAS: California Academy of Sciences, San Francisco, USA; MNHN: Muséum National d'Histoire Naturelle, Paris, France; USNM: United States National Museum, Smithsonian Institution, Washington, USA. See also Fig. 1.

**Indian Ocean:** MA14, MA16: Beau Vallon, Mahé, Seychelles; MA21, MA22: Victoria, Mahé, Seychelles; 2MA10: Glacis, Mahé, Seychelles; 2MA16: North, Mahé, Seychelles; 2MA52: La Reserve, Mahé, Seychelles; 38713, 38714: La Passe, Silhouette, Seychelles; PL16: Anse Volbert, Praslin, Seychelles; AD1, AD25, AD26: Picard, Aldabra, Seychelles; APH8, APH9, APH11, APH12, APH13: Alphonse, Seychelles; GmDer1: Desroches, Seychelles; GmReu1, GmReu3: Réunion, Mascarenes; B-16 (DQ857338): Antananarivo, Madagascar; P-25 (DQ270549): Gorges de la Rivière Noire, Mauritius; D25 (AY517559): Mauritius. **Southern Asia:** USNM-FS 499244, USNM-FS 499246: Lasam, Luzon Is., Philippines; CAS229883, CAS229884: Da Wei Dist., Taninthayi Div., Myanmar; CAS232241, CAS232242, CAS232246, CAS232250, CAS232251, CAS232253, CAS232255: Khandi Dist., Sagaiang Div., Myanmar; CAS232804, CAS232809: Myitkyna Dist, Kachin St., Myanmar; K6 (AY217956): Pulau Pinang, Malaysia. **Pacific Islands:** USNM-FS 220248: Babeldaob, Airai, Palau; USNM-FS 220257: Malakal, Palau; USNM-FS 220441: Koror, Ngerbeched, Palau; USNM-FS 220541: Angaur, Palau; USNM-FS 536087: Saipan, San Vicente, Mariana Islands; BPBM 19753: Rossel Island, Damunu, Papua New Guinea; BPBM 15436: Duabo, Pini Range, Milne Bay Province, Papua New Guinea; USNM-FS 220293: Pohnpei, Kolonia, Caroline Islands; USNM-FS 224263: Yap, Colonia, Caroline Islands; 690: Torres Islands, Vanuatu; USNM-FS 221195: Oahu, Lanikai, Hawaii; USNM-FS 221051: Maui, Wailuku, Hawaii; MNHN 2007-0098: Gambier, Vahanga Island, French Polynesia; Gm1625, Gm1659: Clipperton Atoll.

# On the diversity, colonization patterns and status of *Hemidactylus* spp. (Reptilia: Gekkonidae) from the Western Indian Ocean islands

Sara Rocha[1,2,3], Miguel A. Carretero[1,2] & D. James Harris[1,2]

[1]CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, Portugal
[2]Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Portugal
[3]Departamento de Bioquímica, Genética e Inmunología, Facultad de Biología, Universidad de Vigo, Spain

*Hemidactylus* geckos are probably the most widespread genus of reptiles, with a world-wide distribution and multiple cases of range expansion and transmarine colonization. With an almost cosmopolitan distribution and many species being morphologically similar it has proved difficult to delimit species diversity and distributions. Using a comprehensive analysis of individuals collected across the Western Indian Ocean islands and some locations along the East African coast, we further assess their diversity and the origin of insular populations. Despite four species of *Hemidactylus* being widespread across the Western Indian Ocean islands, most of their range in this area may actually be the result of very recent (possibly human-aided) dispersal events. Most probably, all *Hemidactylus* species occurring in the Comoros and granitic Seychelles archipelagoes are not native. Instances of natural colonization seem to be only the ones of *H. mabouia* to Madagascar and from there to the coralline archipelago of Aldabra. Surprisingly, Aldabra populations reveal a remarkable diversity and structure. Given the degree of divergence observed we propose that insular (Gulf of Guinea, Comoros, Madagascar, Seychelles) populations of *H. mabouia* are recognized as *H. mercatorius*. Cryptic variation is further uncovered in all species in their native range, with *H. platycephalus* and *H. mabouia* harbouring several highly divergent lineages, but further taxonomic assignments should await detailed assessments of distribution and molecular variation.

*Key words:* 16s rRNA, Comoros, geckos, Seychelles, Madagascar

## INTRODUCTION

*Hemidactylus* geckos occur naturally through much of tropical Asia and Africa, the more arid areas of north-east Africa and southwest Asia, the Mediterranean region and South America, which they reached by natural transmarine colonization (Kluge, 1969). Both naturally and anthropogenically, they also reached various islands of the Americas, West Indies, Australia and the Indian, Pacific and Atlantic oceans, demonstrating more cases of range expansion through transmarine colonization than any other reptile genus (Carranza & Arnold, 2006).

Across the Western Indian Ocean islands at least four species belonging to three deeply differentiated clades (Carranza & Arnold, 2006) are known to occur (*H. mabouia/mercatorius*, *H. platycephalus*, *H. frenatus* and *H. brooki*). The origin and status of Malagasy and Comoran populations has been explored in recent years using molecular data (Vences et al., 2004; Rocha et al., 2005; Boumans et al., 2007). Summarizing, both *H. frenatus* and *H. brooki*, which belong to the tropical Asian clade (Carranza & Arnold, 2006), are present in Madagascar and the Comoros (*H. brooki* only in the Comoros) with patterns that better fit anthropogenic introduction scenarios. *Hemidactylus platycephalus* was shown to be the most abundant species in the Comoros but the lack of variation observed across these islands (and Madagascar) pointed also to a very recent range expansion, either natural or human-aided. *Hemidactylus mabouia/mercatorius* exhibited the most complex variation pattern and data clearly indicate that the exact species delimitation and phylogeographic structure within this putative "species-complex" is far from being understood. Vences et al. (2004), based on the deep divergence observed between African (*H. mabouia*) and Malagasy individuals, used the *H. mercatorius* (Gray, 1842) designation for the Malagasy populations but results from Rocha et al. (2005) showed that species delimitation is unclear, given that *H. mabouia* haplotypes from the Gulf of Guinea populations cluster with one of the clades of both Malagasy and Comoran populations. Madagascar harbours two distinct clades of this "species" that were further explored by Boumans et al. (2007), in a more comprehensive analysis across its Malagasy distribution. These authors argued in favour of its native status (at least across most of its range) in Madagascar.

Here we use comprehensive sampling across the Western Indian Ocean islands of the Seychelles (both coralline and granitic archipelagos), Zanzibar and Pemba (off Tanzania), some locations across the East African

*Correspondence:* Sara Rocha, CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, 4485-661 Vairão, Portugal. *E-mail:* sara.rocha@mail.icav.up.pt

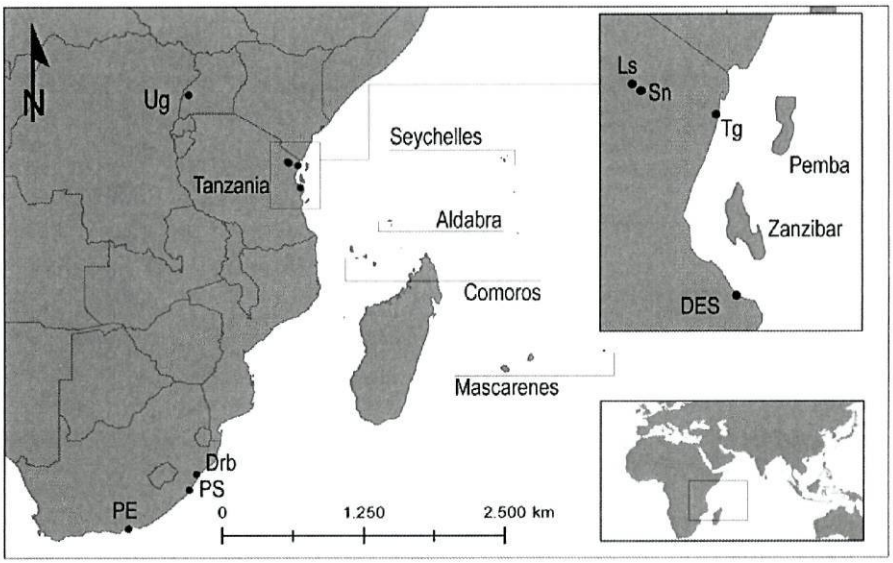



**Fig. 1.** Map of main sample localities (see Electronic Appendix for detailed information). New localities in continental Africa are highlighted (Ug: Kibale, Uganda; Ls, Sn, Tg, DES: Lushoto, Soni, Tanga and DarEsSalaam, Tanzania; Drb, PS, PE: Durban, Port Shepstone and Port Elizabeth, South Africa). For detailed locations of Comoran and Seychellois samples, see Carretero et al. (2005) and Rocha et al. (2010).

coast (Tanzania, South Africa) and Uganda, together with the already published sequences of these species, to further assess their diversity and the origin of these insular populations and to try to provide some insights into the origins and status of all insular populations of both *H. mabouia* and *H. platycephalus* by the inclusion of continental samples from Africa.

## MATERIALS AND METHODS

We collected 130 new samples from the four target species across South Africa, Uganda, Tanzania mainland, Zanzibar and Pemba islands and the Seychelles coralline and granitic archipelagos (Fig. 1 and Electronic Appendix, downloadable at http://www.thebhs.org/pubs_journal_online_appendices. html).

Genetic samples were taken in the field by preserving muscle tissue (autotomized tails) in pure ethanol and total genomic DNA was extracted using standard high-salt (Sambrook et al., 1989) or phenol-chloroform (Taggart et al., 1992) protocols. We used primers 16SA-L and 16SB-H (Palumbi et al., 1991) to amplify a section of approximately 500 bp of the mitochondrial 16S ribosomal RNA gene, using conditions described in Harris et al. (1998). Sequences were produced by a commercial facility (Macrogen, Seoul, Korea).

New sequences were then combined with previously published ones from Vences et al. (2004), Rocha et al. (2005) and Boumans et al. (2007) and aligned in MAFFT v. 6 under the G-INS-I strategy (Katoh et al., 2005), an iterative approach that assumes that the total region can be aligned and tries to align them globally. It is appropriate for gene regions like these, where, despite indel-rich regions, global homology can be observed, and has been shown to perform better than most commonly used pairwise approaches (Wilm et al., 2006). Haplotype networks for all species were constructed using the median-joining method (Bandelt et al., 1999) with MP optimization (Polzin & Daneschmand, 2003) in NETWORK v. 4.510 (www.fluxus-engineering.com). A maximum likelihood (ML) tree was also built for all unique haplotypes of both *H. mabouia–mercatorius* and *H. platycephalus*. The best fitting model was found through jModeltest (Guindon & Gascuel, 2003; Posada, 2008) under the AICc criteria and an ML search with 1000 bootstrap replicates was performed in PhyML (Guindon & Gascuel, 2003).

The software package BEAST v. 1.4.8 (Drummond & Rambaut, 2007) was used to obtain Bayesian estimates (posterior distributions) of the substitution rates underlying the data, assuming the Aldabra group clade of *H. mabouia–mercatorius* to be approximately 125,000 years old (Thomson & Walton, 1972) (see Results and Discussion). For this we again found the best fitting model for the respective dataset (all Aldabra group individuals) using jModeltest and chose the one with the best AICc score (GTR+I) among those available in BEAST. Because we were dealing with a short intraspecific dataset (low information content and little reason to expect rate heterogeneity), we used a strict clock model with a coalescent tree prior and an exponential growth population model. We used a uniform prior on the tree root of 118,000 yr to 140,000 yr (according to Thomson & Walton, 1972) as well as a uniform prior on the mutation rate, which we allowed to vary from 0.005 substitutions per site per mil-

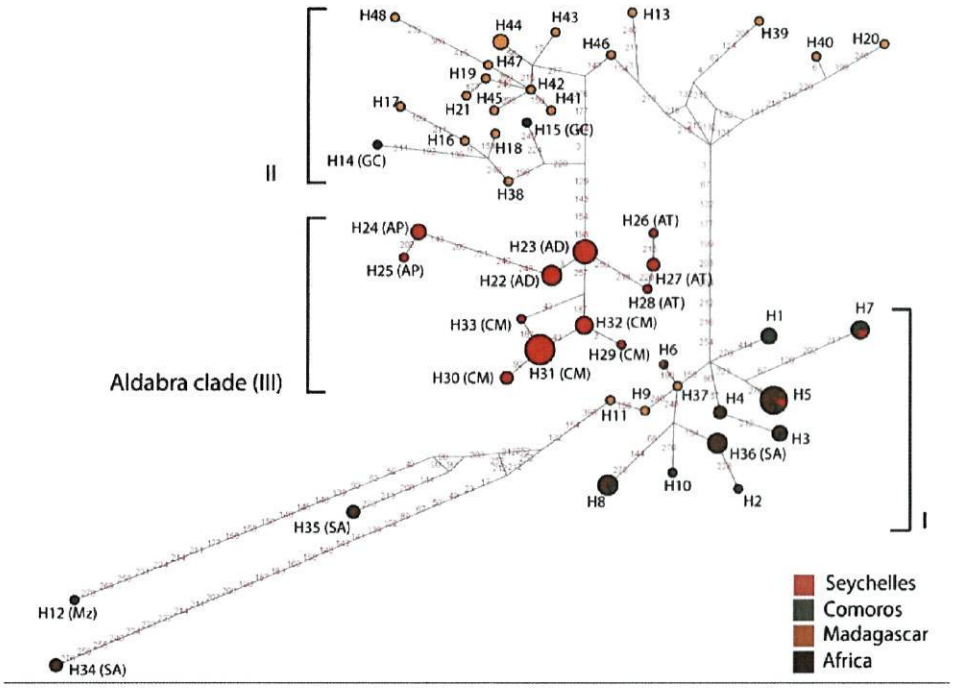



**Fig. 2.** MJ network for *H. mabouia–mercatorius* individuals. Haplotypes are colour-coded geographically and positions presenting substitutions are represented along the branches. The correspondence between haplotypes and individuals can be found in the Electronic Appendix.

lion years (half of the assumed mitochondrial vertebrate long-term mutation rate of 2%; Wilson, 1985) and 0.2 (a twenty-fold increase on the latter rate). Default priors were used for population size, modern day population size and exponential growth rate parameters. The analysis was run at least two times for $50\times10^6$ steps, sampled each 1000 steps, and 10% were discarded as burn-in. The software TRACER v. 1.4.1 (Rambaut & Drummond, 2007) was used to visualize the runs, checking for stationarity, convergence and adequacy of effective sample sizes (ESS) of parameters and to obtain posterior estimates and 95% credibility intervals for estimates.

## RESULTS AND DISCUSSION

### *Hemidactylus mabouia* "complex"

Examination of the haplotype networks concerning the *H. mercatorius–mabouia* "complex" reveal even more complicated patterns of variation than those previously described (Fig. 2). Clearly multiple cryptic lineages exist within this "species", and the current taxonomic arrangement is completely inadequate. Haplotypes from Mozambique and South Africa define three deep, highly divergent lineages from the remaining group (clade A in Figure 4). Both PCR amplification and sequences were clean, without evidence of multiple bands or peaks, so we have no reason to be suspicious about the possibility of analysing paralogous fragments in these individuals (nuclear copies). Furthermore, base composition of these sequences was similar to the remaining ones in the dataset (average 18%T, 26%C, 31%A, 22%G), with values that fit well with those expected for vertebrate mitochondrial genomes (Asakawa et al., 1991). Differentiation between these main lineages (4) is high: all distance comparisons involving the South African lineages (H35 and H34) and the individual from Mozambique (H12) reveal distances of 2.7–8.3%, 7.3–8.5% and 5.8–7.7%, respectively (uncorrected average distances between groups) towards remaining individuals. If we invoke the 3% cut-off value for this same fragment used by Vieites et al. (2009) we may be in the presence of (unconfirmed) candidate species (UCS, *sensu* Vieites et al., 2009).

Within clade A, at least three further groups can tentatively be defined, each with considerable levels of diversity. One of the sub-clades (I in Figures 2 and 4) harbours haplotypes from the north of Madagascar, the Comoros (Mayotte), Tanzania including the islands of Zanzibar and Pemba, South Africa and the Seychelles (Mahé), as well as Uganda and the Gulf of Guinea islands, without any geographic structure. This species is not abundant either in the Comoros, or in the granitic Seychelles – just a few individuals were found during several weeks of fieldwork, on the island of Mahé, and they all belong to this clade, sharing haplotypes with African and Comoran individuals. From the distribution of the African haplotypes, it may be that both Mayotte and Mahé (and

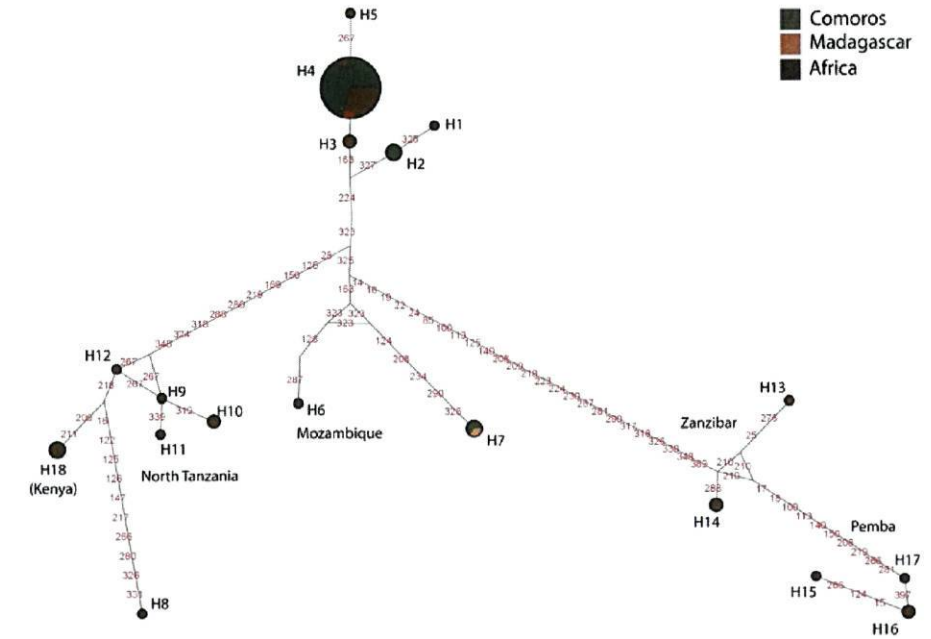



**Fig. 3.** MJ network for *H. platycephalus* individuals. Haplotypes are colour coded geographically and positions presenting substitutions represented along the branches. The correspondence between haplotypes and individuals can be found in the Electronic Appendix.

north Madagascar) were colonized recently multiple times from the East African coast or/and that dispersals from Mayotte, Mahé or North Madagascar back to Africa also do happen (possibly all corresponding to anthropogenic movements). It is also clear that cryptic diversity is present along the East African coast (haplotypes from South Africa belong to three different and highly divergent lineages), where variation within this species and its geographic distribution must be further investigated. The second group (II) of this Western Indian Ocean clade is defined mostly by Malagasy samples (and two individuals from Grand Comoro, Comoros), harbouring considerable haplotype diversity and structure, in what could be considered an endemic Malagasy clade (Boumans et al., 2007). Comoran haplotypes from this clade (two, from Grand Comoro) may again result from occasional introductions.

Closely related to this, haplotypes from the coralline archipelago of Aldabra (Aldabra, Cosmoledo, Astove, Assumption) define a well-supported monophyletic group (III) with considerable diversity and a remarkable phylogeographic structure, without any of these islands sharing any haplotype. This is a remarkable and surprising result given their geographical proximity and that Aldabra was supposedly completely submerged only about 125,000 years ago (Thomson & Walton, 1972) and the remaining islands in this group, lower than Aldabra itself, were also most probably submerged during this last interglacial period. The geographic structure of the distribution of the haplotypes nevertheless clearly supports the native status of this species in these islands. As a test of the likelihood of this clade being native to the Aldabra archipelago we estimated the mutation rate needed to invoke if we assume a colonization of the island right after its emergence, implementing the 118,000–140,000-year interval from Thomson & Walton (1972) as a uniform prior on the root height and a uniform prior on the mutation rate from 0.005 to 0.2 substitutions/site/my. We recovered a roughly "normal-shaped" posterior distribution on the substitution rate, differing considerably from the prior used, with a mean of 0.07 substitutions/site/my but with a large 95% credibility interval (0.019–0.1457), which although higher than the traditional phylogenetic estimate of the rate of evolution, could correspond to the higher reported values of the mutation rate observed in "recent" (= low divergence) intraspecific datasets (Howell et al., 2003; Burridge et al., 2008), regardless of whether they are real or dataset or analysis artefacts (for discussion of this topic, see Ho et al., 2005; Emerson, 2007; Debruyne & Poinar, 2009). Explorative estimates using a relaxed clock model led to similar results (though with an even wider CI).

We thus have no reason to reject the hypothesis that this is a real endemic clade of the Aldabra archipelago, which is notable, given the recent age of these islands and the overall evidence for an otherwise anthropogenic-driven distribution of this species across the Western Indian Ocean islands.

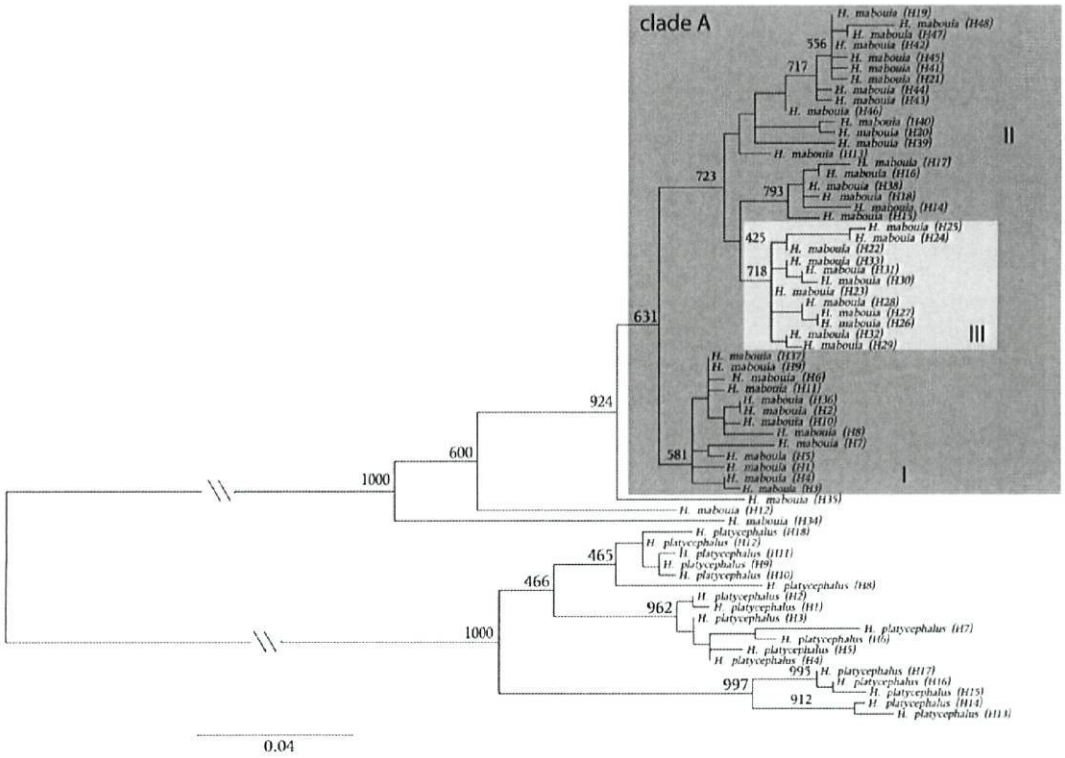



**Fig. 4.** Maximum likelihood tree for all haplotypes of *H. mabouia-mercatorius* and *H. platycephalus*. Relevant bootstrap values are indicated. Haplotype labelling is the same as in Figures 2 and 3. Clade A of *H. mabouia-mercatorius* and its subgroups are highlighted.

A comprehensive clarification of *H. mabouia*-complex taxonomy is not yet possible, but, given the clear differentiation observed between the basal (South African and Mozambique) lineages and the now well sampled insular populations, we propose that *H. mercatorius* becomes the designation applied to Gulf of Guinea, Comoran, Malagasy and Seychellois populations, with the status of eastern African populations remaining unchanged pending further studies. *Hemidactylus mercatorius* has most probably an extensive distribution across East Africa, possibly sympatric with *H. mabouia* across at least part of its range, but any taxonomic assignation of continental populations should wait until the distinction and distribution of these lineages across continental Africa is properly investigated.

### *Hemidactylus platycephalus*

*Hemidactylus platycephalus* was not found in the Seychelles, but was the most common species across the Comoros (Rocha et al., 2005), inhabiting both natural and humanized environments (Carretero et al., 2005), and very abundant also along the East African coast and on the islands of Zanzibar and Pemba. Again, cryptic diversity is unveiled across East Africa with haplotypes from Zanzibar, Pemba, Tanzania (mainland), Mozambique and Kenya defining at least three, highly differentiated, clades (Figs 3 and 4). Distances between these clades vary between 2.6 and 3.7%.

Again, for this species, considering the distribution of the African haplotypes (scattered across the three lineages) and the low or lack of diversity observed respectively in the Comoros and Madagascar, it is most probable that its presence on these islands is the result of recent (possible human-aided) dispersal events. Interestingly, some geographically coherent variation is observed in East Africa, with north Tanzania and Kenya and Zanzibar and Pemba harbouring exclusive lineages. Again, it is possible that multiple species occur under the current designation of *H. platycephalus*, and this should be further investigated through extended sampling and the use of additional nuclear data.

### *Hemidactylus frenatus*

Finally, *H. frenatus*, not found on the East African coast, was the most abundant species of this genus in the granitic Seychelles (Mahé) and was also found across some of the low coralline islands of the Seychelles (Bird, Poivre and Desroches). Several distinct haplotypes were found across the Seychelles, Madagascar, the Mascarenes and the Comoros, but without any clear phylogeographic rela-

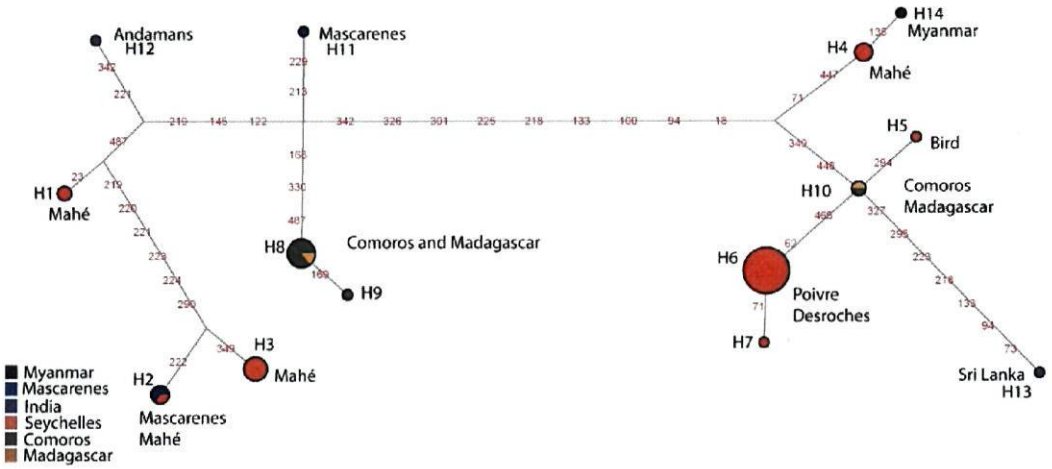



**Fig. 5.** MJ network for *H. frenatus* individuals. Haplotypes are colour-coded geographically and positions presenting substitutions are represented along the branches. The correspondence between haplotypes and individuals can be found in the Electronic Appendix.

tionship (Fig. 5), showing again, and also for the Seychelles, that its presence is most probably attributable to multiple, most likely human-aided, dispersal events. Of the two new Asian samples from this species included in this study, from Myanmar, one clearly clustered with this group of haplotypes (CAS885; H14) but another one (not included in the haplotype network) exhibited a highly divergent haplotype from these (around 10%), pointing again to cryptic diversity within this species in its native range (Carranza & Arnold, 2006).

In sum, despite the four species of *Hemidactylus* being generally widespread across the Western Indian Ocean islands, most of their range in this area may actually be the result of very recent (possibly human-aided) dispersal events. It now seems most probable that none of the *Hemidactylus* species occurring in the Comoros archipelago is actually native, the same being true for the granitic Seychelles. The only instances of natural colonization seem to be those of *H. mabouia* to Madagascar and from there to the coralline archipelago of Aldabra. Given the degree of differentiation observed, we propose that insular (Gulf of Guinea, Comoros, Madagascar, Seychelles) populations of *H. mabouia* are recognized as *H. mercatorius* (as also proposed for the Malagasy populations by Vences et al., 2004), with the status of continental populations pending further studies. Cryptic variation is depicted for the three species studied in their native range, with the cases of *H. platycephalus* and *H. mabouia* being remarkable in the sense that several highly divergent mitochondrial lineages are present along the eastern African coast. Nevertheless, extensive nuclear molecular data should also be collected and the geographical distributions of these lineages clarified before further taxonomic changes are implemented.

## ACKNOWLEDGEMENTS

We are grateful to SBS of Seychelles for permits for fieldwork and tissue collection and to the Ministry of Environment and Natural Resources for logistic support during fieldwork. We thank Justin Gerlach from NPTS for support and information and to Raquel Vasconcelos and Ana Perera for help during fieldwork. Raquel Vasconcelos and Salvador Carranza kindly provided samples from Uganda and Kenya for this study, and Jens Vindum at the California Academy of Sciences (CAS) of *H. frenatus* from Myanmar. This research was supported by grants from Fundação para a Ciência e Tecnologia (FCT) SFRH/BD/1745/2004 (to SR), SFRH/BPD/27025/2006 (to MAC), POCTI/BSE/46647/2000 and PTDC/BIA-BDE/65745/2006 and by the Calouste Gulbenkian Foundation. The authors further acknowledge two anonymous reviewers for useful comments and remarks.

## REFERENCES

Asakawa, S., Kumazawa, Y., Araki, T., Himeno, H., Miura, K. & Watanabe, K. (1991). Strand-specific nucleotide composition bias in echinoderm and vertebrate mitochondrial genomes. *Journal of Molecular Evolution* 32, 511–520.

Bandelt, H.J., Forster, P. & Rohl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16, 37–48.

Boumans, L., Vieites, D.R., Glaw, F. & Vences, M. (2007). Geographical patterns of deep mitochondrial differentiation in widespread malagasy reptiles. *Molecular Phylogenetics and Evolution* 45, 822–839.

Burridge, C.P., Craw, D., Fletcher, D. & Waters, J.M. (2008). Geological dates and molecular rates: fish DNA

sheds light on time dependency. *Molecular Biology and Evolution* 25, 624–633.

Carranza, S. & Arnold, E.N. (2006). Systematics, biogeography, and evolution of *Hemidactylus* geckos (Reptilia: Gekkonidae) elucidated using mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* 38, 531–545.

Carretero, M.A., Harris, D.J. & Rocha, S. (2005). Recent observations of reptiles in the Comoro islands (Western Indian Ocean). *Herpetological Bulletin* 91, 19–28.

Debruyne, R. & Poinar, H. (2009). Time dependency of molecular rates in ancient DNA data sets, a sampling artifact? *Systematic Biology* 58, 348–360.

Drummond, A.J. & Rambaut, A. (2007). Beast: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7, 214.

Emerson, B.C. (2007). Alarm bells for the molecular clock? No support for Ho et al.'s model of time-dependent molecular rate estimates. *Systematic Biology* 56, 337–345.

Gray, J.E. (1842). Description of some new species of reptiles, chiefly from the British Museum collection. *The Zoological Miscellany*, 57–59.

Guindon, S. & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52, 696–704.

Harris, D.J., Arnold, E.N. & Thomas, R.H. (1998). Relationships of lacertid lizards (Reptilia: Lacertidae) estimated from mitochondrial DNA sequences and morphology. *Proceedings of the Royal Society B* 265, 1939–1948.

Ho, S.Y., Phillips, M.J., Cooper, A. & Drummond, A.J. (2005). Time dependency of molecular rate estimates and systematic overestimation of recent divergence times. *Molecular Biology and Evolution* 22, 1561–1568.

Howell, N., Smejkal, C.B., Mackey, D.A., Chinnery, P.F., Turnbull, D.M. & Herrnstadt, C. (2003). The pedigree rate of sequence divergence in the human mitochondrial genome: there is a difference between phylogenetic and pedigree rates. *American Journal of Human Genetics* 72, 659–670.

Katoh, K., Kuma, K., Miyata, T. & Toh, H. (2005). Improvement in the accuracy of multiple sequence alignment program MAFFT. *Genome Information* 16, 22–33.

Kluge, A.G. (1969). The evolution and geographic origin of the new world *Hemidactylus mabouia–brookii* complex (Gekkonidae, Sauria). *Miscellaneous Publications of the Museum of Zoology, University of Michigan* 138, 1–78.

Palumbi, S.R., Martin, A., Romano, S., McMillan, W.O., Stice, L. & Grabowski, G. (1991). *The Simple Fool's Guide to PCR, Version 2.0*. Hawaii: Privately published.

Polzin, T. & Daneschmand, S.V. (2003). On Steiner trees and minimum spanning trees in hypergraphs. *Operations Research Letters* 31, 12–20.

Posada, D. (2008). JModeltest: phylogenetic model averaging. *Molecular Biology and Evolution* 25, 1253–1256.

Rambaut, A. & Drummond, A.J. (2007). *TRACER v1.4*. Available from http://beast.Bio.Ed.Ac.Uk/tracer

Rocha, S., Carretero, M.A. & Harris, D.J. (2005). Diversity and phylogenetic relationships of *Hemidactylus* geckos from the Comoro islands. *Molecular Phylogenetics and Evolution* 35, 292–299.

Rocha, S., Harris, D.J., Perera, A., Silva, A., Vasconcelos, R. & Carretero, M.A. (2010). Recent data on the distribution of lizards and snakes of the Seychelles. *Herpetological Bulletin* 110, 20–32.

Sambrook, J., Fritsh, E.F. & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Press.

Taggart, J.B., Hynes, R.A., Prodoul, P.A. & Ferguson, A. (1992). A simplified protocol for routine total DNA isolation from salmonid fishes. *Journal of Fish Biology* 40, 963–965.

Thomson, J. & Walton, A. (1972). Redetermination of the chronology of the Aldabra atoll by th/u dating. *Nature* 240, 145–146.

Vences, M., Stefan, W., David, R.V., William, R.B., Frank, G. & Axel, M. (2004). Natural colonization or introduction? Phylogeographical relationships and morphological differentiation of house geckos (*Hemidactylus*) from Madagascar. *Biological Journal of the Linnean Society* 83, 115–130.

Vieites, D.R., Wollenberg, K.C., Andreone, F., Kohler, J., Glaw, F. & Vences, M. (2009). Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. *Proceedings of the National Academy of Sciences of the United States of America* 106, 8267–8272.

Wilm, A., Mainz, I. & Steger, A. (2006) An enhanced RNA alignment benchmark for sequence alignment programs. *Algorithms for Molecular Biology* 1, 19. doi:10.1186/1748-7188-1-19.

Wilson, A.C. (1985). The molecular basis of evolution. *Scientific American* 253, 164–173.

*Accepted: 22 February 2010*

**Electronic Appendix**

(S. Rocha et al., 2010. *Herpetological Journal* 20, 83-89)

List of individuals used in this study, haplotype codes and source. For the new specimens locations and Genbank accession nos. are given. Detailed GPS coordinates for Comoran and Seychellois samples can be found in Carretero *et al.* (2005) and Rocha *et al.* (2009). Reference sources for the sequences used: [a]Vences et al. (2004); [b]Rocha et al., 2005; [c]Boumans et al. (2007).

| Species | Haplot. | Individual | Acc. No. | Locality | Source |
|---|---|---|---|---|---|
| *H. mabouia-mercatorius* | H1 | MY9 | | Mamoutzu, urban, Mayotte | b |
| *H. mabouia-mercatorius* | H1 | MY46 | | Chiroungoui, Mayotte | b |
| *H. mabouia-mercatorius* | H1 | MY60 | | Bambo Est Plage, Mayotte | b |
| *H. mabouia-mercatorius* | H2 | 668Ann | | Annobon, Gulf of Guinea | b |
| *H. mabouia-mercatorius* | H3 | MY10 | | Mamoutzu, urban, Mayotte | b |
| *H. mabouia-mercatorius* | H3 | TZ12 | HM192543 | Dar Es Salaam, Tanzania | This study |
| *H. mabouia-mercatorius* | H3 | UG | HM192544 | Kibale, Uganda | This study |
| *H. mabouia-mercatorius* | H4 | TZ23 | HM192545 | Tanga, Tanzania | This study |
| *H. mabouia-mercatorius* | H4 | TZ24 | HM192546 | Tanga, Tanzania | This study |
| *H. mabouia-mercatorius* | H5 | MY27 | | Bouéni (road to), Mayotte | b |
| *H. mabouia-mercatorius* | H5 | PB6 | HM192547 | Mkoani, Pemba Island, Tanzania | This study |
| *H. mabouia-mercatorius* | H5 | TZ35 | HM192548 | Lushoto, Tanzania | This study |
| *H. mabouia-mercatorius* | H5 | Z31 | HM192549 | Kiwengwa, Zanzibar Island, Tanzania | This study |
| *H. mabouia-mercatorius* | H5 | Z47 | HM192550 | Stone Town, Zanzibar Island, Tanzania | This study |
| *H. mabouia-mercatorius* | H5 | SA20 | HM192551 | Durban, South Africa | This study |
| *H. mabouia-mercatorius* | H5 | SA31 | HM192552 | Port Shepstone, South Africa | This study |
| *H. mabouia-mercatorius* | H5 | SA32 | HM192553 | Port Shepstone, South Africa | This study |
| *H. mabouia-mercatorius* | H5 | SA33 | HM192554 | Port Shepstone, South Africa | This study |
| *H. mabouia-mercatorius* | H6 | MY66 | | Dzaouzi road airport, Mayotte | b |
| *H. mabouia-mercatorius* | H7 | MY45 | | Chiroungoui, Mayotte | b |
| *H. mabouia-mercatorius* | H7 | MY58 | | Sada road, Mayotte | b |
| *H. mabouia-mercatorius* | H7 | SA30 | HM192555 | Port Shepstone, South Africa | This study |
| *H. mabouia-mercatorius* | H7 | MA5 | HM192556 | Airport, Mahé Island, Seychelles | This study |
| *H. mabouia-mercatorius* | H8 | MY30 | | Bouéni, Mayotte | b |
| *H. mabouia-mercatorius* | H8 | Z1 | HM192557 | Stone Town, Zanzibar Island, Tanzania | This study |
| *H. mabouia-mercatorius* | H8 | Z40 | HM192558 | Upenja, Zanzibar Island, Tanzania | This study |
| *H. mabouia-mercatorius* | H8 | PB5 | HM192559 | Mkoani, Pemba Island, Tanzania | This study |
| *H. mabouia-mercatorius* | H8 | TZ6 | HM192560 | Dar Es Salaam, Tanzania | This study |
| *H. mabouia-mercatorius* | H9 | .12 | | Sambava, Madagascar | a |
| *H. mabouia-mercatorius* | H10 | Z19 | HM192561 | Chwaka, Zanzibar Island, Tanzania | This study |
| *H. mabouia-mercatorius* | H11 | .11 | | Montagne des Francais, Madagascar | a |
| *H. mabouia-mercatorius* | H12 | .13 | | Zambezia, Mozambique | a |
| *H. mabouia-mercatorius* | H13 | .9 | | Andranofotsy, Madagascar | a |
| *H. mabouia-mercatorius* | H14 | .7 | | Mamoudzou, Mayotte | a |
| *H. mabouia-mercatorius* | H15 | GC3 | | Moroni, urban, Grand Comore | b |
| *H. mabouia-mercatorius* | H16 | .1 | | Ampirojoa (Ankarafantsika), Madagascar | a |
| *H. mabouia-mercatorius* | H17 | .6 | | Antananarivo, Madagascar | a |
| *H. mabouia-mercatorius* | H18 | .8 | | Ankarafantsika, Madagascar | a |
| *H. mabouia-mercatorius* | H19 | .2 and .4 | | Tolagnaro, Madagascar | a |
| *H. mabouia-mercatorius* | H20 | .10 | | Nosy Be, Madagascar | a |
| *H. mabouia-mercatorius* | H21 | .3 | | Tolagnaro, Madagascar | a |
| *H. mabouia-mercatorius* | H22 | AD9 | HM192562 | Picard, Aldabra, Seychelles | This study |
| *H. mabouia-mercatorius* | H22 | AD29 | HM192563 | Malabar, Aldabra, Seychelles | This study |
| *H. mabouia-mercatorius* | H22 | AD30 | HM192564 | Grand Terre, Aldabra, Seychelles | This study |
| *H. mabouia-mercatorius* | H22 | AD32 | HM192565 | Grand Terre, Aldabra, Seychelles | This study |

| | | | | | |
|---|---|---|---|---|---|
| *H. mabouia-mercatorius* | H22 | AD34 | HM192566 | Grand Terre, Aldabra, Seychelles | This study |
| *H. mabouia-mercatorius* | H23 | AD10 | HM192567 | Picard, Aldabra, Seychelles | This study |
| *H. mabouia-mercatorius* | H23 | AD11 | HM192568 | Picard, Aldabra, Seychelles | This study |
| *H. mabouia-mercatorius* | H23 | AD12 | HM192569 | Picard, Aldabra, Seychelles | This study |
| *H. mabouia-mercatorius* | H23 | AD16 | HM192570 | Polymnie, Aldabra, Seychelles | This study |
| *H. mabouia-mercatorius* | H23 | AD22 | HM192571 | Picard, Aldabra, Seychelles | This study |
| *H. mabouia-mercatorius* | H23 | AD23 | HM192572 | Picard, Aldabra, Seychelles | This study |
| *H. mabouia-mercatorius* | H23 | AD24 | HM192573 | Picard, Aldabra, Seychelles | This study |
| *H. mabouia-mercatorius* | H24 | AP7 | HM192574 | Assumption, Seychelles | This study |
| *H. mabouia-mercatorius* | H24 | AP10 | HM192575 | Assumption, Seychelles | This study |
| *H. mabouia-mercatorius* | H24 | AP13 | HM192576 | Assumption, Seychelles | This study |
| *H. mabouia-mercatorius* | H25 | AP11 | HM192577 | Assumption, Seychelles | This study |
| *H. mabouia-mercatorius* | H26 | AT1 | HM192578 | Astove, Seychelles | This study |
| *H. mabouia-mercatorius* | H27 | AT3 | HM192579 | Astove, Seychelles | This study |
| *H. mabouia-mercatorius* | H27 | AT5 | HM192580 | Astove, Seychelles | This study |
| *H. mabouia-mercatorius* | H28 | AT10 | HM192581 | Astove, Seychelles | This study |
| *H. mabouia-mercatorius* | H29 | CM1 | HM192582 | Menai Island, Cosmoledo, Seychelles | This study |
| *H. mabouia-mercatorius* | H30 | CM2 | HM192583 | Menai Island, Cosmoledo, Seychelles | This study |
| *H. mabouia-mercatorius* | H30 | CM4 | HM192584 | Menai Island, Cosmoledo, Seychelles | This study |
| *H. mabouia-mercatorius* | H31 | CM12 | HM192585 | Northwest Island, Cosmoledo, Seychelles | This study |
| *H. mabouia-mercatorius* | H31 | CM15 | HM192586 | Northwest Island, Cosmoledo, Seychelles | This study |
| *H. mabouia-mercatorius* | H31 | CM16 | HM192587 | Northwest Island, Cosmoledo, Seychelles | This study |
| *H. mabouia-mercatorius* | H31 | CM17 | HM192588 | Wizard Island, Cosmoledo, Seychelles | This study |
| *H. mabouia-mercatorius* | H31 | CM24 | HM192589 | Wizard Island, Cosmoledo, Seychelles | This study |
| *H. mabouia-mercatorius* | H31 | CM29A | HM192590 | Wizard Island, Cosmoledo, Seychelles | This study |
| *H. mabouia-mercatorius* | H31 | CM18 | HM192591 | Wizard Island, Cosmoledo, Seychelles | This study |
| *H. mabouia-mercatorius* | H31 | CM23 | HM192592 | Wizard Island, Cosmoledo, Seychelles | This study |
| *H. mabouia-mercatorius* | H31 | CM25 | HM192593 | Wizard Island, Cosmoledo, Seychelles | This study |
| *H. mabouia-mercatorius* | H31 | CM26A | HM192594 | Wizard Island, Cosmoledo, Seychelles | This study |
| *H. mabouia-mercatorius* | H31 | CM28A | HM192595 | Wizard Island, Cosmoledo, Seychelles | This study |
| *H. mabouia-mercatorius* | H32 | CM28 | HM192596 | Northeast Island, Cosmoledo, Seychelles | This study |
| *H. mabouia-mercatorius* | H32 | CM34 | HM192597 | Northeast Island, Cosmoledo, Seychelles | This study |
| *H. mabouia-mercatorius* | H32 | CM35 | HM192598 | Northeast Island, Cosmoledo, Seychelles | This study |
| *H. mabouia-mercatorius* | H32 | CM36 | HM192599 | Northeast Island, Cosmoledo, Seychelles | This study |
| *H. mabouia-mercatorius* | H33 | CM27A | HM192600 | Wizard Island, Cosmoledo, Seychelles | This study |
| *H. mabouia-mercatorius* | H34 | SA21 | HM192601 | Durban, South Africa | This study |
| *H. mabouia-mercatorius* | H34 | SA22 | HM192602 | Durban, South Africa | This study |
| *H. mabouia-mercatorius* | H35 | SA18 | HM192603 | Durban, South Africa | This study |
| *H. mabouia-mercatorius* | H35 | SA34 | HM192604 | Port Elizabeth, South Africa | This study |
| *H. mabouia-mercatorius* | H36 | SA19 | HM192605 | Durban, South Africa | This study |
| *H. mabouia-mercatorius* | H36 | SA23 | HM192606 | Durban, South Africa | This study |
| *H. mabouia-mercatorius* | H36 | SA27 | HM192607 | Port Shepstone, South Africa | This study |
| *H. mabouia-mercatorius* | H36 | SA28 | HM192608 | Port Shepstone, South Africa | This study |
| *H. mabouia-mercatorius* | H36 | SA29 | HM192609 | Port Shepstone, South Africa | This study |
| *H. mabouia-mercatorius* | H37 | MA-77 | | Andapa, Madagascar | c |
| *H. mabouia-mercatorius* | H38 | MA-70 | | Maroantsetra, Madagascar | c |
| *H. mabouia-mercatorius* | H39 | MA78 | | Andapa, Madagascar | c |
| *H. mabouia-mercatorius* | H40 | MA-83 | | Sambava, Madagascar | c |
| *H. mabouia-mercatorius* | H41 | MA-69 | | Maroantsetra, Madagascar | c |
| *H. mabouia-mercatorius* | H42 | MVB45 | | Tolagnaro, Madagascar | c |
| *H. mabouia-mercatorius* | H43 | MA-73 | | Tranomaro, Madagascar | c |
| *H. mabouia-mercatorius* | H44 | MA-75 | | Esomony, Madagascar | c |
| *H. mabouia-mercatorius* | H44 | MA-80 | | Esomony, Madagascar | c |
| *H. mabouia-mercatorius* | H44 | MA-82 | | Esomony, Madagascar | c |
| *H. mabouia-mercatorius* | H45 | MAMano | | Manombo, Madagascar | c |
| *H. mabouia-mercatorius* | H46 | MARanomaf | | Ranomafana, Madagascar | c |
| *H. mabouia-mercatorius* | H47 | MA-81 | | Tranomaro, Madagascar | c |
| *H. mabouia-mercatorius* | H48 | MA-84 | | Tranomaro, Madagascar | c |
| | | | | | |
| *H. platycephalus* | H1 | AJ24 | | Pomoni, Anjouan | b |
| *H. platycephalus* | H2 | GC27 | | Hantoindzi (Chezani), Grand Comore | b |
| *H. platycephalus* | H2 | GC33 | | Itsoundzou, Grand Comore | b |
| *H. platycephalus* | H2 | .22 | | Chomoni, Grand Comoro | a |
| *H. platycephalus* | H3 | Z11 | HM192610 | Stone Town, Zanzibar Island, Tanzania | This study |
| *H. platycephalus* | H3 | Z14 | HM192611 | Stone Town, Zanzibar Island, Tanzania | This study |
| *H. platycephalus* | H4 | MY11 | | Chirongoui, Mayotte | b |
| *H. platycephalus* | H4 | MY13 | | Chirongoui, Mayotte | b |
| *H. platycephalus* | H4 | MY14 | | Chirongoui, Mayotte | b |
| *H. platycephalus* | H4 | MY21 | | Kani-Kéli, Mayotte | b |

| | | | | | |
|---|---|---|---|---|---|
| *H. platycephalus* | H4 | MY29 | | Bouéni, Mayotte | b |
| *H. platycephalus* | H4 | MY36 | | Bouéni (road to), Mayotte | b |
| *H. platycephalus* | H4 | MY38 | | Bambo Ouest, Mayotte | b |
| *H. platycephalus* | H4 | MY39 | | M'bouanatsa, Mayotte | b |
| *H. platycephalus* | H4 | MY40 | | Chirongoui (Plage Mtihi), Mayotte | b |
| *H. platycephalus* | H4 | MY41 | | Chirongoui (Plage Mtihi), Mayotte | b |
| *H. platycephalus* | H4 | MY42 | | Chirongoui (crossroads), Mayotte | b |
| *H. platycephalus* | H4 | MY52 | | Sada road, Mayotte | b |
| *H. platycephalus* | H4 | MY53 | | Sada road, Mayotte | b |
| *H. platycephalus* | H4 | MY78 | | Majicavo | b |
| *H. platycephalus* | H4 | .21 | | Chissioua Mtsamboro, Mayotte | a |
| *H. platycephalus* | H4 | MH9 | | Fomboni, urban, Moheli | b |
| *H. platycephalus* | H4 | MH15 | HM192612 | Fomboni, urban, Moheli | This study |
| *H. platycephalus* | H4 | MH31 | | Fomboni, urban, Moheli | b |
| *H. platycephalus* | H4 | MH32 | | Fomboni, urban, Moheli | b |
| *H. platycephalus* | H4 | GC4 | | Moroni, urban, Grand Comore | b |
| *H. platycephalus* | H4 | GC26 | | Hantoindzi (Chezani), Grand Comore | b |
| *H. platycephalus* | H4 | GC35 | | Mouadzazi, Grand Comore | b |
| *H. platycephalus* | H4 | GC43 | | Ifoundihé Chambouani, Grand Comore | b |
| *H. platycephalus* | H4 | GC45 | | Foumbouni, Grand Comore | b |
| *H. platycephalus* | H4 | GC46 | | Foumbouni, Grand Comore | b |
| *H. platycephalus* | H4 | GC52 | | Konbani, Grand Comore | b |
| *H. platycephalus* | H4 | GC57 | | Mvouni, Grand Comore | b |
| *H. platycephalus* | H4 | GC58 | | Mvouni, Grand Comore | b |
| *H. platycephalus* | H4 | GC67 | | Hantoindzi (Chezani), Grand Comore | b |
| *H. platycephalus* | H4 | GC68 | | Hantoindzi (Chezani), Grand Comore | b |
| *H. platycephalus* | H4 | Z3 | HM192613 | Stone Town, Zanzibar Island, Tanzania | This study |
| *H. platycephalus* | H4 | Z4 | HM192614 | Stone Town, Zanzibar Island, Tanzania | This study |
| *H. platycephalus* | H4 | Z13 | HM192615 | Stone Town, Zanzibar Island, Tanzania | This study |
| *H. platycephalus* | H4 | Z21 | HM192616 | Dungo, Zanzibar Island, Tanzania | This study |
| *H. platycephalus* | H4 | Z22 | HM192617 | Juzani, Zanzibar Island, Tanzania | This study |
| *H. platycephalus* | H4 | Z24 | HM192618 | Mahonda, Zanzibar Island, Tanzania | This study |
| *H. platycephalus* | H4 | Z44 | HM192619 | Mena Bay, Zanzibar Island, Tanzania | This study |
| *H. platycephalus* | H4 | PB7 | HM192620 | Mkoani, Pemba Island, Tanzania | This study |
| *H. platycephalus* | H4 | PB11 | HM192621 | Chake, Pemba Island, Tanzania | This study |
| *H. platycephalus* | H4 | PB30 | HM192622 | Airport, Pemba Island, Tanzania | This study |
| *H. platycephalus* | H4 | TZ13 | HM192623 | Dar Es Salaam, Tanzania | This study |
| *H. platycephalus* | H4 | TZ14 | HM192624 | Dar Es Salaam, Tanzania | This study |
| *H. platycephalus* | H5 | Z25 | HM192625 | Upenja, Zanzibar Island, Tanzania | This study |
| *H. platycephalus* | H6 | .18 | | Zambezia, Mozambique | a |
| *H. platycephalus* | H7 | .15 | | Nosy Be, Madagascar | a |
| *H. platycephalus* | H7 | GC34 | | Mouadzazi, Grand Comore | b |
| *H. platycephalus* | H7 | AJ1 | | Moutsamoudu, urban, Anjouan | b |
| *H. platycephalus* | H8 | TZ22 | HM192626 | Tanga, Tanzania | This study |
| *H. platycephalus* | H9 | TZ40 | HM192627 | Lushoto, Tanzania | This study |
| *H. platycephalus* | H10 | TZ39 | HM192628 | Lushoto, Tanzania | This study |
| *H. platycephalus* | H10 | TZ41 | HM192629 | Lushoto, Tanzania | This study |
| *H. platycephalus* | H11 | TZ44 | HM192630 | Soni, Tanzania | This study |
| *H. platycephalus* | H12 | TZ45 | HM192631 | Soni, Tanzania | This study |
| *H. platycephalus* | H13 | Z42 | HM192632 | Kiwengwa, Zanzibar Island, Tanzania | This study |
| *H. platycephalus* | H14 | Z30 | HM192633 | Kiwengwa, Zanzibar Island, Tanzania | This study |
| *H. platycephalus* | H14 | Z39 | HM192634 | Mkokotoni, Zanzibar Island, Tanzania | This study |
| *H. platycephalus* | H15 | PB2 | HM192635 | Mkoani, Pemba Island, Tanzania | This study |
| *H. platycephalus* | H16 | PB21 | HM192636 | Wete, Pemba Island, Tanzania | This study |
| *H. platycephalus* | H16 | PB24 | HM192637 | Konde, Pemba Island, Tanzania | This study |
| *H. platycephalus* | H17 | PB3 | HM192638 | Mkoani, Pemba Island, Tanzania | This study |
| *H. platycephalus* | H18 | 7 | HM192639 | Kajiado District, Kenya | This study |
| *H. platycephalus* | H18 | 8 | HM192640 | Kajiado District, Kenya | This study |
| *H. platycephalus* | H18 | 9 | HM192641 | Kajiado District, Kenya | This study |
| | | | | | |
| *H. frenatus* | H1 | MA13 | HM192642 | Beau Vallon, Mahé, Seychelles | This study |
| *H. frenatus* | H1 | 38722 | HM192643 | Mont Fleuri, Victoria, Mahé, Seychelles | This study |
| *H. frenatus* | H2 | .32-.36 | AY517561 | Mauritius/Rodrigues, Mascarenes | a |
| *H. frenatus* | H2 | 2MA21 | HM192644 | Anse Etoile, Mahé, Seychelles | This study |
| *H. frenatus* | H2 | GmReu2 | HM192645 | Reunion, Mascarenes | This study |
| *H. frenatus* | H3 | MA11 | HM192646 | Beau Vallon, Mahé, Seychelles | This study |
| *H. frenatus* | H3 | MA12 | HM192647 | Beau Vallon, Mahé, Seychelles | This study |
| *H. frenatus* | H3 | MA15 | HM192648 | Beau Vallon, Mahé, Seychelles | This study |
| *H. frenatus* | H3 | 3MA16 | HM192649 | Anse a la Mouche, Mahé, Seychelles | This study |

| | | | | | |
|---|---|---|---|---|---|
| *H. frenatus* | H3 | 3MA38 | HM192650 | Baie Lazare, Mahé, Seychelles | This study |
| *H. frenatus* | H4 | 3MA40 | HM192651 | Baie Lazare, Mahé, Seychelles | This study |
| *H. frenatus* | H4 | 3MA91 | HM192652 | Victoria, Mahé, Seychelles | This study |
| *H. frenatus* | H4 | 3MA100 | HM192653 | Beau Vallon, Mahé, Seychelles | This study |
| *H. frenatus* | H5 | HfBird | HM192654 | Bird Island, Seychelles | This study |
| *H. frenatus* | H6 | PV1 | HM192655 | Poivre, Amirantes, Seychelles | This study |
| *H. frenatus* | H6 | PV2 | HM192656 | Poivre, Amirantes, Seychelles | This study |
| *H. frenatus* | H6 | PV4 | HM192657 | Poivre, Amirantes, Seychelles | This study |
| *H. frenatus* | H6 | PV5 | HM192658 | Poivre, Amirantes, Seychelles | This study |
| *H. frenatus* | H6 | PV6 | HM192659 | Poivre, Amirantes, Seychelles | This study |
| *H. frenatus* | H6 | PV8 | HM192660 | Poivre, Amirantes, Seychelles | This study |
| *H. frenatus* | H6 | DR1 | HM192661 | Descroches, Amirantes, Seychelles | This study |
| *H. frenatus* | H6 | DR2 | HM192662 | Descroches, Amirantes, Seychelles | This study |
| *H. frenatus* | H6 | DR3 | HM192663 | Descroches, Amirantes, Seychelles | This study |
| *H. frenatus* | H6 | DR4 | HM192664 | Descroches, Amirantes, Seychelles | This study |
| *H. frenatus* | H6 | DR5 | HM192665 | Descroches, Amirantes, Seychelles | This study |
| *H. frenatus* | H6 | DR7 | HM192666 | Descroches, Amirantes, Seychelles | This study |
| *H. frenatus* | H6 | DR8 | HM192667 | Descroches, Amirantes, Seychelles | This study |
| *H. frenatus* | H6 | DR10 | HM192668 | Descroches, Amirantes, Seychelles | This study |
| *H. frenatus* | H6 | HBDER1 | HM192669 | Descroches, Amirantes, Seychelles | This study |
| *H. frenatus* | H6 | HFDER1 | HM192670 | Descroches, Amirantes, Seychelles | This study |
| *H. frenatus* | H6 | HFDER2 | HM192671 | Descroches, Amirantes, Seychelles | This study |
| *H. frenatus* | H6 | HFDER3 | HM192672 | Descroches, Amirantes, Seychelles | This study |
| *H. frenatus* | H6 | HFDER4 | HM192673 | Descroches, Amirantes, Seychelles | This study |
| *H. frenatus* | H7 | DR6 | HM192674 | Descroches, Amirantes, Seychelles | This study |
| *H. frenatus* | H8 | MY26 | | Boueni (road to), Mayotte | b |
| *H. frenatus* | H8 | MY28 | | Boueni (road to), Mayotte | b |
| *H. frenatus* | H8 | MH28 | | Fomboni, urban, Moheli | b |
| *H. frenatus* | H8 | MH1 | HM192675 | Fomboni, Moheli | This study |
| *H. frenatus* | H8 | MH3 | HM192676 | Fomboni, Moheli | This study |
| *H. frenatus* | H8 | GC1 | HM192677 | Moroni (urban), Grand Comoro | This study |
| *H. frenatus* | H8 | .44 | | Ankarafantsika, Madagascar | a |
| *H. frenatus* | H9 | .43 | | Moroni, Grand Comoro | a |
| *H. frenatus* | H10 | .39 | | Montagne des Francais, Madagascar | a |
| *H. frenatus* | H10 | GC29 | HM192678 | Hantoindzi (Chezani) | This study |
| *H. frenatus* | H11 | .41 | | La Saline des Baines, Reúnion, Mascarenes | a |
| *H. frenatus* | H12 | .38 | | Port Blair, Andaman Islands | a |
| *H. frenatus* | H13 | .40 | | Negombo, Sri Lanka | a |
| *H. frenatus* | H14 | CAS885 | HM192679 | Myanmar | This study |
| *H. frenatus* | | CAS221 | HM192680 | Myanmar | This study |

## References


Boumans, L., Vieites, D.R., Glaw, F. & Vences, M. (2007). Geographical patterns of deep mitochondrial differentiation in widespread malagasy reptiles. *Molecular Phylogenetics and Evolution* 45, 822–839.

Carretero, M.A., Harris, D.J. & Rocha, S. (2005) Recent observations of reptiles in the Comoro islands (Western Indian Ocean). Herpetological Bulletin 91, 19-28.

Rocha, S., Carretero, M.A. & Harris, D.J. (2005). Diversity and phylogenetic relationships of *Hemidactylus* geckos from the Comoro islands. *Molecular Phylogenetics and Evolution* 35, 292–299.

Rocha, S., Harris, D.J., Perera, A., Silva, A., Vasconcelos, R. & Carretero, M.A. (2009) Recent data on the distribution of lizards and snakes of the Seychelles. Herpetological Bulletin 11, 20-32.

Vences, M., Stefan, W., David, R.V., William, R.B., Frank, G. & Axel, M. (2004). Natural colonization or introduction? Phylogeographical relationships and morphological differentiation of house geckos (*Hemidactylus*) from Madagascar. *Biological Journal of the Linnean Society* 83, 115–130.

Amphibia-Reptilia 31 (2010): 375-385

# Genetic diversity and phylogenetic relationships of *Mabuya* spp. (Squamata: Scincidae) from western Indian Ocean islands

Sara Rocha[1,2,3,*], Miguel A. Carretero[1,2], D. James Harris[1,2]

**Abstract.** Western Indian Ocean islands (excluding Madagascar) are inhabited by at least five species of the lizard genus *Mabuya*, for which diversity and inter-island phylogenetic relationships are still unclear, particularly concerning island populations of *M. maculilabris*, *M. comorensis* and *M. striata*. With a comprehensive sampling across the Comoros archipelago and the islands of Zanzibar and Pemba (Tanzania) we use molecular data and previously published sequences of these species to describe genetic variation across their insular range and explore the possible distinction of insular populations of the different species, contributing to the clarification of their status and knowledge of their colonization patterns. We describe patterns of cryptic diversity within *M. comorensis*, revealing a West to East colonization of the Comoros archipelago and clarify the status of Malagasy populations of this species. Cryptic variation is also observed within *M. maculilabris* and *M. striata*, and the status of several continental and island populations discussed. *M. sechellensis* is shown to be paraphyletic relative to *M. wrightii*. Available data for all Afro-Malagasy *Mabuya* reveals additional possible instances of paraphyly within some species but does not allow for further resolution of basal relationships within this group, pointing to rapid and simultaneous divergences within this group and the need for multiple independent markers to recover the history of this group.

*Keywords*: Africa, Comoros, cryptic diversity, island colonisation, *Mabuya*, Madagascar, Seychelles, *Trachylepis*, Western Indian Ocean islands.

## Introduction

Five species of the genus *Mabuya* (Squamata: Scincidae) are known to occur across the western Indian Ocean archipelagos of the Comoros, the Seychelles, Zanzibar and Pemba (following Spawls et al., 2002; Carretero, Rocha and Harris, 2005; Gerlach, 2007) with several others inhabiting exclusively the island of Madagascar (Glaw and Vences, 2004). It has been hypothesised that the Malagasy species have multiple origins, and molecular data does not contradict this (Mausfeld et al., 2000; but see Whiting et al., 2006). However, little is known concerning the variation and inter-island relationships within the Comoran species *M. comorensis*, on the relationship of Comoran populations of *M. striata* with its continental conspecifics or on the Zanzibar and Pemba populations of both *M. striata* and *M. maculilabris*. The Seychelles archipelago is also inhabited by two endemic species, *M. wrightii* and *M. sechellensis*, that are known to belong to the Afro-Malagasy clade of *Mabuya* (Carranza et al., 2001; Jesus, Harris and Brehm, 2005), now usually referred to as *Trachylepis* (although see Jesus, Harris and Brehm, 2005 and Whiting et al., 2006 for arguments against the use of this nomenclature) but their precise phylogenetic relationships are not known.

*Mabuya maculilabris* has an impressive distribution encompassing Cameroon and the Gulf of Guinea islands of S. Tomé and Principe in West Africa, to Mozambique, Tanzania and the offshore islands of Mozambique, Europa, Zanzibar and Pemba in East Africa (Biodiversity occurrence data accessed through GBIF Data Portal, www.gbif.net, 2009-08-12). Possible intraspecific variation has long been recognized within this species, and led to the definition of the "*Mabuya maculilabris*-group" (sensu

1 - CIBIO-UP, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, 4485-661 Vairão, Portugal
2 - Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, FC4, 4169-007, Porto, Portugal
3 - Departamento de Bioquímica, Genética e Inmunología, Facultad de Biología, Universidad de Vigo, Vigo 36310, Spain
*Corresponding author; e-mail: sara.rocha@mail.icav.up.pt

© Koninklijke Brill NV, Leiden, 2010. Also available online - www.brill.nl/amre

Broadley, 1974). Although several taxa were assigned to this group its composition remained unstable (see Mausfeld-Lafdhiya et al., 2004). Mausfeld-Lafdhiya et al. (2004) used molecular data to explore genetic variation within this group and reported two significantly distinct clades defined by Western and Eastern African specimens, respectively, but the use of a very reduced dataset (a few samples from Tanzania and Cameroon) inhibited the clarification of the status of the several described species and subspecies, mainly of most island populations such as *M. maculilabris casuarinae* (from Mozambique island); *M.* ssp. *infralineata* (Boettger, 1913; Broadley, 1974; Brygoo, 1981) (from Europa island) and *M. m. albotaeniata* (from Pemba island, Tanzania). By comparing their sequences from Tanzania with one sequence from Carranza et al., (2001), from a specimen from Mozambique, these authors further hypothesized on another cryptic species within this group, but further exploration was not performed. Also Jesus, Harris and Brehm (2005) argued that *M. maculilabris* is most probably a species complex, as at least five lineages could be identified using a 12s rRNA gene dataset. However, again, sampling was limited, and only three east African published sequences were used (*M. comorensis* from Madagascar, *M. maculilabris casuarinae* and *M. maculilabris maculilabris* from Mozambique).

*Mabuya comorensis* also has an unstable taxonomic history. It is mostly confined to island populations, from the Comoros archipelago, but some specimens from Tanzania and Kenya were described as *M. comorensis* and *M. maculilabris comorensis* (Loveridge, 1929), indicating an unclear distinction between *M. maculilabris* and *M. comorensis*, and a close relationship, recently confirmed by molecular data (Mausfeld-Lafdhiya et al., 2004). Variation has also been recognised within *M. comorensis*, with Peters (1854) describing *Euprepes comorensis* from Anjouan, Comoros, and naming a new species from Grand Comoro *E. angasijanus* (Peters, 1882), although it was considered a variant of *M. comorensis*. To date, relationships of specimens from the Comoros were never investigated using molecular data. Used in earlier phylogenetic analyses was instead an individual from Nosy Tanikely, a small offshore island in Northwest Madagascar, where this species is known to occur. The relationship between this and the Comoran forms is also of interest.

*Mabuya striata* is another species of the Afro-Malagasy clade with an impressively wide distribution, from Congo and Namibia in West Africa to Tanzania, Kenya, and Mozambique in East Africa (Biodiversity occurrence data accessed through GBIF Data Portal, www.gbif.net, 2009-08-12) and with several island populations (Zanzibar, Pemba, Anjouan – Comoros) whose status and possible distinction from continental forms was also never assessed using molecular data.

We sampled across the archipelago of the Comoros (*Mabuya comorensis* and *M. striata*) and the islands of Zanzibar and Pemba (*M. striata* and *M. maculilabris*) and also from the Tanzanian coast (*M. maculilabris*) and analysed these samples together with published sequences of *M. maculilabris* from the West African islands of the Gulf of Guinea and from Mozambique (East Africa) and of continental *M. striata*. A sample from *Mabuya* spp. from Europa Island was also obtained. We thus aimed to describe genetic variation across the (eastern African) insular range of these species and explore the possible distinction of insular populations of the different species, contributing to the clarification of their status and the knowledge of their colonization patterns. Furthermore, we included both Seychelles' species in a comprehensive phylogenetic analysis together with all available sequences of the "Afro-Malagasy" clade to re-examine their phylogenetic relationships.

## Material and methods

Specimens used in this study and respective locations can be found in table 1. Sampling was carried out across the

**Table 1.** Specimens used in this study, respective locations (detailed GPS coordinates for Comoran samples can be found in Carretero et al., 2005) and Genbank accession nos.

| Species | Individual | Location | Genbank accession nos. | |
|---|---|---|---|---|
| | | | Cyt-*b* | 16s |
| *M. comorensis* | MY1 | Dzaozi (airport road), Mayotte, Comoros | HM192681 | HM192751 |
| *M. comorensis* | MY12 | Chirongui, Mayotte, Comoros | HM192682 | HM192750 |
| *M. comorensis* | MY20 | Kani-Kéli, Mayotte, Comoros | HM192683 | |
| *M. comorensis* | MY25 | M'zouazia, Mayotte, Comoros | HM192684 | |
| *M. comorensis* | MY37 | Bouéni (road to), Mayotte, Comoros | HM192685 | HM192749 |
| *M. comorensis* | MY49 | Ouangani, Mayotte, Comoros | HM192686 | |
| *M. comorensis* | MY51 | Mangajou, Mayotte, Comoros | HM192687 | HM192748 |
| *M. comorensis* | MY55 | Sada, Mayotte, Comoros | HM192688 | |
| *M. comorensis* | MY59 | Bambo Est, Mayotte, Comoros | HM192689 | |
| *M. comorensis* | MY61 | Bandrélé, Mayotte, Comoros | HM192690 | |
| *M. comorensis* | MY81 | Trévani, Mayotte, Comoros | HM192691 | |
| *M. comorensis* | AJ3 | Moutsamoudou, Anjouan, Comoros | HM192692 | HM192752 |
| *M. comorensis* | AJ7 | Bazimini, Anjouan, Comoros | HM192693 | |
| *M. comorensis* | AJ14 | Mboúeladoungou, Anjouan, Comoros | HM192694 | |
| *M. comorensis* | AJ17 | Adda-Douéni, Anjouan, Comoros | HM192695 | |
| *M. comorensis* | AJ18 | Foret de Moya, Anjouan, Comoros | HM192696 | HM192753 |
| *M. comorensis* | AJ22 | Pomoni, Anjouan, Comoros | HM192697 | |
| *M. comorensis* | AJ25 | Dindi, Anjouan, Comoros | HM192698 | |
| *M. comorensis* | AJ26 | Mchakojou, Anjouan, Comoros | HM192699 | |
| *M. comorensis* | AJ29 | Haiko, Anjouan, Comoros | HM192700 | |
| *M. comorensis* | AJ33 | Koni-Djodjo, Anjouan, Comoros | HM192701 | |
| *M. comorensis* | MH7 | Fomboni, Moheli, Comoros | HM192702 | HM192758 |
| *M. comorensis* | MH11 | Badjo, Moheli, Comoros | HM192703 | HM192756 |
| *M. comorensis* | MH20 | Gnombéni, Moheli, Comoros | HM192704 | |
| *M. comorensis* | MH21 | Ouanani, Moheli, Comoros | HM192705 | |
| *M. comorensis* | MH23 | Ouhoni, Moheli, Comoros | HM192706 | HM192757 |
| *M. comorensis* | MH25 | Sambia, Moheli, Comoros | HM192707 | |
| *M. comorensis* | MH35 | Mbouerani,Moheli, Comoros | HM192708 | |
| *M. comorensis* | MH37 | Mbatsé, Moheli, Comoros | HM192709 | |
| *M. comorensis* | GC5 | Moroni, Grand Comoro, Comoros | HM192712 | HM192754 |
| *M. comorensis* | GC16 | N of Moroni airport, Grand Comoro, Comoros | HM192713 | |
| *M. comorensis* | GC18 | Mouadja, Grand Comoro, Comoros | HM192714 | |
| *M. comorensis* | GC36 | Mouadzaza, Grand Comoro, Comoros | HM192715 | |
| *M. comorensis* | GC42 | Mbambani, Grand Comoro, Comoros | HM192716 | HM192755 |
| *M. comorensis* | GC47 | Bandanadji, Grand Comoro, Comoros | HM192717 | |
| *M. comorensis* | E | Moroni, Grand Comoro, Comoros | HM192710 | |
| *M. comorensis* | G | Itzandra, Grand Comoro, Comoros | HM192711 | |
| *M. comorensis* | | Nosy Tanikely, Madagascar | | AY153565 |
| *M. maculilabris albotaeniata* | PB4 | Mkoani, Pemba Island, Tanzania | HM192718 | |
| *M. maculilabris albotaeniata* | PB8 | Chake, Pemba Island, Tanzania | HM192719 | |
| *M. maculilabris albotaeniata* | PB10 | East Chake, Pemba Island, Tanzania | HM192720 | |
| *M. maculilabris albotaeniata* | PB12 | East Chake, Pemba Island, Tanzania | HM192721 | |
| *M. maculilabris albotaeniata* | PB18 | Jondeni, Pemba Island, Tanzania | HM192722 | |
| *M. maculilabris albotaeniata* | PB20 | Wete, Pemba Island, Tanzania | HM192723 | |
| *M. maculilabris albotaeniata* | PB22 | Wete, Pemba Island, Tanzania | HM192724 | |
| *M. maculilabris albotaeniata* | PB23 | Konde, Pemba Island, Tanzania | HM192725 | |
| *M. maculilabris albotaeniata* | PB27 | Ngezi, Pemba Island, Tanzania | HM192726 | |
| *M. maculilabris albotaeniata* | PB29 | Airport, Pemba Island, Tanzania | HM192727 | |
| *M. maculilabris maculilabris* | | Mozambique | AF280269 | |
| *M. maculilabris casuarinae* | | | AF280270 | AY151474 |
| *M. (maculilabris) infralineata* | Mab4Eur | Europa Island | HM192728 | |
| *M. maculilabris maculilabris* | | East Africa | | AY070356 |

**Table 1.** (Continued).

| Species | Individual | Location | Genbank accession nos. | |
|---|---|---|---|---|
| | | | Cyt-*b* | 16s |
| *M. maculilabris maculilabris* | TZ10 | Dar Es Salaam,Tanzania | HM192729 | |
| *M. maculilabris maculilabris* | TZ11 | Dar Es Salaam, Tanzania | HM192730 | |
| *M. maculilabris maculilabris* | TZ16 | Tanga, Tanzania | HM192731 | |
| *M. maculilabris maculilabris* | TZ36 | Lushoto, Tanzania | HM192732 | |
| *M. maculilabris maculilabris* | Z20 | Zanzibar Island, Tanzania | HM192733 | |
| *M. maculilabris maculilabris* | Z36 | Zanzibar Island, Tanzania | HM192734 | |
| *M. maculilabris maculilabris* | | S. Tomé Island, West Africa | AY997770 | |
| *M. maculilabris maculilabris* | | Rolas, S. Tomé Is., West Africa | AY997769 | |
| *M. maculilabris maculilabris* | 599 | Terra Velha, Principe | HM192735 | |
| *M. striata* | PB1 | Mkoani, Pemba Island, Tanzania | HM192736 | |
| *M. striata* | Z18 | Cwaka bay, Zanzibar Island, Tanzania | HM192737 | |
| *M. striata* | Z29 | Kiwengwa, Zanzibar Island, Tanzania | HM192738 | |
| *M. striata* | TZ5 | Dar Es Salaam, Tanzania | HM192739 | |
| *M. striata* | TZ9 | Dar Es Salaam, Tanzania | HM192741 | |
| *M. striata* | AJ28 | Haiko, Anjouan, Comoros | HM192740 | |
| *M. striata* | | Namibia | AY217812 | |
| *M. margaritifera* | | Probably Tanzania (pet trade) | AF153591 | |
| *M. margaritifera* | | Mozambique | AF280268 | |
| *M. hoeschi* | | Namibia | AY217809 | |
| *M. varia* | TZ25 | Lushoto, Tanzania | HM192742 | |
| *M. varia* | TZ33 | Lushoto, Tanzania | HM192743 | |
| *M. gravenhorstii* | | Madagascar | DQ239123 | |
| *M. madagascariensis* | | Madagascar | DQ239124 | |
| *M. elegans* | | Madagascar | DQ239122 | |
| *M. perrotetii* | | Ghana | DQ239146 | |
| *M. capensis* | | South Africa | DQ239178 | |
| *M. occidentalis* | | South Africa | DQ239182 | |
| *M. quinquetaeniata* | | unknown | DQ239183 | |
| *M. quinquetaeniata* | | unknown | DQ239115 | |
| *M. quinquetaeniata* | | Ghana | DQ239148 | |
| *M. quinquetaeniata* | | unknown | EU443143 | |
| *M. quinquetaeniata* | | unknown | AF153593 | |
| *M. socotrana* | | Socotra Island | AF280273 | |
| *M. acutilabris* | | Namibia | DQ239181 | |
| *M. sechellensis* | MA2 | Mahé Island, Seychelles | HM192744 | |
| *M. sechellensis* | 27CUR | Curieuse Island, Seychelles | HM192745 | |
| *M. wrightii* | 15SP | Saint Pierre Island, Seychelles | HM192746 | |
| *M. boettgeri* | | Madagascar | DQ239120 | |
| *M. vato* | | Madagascar | DQ239125 | |
| *M. aureopunctata* | | Madagascar | DQ239119 | |
| *M. dumasi* | | Madagascar | DQ239121 | |
| *M. homalocephala* | | South Africa | DQ239116 | |
| *M. atlantica* | | Fernando de Noronha, Brazil | DQ239155 | |
| *M. affinis* | | unknown | AF153587 | |
| *M. ozorri* | 625 | Annobon Island, West Africa | HM192747 | |
| *M. multifasciata* | | Lao PDR, Asia | DQ239138 | |

western Indian Ocean islands of Mayotte, Anjouan, Moheli and Grand Comoro (Comoros), Europa, Zanzibar and Pemba and the Seychelles, and a few locations on the Tanzanian coast (fig. 1). From the Genbank database (17-07-09) all the available sequences of *Mabuya* spp. currently attributed to the Afro-Malagasy clade (sensu Mausfeld et al., 2002; Bauer, 2003) except *M. vittata* (see Jesus, Harris and Brehm, 2005 for a justification) were downloaded, amounting to 20 species, out of the ca. 70 that supposedly belong to this clade (assessed through the TIGR reptile database, Uetz, 2009, under *Trachylepis*).

From the tissues collected, genomic DNA from each individual was extracted using a standard high-salt (Sambrook, Fritsh and Maniatis, 1989) or phenol-chloroform

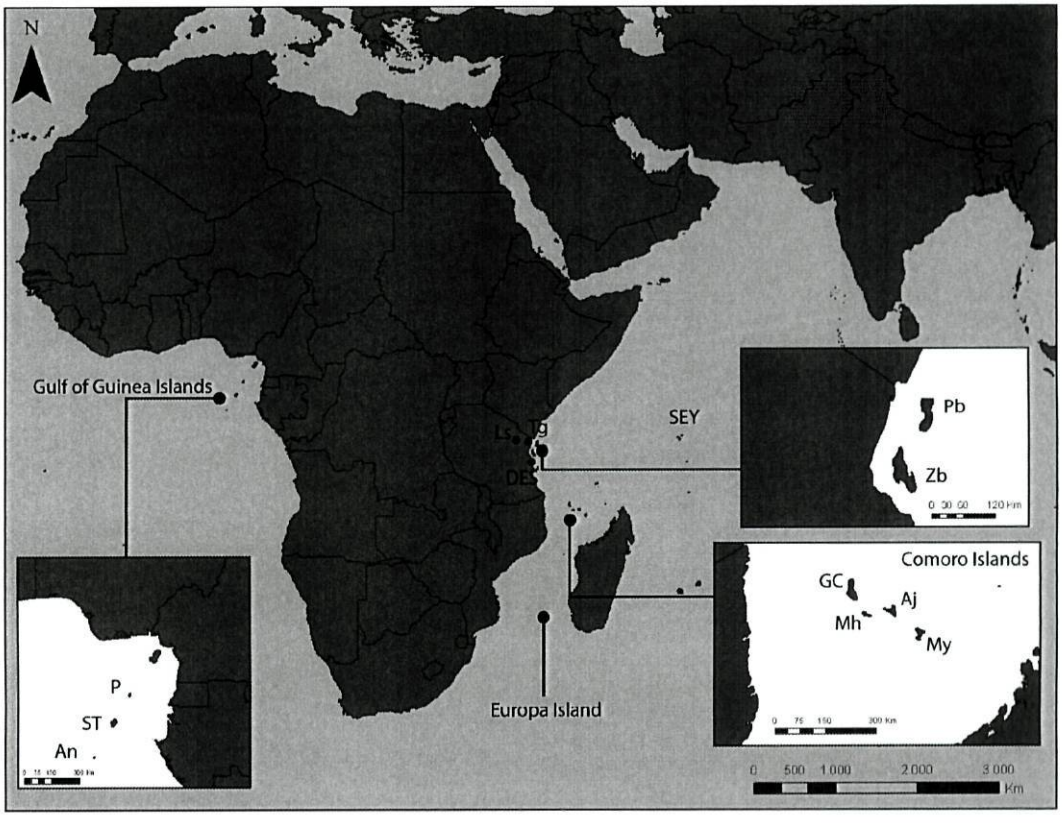



**Figure 1.** Map showing main sampling regions for *M. maculilabris* and *M. comorensis*. P: Príncipe; ST: São Tomé; An: Annobon (Gulf of Guinea Islands); Tg: Tanga; Ls: Lushoto; DES: Dar Es Salamm; Zb: Zanzibar; Pb: Pemba (Tanzania); GC: Grand Comoro; Mh: Moheli; Aj: Anjouan; My: Mayotte (Comoros); SEY: Seychelles. See table 1 for detailed locations.

(Taggart et al., 1992) protocol. A fragment with approximately 700 bp of the cytochrome *b* (Cyt-*b*) mitochondrial gene was amplified via PCR for most individuals with forward primer CB1 (5′-CCATCCAACATCTCAGCATGAT GAAA-3′), modified from the original CBL14841 from Kocher et al. (1989), and reverse CB3H (Palumbi et al., 1991). For some individuals for which amplification was difficult, forward primers designed specifically for *M. sechellensis* and *M. wrightii* were used (CBMabF: 5′-TCTCAGCATGATGAAAYTTTGG-3′) and CBMabPLF3 (5′-AGCATGATGAAATTTTGGTTCC-3′) and successfully amplified an internal region of approximately 650 bp. PCR reaction conditions are given in Rocha et al. (2009), and sequences were determined by a commercial facility (Macrogen, Seoul, Korea). In the same way, 16s rRNA gene sequences were obtained for a subset of *M. comorensis* individuals (2 to 4 individuals from each island), using primers 16sA-L and 16sB-H (Palumbi et al., 1991) and standard conditions. Sequences have been deposited in GenBank under accession nos. HM192681-HM192755.

Cytochrome-*b* sequences obtained were aligned using BioEdit (Hall, 1999) with remaining Afro-Malagasy *Mabuya spp.* from Genbank (alignment was unambiguous) and with *M. multifasciata*, which was used as outgroup, as it is clearly phylogenetically distant from Afro-Malagasy species, belonging instead to the Asian clade (Mausfeld et al., 2002). Only a subset of *M. comorensis* samples (2-3 individuals from each island) was used for the phylogenetic analysis and identical haplotypes from any species were removed, resulting in a 58 taxa (and 623 bp) dataset. The appropriate models of evolution were determined through jModeltest (Guindon and Gascuel, 2003; Posada, 2008), both for the complete dataset and for the 1st, 2nd and 3rd codon positions independently, using the AICc criteria (following Posada and Buckley, 2004), and were used to run both Maximum-Likelihood (ML) and Bayesian Analysis using respectively PhyML 3.0 (Guindon and Gascuel, 2003) and MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001). Support for ML analysis was assessed through 1000 bootstrap replicates also performed in PhyML. Bayesian analyses (BI) were run for 11 million generations and stationarity and convergence of independent runs were checked using AWTY (Nylander et al., 2008). Analyses were performed both considering a single model for the whole dataset or an independent model for each codon position (partitioned Bayesian analysis, sensu Brandley, Schmitz and Reeder, 2005) and Bayes Factor (BF) used to identify the most appropriate partitioning strategy. Substitution model parameters were always unlinked across partitions, while topology and branch lengths were linked across partitions.

Maximum-parsimony (MP) analysis was carried out through a heuristic search (10 replicates) using TBR branch-swapping algorithm and support for nodes estimated by 1000 bootstrap replicates.

Within *M. comorensis*, in order to visualize the diversity and relationships between the haplotypes within each island, statistical parsimony (Templeton, Crandall and Sing, 1992), implemented in the program TCSv1.2.1 (Clement, Posada and Crandall, 2000) was used and the 95% parsimony connection limit applied. The obtained 16s rRNA gene sequences of *M. comorensis* were used for comparison with the published sequence from a Malagasy individual. For this they were aligned in MAFFT ver.6 under the G-INS-I strategy (Katoh et al., 2005) together with the published sequence of the Malagasy *M. comorensis* (Mausfeld et al., 2002) and of *M. m. casuarinae* (Carranza et al., 2001). Only Maximum-likelihood analysis was performed in this dataset, using the same methodology as above.

## Results

The final dataset for phylogenetic analysis (cyt-*b*) consisted of 58 taxa and a 623 bp alignment, from which 293 sites were variable and 274 were parsimony informative. Judging from the Bayes factor (2lnBF = 722, see Kass and Raftery, 1995) the tree obtained when allowing codon positions to have independent models was identified as the preferred inference (Bayesian) of phylogenetic relationships within this group. Maximum-likelihood and MP analysis recovered similar topologies (not shown) and support values were also largely concordant (fig. 2). Haplotype networks are represented in fig. 3.

*M. comorensis* is a monophyletic group, with specimens from Grand Comoro, Moheli and Mayotte/Anjouan defining also well-supported clades, harbouring considerable levels of diversity (figs 2 and 3). Anjouan and Mayotte populations of *M. comorensis* are not recovered as reciprocally monophyletic in any of the phylogenetic analyses, although it's clear from the haplotype network (fig. 3) that each island harbours an exclusive group of haplotypes, separated by a minimum of 6 mutational steps. The close relationship of *M. comorensis* with *M. maculilabris* is also confirmed, with *M. comorensis* making *M. maculilabris* paraphyletic. Both *M. maculilabris casuarinae* (Mozambique Island, Mozambique) and *M. comorensis* from Madagascar (Nosy Tanikely) cluster within the Moheli clade of *M. comorensis* (for *M. comorensis* from Madagascar we compared the published 16s sequence with 16s sequences of Comoran specimens – fig. 2, right). Four additional lineages within *M. maculilabris* are evident from our analysis, with some of them harbouring considerable diversity. *Mabuya m. infralineata* from Europa Island clusters with a sample from coastal Mozambique (2.4% diff.) and both define a well-supported clade together with individuals from the island of Pemba, Tanzania. Individuals from the Gulf of Guinea islands of S. Tomé and Príncipe did not formed a monophyletic group. The fourth *M. maculilabris* lineage observed comprised individuals from continental Tanzania and from the island of Zanzibar, with some variation observed between Northern (TZ16; TZ36) and southern (TZ10; TZ11) Tanzania and Zanzibar.

Species from the Seychelles Islands formed a monophyletic lineage but *M. sechellensis* revealed to be paraphyletic relative to *M. wrightii*. Their phylogenetic affinities are uncertain, as basal relationships within this whole clade, in general, are unresolved. This analysis further revealed some additional possible instances of paraphyly (involving *M. quinquetaeniata*; *M. margaritifera* and *M. striata* – fig. 2). Within *M. striata*, deep differentiation (and possible paraphyly) was found between a Western African sample (Namibia) and remaining specimens from Eastern Africa and Indian Ocean Islands (Tanzania, Zanzibar, Pemba and Anjouan). Little or no differentiation however was found within this later group (fig. 2). Contrary to most recent previous estimates (Whiting et al., 2006), Malagasy *Mabuya* spp. formed two well-supported but unrelated clades, both in ML, BI and MP analysis.

## Discussion

*Mabuya comorensis* was found to be a highly diverse clade, with each of the Comoro islands

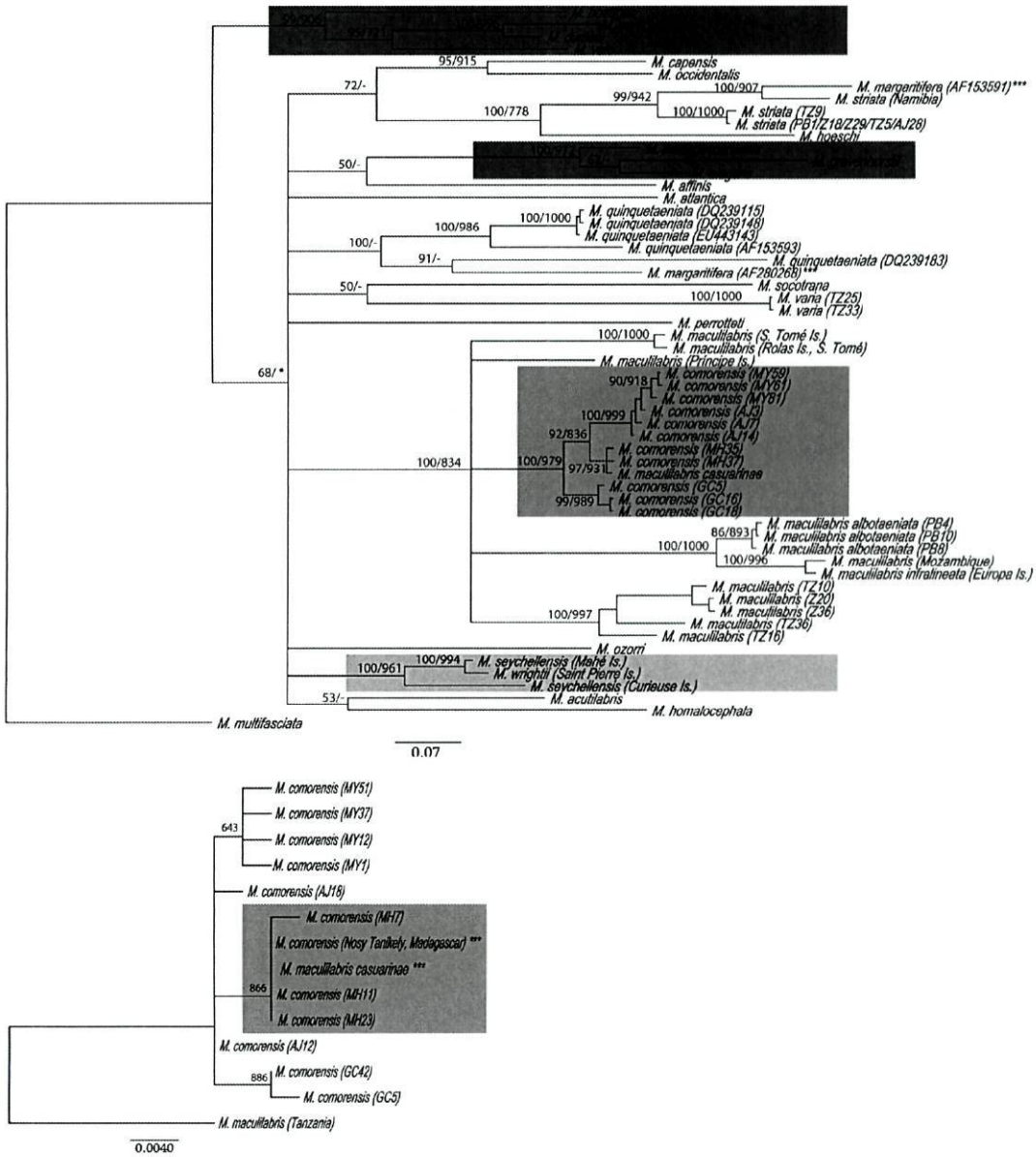



**Figure 2.** Bayesian 50% majority-rule consensus phylogenetic tree from partitioned analysis. Posterior probabilities (PP) and ML bootstrap (BS) values (PP/BS) are given above branches when higher than 0.5/500 (- represents BS values below 500 and an * signs nodes not recovered in the ML and MP analysis). MP bootstrap values above 500 are given below the branches (or in 3rd place). Relevant clades are highlighted: Malagasy groups (dark grey); *M. comorensis* and the Seychelles Islands clade (lighter greys). Individuals of *M. margaritifera* (paraphyletic) are signed with ***. On the right: ML tree of the 16s rRNA gene data for the subset of *M. comorensis* individuals. BS support values (higher than 500) are given above branches. Clade from Moheli is highlighted and individuals from Madagascar and *M. maculilabris casuarinae* signed with ***.

(Grand Comoro, Moheli, Anjouan and Mayotte) harbouring an exclusive haplotype lineage. Although further sampling is still needed to determine its putative sister group along the East African coast, the well supported tree topology for the Comoros samples clearly suggests a colonisation from West to East within the archipelago. Differentiation between lineages is not particularly high (1% – between Anjouan and Mayotte – to 7.4% between Mayotte and

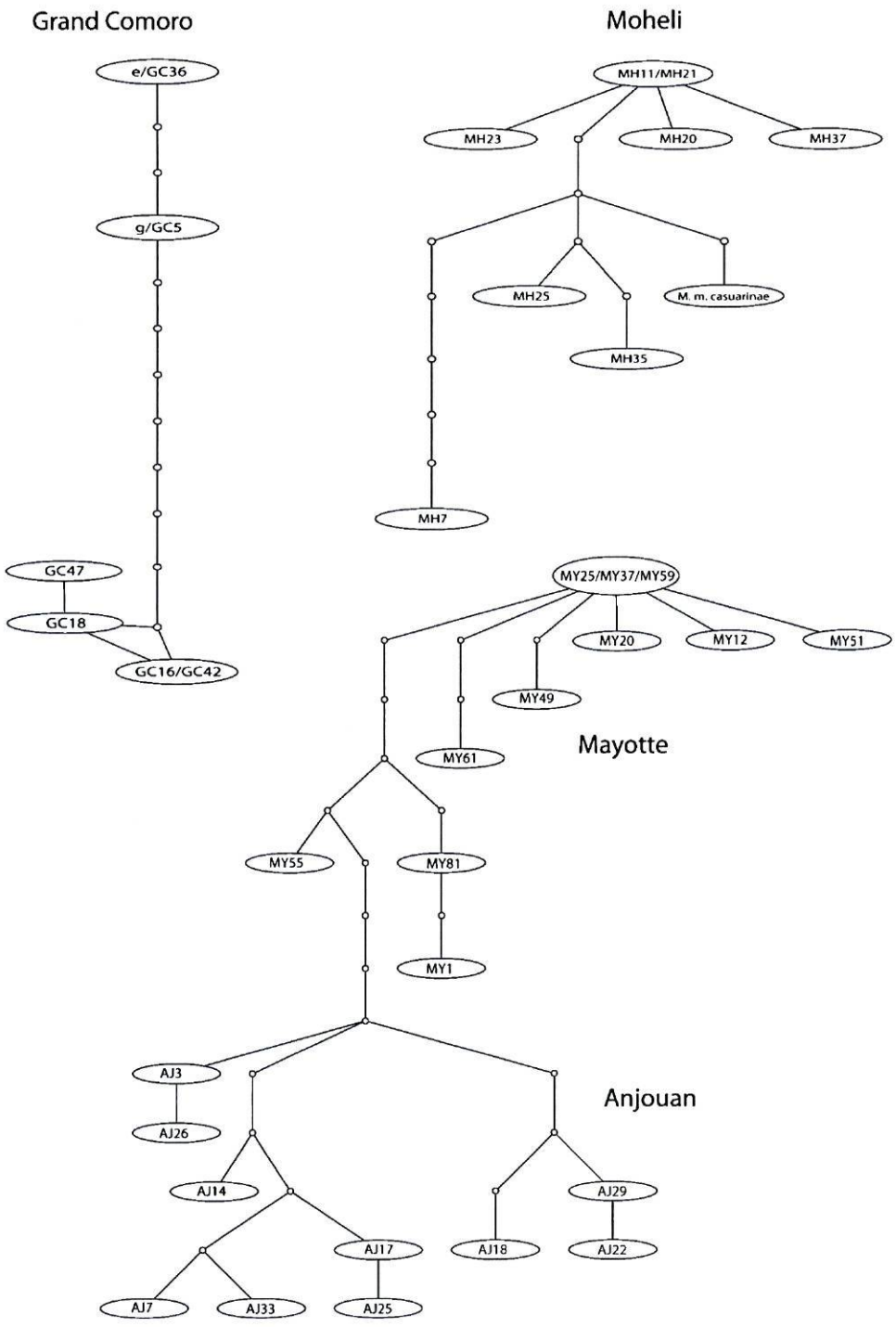



**Figure 3.** Haplotype networks representing variation within *M. comorensis*. Oval size is proportional to haplotype frequency (sample code is given within each and respective locations can be found on table 1). Each branch represents one substitution and each open circle a missing haplotype. Each group of haplotypes (3) is given as defined by the 95% parsimony connection limit.

Grand Comoro; number of base substitutions per site from net average between groups of sequences) when compared to the 13.5% average distance between species within genera reported by Harris (2002), but it is higher or at the same level of the one seen between many sister species of Cape Verde *Mabuya* for this same fragment (0.033 to 0.107, range of average net distances between species, from Carranza et al., 2001). Given their geographical isolation, it is probable that these populations do not have the opportunity to interbreed and that each mtDNA lineage defines an independent evolutionary unit. Though sampling is limited, the diversity within each *M. comorensis* lineage does not appear to be correlated with island age, as all islands seem to harbour similar levels of haplotype diversity (see haplotype networks), despite their significant age differences – Mayotte, 10-15 Myr; Anjouan, 11.5 Myr; Moheli, c. 5 Myr and Grand Comoro 0.5 Myr (Montaggioni and Nougier, 1981; Emerick and Duncan, 1982; Nougier et al., 1986). *Mabuya comorensis* from Madagascar clearly clusters within the Moheli Island clade, without differentiation relative to Moheli individuals, suggesting a very recent origin of this species in Madagascar probably due to human introduction. A similar situation occurs with *M. maculilabris casuarinae*. Broadley (1974) had already stated its similarity to *M. comorensis* when he described this subspecies (Mausfeld-Lafdhiya et al., 2004) from Casuarina Island off the coast of North Mozambique. Using molecular data, Jesus, Harris and Brehm (2005) confirmed its lack of differentiation relative to *M. comorensis* (from Madagascar). Here, using the faster-evolving mtDNA marker cytochrome-*b* and a comprehensive sampling of *M. comorensis*, this "sub-species" is clearly placed within the Moheli island clade of *M. comorensis*, and, again, supports the hypothesis of a human-aided introduction, or at least very recent dispersal. This should definitely lead to a taxonomic reclassification of this "species" in order to better reflect its phylogenetic position.

More remarkably, *M. maculilabris* harbours multiple highly divergent lineages. In addition to the already described (Mausfeld-Lafdhiya et al., 2004) eastern and western African clades and the multiple western African lineages (Jesus, Harris and Brehm, 2005), further cryptic lineages are present within East Africa. Individuals from Pemba Island, Tanzania (*M. maculilabris albotaeniata*), form a well-supported clade, closely related to *M. maculilabris infralineata* from Europa Island and a continental sample from Mozambique. Deeply divergent from these, another well-supported clade is defined by samples from Zanzibar and Tanzania, within which, furthermore, slight differentiation is observed between individuals from central (Dar Es Salaam) and northern (Tanga, Lushoto) Tanzania. Considering the high number of lineages observed and the limited range of this species sampled, this is certainly a species-complex that probably harbours yet more lineages within both continental East and West Africa. In particular, specimens assigned to the originally described *Euprepes anchietae*, *Euprepes notabilis* (Angola), *Mabuia boulangeri* (Makonde Plateau, Tanzania), as well as to *Mabuia maculilabris major* and its several varieties from D.R. Congo, Tanzania and Uganda described by Sternfeld in 1912 (see Mausfeld-Lafdhiya et al., 2004, appendix 2), were never to our knowledge included in a molecular phylogeny. The status of both western (Jesus, Harris and Brehm, 2005) and eastern (this study) insular populations is, on the contrary, now quite well known.

*Mabuya striata* demonstrated both similarities and differences from the phylogeographic patterns recovered in *M. maculilabris*. In common, there is also a deep East-West Africa divergence (in this case *M. striata* may even be paraphyletic), with samples from Tanzania and the Western Indian Ocean having a 12% *p*-distance from the individual from Namibia available on Genbank. Nevertheless, no differentiation was observed across the insular range of this species: individuals from the Co-

moros (Anjouan), Zanzibar, Pemba, and Tanzania shared the same or very closely related haplotypes, pointing to a very recent spread of this species across these Western Indian Ocean Islands.

Concerning the Seychelles species, *M. sechellensis* and *M. wrightii*, their monophyly (as a lineage) is still corroborated, but no evident close relationship to other species of this Afro-Malagasy clade was recovered. Contrary to a previous hypothesis (Peters 1882 in Gerlach, 2005), they do not appear to be closely related to *M. maculilabris*. The paraphyletic status of *M. sechellensis* is noteworthy and warrants further investigation on the intraspecific structure of both these species.

The inclusion of all available Afro-Malagasy clade sequences revealed two additional instances of paraphyly, both involving *M. margaritifera* with (1) *M. quinquetaeniata* and (2) *M. striata* that should be further investigated. They also support the (at least) two colonizations of Madagascar by this genus, with two well-supported clades defined by *M. madagascariensis*, *M. elegans* and *M. gravenhorsti* (1) and *M. boettgeri*, *M. vato*, *M. aureopunctata* and *M. dumasi* (2), this latter one recovered, in the Bayesian analysis, as basal to the entire Afro-Malagasy group (although with low PP). This result is not in agreement with the Malagasy monophyletic clade recovered by Whiting et al. (2006) using considerably more data (~5000 bp, of mitochondrial and nuclear genes), but we included, however, a higher number of African species in the analysis (17 contra 11). Considering that more than 70 species are estimated to belong to this clade, the question of the single or multiple colonization of Madagascar must still remain open pending a more comprehensive sampling effort.

The overall tree topology points to rapid and simultaneous divergences within this group and to the need of multiple independent markers to recover the order of the branching events within this apparently rapid radiation.

**Acknowledgements.** This research was partially supported by grants from Fundação para a Ciência e Tecnologia (FCT) SFRH/BD/1745/2004 (to SR), POCTI/BSE/46647/2000 and PTDC/BIA-BDE/65745/2006. We are grateful to Darren Peck for the sample from Europa Island and to José Jesus for providing unpublished sequences of *M. maculilabris* from Gulf of Guinea Islands. We further acknowledge Dr. Denis Matatiken and Wilna Accouche (at the Ministry of Environment and Natural Resources, Seychelles) and the Seychelles Bureau of Standards for the permit to obtain samples from the Seychelles.

## References

Bauer, A. (2003): On the identity of *Lacerta punctata* Linnaeus 1758, the type species of the genus *Euprepis* Wagler 1830, and the generic assignment of Afro-Malagasy skinks. Afr. J. Herpetol. **52**: 1-7.

Boettger, O. (1913): Reptilien und Amphibien von Madagaskar, den Inseln und dem Festland Ostafrikas (Sammlung Voeltzkow 1889-1895 und 1903-1905). In: Reise in Ostafrika in den Jahren 1903-1905 mit Mitteln der Hermann und Elise geb. Heckmann Wentzel-Stiftung ausgeführt von Professor Dr. Alfred Voeltzkow, Band III. Systematische Arbeiten Heft IV, p. 269-375. Voeltzkow, A., Ed., Wissenschaftliche Ergebnisse, Stuttgart.

Brandley, M.C., Schmitz, A., Reeder, T.W. (2005): Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. Syst. Biol. **54**: 373-390.

Broadley, D.J. (1974): A review of the *Mabuya striata* complex in South-Eastern Africa (Sauria: Scincidae). Occasional Papers of the National Museums and Monuments of Rhodesia **B6**: 45-79.

Brygoo, É.R. (1981): Systématique des Lézards Scincidés de la région malgache. VIII. Les *Mabuya* des îles de l'Ocean Indien occidental: Comores, Europa, Séchelles. Bulletin du Museum National d'Histoire Naturelle (Sér. 4) **3**: 911-930.

Carranza, S., Arnold, E.N., Mateo, J.A., Lopez-Jurado, L.F. (2001): Parallel gigantism and complex colonization patterns in the Cape Verde scincid lizards *Mabuya* and *Macroscincus* (Reptilia: Scincidae) revealed by mitochondrial DNA sequences. Proc. Biol. Sci. **268**: 1595-1603.

Carretero, M.A., Rocha, S., Harris, D.J. (2005): Recent observations of reptiles in the Comoro islands (Western Indian Ocean). Herp. Bull. **91**: 19-28.

Clement, M., Posada, D., Crandall, K.A. (2000): TCS: A computer program to estimate gene genealogies. Mol. Ecol. **9**: 1657-1659.

Emerick, C.M., Duncan, R.A. (1982): Age progressive volcanism in the Comores Archipelago, western Indian Ocean and implications for Somali plate tectonics. Earth Planet Sci. Lett. **60**: 415-428.

Gerlach, J. (2005): Inter-island variation and taxonomy of Seychelles *Trachylepis*. Afr. J. Herpetol. **54**: 31-42.

Gerlach, J. (2007): Terrestrial and Freshwater Vertebrates of the Seychelles Islands. Backhuys Publishers, Leiden, The Netherlands.

Glaw, F., Vences, M. (2007): A Field Guide to the Amphibians and Reptiles of Madagascar, 3rd Edition. Vences & Glaw, Köln.

Guindon, S., Gascuel, O. (2003): A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. **52**: 696-704.

Hall, T.A. (1999): Bioedit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. **41**: 95-98.

Harris, D.J. (2002): Reassessment of comparative genetic distance in reptiles from the mitochondrial cytochrome b gene. Herpetol. J. **12**: 85-86.

Huelsenbeck, J.P., Ronquist, F. (2001): MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics **17**: 754-755.

Jesus, J., Harris, D.J., Brehm, A. (2005): Phylogeography of *Mabuya maculilabris* (Reptilia) from São Tomé island (gulf of Guinea) inferred from mtDNA sequences. Mol. Phylogenet. Evol. **37**: 503-510.

Kass, R.E., Raftery, A.E. (1995): Bayes factors. J. Amer. Statist. Assoc. **90**: 773-795.

Katoh, K., Kuma, K., Miyata, T., Toh, H. (2005): Improvement in the accuracy of multiple sequence alignment program MAFFT. Genome Inform. **16**: 22-33.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C. (1989): Dynamics of mitochondrial-DNA evolution in animals – amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA **86**: 6196-6200.

Loveridge, A. (1929): East African reptiles and amphibians in the United States National Museum. Bull. United States Natl. Mus. **151**: 1-129.

Mausfeld, P., Vences, M., Schmitz, A., Veith, M. (2000): First data on the molecular phylogeography of scincid lizards of the genus *Mabuya*. Mol. Phylogenet. Evol. **17**: 11-14.

Mausfeld, P., Schmitz, A., Böhme, W., Misofa, A., Vrcibradicb, D., Rocha, C.D.F. (2002): Phylogenetic affinities of *Mabuya atlantica* Schmidt, 1945, endemic to the Atlantic Ocean archipelago of Fernando de Noronha (Brazil): Necessity of partitioning the genus *Mabuya* Fitzinger, 1826 (Scincidae: Lygosominae). Zool. Anz. **241**: 281-293.

Mausfeld-Lafdhiya, P., Schmitz, A., Ineich, I., Chirio, L. (2004): Genetic variation in two African *Euprepis* species (Reptilia, Scincidae), based on maximum-likelihood and Bayesian analyses: Taxonomic and biogeographic conclusions. Bonner Zoologische Beiträge **52**: 159-177.

Montaggioni, L., Nougier, J. (1981): Les enclaves de roches dé tritiques dans les volcans d'Anjouan (Archipel des Comores): origine et interprétation dans le cadre de l'évolution du canal de Mozambique. Bull. Soc. Géol. France **23**: 595-601.

Nougier, J., Cantagrel, J.M., Karche, J.P. (1986): The Comores Archipelago in the western Indian Ocean: volcanology, geochronology, and geodynamic setting. J. Afr. Earth Sci. **5**: 135-145.

Nylander, J.A., Wilgenbusch, J.C., Warren, D.L., Swofford, D.L. (2008): AWTY (are we there yet?): A system for graphical exploration of mcmc convergence in Bayesian phylogenetics. Bioinformatics **24**: 581-583.

Palumbi, S.R., Martin, A., Romano, S., McMillan, W.O., Stice, L., Grabowski, G. (1991): The simple fool's guide to pcr, version 2.0. Privately published, Hawaii.

Peters, W.C.S. (1854): Diagnosen neuer Batrachier, welche zusammen mit der früher (24. Juli und 17. August) gegebenen Übersicht der Schlangen und Eidechsen mitgetheilt werden. Bericht über die zur Bekanntmachung geeigneten Verhandlungen der Königlichen-Preussischen Akademie der Wissenschaften zu Berlin **1854**: 614-628.

Peters, W.C.S. (1882): Zoologie III. Amphibien. Naturwissenschaftliche Reise nach Mossambique auf Befehl seiner Majestät des Königs Friedrich Wilhelm IV. In den Jahren 1842 bis 1848 ausgeführt von Wilhelm C.H. Peters. G. Reimer, Berlin.

Posada, D. (2008): jModeltest: Phylogenetic model averaging. Mol. Biol. Evol. **25**: 1253-1256.

Posada, D., Buckley, T.R. (2004): Model selection and model averaging in phylogenetics: Advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Syst. Biol. **53**: 793-808.

Rocha, S., Vences, M., Glaw, F., Posada, D., Harris, D.J. (2009): Multigene phylogeny of Malagasy day geckos of the genus *Phelsuma*. Mol. Phylogenet. Evol. **52**: 530-537.

Sambrook, J., Fritsh, E.F., Maniatis, T. (1989): Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, New York.

Spawls, S., Howell, K., Drewes, R., Ashe, J. (2002): A Field Guide to the Reptiles of East Africa. Academic Press, San Diego.

Sternfeld, R. (1912): Reptilia. In: Ergebnisse der Deutschen Zentral-Afrika-Expedition 1907-1908. Klindkhart & Biermann, Leipzig.

Taggart, J.B., Hynes, R.A., Prodoul, P.A., Ferguson, A. (1992): A simplified protocol for routine total DNA isolation from salmonid fishes. J. Fish Biol. **40**: 963-965.

Templeton, A.R., Crandall, K.A., Sing, C.F. (1992): A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics **132**: 619-633.

Uetz, P. (2009): The reptile database. Available at http://www.reptile-database.org/. Accessed in August 2009.

Whiting, A.S., Sites, J.W.S., Pellegrino, K.C.M., Rodrigues, M.T. (2006): Comparing alignment methods for inferring the history of the new world lizard genus *Mabuya* (Squamata: Scincidae). Mol. Phylogenet. Evol. **38**: 719-730.

*Received: October 19, 2009. Accepted: February 11, 2010.*

# CHAPTER 4

## DIVERSITY AND DIVERSIFICATION ON THE SEYCHELLES ARCHIPELAGO: *Urocotyledon, Phelsuma* AND *Mabuya spp.*

**One or more?**

# Cryptic diversity within the endemic Prehensile-tailed gecko *Urocotyledon inexpectata* across the Seychelles Islands: patterns of phylogeographic structure and isolation at the multilocus level

Rocha S.[1,2,3], Harris D.J.[1,2] and Posada D.[3]

[1]*CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, 4485-661 Vairão, Portugal*

[2]*Departamento de Biologia, Faculdade de Ciências, Rua do Campo Alegre FC4 4169-007 Porto, Portugal*

[3]*Departamento de Bioquímica, Genética e Inmunología, Facultad de Biología, Universidad de Vigo, Vigo 36310, Spain*

## Abstract

We examine patterns of genetic structure and isolation within *Urocotyledon inexpectata* (Reptilia: Geckkonidae), an endemic species from the Seychelles islands. Genetic diversity was screened from populations across the archipelago for both mitochondrial and nuclear genes. Gene genealogies and model-based inference were used to explore patterns and timings of isolation between the main lineages. High levels of genetic diversity were found for the mitochondrial and some of the nuclear markers. This species harbours at least two highly differentiated lineages, exclusively distributed across the northern and southern groups of islands. The main split between these lineages was dated back to the Miocene/Late Pliocene, but isolation events throughout the Pliocene and Pleistocene were also inferred. Migration between groups of islands was apparently non-existent, except for one case. The low dispersal capabilities of this species, together with the intrinsic fragmented nature of its geographic distribution and its dynamic biogeographical setting - cycles of eustatic sea-level changes - seem to have resulted into highly structured populations, which may currently represent more than one species. The observed pattern of genetic structure suggests also a hypothetical biogeographic scenario for the Seychelles that can be further tested with the exploration of the phylogeographic structure of other Seychellois taxa.

Additional keywords: cryptic variation - islands – phylogeography - Seychelles - *Urocotyledon* – vicariance – western Indian Ocean.

## Introduction

Lying on a shallow plateau, the granitic islands of the Seychelles archipelago are a group of about 40 islands clustered together on the undersea shelf of granite that is the Seychelles bank. They comprise the islands of North, Silhouette, Mahé, Fregate, Praslin, La Digue, Curieuse and several smaller ones encircling them (Figure 1). Considerable oscillations of the sea-level throughout the Pleistocene (and back along the Miocene) occurred, with low stands of often more than 80m below present sea level (bpsl), and up to 145m bpsl during the Pleistocene (Colonna *et al.*, 1996; Miller *et al.*, 2005; Siddall *et al.*, 2003) that must have played a major role in shaping diversity patterns across the archipelago. All the islands in the Seychelles plateau would have been united by sea levels of roughly 60m bpsl, but even less pronounced minima should have greatly increased the size of each island and their connectivity (Figure 1). Above sea-level (asl) stands during the Pleistocene (~11 and 17m, according to Siddall et al., 2003) were much less dramatic and most granitic islands maintained a considerable size. It is probable that these cycles of allopatry and contact between islands have driven organismal divergence and diversification within the granitic Seychelles, and that they are reflected today in the genetic structure of most species, even in the absence of evident morphological or ecological differentiation.

Very little is known about the intra-archipelago structure of Seychellois taxa. Only a few studies have examined the patterns of variation and diversification within the Seychelles. Historically, Mahé and surrounding islands plus Silhouette and North have been considered to form a biogeographic unit separated from the Praslin-La Digue group (plus adjacent islands). Apart from the rest, Fregate is generally viewed as an isolated or intermediate unit (Cheke, 1984; Radtkey, 1996; Scott, 1933). Concordant differentiation with this geographical partition has been detected in some faunal lists for insects (Scott, 1933), molluscs (Gerlach & van Bruggen, 1999) and also in (the few) studies of intraspecific variability within amphibians (Nussbaum & Wu, 1995) and reptiles (Gardner, 1987; Radtkey, 1996), but it does not appear to stand for other groups, such as land snails and other amphibians (Gerlach, 1999; Nussbaum, 1984). Indeed, the similarity observed in the faunal lists between Mahé and Silhouette - the highest islands in the group - apparent in faunal lists could be also a consequence of their similar habitats, consisting of extensive areas of mist forest, while lowland and palm forests predominantly cover (or covered until recently) Praslin, La Digue and Fregate. This ecological variation may also have contributed to the differentiation of some organisms by local adaptation to particular habitats.

In general, *Urocotyledon* species are rarely encountered, remaining the most poorly known of all gecko genera (Bauer & Menegon, 2006). *Urocotyledon inexpectata* (Stejneger 1893) - endemic from the Seychelles Islands - was previously thought to be very rare, though recent data (Gerlach, 2008; Rocha *et al.*, 2009a) suggest this is not the case; they may be abundant, but cryptic. Found in varied habitat types on different islands, though always within crevices in rocks or trees, and at altitudes from sea level up to 700m (Gerlach, 2008), it is probable that they are widespread, although no population density estimates have been made.

Our aim was to investigate the genetic diversity and structure within the gecko *Urocotyledon inexpectata* across the Seychelles islands and to decipher the evolutionary processes underlying the patterns observed. Due to its specialized habitat requirements we expect it to be amongst the Seychelles reptile species with lowest dispersal capacity, although no formal studies have been conducted. It is therefore a good model species to gain insights into the contribution of eustatic sea-level changes to lineage diversification in this biodiversity hotspot (see Warren *et al.*, 2010 for an extensive review of sea-level fluctuations in the Western Indian Ocean region). Here, we have explored the genetic structure and diversity of this species using both nuclear and mitochondrial DNA fragments. We used model-based methods to characterize the processes of divergence between lineages and to obtain estimates of genetic diversity and demographic parameters. With up to 90% of reptile extinctions being island endemics (WCMC, 1992), a basic knowledge on alpha-taxonomy and intraspecific genetic variation is vital, and we discuss our results in the light of their taxonomic, biogeographic and conservation implications.

## Material and Methods

### *Sampling and molecular methods*

Tail-tips from 51 individuals were collected during 2007 and 2008 across nine of the granitic islands of the Seychelles archipelago, approximately covering the entire range of the distribution of this species, and stored in 100% ethanol. The number of individuals sampled per island varied between one and 23 (see Figure 1 and Appendix 1 for detailed information).

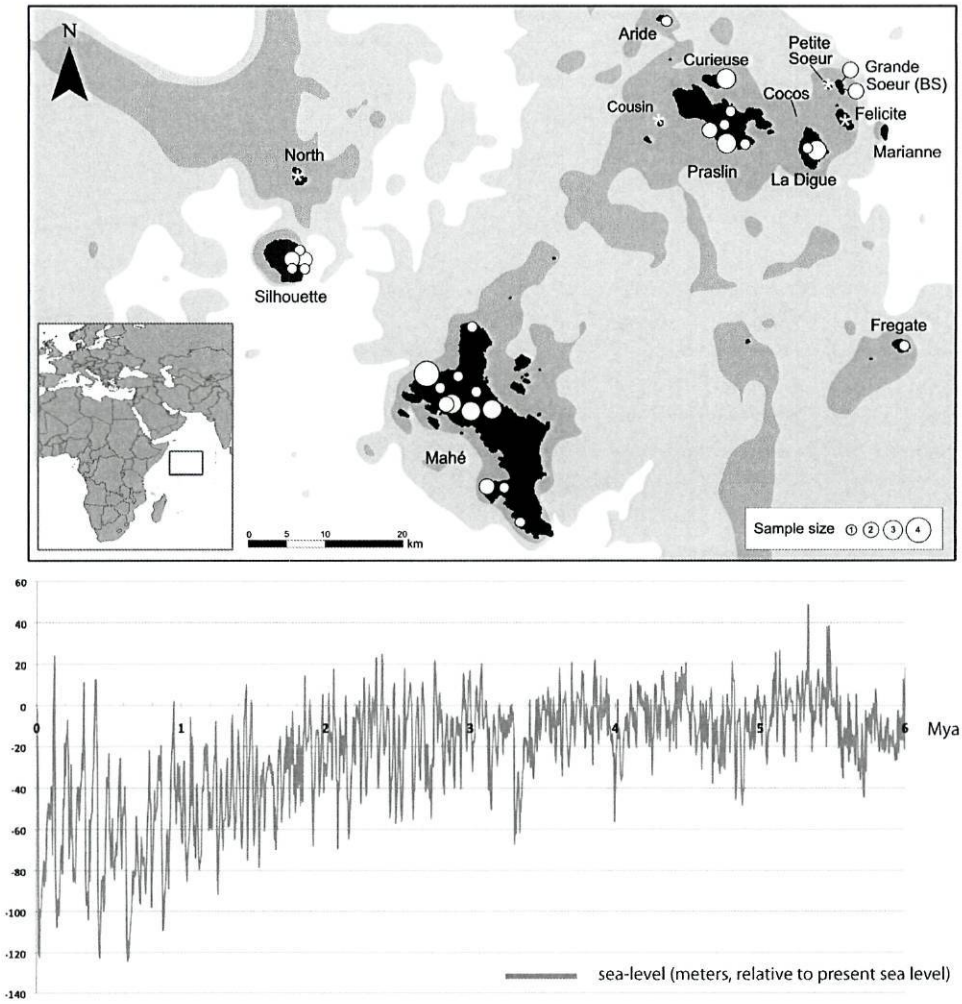



**Figure 1 -** Known distribution of *Urocotyledon inexpectata* with localities and number of individuals sampled. Islands for which the species is recorded but was not sampled are marked with an asterisk. Along the paper, the southern group of islands (Mahé and Silhouette) plus Fregate is also referred to as the "Mahé-group" and the northern group (Praslin, La Digue and surrounding islands) as the "Praslin-La Digue group". Different shadings show areas that would be emerged at -30m (dark grey) and -50m (light grey) below present sea level (bpsl) stands. The graph shows a global sea level estimate derived from $\partial^{18}O$ for the last six Myr (from Miller et al., 2005; supplementary Table S1).

Genomic DNA was extracted following standard salt or phenol-chlorophorm protocols (Kocher *et al.*, 1989; Sambrook, Fritsh & Maniatis, 1989). All but one individual were genotyped for a fragment of the mitochondrial gene cytochrome-*b*, and a subset of these (see Appendix 1) for five nuclear fragments (C-mos, Rag2, MC1R, PDC and RELNint61). For cyt-*b*, a fragment of circa 800 bp was initially amplified with primers CBL14753 (Austin, Arnold & Jones, 2004) and CB3H (Palumbi *et al.*, 1991) and conditions as in Rocha *et al.* (2009b). Specific primers for this species were later designed (UroF; 5'-CAAAAACCTAATGACCCCACTACG-3' and UroR; 5'-TAAAGTTTTCTGGGTCTCCTAG-3', forward and reverse, respectively) and used to amplify a fragment of 730 bp. Amplifications were conducted in 15-25 µl reactions using standard conditions with annealing temperatures of 50-52ºC and magnesium concentration of 1-1.5mM. For C-mos we used primers Mos-F and Mos-R and conditions as in Godinho *et al.* (2005); for Rag-2, amplification was as in Rocha *et al.* (2009b) and for PDC we used primers and conditions as in Gamble *et al.* (2008). Primers and conditions for MC1R and RELN*int*61 are given in Pinho *et al.* (2009). Annealing temperature adjustments were made when necessary.

PCR products were purified using ExoSAP-IT and sequenced either in a local automated DNA sequencing apparatus or by a commercial facility (Macrogen, Seoul, Korea). Sequences obtained were deposited in GenBank under accession numbers #####-##### (to be added upon manuscript acceptance).

*Alignment and haplotype determination*

Alignments were unambiguous, and were hand-made and inspected by-eye using BioEdit (Hall, 1999). Protein coding regions were translated and a correct reading frame was observed in all cases, so mitochondrial sequences were thus assumed to be genuine mitochondrial copies and not nuclear paralogues. The cytochrome-*b* alignment was trimmed to 700 bp. For most individuals nuclear fragments were sequenced for both strands to assure double peaks were real and not basecalling errors. Haplotype phases were determined using two different strategies. For sequences that were heterozygous for insertions or deletions (some individuals for the RELN*int*61 fragment) we used the method described by Flot *et al.* (2006), while for the remaining sequences we used the Bayesian algorithm implemented in PHASE (Stephens, Smith & Donnelly, 2001), using known phases determined by the previous method, when available. Multiple runs of PHASE were performed for each dataset and phase calls checked for

consistency. For the RELN*int*61 dataset, few of the phase calls were not identical across runs, and the PHASE run with the highest likelihood score was used. We kept all positions for which phases where resolved with a posterior probability higher than 0.8, though the majority of them were actually resolved with >0.9 probability. Remaining positions were coded as missing data (N) for the reconstruction of haplotype networks, calculation of summary statistics and IM analyses, and with IUPAC ambiguity codes for the remaining analyses (See below), but whole individuals were never excluded. Because ~23% of the RELN*int*61 variable positions did not meet the 0.8 threshold, downstream IM analyses in this case were made treating these sites as missing data and also using the inferred phase estimates regardless of probability, to check for consistency of the estimates (see Garrick, Sunnucks & Dyer, 2010). Contiguous multi-base indels were treated as single events.

*Gene genealogies and age estimation*

The mitochondrial dataset was collapsed into haplotypes using ALTER (Glez-Peña *et al.*, 2010). The best-fit model of nucleotide substitution was estimated with jModeltest (Posada, 2008) and PhyML (Guindon & Gascuel, 2003) using the corrected Akaike Information Criteria (Posada & Buckley, 2004; Sugiura, 1978). Phylogenetic relationships among haplotypes were estimated with MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003) under the best-fit model. Two runs of five million generations were performed with default heating parameters, sampled each 1000$^{th}$ sample. Runs were checked for convergence and consistency using AWTY (Nylander *et al.*, 2008). Median-joining networks (Bandelt, Forster & Rohl, 1999) with maximum-parsimony (MP) posterior optimization (Polzin & Daneschmand, 2003) were constructed using NETWORK v4.510 (available from http://fluxus-engineering.com) and used to depict closest relationships among haplotypes. Net distances between main groups (Da) identified from cyt-*b* were calculated using MEGAv4 (Kumar *et al.*, 2008).

We used the Bayesian MCMC method from Heled & Drummond (2009), implemented in BEASTv1.5.3 (Drummond & Rambaut, 2007), to obtain an estimate of the age of the root of the tree. This method makes use of the multispecies coalescent to co-estimate the gene trees and the underlying "species-tree", together with splitting times and effective population sizes of extant and ancestral "species" (the term species being applied to any taxonomic rank or any divergent "population"). Using multiple loci and individuals per "species", these estimates are more

accurate than the ones based on single locus or concatenated data (Heled & Drummond, 2009). We defined two groups of individuals (based on the results from the mtDNA phylogeny) and estimated the appropriate model of evolution for each gene fragment using the same procedure as above. An uncorrelated relaxed clock model was assumed for the cyt-*b* dataset, whereas a strict clock model was assumed for the nuclear gene fragments, given the low variability that characterized them (causing runs under relaxed clock models to fail to converge). The substitution rate for cyt-*b* was assumed to be normally distributed, with a 0.01 (mutations/site/million years) mean and 0.0027 standard deviation, as in Paulo *et al.*, (2008). Similar rates (~1%) have been consistently estimated for mtDNA fragments of other lizards at the intrageneric level (Carranza *et al.*, 2008; Chapple, Ritchie & Daugherty, 2009; Cox, Carranza & Brown, 2010; Miralles & Carranza, 2010). Substitution rates for the nuclear loci were co-estimated along the run, relative to the mitochondrial rate. Multiple runs of 100 million steps were performed, sampled at appropriate intervals to obtain 10,000 final samples (trees). The initial 10% of the trees were discarded as burnin. Tracer v1.5 (Rambaut & Drummond, 2007) was used to visualize the results of each run and to calculate the effective sample size of each parameter to check for convergence.

*Gene variability, neutrality tests and demography*

DNAsp v5.00.7 (Librado & Rozas, 2009) was used to calculate diversity summary statistics for each marker and mtDNA clade/island group (haplotype and nucleotide diversity; number of segregating sites and the population mutation parameter, $\theta w$) and also to calculate Tajima's D, Fu's (1997) Fs and Ramos-Onsins & Rozas (2002) $R_2$ statistics and assess their significance using 10,000 coalescent simulations.

Additionally, the extended Bayesian skyline plot model (EBSP), implemented in BEASTv.1.5.3 (Heled & Drummond, 2008) was used to explore the pattern of demographic change through time within each mtDNA clade at the multilocus level. To allow for a continuous population size dynamics, a linear model, where population size is allowed to grow or decline between change points was used. The appropriate model of nucleotide substitution was estimated for each locus and implemented in BEAST as previously. An uncorrelated relaxed clock model was used for cytochrome-*b* and a strict clock model for the remaining nuclear genes. A substitution rate of 1% was assumed as before. Multiple runs were performed and sampled at

appropriate intervals. Tracer v1.5 was used to check for convergence (Rambaut & Drummond, 2007).

*Inference under the Isolation-with-migration model*

To better understand how this species responded to the cyclic fluctuations in the connectivity of the islands, and to decipher if dispersal and gene flow or rather isolation and drift were predominant throughout its evolutionary history, we used the multilocus coalescent approach implemented in IMa2 (Hey, 2010; Hey & Nielsen, 2007). This model allows for the estimation of mutation-scaled divergence times, population sizes and post-divergence rates of gene flow (Hey & Nielsen, 2004; Nielsen & Wakeley, 2001). For this, the method makes several assumptions including no recombination within each locus and neutrality of the markers used. We tested for recombination and selection through the Phi statistic (Bruen, Philippe & Bryant, 2006) and multiple loci HKA tests (Hudson, Kreitman & Aguade, 1987) (available respectively at www.math.auckland.ac.nz/~bryant/software/ and http:/genfaculty.rutgers.edu/hey/software). A significant P-value for recombination was detected at the RELN*int*61 dataset, that was reduced to the maximum block free of evidence from recombination (485 bp), according to the Hudson and Kaplan's (1985) four-gamete test, using IMgc (Woerner, Cox & Hammer, 2007) with default parameters. The IM model was fitted to the whole dataset (mtDNA + nuclear loci) and also to the nuclear data only. Two population groups were defined based on the geography and the mtDNA phylogeny, corresponding to the southern (plus Fregate) and the northern groups of islands. We further fitted the IM model to the two divergent mtDNA lineages within the northern group of islands (Praslin, Curieuse and Aride versus La Digue and Grande Soeur) observed from the mtDNA phylogeny, to infer their putative time of split and migration rates. Additionally, to test the hypothesis of significant isolation within the southern group, between Mahé and Silhouette islands, given the higher sea-depths that separate them, we also fitted the IM model to mitochondrial and nuclear DNA data from these populations solely. The HKY (Hasegawa, Kishino & Yano, 1985) model was used for the mtDNA dataset and also for Rag-2 and MC1R nuclear gene fragments – where homoplasic positions were observed - and the infinite sites (IS) model (Kimura, 1969) for remaining nuclear gene fragments. Prior bounds were first selected based on the summary statistics and optimized and adjusted in subsequent runs. Multiple runs of each dataset were performed and effective sample size of parameters (ESS) and trend plots examined

to assure convergence. The significance of the migration rate estimates was measured using the log-likelihood-ratio (*LLR*) statistic of Nielsen & Wakeley (2001). A substitution rate of 1% (mtDNA) and a generation time of two years (Rösler, 1995) were used to convert parameter estimates into absolute demographic and time quantities.

**Results**

*Genetic structure*

Concerning mitochondrial DNA variation, individuals from different islands formed two deeply divergent clades (~9% uncorrected p-distance), with a strong phylogeographic structure (Figure 2). Each clade was composed by individuals from the northern (Praslin-La Digue) and southern (Mahé) groups of islands, exclusively. The population from Fregate island was closely related to - and herein included in – the southern group. Further structure within each clade was also observed. Within the northern clade, haplotypes could be assigned to two groups, of circa 3% divergence; one of the groups was formed by haplotypes from La Digue and Grande Soeur islands (no haplotype sharing between islands was observed), and the other group consisted of haplotypes from Praslin, Curieuse, Aride, plus one individual from Grande Soeur (again, no haplotype was shared across islands). Differentiation within the southern clade was lower (1.6%), but again, no haplotypes were shared across islands.

Nuclear gene genealogies also reflected the northern/southern genetic structure but varied in the divergence levels between the two main mtDNA lineages (Figure 3). Haplotype sharing between individuals belonging to different mtDNA lineages happened only in two instances (PDC and MC1R), both involving a central, highly frequent, haplotype. The multispecies coalescent estimate of the age of the split between the northern and southern clades was of 9.9 [5.04 – 15.02] Mya (million years ago; mean and 95% HPD interval).

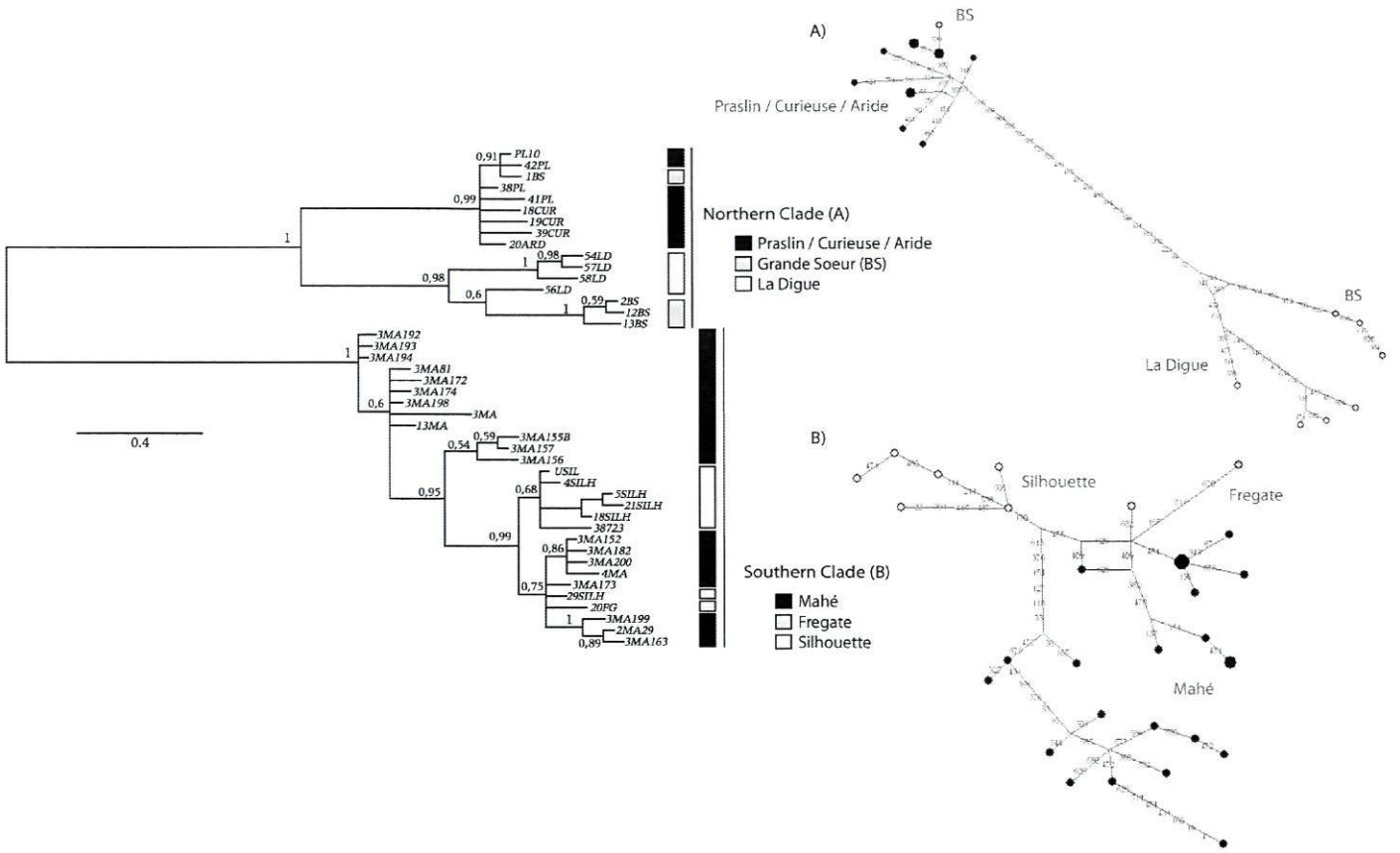



**Figure 2 –** Midpoint rooted Bayesian inference (BI) tree representing the phylogenetic relationships between mtDNA haplotypes (left), and MJ networks for each mtDNA clade (right; A and B). Circle size is proportional to haplotype frequency and numbers on branches indicate the number (and position) of mutations between haplotypes. Posterior probabilities > 0.5 above are shown above branches.

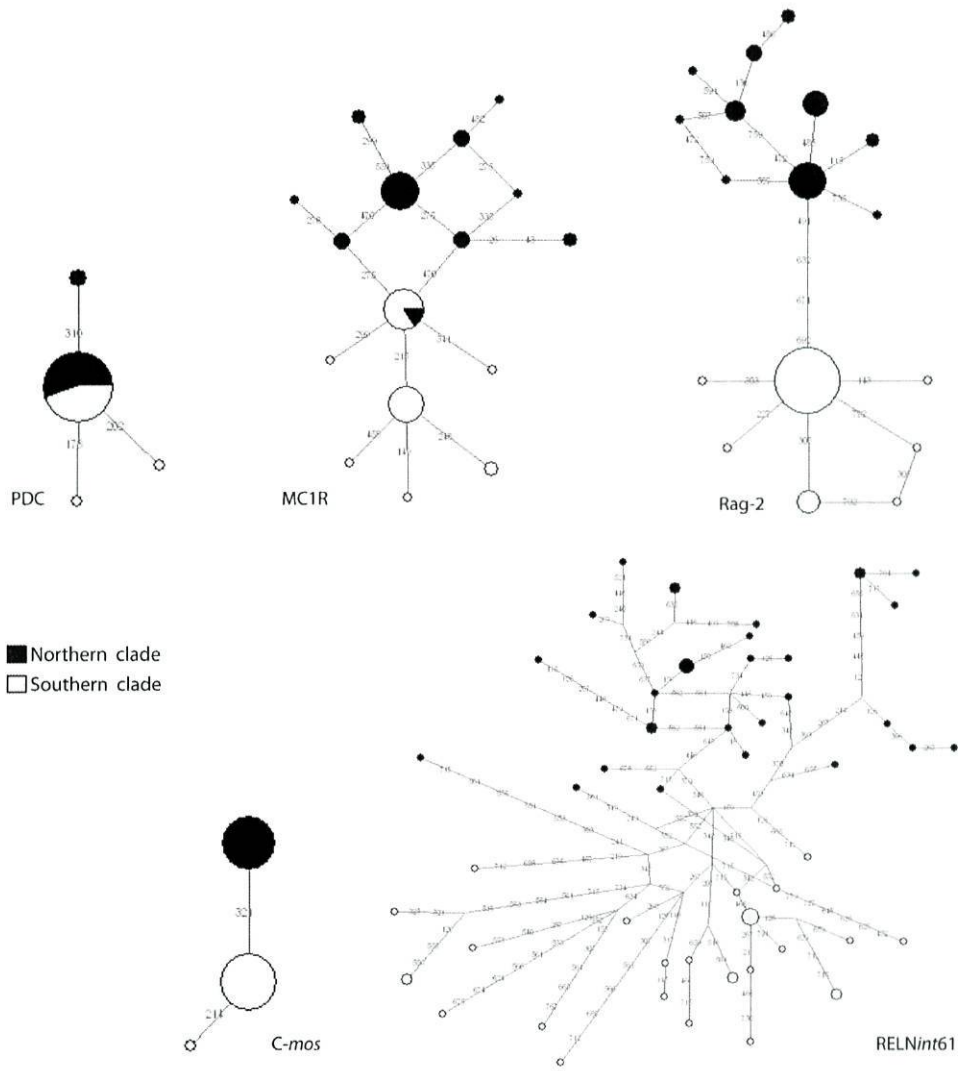



**Figure 3 –** MJ networks for nuclear markers. Mitochondrial DNA clades are colour coded. Circle size is proportional to haplotype frequency and numbers on branches indicate the number (and position) of mutations between haplotypes

*Genetic diversity and demographic changes*

Detailed information on the length, polymorphism levels and summary statistics for all gene regions analysed are given in Table 1. Haplotype and nucleotide diversity varied considerably among fragments, with the higher values harboured by Cyt-*b* and by the nuclear intron RELN*int*61. Diversity values were similar across markers for both northern and southern clades. Tajima D values were usually negative, Fu's Fs were negative and significant, and $R_2$ values were generally low. Although simultaneous significant values for the three statistics were rarely achieved, taken together they seem to reflect signs of a recent and moderate population expansion, in concordance with the Bayesian skyline plot (Figure 4).

**Table 1 –** Summary statistics and neutrality tests for the two major lineages observed at the mtDNA level.
N, number of individuals (mtDNA) / chromosomes (nucDNA); *H*, number of haplotypes; *Hd*, haplotype diversity; *S*, number of segregating sites; $\pi$, nucleotide diversity; $\theta w$, population mutation parameter, calculated according to Watterson (1975). D, Tajima's D; *Fs*, Fu's (1997) *Fs*; $R_2$, Ramos-Onsins and Rozas's (2002) $R_2$. Summary statistics were calculated excluding indels and positions coded as missing data. *Phi P-value* represents the p-value for observing the tested sequences under the null hypothesis of no recombination, calculated using the Phi statistic (Bruen et al., 2006). Values not given (-) are impossible to calculate due to too few informative sites. P-value of HKA test for selection at multiple loci is also indicated. ** significant at P< 0.01; * significant at P<0.05.

| Gene | Clade | Length | N | Polymorphism | | | | | *D* | *Fs* | $R_2$ | Recombination |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *H* | *Hd* | *S* | $\pi$ | $\theta w$ | | | | *Phi P-value* |
| Cyt-b | Southern Is. | 700 | 31 | 28 | 0.991 | 50 | 0.00013 | 0.01788 | -0.7158 | -16.343** | 0.0902 | |
| | Northern Is. | 700 | 19 | 16 | 0.982 | 61 | 0.02770 | 0.02657 | -0.1754 | -1.807 | 0.0145 | |
| C-mos | Southern Is. | 342 | 48 | 2 | 0.082 | 1 | 0.00024 | 0.00064 | -0.8664 | -0.847 | -0.0408* | - |
| | Northern Is. | 342 | 40 | 1 | - | | - | - | - | - | - | |
| Rag-2 | Southern Is. | 796 | 60 | 7 | 0.327 | 5 | 0.00047 | 0.00135 | -1.5183* | -5.707** | 0.0515 | 0.198 |
| | Northern Is. | 796 | 40 | 9 | 0.760 | 6 | 0.00137 | 0.00178 | -0.6191 | -4.223** | 0.0896 | |
| Reln*int*61 | Southern Is. | 794 | 30 | 19 | 0.940 | 35 | 0.00532 | 0.01113 | -1.9058* | -9.724** | 0.0564* | 0.0039** |
| | Northern Is. | 804 | 32 | 23 | 0.970 | 37 | 0.00826 | 0.01174 | -1.0771 | -10.65** | 0.0770 | |
| MC1R | Southern Is. | 621 | 36 | 7 | 0.663 | 6 | 0.00136 | 0.00233 | -1.13770 | -3.066* | 0.0736 | 0.536 |
| | Northern Is. | 621 | 36 | 10 | 0.762 | 9 | 0.00241 | 0.00349 | -0.92678 | -4.055* | 0.0811 | |
| PDC | Southern Is. | 360 | 32 | 3 | 0.123 | 2 | 0.00035 | 0.00138 | -1.5037* | -2.437* | 0.1210 | - |
| | Northern Is. | 360 | 40 | 2 | 0.097 | 1 | 0.00027 | 0.00065 | -0.8360 | -0.688 | 0.0487* | |
| *P-value HKA = 0.87445* | | | | | | | | | | | | |

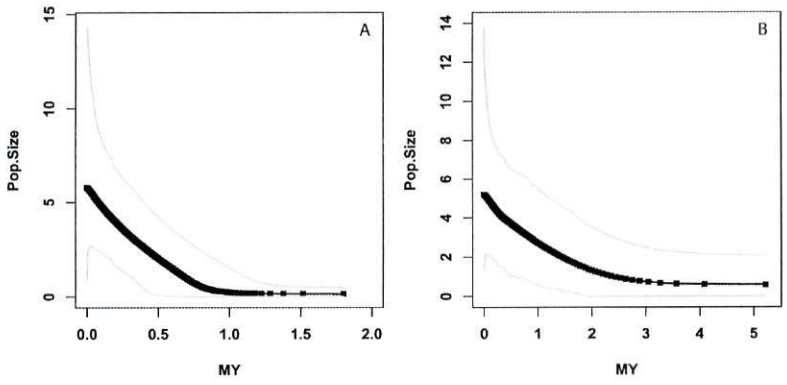



**Figure 4 –** Extended Bayesian skyline plots for the southern (A) and northern (B) mtDNA clades. The x-axis indicates million years (MY) while the y-axis represents the product of the effective population size and the generation length in million years) (Ne*t) Black dotted lines correspond to median estimates and grey lines the 95% upper and lower intervals.

*Isolation and migration*

Independent runs of IMa2 across datasets converged on similar marginal posterior probability distributions, providing reliable posterior estimates for all parameters across all analyses (Table 2, Figure 5 and Supplementary Figure 1). Post divergence gene-flow rates both between the two main mtDNA lineages (northern and southern groups) and also between populations from Mahé and Silhouette islands do not differed significantly from zero. Results were similar both when using mtDNA+nuclear data or only nuclear data (not shown). Results were also very alike both when excluding uncertain phases and when using best guesses, though population size estimates were slightly higher in the latter case (results not shown). The single case where a significant migration rate was recovered was between the two northern lineages, from the Praslin+Curieuse+Aride group into the La Digue+Grande Soeur group (Table 2).

The IMa estimate of the splitting time between the northern and southern clades was of 6.24 [3.44-13.92] Mya, with a posterior density interval fairly coincident with that previously estimated using the multispecies coalescent model. The splits within the northern group and between Mahé and Silhouette populations seem considerably younger; 1.6 [0.29-27.5] and 0.47 [0.18-1.22] Mya respectively (Table 2, Figure 5).

**Table 2 –** IMa derived estimates of peak posterior distribution and 95% highest posterior densities (HPD) of population sizes (θ), effective number of migrants (*2Nm*) and time since divergence (*T*) across comparisons. Significant values for migration (LLR test, Nielsen and Wakeley 2001) are indicated with an asterisk. A substitution rate of 1% per site per million year and a generation time of two years were assumed to convert the estimates into absolute demographic and time quantities.

| Parameter Estimates | | | | | |
|---|---|---|---|---|---|
| Population | Population Size *θ* [95%HPD] | | Effective Number of Migrants *into the population* *2Nm* [95%HPD] | Time Since Divergence *T* [95%HPD] | |
| | relative | absolute (millions of individuals) | absolute | relative | absolute (in million years) |
| Southern-group | 2.398 [1.502-3.602] | 1.628 [1.019-2.445] | 0.00029 [0-0.14] | 1.148 [0.63-2.56] | 6.24 [3.44-13.92] |
| Northern-group | 2.498 [1.586-3.782] | 1.695 [1.076-2.569] | 0.00069 [0-0.28] | | |
| Mahé | 4.385 [2.145-8.985] | 1.533 [0.750-3.143] | 0.0199 [0-11.13] | 0.1625 [0.065-0.422] | 0.47[0.18-1.22] |
| Silhouette | 1.403 [0.518-4.148] | 0.490 [0.181-1.451] | 0.4948 [0-8.171] | | |
| Praslin+Curieuse+Aride | 0.3025 [0.05-2.34] | 0.319 [0.052-2.472] | 0.01249 [0.0-2.611] | 0.24 [0.042-3.998][a] | 1.6 [0.289-27.5][a] |
| La Digue+Grande Soeur | 0.2575 [0.05-2.1] | 0.272 [0.052-2.218] | **1.943 [0.1495-8.121] ***** | | |

*** indicates a significant value for migration according to likelihood ratio test from Nielsen and Wakeley (2001) ($p<0.001$)

[a] indicates a HPD interval which may not be useful as the posterior density does not reach low levels near the upper limito of the prior

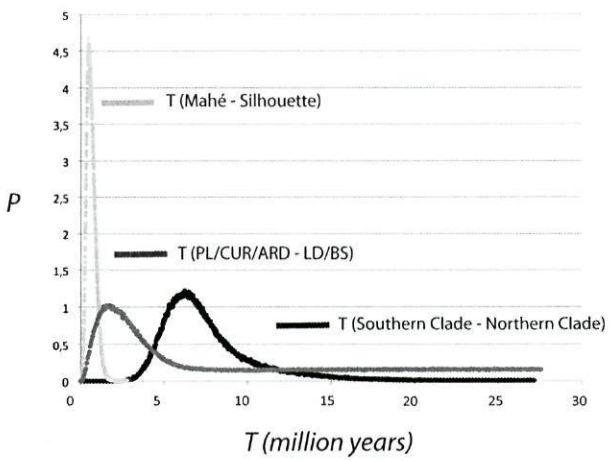



**Figure 5 –** Posterior probability density estimates (*P*) for the time since divergence between 1) the southern and northern groups of islands (black); 2) populations from Mahé and Silhouette islands (light grey); and 3) populations from Praslin+Curieuse+Aride and La Digue+Grande Soeur (dark grey). X axis (*T*) in million years before present. Translation of estimates of time into years was made assuming a 1% rate of substitution per million years for the cytochrome-*b* and a generation time of two years in all cases.

**Discussion**

Given the old isolation and the particularly dynamic biogeographic history of the Seychelles, it is to be expected that its endemic taxa reflect those factors, both in terms of diversity and geographic structure. *Urocotyledon inexpectata*, the endemic prehensile tailed gecko, is a clear example. Poorly known, and previously thought to be rare, exhibits in fact levels of genetic variability comparable to – or even higher than, for some markers - the ones observed in other Seychellois lizard species such as *Mabuya sechellensis* or *Phelsuma spp* (Rocha, 2010), known to be very abundant throughout the islands. Possibly *Urocotyledon inexpectata* has similar population sizes, but it is more difficult to see. In fact we did not appreciate any signs of population decline, but a slight recent demographic growth.

This species harbours two deeply divergent lineages, and further structure within each lineage is present, and in the case of the northern clade, with considerable divergence. The distribution of these lineages is strongly correlated with geography, and each group of islands harbours unique mitochondrial clades. The deepest split is observed between the southern (Mahé+Silhouette)+Fregate and the northern (Praslin, Curieuse, Aride, La Digue and Grande Soeur) groups of islands and not directly correlates with sea-level depths (which would make Silhouette the most isolated island relative to all others – see Figure 1), suggesting that geographical distance was more important than sea-level depths in shaping the genetic structure of this species. Nevertheless, populations seem to be highly structured, as they did not shared mtDNA haplotypes between any islands. Within the northern group, two differentiated lineages could be distinguished, one inhabiting the islands of Aride, Praslin and Curieuse, and the second in La Digue and Grande Soeur, though one individual from Grande Soeur exhibits a mtDNA haplotype that clusters within haplotypes from Praslin. This geographic structure is mirrored by the distribution of the two lineages of *Phelsuma sundbergi* in the same island group *(P. s. sundbergi* and *P. s. ladiguensis*, Gerlach, 2007). This distribution is logical in terms of biogeography, as islands within each group are closer to each other than to other groups' islands. Nevertheless, they are only separated by sea-depths of circa of 20m (Figure 1), having been putatively connected during most of the Pleistocene (Warren et al., 2010). It is interesting therefore, to consider that two slightly differentiated lineages were formed and maintained despite so many opportunities for migration. In this context, it would be relevant to determine the genetic constitution of populations in the remaining islands in the group, primarily Cocos,

Marianne, and Petite Soeur, and to use more variable nuclear markers to accurately depict the patterns of connectivity between populations. While in theory it might be possible that the particularly strong geographic structure detected at the mtDNA level is also partly due to gender-biased dispersal, with females dispersing less than males, we found no particular evidence for this, and it seems instead likely that the different mutation rates and effective population sizes of nuclear and mitochondrial markers provide a better explanation of this result. Haplotypes from Curieuse cluster within the ones from Praslin, which is not surprising given that Curieuse can be viewed as an extension of the Praslin island, with which it is united with a lowering of the sea-level of less than 10m (Figure 1). The close relationship of the Fregate population to populations from the southern group is clear, and it will be particularly interesting to see if this pattern stands for other taxa.

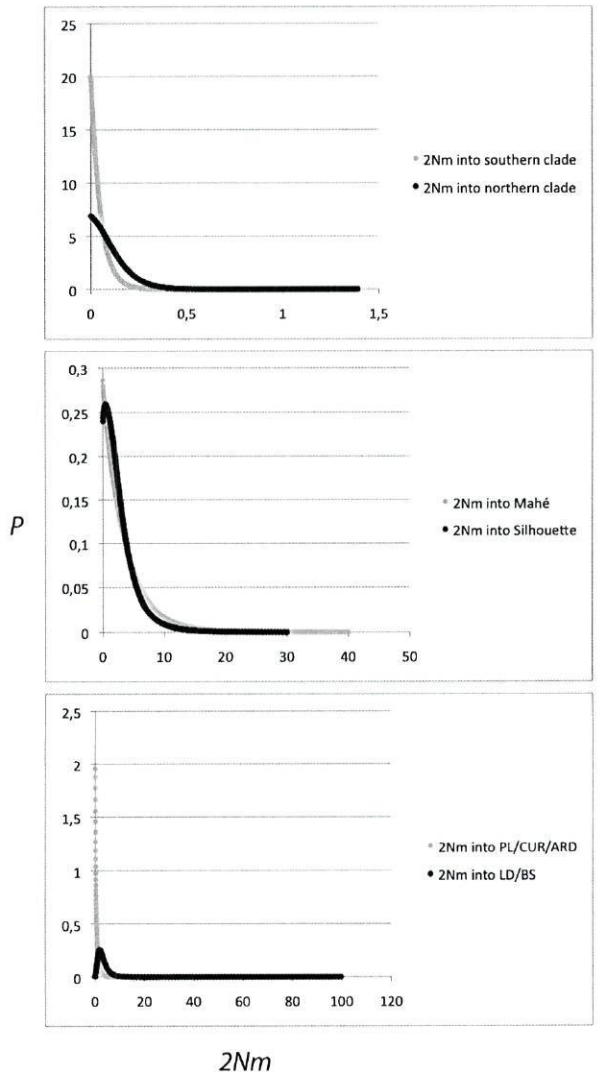
The fact that the levels of gene flow between most groups of islands are not significantly different from zero, and considering the estimated splitting times, suggests that these groups may represent distinct evolutionary lineages, even in the absence of evident phenotypic differentiation. This should be especially true for the two most divergent lineages, which correspond to the two most isolated groups of islands: their possibly Miocenic divergence (~6 Mya) together with an absence of post-divergence gene flow, makes them good candidates to be considered full species (see Hey, 2009; Hickerson, Meyer & Moritz, 2006), and their apparently cryptic differentiation should be the subject of further research. The isolation between other lineages is more recent (Figure 5) and although significant gene flow was only detected in one instance, from the Praslin/Curieuse/Aride clade into Grande Soeur (and actually caused by a single individual, thus being debatable whether it reflects an historical or an anthropogenic event), it is less likely that intrinsic barriers to gene flow have evolved in such a short time, under a simple allopatric model (Hickerson et al., 2006), though local adaptation may be promoting populations divergence along other axis.

## Conclusions

Genetic diversity and structure within this species highlights the importance of intrinsic habitat fragmentation and sea-level fluctuations in shaping the organismal diversity of the Seychelles archipelago early since its colonization, even though habitat connectivity and opportunities for migration and gene flow occurred. The initial divergence was dated to the Plio-Miocene, while further instances of higher sea-level, both along the Miocene and Pleistocene, together with geographic isolation and the likely reduced dispersal of this species continued to create opportunities for divergence, leading to the observed high lineage diversity and structure. A main north-south biogeographic division is depicted, with the Fregate population exhibiting a close link to the southern populations. This biogeographic scenario should now be explored across different groups from the archipelago, as cryptic diversity may be the case for other taxa also.

**Acknowledgments**

We are grateful to SBS of Seychelles for permits for fieldwork and tissue collection and to the Ministry of Environment and Natural Resources for logistic support during fieldwork. This research was supported by grants from Fundação para a Ciência e Tecnologia SFRH/BD/1745/2004 (to SR) and PTDC/BIA-BDE/65745/2006. We wish to thank Raquel Vasconcelos and Ana Perera for help during fieldwork and also Justin and Ron Gerlach from NPTS for support and information. Thanks to Ben Warren for sea-level data, to Ana Perera for Figure 1 and to Catarina Pinho, Rui Faria and Miguel Carneiro (CIBIO) for advice with IM analyses. We further thank Nancy Bunbury, Graciela Sotelo and Raquel Vasconcelos for comments on previous versions of the manuscript.




**Supplementary Figure 1 -** Posterior probability density estimates (*P*) for the effective number of migrants (*2Nm*) between the southern and northern groups of islands (top); between the populations from Mahé and Silhouette islands (center); and between the populations from Praslin+Curieuse+Aride and La Digue+Grande Soeur (down). Curve height is an estimate of the posterior probability.

**Appendix 1** - Locality information for the studied specimens and Genbank accession numbers. For detailed geographical coordinates refer to Location ID in Rocha et al. (2009a).

| Individual ID | Island | Location | Cyt-b | Rag-2 | C-mos | Reln | MC1R | PDC |
|---|---|---|---|---|---|---|---|---|
| 2MA29 | Mahé | 41 Casse Dent, Mahé | # | # | | | | |
| 3MA81 | Mahé | 46 Trois Freres 1, Mahé | # | # | # | # | | # |
| 3MA152 | Mahé | 32 Danzil2, Mahé | # | # | # | | # | # |
| 3MA155B | Mahé | 32 Danzil2, Mahé | # | # | # | | | |
| 3MA156 | Mahé | 32 Danzil2, Mahé | # | # | # | # | # | |
| 3MA157 | Mahé | 32 Danzil2, Mahé | # | # | # | # | # | |
| 3MA163 | Mahé | 41 Casse Dent, Mahé | # | # | # | | | |
| 3MA165 | Mahé | 41 Casse Dent, Mahé | # | # | # | | | |
| 3MA172 | Mahé | 45 Copolia, Mahé | # | # | # | | | |
| 3MA173 | Mahé | 45 Copolia, Mahé | # | # | # | | # | # |
| 3MA174 | Mahé | 45 Copolia, Mahé | # | # | | | | |
| 3MA182 | Mahé | 36 Port Glaud 2, Mahé | # | # | # | | | |
| 3MA183 | Mahé | 36 Port Glaud 2, Mahé | # | # | | | | |
| 3MA192 | Mahé | 60 To Anse Soleil, Mahé | # | # | # | # | # | # |
| 3MA193 | Mahé | 61 Anse Soleil, Mahé | # | # | # | | # | |
| 3MA194 | Mahé | 61 Anse Soleil, Mahé | # | # | | # | # | |
| 3MA198 | Mahé | 37 Port Glaud 3, Mahé | # | # | # | # | | # |
| 3MA199 | Mahé | 37 Port Glaud 3, Mahé | # | # | | | | |
| 3MA200 | Mahé | 37 Port Glaud 3, Mahé | # | # | # | # | # | # |
| 3MA | Mahé | 58 Anse Intendance, Mahé | # | # | # | # | # | # |
| 4MA | Mahé | 27 Danzil 3, Mahé | # | # | # | # | # | # |
| 7MA | Mahé | 29 Mare aux Cochons, Mahé | # | # | # | # | # | # |
| 13MA | Mahé | 19 North, Mahé | # | # | | | # | # |
| 38723 | Silhouette | 73 La Passe 3, Silhouette | # | # | # | | | |
| USIL | Silhouette | 76 around GB rock, Silhouette | # | | | | | |
| 4SILH | Silhouette | 72 La Passe 2, Silhouette | # | # | # | | # | # |
| 5SILH | Silhouette | 73 La Passe 3, Silhouette | # | # | # | # | # | # |
| 18SILH | Silhouette | 75 Anse Lascars, Silhouette | # | # | # | # | # | # |
| 21SILH | Silhouette | 76 around GB rock, Silhouette | # | # | # | # | # | # |
| 29SILH | Silhouette | 77 Gratte Fesse, Silhouette | # | # | # | # | # | # |
| PL10 | Praslin | 99 Anse Volbert | # | # | # | # | # | # |
| 38PL | Praslin | 110 Fonde de L'Anse, Praslin | # | # | # | # | # | # |
| 39PL | Praslin | 110 Fonde de L'Anse, Praslin | # | # | # | # | # | # |
| 41PL | Praslin | 111 Anse Bois de Rose | # | # | # | | | # |
| 42PL | Praslin | 111 Anse Bois de Rose | # | # | # | | # | # |
| 43PL | Praslin | 111 Anse Bois de Rose | # | # | # | # | # | # |
| 44PL | Praslin | 112 Anse Consolation | | # | # | | | # |
| 67PL | Praslin | 101 Salazie, Praslin | # | # | # | # | # | # |
| 18CUR | Curieuse | 80 Point1, Curieuse | # | # | # | # | # | # |
| 19CUR | Curieuse | 80 Point1, Curieuse | # | # | # | # | # | # |
| 39CUR | Curieuse | 80 Point1, Curieuse | # | # | # | # | # | # |
| 54LD | La Digue | 94 La Veuve Reserve 2, La Digue | # | # | # | # | # | # |
| 56LD | La Digue | 95 Belle Vue | # | # | # | # | # | # |
| 57LD | La Digue | 95 Belle Vue | # | # | # | # | # | # |
| 58LD | La Digue | 95 Belle Vue | # | # | # | # | # | # |
| 1BS | Grande Soeur | 90 Point 1, Grande Soeur | # | # | # | | # | # |
| 2BS | Grande Soeur | 90 Point 1, Grande Soeur | # | # | # | # | # | # |
| 12BS | Grande Soeur | 92 north path 2, Grande Soeur | # | # | # | # | # | # |
| 13BS | Grande Soeur | 92 north path 2, Grande Soeur | # | # | # | | # | # |
| 20FG | Fregate | 114 Fregate | # | # | # | # | # | # |
| 20ARD | Aride | 79 Aride Island | # | # | # | # | # | # |

## References


**Austin JJ, Arnold EN, Jones CG. 2004.** Reconstructing an island radiation using ancient and recent DNA: the extinct and living day geckos (*Phelsuma*) of the Mascarene islands. *Molecular Phylogenetics and Evolution* **31:** 109-122.

**Bandelt HJ, Forster P, Rohl A. 1999.** Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16:** 37-48.

**Bauer A, Menegon M. 2006.** A new species of Prehensile-tailed gecko, *Urocotyledon* (Squamata: Gekkonidae), from the Udzungwa Mountains, Tanzania. *African Journal of Herpetology* **55:** 13-22.

**Bruen TC, Philippe H, Bryant D. 2006.** A simple and robust statistical test for detecting the presence of recombination. *Genetics* **172:** 2665-2681.

**Carranza S, Arnold EN, Geniez P, Roca J, Mateo JA. 2008.** Radiation, multiple dispersal and parallelism in the skinks, *Chalcides* and *Sphenops* (Squamata: Scincidae), with comments on *Scincus* and *Scincopus* and the age of the Sahara Desert. *Molecular Phylogenetics and Evolution* **46:** 1071-1094.

**Chapple DG, Ritchie PA, Daugherty CH. 2009.** Origin, diversification, and systematics of the New Zealand skink fauna (Reptilia: Scincidae). *Molecular Phylogenetics and Evolution* **52:** 470-487.

**Cox SC, Carranza S, Brown RP. 2010.** Divergence times and colonization of the Canary Islands by *Gallotia* lizards. *Molecular Phylogenetics and Evolution* **52:** 745-757.

**Drummond AJ, Rambaut A. 2007.** BEAST: Bayesian evolutionary analysis by sampling trees. *Bmc Evolutionary Biology* **7:** 214.

**Flot JF, Tillier A, Samadi S, Tillier S. 2006.** Phase determination from direct sequencing of length-variable DNA regions. *Molecular Ecology Notes* **6:** 627-630.

**Fu YX. 1997.** Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* **147:** 915-925.

**Gamble T, Bauer AM, Greenbaum E, Jackman TR. 2008.** Evidence for Gondwanan vicariance in an ancient clade of gecko lizards. *Journal of Biogeography* **35:** 88-104.

**Gardner AS. 1987.** The systematics of the *Phelsuma madagascariensis* species group of day geckos (Reptilia: Gekkonidae) in the Seychelles. *Zoological Journal of the Linnean Society* **91:** 93-105.

**Garrick RC, Sunnucks P, Dyer RJ. 2010.** Nuclear gene phylogeography using PHASE: dealing with unresolved genotypes, lost alleles, and systematic bias in parameter estimation. *Bmc Evolutionary Biology* **10:** 118.

**Gerlach J. 1999.** Snails of the genus *Pachnodus* (Mollusca; Gastropoda; Enidae): their origins and evolution. *Journal of Biogeography* **26:** 251-255.

**Gerlach J. 2007.** *Terrestrial and freshwater vertebrates of the Seychelles islands*. Backhuys Publishers: Leiden, The Netherlands.

**Gerlach J. 2008.** Population and conservation status of the reptiles of the Seychelles islands. *Phelsuma* **16:** 30-48.

**Gerlach J, van Bruggen AC. 1999.** Streptaxidae (Mollusca: Gastropoda: Pulmonata) of the Seychelles Islands, western Indian Ocean. *Zoologische Verhandelingen* **328:** 1-60.

**Glez-Peña D, Gomez-Blanco D, Reboiro-Jato M, Fdez-Riverola F, Posada D. 2010.** ALTER: program-oriented conversion of DNA and protein alignments. *Nucleic Acids Research*: 1-5.

**Godinho R, Crespo EG, Ferrand N, Harris DJ. 2005.** Phylogeny and evolution of the green lizards, *Lacerta spp.* (Squamata: Lacertidae) based on mitochondrial and nuclear DNA sequences. *Amphibia-Reptilia* **26:** 271-285.

**Guindon S, Gascuel O. 2003.** A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52:** 696-704.

**Hall TA. 1999.** BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41:** 95-98.

**Hasegawa M, Kishino H, Yano T. 1985.** Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **22:** 160-174.

**Heled J, Drummond AJ. 2008.** Bayesian inference of population size history from multiple loci. *Bmc Evolutionary Biology* **8:** 289.

**Heled J, Drummond AJ. 2009.** Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution* **27:** 570-580.

**Hey J. 2010.** Isolation with Migration Models for more than Two Populations. *Molecular Biology and Evolution* 27: 905-920.

**Hey J. 2009.** On the arbitrary identification of real species. In: Butlin RK, Bridle J and Schluter D, eds. *Speciation and Patterns of Diversity*. Cambridge: Cambridge University Press. 15-28.

**Hey J, Nielsen R. 2004.** Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura* and *D. persimilis*. *Genetics* **167.**

**Hey J, Nielsen R. 2007.** Integration within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. *Proceedings of the National Academy of Sciences of the United States of America* **104:** 2785-2790.

**Hickerson MJ, Meyer CP, Moritz C. 2006.** DNA barcoding will often fail to discover new animal species over broad parameter space. *Systematic Biology* **55:** 729-739.

**Hudson RR, Kaplan NL. 1985.** Statistical properties of the number of recombination events in the history of a sample of DNA sequences. *Genetics*: 147-164.

**Hudson RR, Kreitman M, Aguade M. 1987.** A test of neutral molecular evolution based on nucleotide data. *Genetics* **116:** 153-159.

**Kimura M. 1969.** The number of heterozygous nucleotide sites maintained in a finite population due to steady flux of mutations. *Genetics* **61:** 893-903.

**Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, Wilson AC. 1989.** Dynamics of mitochondrial DNA evolution in animals - amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences of the United States of America* **86:** 6196-6200.

**Kumar S, Nei M, Dudley J, Tamura K. 2008.** MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. *Briefings in Bioinformatics* **9:** 299-306.

**Librado P, Rozas J. 2009.** DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25:** 1451-1452.

**Miralles A, Carranza S. 2010.** Systematics and biogeography of the Neotropical genus *Mabuya*, with special emphasis on the Amazonian skink *Mabuya nigropunctata* (Reptilia, Scincidae). *Molecular Phylogenetics and Evolution* **54:** 857-869.

**Nielsen R, Wakeley J. 2001.** Distinguishing migration from isolation: a Markov chain Monte Carlo approach. *Genetics* **158:** 885-896.

**Nussbaum RA. 1984.** The amphibians of the Seychelles. In: Stoddart DR, ed. *Biogeography invertebrate and vertebrate taxonomy, ecology and and ecology of the Seychelles Islands*. The Hague: Dr. W. Junk. 378-415.

**Nussbaum RA, Wu SH. 1995.** Distribution, variation, and systematics of the Seychelles treefrog, *Tachycnemis seychellensis* (Amphibia: Anura: Hyperoliidae). *Journal of Zoology* **236:** 383-406.

**Nylander JA, Wilgenbusch JC, Warren DL, Swofford DL. 2008.** AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics* **24:** 581-583.

**Palumbi SR, Martin A, Romano S, McMillan WO, Stice L, Grabowski G. 1991.** *The simple fool's guide to PCR, Version 2.0.* Privately published: Hawaii.

**Paulo OS, Pinheiro J, Miraldo A, Bruford MW, Jordan WC, Nichols RA. 2008.** The role of vicariance vs. dispersal in shaping genetic patterns in ocellated lizard species in the western Mediterranean. *Molecular Ecology* **17:** 1535-1551.

**Pinho C, Rocha S, Carvalho BM, Lopes S, Mourão S, Vallinoto M, Brunes TO, Haddad CFB, Gonçalves H, Sequeira F, Ferrand N. 2009.** New primers for the amplification and sequencing of nuclear loci in a taxonomically wide set of reptiles and amphibians. *Conservation Genetics Resources* **2:** 181-185.

**Polzin T, Daneschmand SV. 2003.** On Steiner trees and minimum spanning trees in hypergraphs. *Operations Research Letters* **31:** 12-20.

**Posada D. 2008.** jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* **25:** 1253-1256.

**Posada D, Buckley TR. 2004.** Model selection and model averaging in phylogenetics: advantages of akaike information criterion and bayesian approaches over likelihood ratio tests. *Systematic Biology* **53:** 793-808.

**Radtkey RR. 1996.** Adaptive radiation of day-geckos (*Phelsuma*) in the Seychelles archipelago: a phylogenetic analysis. *Evolution* **50:** 604-623.

**Rambaut A, Drummond AJ. 2007.** Tracer v1.5, Available from http://beast.bio.ed.ac.uk/Tracer

**Ramos-Onsins SE, Rozas J. 2002.** Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution* **19:** 2092-2100.

**Rocha S. 2010.** Phylogeny, Phylogeography and Colonization Patterns of Reptiles in Indian Ocean Islands. Unpublished PhD Thesis, University of Porto.

**Rocha S, Harris DJ, Perera A, Silva A, Vasconcelos R, Carretero MA. 2009a.** Recent data on the distribution of lizards and snakes of the Seychelles. *Herpetological Bulletin* **110:** 20-32.

**Rocha S, Vences M, Glaw F, Posada D, Harris DJ. 2009b.** Multigene phylogeny of Malagasy day geckos of the genus *Phelsuma*. *Molecular Phylogenetics and Evolution* **52:** 530-537.

**Ronquist F, Huelsenbeck JP. 2003.** MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19:** 1572-1574.

**Rösler H. 1995.** *Geckos der Welt, alle Gattungen*. Urania Verlag: Leipzig.

**Sambrook J, Fritsh EF, Maniatis T. 1989.** *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Press: Cold Spring Harbor, NY.

**Scott H. 1933.** General conclusions regarding the insect fauna of the Seychelles and adjacent islands. *Transactions of the Linnean Society of London 2nd series (Zoology)* **19:** 307-391.

**Siddall M, Rohling EJ, Almogi-Labin A, Hemleben C, Meischner D, Schmelzer I, Smeed DA. 2003.** Sea-level fluctuations during the last glacial cycle. *Nature* **423:** 853-858.

**Stephens M, Smith NJ, Donnelly P. 2001.** A new statistical method for haplotype reconstruction from population data.

*American Journal of Human Genetics* **68:** 978-989.

**Sugiura N. 1978.** Further analysis of the data by Akaike's information criterion and the finite corrections. *Communications in Statistics -Theory and Methods*: 13-26.

**Warren BH, Dominique S, Bruggemann JH, Robert PP-J, Christophe T. 2010.** Why does the biota of the Madagascar region have such a strong Asiatic flavour? *Cladistics* **26:** 526-538.

**Watterson GA. 1975.** On the number of segregating sites in genetical models without recombination. *Theoretical Population Biology* **7:** 256-276.

**WCMC. 1992.** *Global biodiversity: status of the Earth's living resources*. Chapman and Hall: London.

**Woerner AE, Cox MP, Hammer MF. 2007.** Recombination-filtered genomic datasets by information maximization. *Bioinformatics* **23:** 1851-1853.

## The Time frame of Diversification of day-gecko genus *Phelsuma* on the Seychelles and its Driving Mechanisms.

Rocha S.[1,2,3], Posada D.[3], Harris D.J.[1,2]

[1]*CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, 4485-661 Vairão, Portugal*

[2]*Departamento de Biologia, Faculdade de Ciências, Rua do Campo Alegre FC4 4169-007 Porto, Portugal*

[3]*Departamento de Bioquímica, Genética e Inmunología, Facultad de Biología, Universidad de Vigo, Vigo 36310, Spain*

### Abstract

Belonging to one of the Earth's biodiversity hotspots, and lying in a shallow continental shelf affected by oscillating sea levels since the Miocene, the Seychelles Islands are a particularly interesting system for evolutionary studies. Yet very little is known regarding the phylogeographic structure of their taxa and on the factors promoting diversification within the archipelago. Here we used multilocus molecular data (one mitochondrial and four nuclear loci) and model-based approaches to decipher the evolution of one of the archipelago's most diverse vertebrate group, the day-geckos of the genus *Phelsuma*, and to test hypotheses concerning the causes of the different degrees of morphological differentiation within species across the archipelago. No evidence of gene flow between (sympatric) species was found while significant values suggest the possibility of past gene flow between (allopatric) subspecies. Time estimates suggest a possibly synchronous divergence within each species, most possibly affected by common historical factors. If true, differential selective pressures and niche differentiation must play an important role in shaping the morphological diversification of these species within the archipelago, instead of it being only the result of size adjustment proportional to time since sympatry, as previously suggested. The hypothesis of body size being influenced by the distribution of native vegetation and social systems within this group is brought again to consideration. These results highlight not only the role of ecology and interspecific interactions in Seychellois *Phelsuma* diversification and community assemblage but also the importance of co-evolutionary mechanisms and their importance for a proper conservation of islands biodiversity.

Keywords: *Phelsuma*, adaptive radiation, Seychelles, phylogeography, diversification, morphological evolution, biogeography.

A common outcome of evolution on islands is species radiation, where a colonizing ancestor rapidly diversifies to produce a wide array of species, either adapted to different ecological niches (adaptive radiation, Schluter, 2000; Simpson, 1953) or not. The term non-adaptive radiation is often applied to cases where allopatric speciation via founder effect and genetic drift mechanisms are predominant, without much niche differentiation (e.g. Kozak et al., 2006). Most groups occupy in fact an intermediate space along this conceptual gradient (Rundell and Price, 2009; Whittaker and Fernández-Palacios, 2007), with speciation due to geographic barriers to gene flow often featuring the ecological divergence of populations (reviewed in Rundle and Nosil, 2005). By reducing or inhibiting gene flow, geographic barriers may ease local adaptation and spur phenotypic differentiation among populations and species, which may later come into secondary contact, resulting in further differentiation (for example through resource competition and character displacement) and/or sometimes, in hybridization.

Many of the most famous examples of adaptive radiations fit to this model of preponderant allopatry and ecological opportunity in the initial stages of diversification (Schluter, 2000), after which selective forces act at the establishment of sympatry, resulting in increased differentiation (Grant and Grant, 2008b; Losos, 2009; Tarr and Fleischer, 1995; Wagner and Funk, 1995). Introgressive hybridization is also not uncommon, (Mallet, 2005; Seehausen, 2004) either due to demographic, behavioural or ecological reasons (Grant and Grant, 1997; Grant et al., 1996; Seehausen et al., 1997). Hybridization may enhance the potential of species for evolutionary change and its outcome is highly correlated with external ecological factors (Carlquist, 1995; Grant and Grant, 2008a; Grant and Grant, 2008b; Seehausen, 2004; Seehausen, 2006; Thorpe et al., 2008). Allopatry, competition in sympatry and hybridization may thus have synergistic or antagonistic effects on the generation of biodiversity within a radiation, which are not straightforward to disentangle.

With at least 46 species distributed across all western Indian Ocean archipelagos, day geckos of the genus *Phelsuma* mostly diversified in Madagascar, from where they colonized surrounding archipelagos and often radiated, to a wider or lesser extent (reviewed in Rocha et al., 2010). Within each radiation, *Phelsuma* species have diverged ecologically and morphologically, evolving a striking variety of forms and occupying a broad range of arboreal habitats (see Harmon et al., 2008 and references therein). Moreover, sympatric species partition the habitat and shift their habitat use in the presence of other species (Gardner, 1984; Harmon et al., 2007). However the relative contribution of geographic isolation, local adaptation and

interspecific interactions in the generation of these species assemblages is, in most of the cases, rather unclear.

In the granitic Seychelles, *Phelsuma* is represented by a small endemic monophyletic radiation (Radtkey, 1996; Rocha et al., 2009). The classification of the Seychelles "*forms*" of *Phelsuma* has been a persistent problem for taxonomists and a source of confusion for those studying ecology and biogeography in the Seychelles. The considerable variation in colour pattern and size across islands and their insular distribution are the causal factors of these difficulties. Cheke (1982) and Gardner (1984; 1986; 1987) specifically assessed the systematics of the Seychelles forms leading to a better understanding of their relationships and intraspecific variation. Two endemic species are currently recognized: *P. sundbergi* and *P. astriata*, each one with an unclear number of subspecies or *forms*. Within *P. sundbergi* up to six subspecies are recognized: *P. sundbergi longinsulae*, inhabiting Mahé and associated islands plus Fregate; *P. (sundbergi) longinsulae rubra* and *umbrae*, respectively from North and Silhouette (sensu Borner and Minuth, 1985); *P. sundbergi sundbergi*, from Praslin, Curieuse, Aride and surrounding islands, and *P. sundbergi ladiguensis*, from La Digue, Mariane, Grande and Petite Soeur and surrounding islands (Fig. 1). Gardner (1986; 1987) examined variation within this species in a comprehensive multivariate morphometric study (based on body size, scalation and colour pattern variables) and found support for three phenetic groups, corresponding to *P. s. longinsulae*, *P. s. sundbergi* and *P. s. ladiguensis* (the latter two being clearly more similar between themselves), but not for the distinction of the North and Silhouette forms, *rubra* and *umbrae*, which he synonymised with *P. sundbergi longinsulae*, as well as *P. (sundbergi) longinsulae menaiensis* (sensu Mertens, 1966), a putative subspecies from the coralline islands of Cosmoledo described in some detail by Cheke (1982). Within *P. astriata* two geographically disjunct subspecies are recognized; *P. astriata astriata* and *P. astriata semicarinata*, from the southern and northern group of islands respectively (Figure 1), with populations from Fregate described as "intermediate forms" and even hypothesized to be hybrids between the two subspecies (Cheke, 1982). Individuals from Astove (coralline islands) are sometimes referred to as *P. astriata astovei*, but were considered by Cheke (1982) to have clear affinities with the nominate *astriata* on Mahé. An additional species occurs on the raised coralline islands of Aldabra and Assumption (*P. abbotti*), but it does not seem to be closely related to the former ones, resulting instead from an independent colonization from Madagascar (Rocha et al., 2009).

The degree of morphological differentiation between and within species and subspecies varies geographically. Notoriously, body size variation shows no clear geographic pattern within *P. astriata*, while clearly increases from south to north within *P. sundbergi*, with *P. s. sundbergi* and *P. s. ladiguensis* individuals being significantly larger than *P. s. longinsulae* (Gardner, 1984; Radtkey, 1996). Radtkey (1996) used body size, ecological and molecular information to explore the diversification of this endemic radiation and proposed allopatric speciation followed by asynchronous dispersals across island groups and resource competition generating character displacement as the drivers of body size evolution and speciation within this group. More specifically, he proposed that, after the invasion of the Seychelles archipelago by a single species of *Phelsuma*, separate eustatic sea-level changes led first to allopatric speciation, with the ancestor of *P. sundbergi* and *P. astriata* differentiating in the southern and northern groups of islands, respectively, and then to the dispersal of these species across island groups. The differences in the relative timing of the dispersal between island groups (with the ancestor of *P. s. sundbergi* and *P. s. ladiguesis* colonizing the northern group of islands much earlier than the ancestor of *P. astriata astriata* colonized the southern group) putatively resulted in *P. sundbergi* evolving an intermediate body size in the group of islands associated with Mahé (southern) and a large body size in the group of islands associated with Praslin and La Digue (northern), due to character displacement, given the longer period of sympatry with *P. astriata*. Nevertheless, this is not the only possible explanation for the different degrees of body size disparity. Body size distributions may be governed instead by different ecological variables and interactions happening within each island group (Gardner, 1984). For example, competition and selective pressures for body size within each island or island group may be distinct. Specifically, Gardner (1984) proposed that body size has probably been influenced by the distribution of vegetation and social systems. The utilization of defensible pollen and nectar food supplies, especially the male inflorescences of *Lodoicea maldivica*, native to (and only found in) the northern islands of Praslin and Curieuse, may have resulted in the evolution of the larger body size of *P. sundbergi* in the northern islands while hardwood forests, scrub and seabird islands vegetation may have lead to smaller sizes. The competitive relationships between the species are then determined by their body size differences and support the selective pressures within the system. Furthermore, sympatric species within each island may be partitioning their habitat differently or diversifying along different axis (eg. Ackerly et al., 2006; Losos, 2010; Noble et al., 2010; Schluter, 2000), resulting in the current pattern of body size distribution, without the need to invoke their

asynchronous sympatry as its causing mechanism. In the Western Indian Ocean archipelago of Mauritius for example, sympatric species of *Phelsuma* often differ across several resource axes at the same time (Harmon et al., 2007). If the times since contact between the different species in the Seychelles are not different, ecological factors and variation on selective pressures across islands must play an important role on diversification of Seychellois *Phelsuma*, which may in turn be an excellent model to study the influence of habitat variability and ecological opportunity in diversification. It is also not known if these species hybridize at any island group, and the relationship between habitat diversity, habitat partitioning and reproductive isolation is also an outstanding question that can potentially be explored using this system.

The biogeographic scenario proposed by Radtkey (1996) was largely based on his estimate of the phylogeny, and on the different pairwise genetic distances between the different intraspecific mtDNA clades, but this variance in gene divergence can also be due to stochasticity and different levels of ancestral polymorphism within each ancestor population predating isolation (Edwards and Beerli, 2000; Hickerson et al., 2006). The degree to which gene divergence predates population divergence can be substantial when the effective population size (*Ne*) of the common ancestor is large relative to the divergence time (Edwards and Beerli, 2000). In fact, recent works emphasize the need for model-based approaches in testing for the effects of shared historical factors (Hurt et al., 2009; Knowles, 2009). Moreover, with only a single non-recombining locus, and especially if reciprocal monophyly has been achieved, it is not possible to differentiate between variance due to divergence times and that resulting from ancestral coalescence. Indeed, incorporating DNA sequence from multiple unlinked loci is the most effective way to improve the accuracy and precision of historical parameter estimates (Edwards and Beerli, 2000; Wakeley and Hey, 1997).

Here we use multilocus DNA data and a comprehensive sampling across the Seychelles archipelago to describe the intraspecific genetic variation within both species and its relation to geography, re-assessing the current taxonomy, possible colonization routes and the geographic origins of outer islands populations. Then we employ coalescent approaches that explicitly model gene genealogies to infer the underlying species phylogeny, to test for gene flow across species and subspecies after initial divergence, and finally to test the previous hypothesis of asynchronous divergence within each species, providing a time scale for the diversification of this radiation.

This phylogeographic approach will help us to better understand the influence of oscillating sea-levels and putative selective pressures throughout the last few million years in shaping diversification across these islands, and provide a valuable framework upon which the ecological determinants of diversification within this group can be further explored.

## Material and Methods

### *Sampling and Molecular Data Collection*

Tissue samples of *Phelsuma sundbergi* and *P. astriata,* covering almost their entire distribution were collected and stored in 100% ethanol. DNA extraction followed standard salt or phenol-chloroform protocols (Kocher et al., 1989; Sambrook et al., 1989). Cytochrome-*b* (*cyt-b*) was amplified in 126 *P. sundbergi* individuals and 73 *P. astriata* individuals, comprising all recognized morphological variation within each species (Fig. 1). For all four nuclear markers (see below), between one and ten individuals per island per species were genotyped (online Appendix 1).

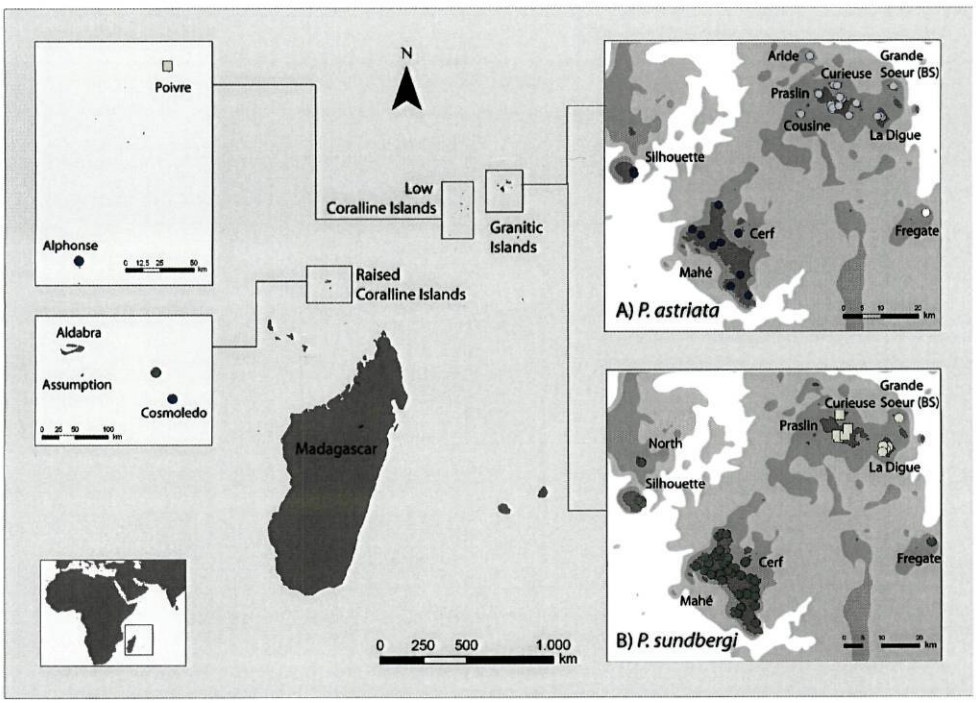



Figure 1 – Sampling sites for *Phelsuma* species along the granitic and coralline Seychelles islands. Detailed locations and accession numbers are given in online Appendix 1. Species and subspecies are colour coded: dark blue circles, *P. astriata astriata*; light blue circles, *P. astriata semicarinata*; white circles, *P. astriata* (Fregate population); dark green circles, *P.*

*sundbergi longinsulae*; light green squares, *P. sundbergi sundbergi*; light green circles, *P. sundbergi ladiguensis*. Within the granitic islands (a and b), different shadings show areas that would be emerged at -30 m (dark grey) and -50 m (light grey) below present sea-level stands. Low-stands below 60 m would allow contact between all islands in this granitic group. Current data on Indian Ocean sea level changes support low-stands of up to 145 ± 5 m below present sea level (BPSL) at six episodes in the last 0.5 Myr, some of which persisted for up to 50,000 years at a time (see Warren et al., 2010, Figure 2a) and geological records going back further suggest low-stands of 80 - 120 m BPSL at 0.64, 0.88, 1.04, 1.26 and 1.54 MA, and eleven additional low-stand episodes of 50 m or more BPSL during the last 5 Myr (Miller et al., 2005 – see also supplementary Fig. 1).

Primers for cytochrome-*b* were CBL14753 (Austin et al., 2004) and CB3H (Palumbi et al., 1991) with amplification conditions as given in Rocha et al. (2009). Phosducin (PDC) amplification was made with primers and conditions from Gamble et al. (2008), oocyte-maturation factor MOS (C-mos) using primers Mos-F and Mos-R and conditions as in (Godinho et al., 2005) recombination-activating gene 2 (Rag2) as in Rocha et al. (2009), melanocortin-1 receptor (MC1R) and intron 61 of the reelin gene (RELN*int*61) using primers and conditions from Pinho et al. (2010). Sequences were determined by a commercial facility (Macrogen, Seoul, Korea). For most individuals both strands of nuclear fragments were sequenced to assure double peaks were real and not basecalling errors.

Sequences were aligned manually using BioEdit (Hall, 1999). For the nuclear genes, we resolved haplotype phases using two complementary methods: first, for sequences that were heterozygous for insertions or deletions (some individuals for the RELNint61 fragment), we used the method described by Flot et al. (2006), after which we applied the Bayesian algorithm implemented in PHASE software (Stephens et al., 2001) using the known phases of haplotypes determined by the previous method, when available. For the remaining genes PHASE was applied directly to the datasets. We considered all positions for which phases where resolved with a posterior probability higher than 0.8 (though the majority of them were actually resolved with 1 or >0.9 probability). Remaining positions were coded as missing data (N) or with ambiguity codes for subsequent sequence analysis as appropriate, but complete individuals were never excluded. Multiple runs of PHASE were performed for each dataset and phase calls checked for consistency.

*Genetic Variability, Neutrality Tests and Gene Genealogies Estimation*

To assess how genetic variability was distributed across and within species we calculated a series of summary statistics for each marker and putative subspecies (Cheke, 1982; Gardner, 1987). As only three individuals of *P. astriata* from the Fregate population (the "intermediate form" sensu Cheke, 1982) were collected, summary statistics were not computed for this small set.

For each molecular marker, we calculated the number of haplotypes, the number of segregating sites, haplotype diversity, nucleotide diversity $\pi$, and population mutation parameter $\theta$ (Watterson, 1975). We tested for non-neutral evolution by computing Tajima's D (Tajima, 1989). Departures from a mutation-drift equilibrium, such as those caused by positive selection or demographic expansions, would lead to negative values of this statistic. We further tested each population for signals of demographic expansions by calculating Fu's Fs (Fu, 1997) and $R_2$ (Ramos-Onsins and Rozas, 2002). Population growth is expected to lead to an excess of rare alleles, resulting in negative values of Fs and low values of $R_2$. We tested significance of the three tests using 10,000 coalescent simulations. All these analyses were conducted in DnaSP v5 (Librado and Rozas, 2009).

We used MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003) to obtain an estimate of the *cyt-b* phylogeny (BI). The dataset was reduced to single haplotypes using ALTER (Glez-Peña et al., 2010) and the best fitting model estimated using jModeltest (Posada, 2008) and PhyML (Guindon and Gascuel, 2003), under the AICc criteria (Posada and Buckley, 2004; Sugiura, 1978). Multiple runs of 11 million generations with default heating parameters were performed. Convergence and congruence across runs were assessed using AWTY (Nylander et al., 2008). MEGA v4 (Kumar et al., 2008) was used to estimate distance between clades. Median-joining networks (Bandelt et al., 1999) with MP posterior optimization (Polzin and Daneschmand, 2003) were constructed using NETWORK v4.510 (available from http://fluxus-engineering.com) and used to illustrate the relationships between nuclear and mitochondrial haplotypes (applied separately for each intraspecific clade in the latter).

*Testing for the Asynchronous Colonization Hypothesis: Estimates of Divergence Times and Gene Flow under the Isolation-with-Migration model*

Our results with respect to genetic variation and structure at the mtDNA level revealed, in agreement with the morphological variation and taxonomy, two distinct clades within each species. These correspond to the northern and southern groups of islands, hypothesized by Radtkey (1996) to have evolved through an history of asynchronous dispersals between island groups (see results for further details). To test this hypothesis with our multilocus data we employed the isolation-with-migration model implemented in IMa2 (Hey, 2010; Hey and Nielsen, 2007). We were particularly interested in testing for the coincidence, or lack of it, in the estimates of time since divergence between the northern and southern clades, but also in testing for the existence of gene flow between them after initial divergence. Therefore, we fitted each species data to a two-populations model (the northern and southern clades of each species, respectively), estimating the six parameters involved: ancestral and current population sizes ($\theta_A$, $\theta_1$, and $\theta_2$), migration in both directions ($m_1$ and $m_2$) and time since the population split ($T$). One individual of *P. sundbergi longinsulae* from Mahé island that carried a haplotype which clustered within *P. sundbergi sundbergi* haplotypes from Praslin was excluded from the analysis, as it could possibly reflect an anthropogenic introduction (see results and discussion). Within *P. astriata*, the Fregate population (N=3) was excluded from the analysis (see discussion).

Because IMa2 assumes no intragenic recombination or selection we tested each locus for recombination using the pairwise homoplasy index (Bruen et al., 2006) implemented in PhiPack, and performed multiple loci HKA tests (Hudson et al., 1987) using SITES and HKA (available respectively at www.math.auckland.ac.nz/~bryant/software/ and http://genfaculty.rutgers.edu/hey/software).

The HKY (Hasegawa et al., 1985) model was used for the mtDNA dataset and also for Rag2, RELN*int*61 and MC1R nuclear gene fragments, where homoplasic positions were detected, and the IS model (Kimura, 1969) for PDC. Prior bounds were first selected based on the summary statistics and optimized and adjusted in subsequent runs. Multiple runs of each dataset were performed and effective sample size of parameters (ESS) and trend plots examined to assure proper mixing and convergence. The significance of the estimated migration rates was measured by the LLR test of Nielsen and Wakeley (Nielsen and Wakeley, 2001). A substitution

rate of 1% (mtDNA) and a generation time of one year (Gardner, 1984) were used to convert parameter estimates into biologically meaningful quantities.

Additionally, to obtain an estimate of the divergence time between *P. sundbergi* and *P. astriata*, and to test for the existence of post divergence gene flow across species, we also fitted the isolation-with-migration model to the whole dataset, treating each species as a separate population.

*Species-Tree Inference*

To obtain a multilocus perspective of the diversification within the whole radiation, we employed the Bayesian MCMC method from Heled and Drummond (2009), *BEAST, implemented in BEASTv1.5.3 (Drummond and Rambaut, 2007). This method makes use of the multispecies coalescent model to co-estimate the multiple gene trees and the "species-tree" they are embedded into, together with splitting times and effective population sizes of extant and ancestral species. Especially at this shallow level of divergence, it is likely that multiple processes such as demography and random sorting influence the gene genealogies and the implementation of a method that properly accommodates this variation, rather than a concatenation approach, becomes especially relevant (Edwards, 2009; Leache, 2009 and references therein). A minimum of two individuals per "species" (the term species not necessarily referring to the taxonomic rank but to any diverging "population structure" – in our case the five subspecies) per loci is needed, while additional individuals increase accuracy (Heled and Drummond, 2009). We therefore constructed a dataset with a subsample of our individuals, using some of those that were genotyped for the five genes: 19 *P. sundbergi longinsulae*, 8 *P. sundbergi sundbergi*, 5 *P. sundbergi ladiguensis*, 20 *P. astriata astriata*, 22 *P. astriata semicarinata* and finally 2 individuals from *P. astriata* from Fregate (online Appendix 1).

We estimated the appropriate model of evolution for each fragment as previously and implemented it choosing the most similar within the ones available in BEASTv1.5.3. A substitution rate of 1% with a 0.27% standard deviation (as in Paulo et al., 2008) was assumed for the mtDNA fragment as a normal prior distribution in order to calibrate the tree. Similar rates (~1%) have been consistently recovered for mtDNA fragments of other lizards at the intrageneric level (Carranza et al., 2008; Chapple et al., 2009; Cox et al., 2010; Miralles and Carranza, 2010), and in the absence of any available external calibration, we feel it as an

appropriate prior. Nuclear rates were co-estimated along the run relative to this. An uncorrelated relaxed clock model was assumed for the cyt-*b* dataset, whereas a strict clock was assumed for the nuclear gene fragments, given their low variability (causing runs under relaxed clock models to fail to converge). We used a Yule prior for the species tree and a population size model of continuous growth and constant root. Multiple runs of 100 million generations were performed and sampled at appropriate intervals to obtain 10,000 final samples (trees). Tracer v1.5 was used to visualize the results of each run, to check the effective sample size of each parameter and chose appropriate burnin values (Rambaut and Drummond, 2007). After discarding the burnin samples, the consensus species tree, node heights and supports were obtained using TreeAnnotator v1.5.3 (Drummond and Rambaut, 2007) and visualized using FigTree v1.2.3 (Rambaut, 2009)

## Results

*Genetic Variability and Neutrality*

The molecular markers used showed considerable variation in the levels of polymorphism observed (Table 1). Overall, *cyt-b* and RELN*int*61 were the most variable fragments. The variability levels within each subspecies were not remarkably different, although they were slightly higher within *P. sundbergi longinsulae*. Tajima D and Fu's Fs were usually negative while $R_2$ values were usually small, suggesting demographic growth across all groups. Only in a few instances (*P. sundbergi longinsulae* for the mitochondrial and Rag-2 markers and *P. astriata semicarinata* for the mitochondrial marker) were values significant for all statistics at once.

**Table 1 -** Summary diversity statistics, tests of neutrality and indexes of populations growth for all subspecies of *P. sundbergi* and *P. astriata* [a]. N, number of individuals (mtDNA) / chromossomes (nucDNA). Polymorphism indexes: H, number of haplotypes detected; S, number of segregating sites; Hd, haplotype diversity; $\pi$, nucleotide diversity; $\theta w$, population mutation parameter (Waterson, 1975). Neutrality test: D, Tajima's D. Population growth tests: $F_S$, Fu's $F_S$; $R_2$, Ramos-Onsins and Rozas' $R_2$.* $p< 0.05$; ** $p< 0.01$

| | | | | Polymorphism | | | | | Neutrality | Population growth | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Subspecies | Locus | Lenght | N | H | S | Hd | $\pi$ | $\theta w$ | D | $F_S$ | $R_2$ |
| *P. sundbergi longinsulae* | Cyt *b* | 761 | 93 | 28 | 30 | 0,875 | 0,00362 | 0,00772 | -1,62892* | -17,813* | 0,0445* |
| | MC1R | 617 | 58 | 13 | 10 | 0,758 | 0,00261 | 0,00358 | -0,76192 | -5,943* | 0,0759 |
| | RELN*int*61 | 877 | 52 | 19 | 21 | 0,903 | 0,00401 | 0,00587 | -1,02213 | -6,903* | 0,0798 |
| | RAG-2 | 796 | 136 | 17 | 15 | 0,554 | 0,00089 | 0,00345 | -1,97263* | -1,97263* | 0,0261* |
| | PDC | 360 | 56 | 7 | 6 | 0,710 | 0,00255 | 0,00363 | -0,74314 | -2,145 | 0,0772 |
| *P. sundbergi sundbergi* | Cyt *b* | 761 | 17 | 6 | 12 | 0,588 | 0,00234 | 0,00466 | -1,87150* | -0,889 | 0,0983 * |
| | MC1R | 617 | 24 | 9 | 7 | 0,853 | 0,00391 | 0,00312 | 0,81826 | -2,265 | 0,1645 |
| | RELN*int*61 | 877 | 22 | 12 | 19 | 0,835 | 0,00364 | 0,00596 | -1,44378 | -4,329* | 0,0839 |
| | RAG-2 | 796 | 34 | 6 | 5 | 0,371 | 0,00051 | 0,00154 | -1,76914* | -4,537* | 0,0611 |
| | PDC | 360 | 22 | 7 | 6 | 0,762 | 0,00422 | 0,00457 | -0,23808 | -1,875 | 0,1186 |
| *P. sundbergi ladiguensis* | Cyt *b* | 761 | 7 | 5 | 6 | 0,857 | 0,00300 | 0,00322 | -0,33869 | -1,262 | 0,1543 |
| | MC1R | 617 | 10 | 5 | 4 | 0,844 | 0,00223 | 0,00229 | -0,09820 | -1,468 | 0,1652 |
| | RELN*int*61 | 877 | 12 | 6 | 8 | 0,758 | 0,00211 | 0,00303 | -1,20688 | -1,540 | 0,1351 |
| | RAG-2 | 796 | 14 | 2 | 1 | 0,143 | 0,00018 | 0,00040 | -1,15524 | -0,595 | 0,2575 |
| | PDC | 360 | 14 | 7 | 6 | 0,890 | 0,00394 | 0,00524 | -0,89259 | -3,209* | 0,1071* |
| *P. astriata astriata* | Cyt *b* | 761 | 20 | 10 | 18 | 0,895 | 0,00541 | 0,00667 | -0,71027 | -1,539 | 0,1044 |
| | MC1R | 617 | 30 | 8 | 8 | 0,660 | 0,00279 | 0,00327 | -0,44757 | -1,813 | 0,1059 |
| | RELN*int*61 | 877 | 30 | 11 | 10 | 0,867 | 0,00356 | 0,00289 | 0,73543 | -2,134 | 0,1555 |
| | RAG-2 | 796 | 40 | 3 | 2 | 0,188 | 0,00024 | 0,00059 | -1,11593 | -1,548 | 0,0858 |
| | PDC | 360 | 30 | 3 | 2 | 0,439 | 0,00132 | 0,00140 | -0,12690 | -0,044 | 0,1184 |
| *P. astriata semicarinata* | Cyt *b* | 761 | 46 | 19 | 20 | 0,865 | 0,00289 | 0,00598 | -1,67207* | -12,687** | 0,0499* |
| | MC1R | 617 | 64 | 6 | 4 | 0,445 | 0,00084 | 0,00137 | -0,82812 | -2,856* | 0,0651 |
| | RELN*int*61 | 877 | 56 | 10 | 11 | 0,728 | 0,00307 | 0,00277 | 0,30059 | -0,596 | 0,1193 |
| | RAG-2 | 796 | 92 | 4 | 3 | 0,184 | 0,00024 | 0,00074 | -1,23597 | -2,748 | 0,0445 |
| | PDC | 360 | 58 | 3 | 2 | 0,101 | 0,00028 | 0,00120 | -1,31498* | -2,516* | 0,0673 |

[a] Statistics were not computed for *P. astriata* population from Fregate as only three individuals were sampled.

*Intraspecific mtDNA Variability*

The two species, *P. sundbergi* and *P. astriata* form two separate mtDNA clades (Fig. 2) (Nei's Da=11%). *Phelsuma sundbergi longinsulae* and the more closely related *P. sundbergi sundbergi* and *P. sundbergi ladiguensis* are also distinct lineages (Da=2.5%). Within *P. sundbergi longinsulae*, the North and Silhouette populations share or carry haplotypes closely related to Mahé. The Silhouette population is paraphyletic relative to Mahé, and North exhibits a single haplotype, shared with individuals from Mahé (Fig. 2b). Furthermore, all Fregate individuals exhibit a unique haplotype just one mutational step distant from the most abundant haplotype from Mahé. The haplotype of one *P. sundbergi* individual from Mahé (2MA206) clusters with *P. sundbergi* haplotypes from Praslin. We provisionally interpreted this as an anthropogenic related pattern (see Discussion), so we excluded this individual for the inference of divergence times and gene flow across main clades. Concerning the *P. sundbergi* populations from the northern group of Praslin, La Digue and associated islands, i.e., *P. sundbergi sundbergi* and *P. sundbergi longinsulae*, they form two slightly divergent mtDNA clades, in concordance with their recognized morphological variation: Praslin and Curieuse populations in one clade and La Digue and Grande Soeur populations in the other clade (Fig. 2). Two individuals from La Digue exhibit haplotypes that are more closely related to haplotypes from Praslin. Again, these were interpreted as anthropogenic dispersals and were excluded from analysis as adequate (see Discussion). Within *P. astriata* the geographic distribution of mitochondrial lineages agrees with the currently recognized subspecies but the divergence between them is lower, with just three mutational steps separating haplotypes from *P. astriata astriata* and *P. astriata semicarinata* and also two and three mutations separating both these subspecies from the "intermediate form" from Fregate, from where the three individuals genotyped exhibit the same mtDNA haplotype. Nevertheless, these subspecies do not share haplotypes in our sample (Fig. 2c).

All the outer island populations share mtDNA haplotypes with the granitic islands populations. The Cosmoledo populations of *P. sundbergi longinsulae* are indistinguishable from those from Mahé, as well as the *P. astriata astriata* haplotypes from Alphonse are shared with Mahé populations. Similarly, the Astove population of *P. astriata astriata* shares its (single) haplotype with Mahé, Cerf and Silhouette individuals, and individuals of *P. sundbergi sundbergi* from Poivre exhibit the same haplotype as most of the individuals from Praslin.

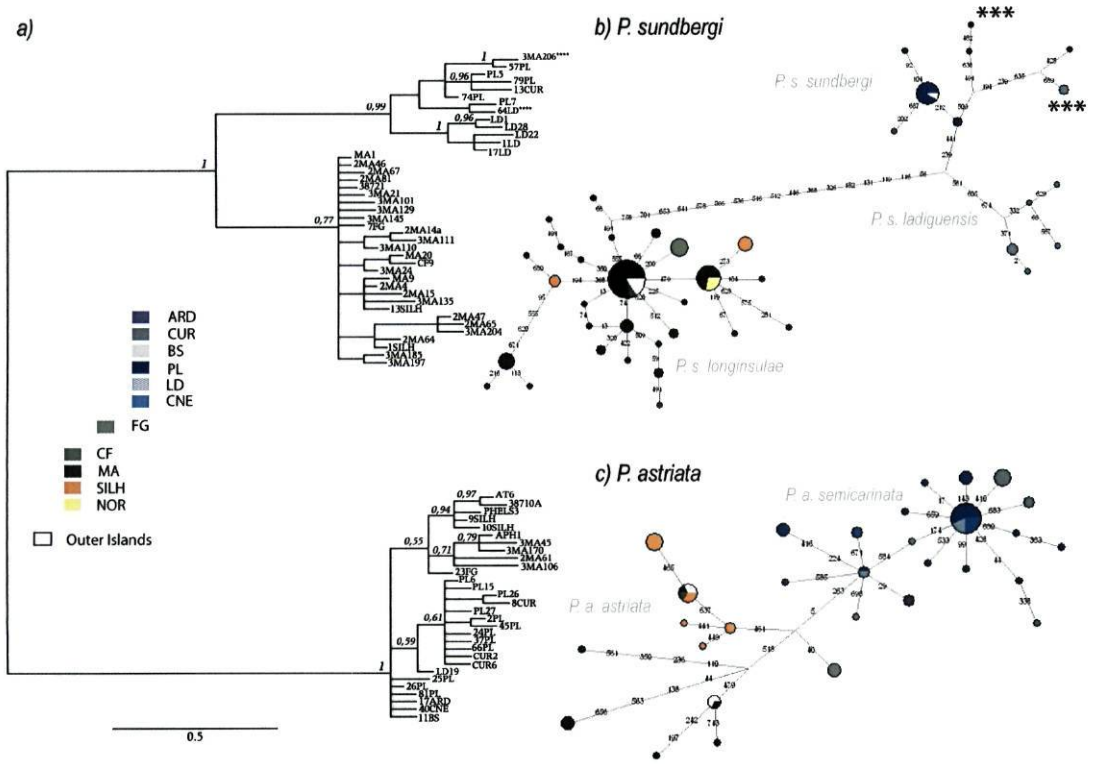



**Figure 2** – a) MtDNA haplotype phylogeny. Midpoint-rooted Bayesian 50% majority-rule consensus tree with posterior probabilities indicated above branches. Highlighted haplotypes (***) most probably represent human-related introductions (see text). Median-Joining networks for *P. sundbergi* (b) and *P. astriata* (c). Islands are colour-coded and circle size is proportional to the number of individuals. Numbers across branches represent mutations (corresponding to alignment position). Islands labelling is as follows: Aride (ARD), Curieuse (CUR), Grande Soeur (BS), Praslin (PL), La Digue (LD), Cousine (CNE), Fregate (FG), Cerf (CF), Mahé (MA), Silhouette (SILH) and North (NOR).

*Nuclear DNA Genealogies*

The nuclear variation is almost completely sorted between species (Fig. 3). *P. astriata* and *P. sundbergi* share only a single haplotype (PDC), central in the network and the most in *P. astriata*. Nevertheless, the overall divergence between species is not high and the different subspecies commonly share haplotypes.

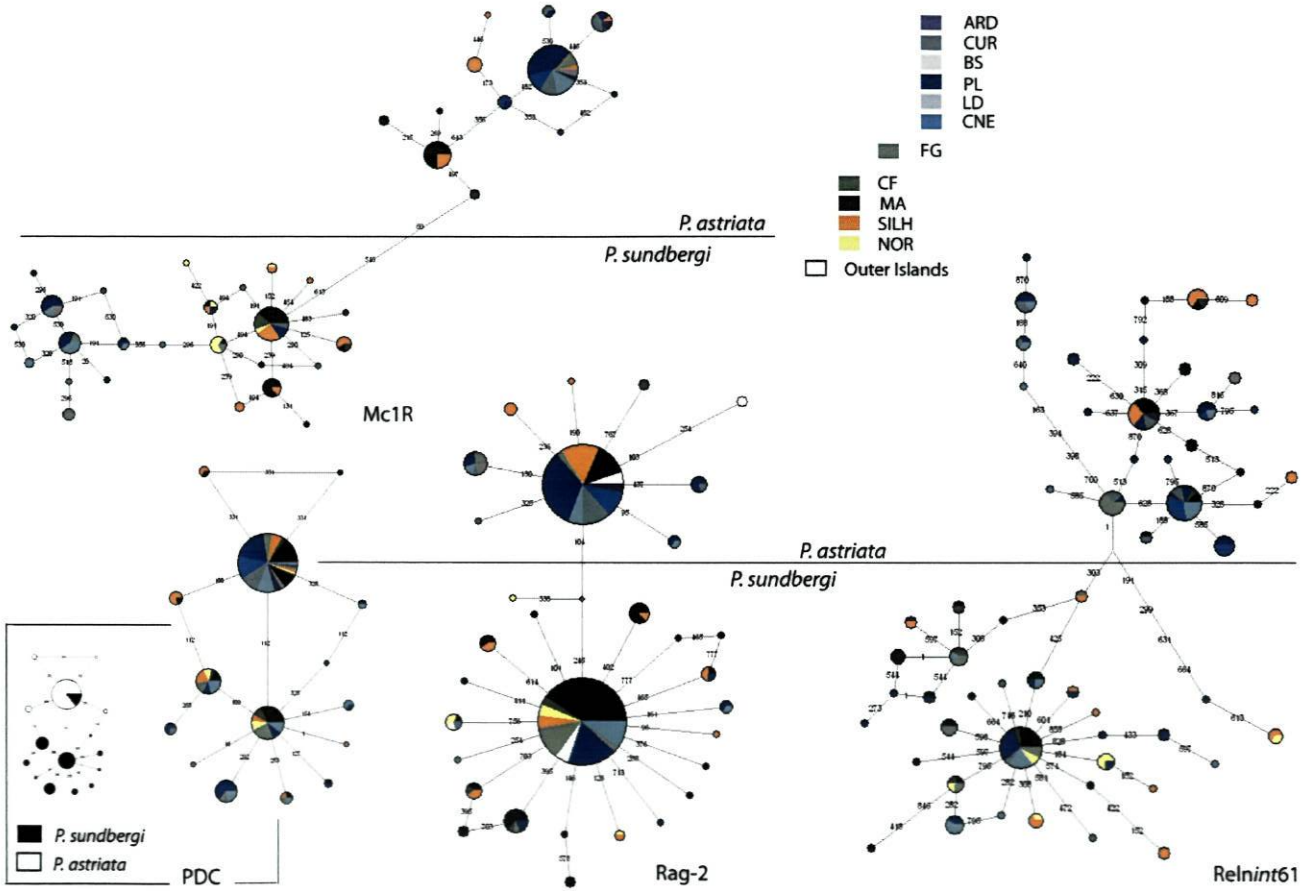



**Figure 3** - MJ networks representing nuclear DNA variation. Islands are colour-coded and circle size is proportional to the number of individuals. Labelling is the same as in Figure 2. Numbers along branches represent mutations (corresponding to alignment position). Clades above and below the horizontal bar correspond to *P. astriata* and *P. sundbergi*, respectively (except for PDC where haplotype sharing is observed – inset network).

*Isolation-with-Migration Analysis*

Neither recombination nor selection was detected at any marker. Using the isolation-with-migration model we obtained consistent estimates of demographic parameters across all runs, with high ESS values, convergence and a good sampling of the parameter space.

The estimated time since divergence between the two species was 6.39 million years (Myr), with a 95% posterior density interval of 4.264 – 8.397. No gene flow was detected between the species and the ancestral population size is very low (Table 2). Estimates of the splitting times between the southern and northern clades of both species that are fairly coincident (at around 500,000 years old) as also are their posterior densities (Table 2 and Fig. 4). Nevertheless, the 95% posterior density intervals reflect a possible variance of the estimates by a factor of 10 (Table 2). The effective population sizes estimates for *P. sundbergi* were more than the double than for *P. astriata*, irrespectively of the group of islands. In agreement, the estimated effective ancestral population sizes were also higher in *P. sundbergi*. Significant migration rates were recovered within both species, although lower for *P. astriata*.

**Table 2** – Isolation-with-migration estimates. Estimates and 95% highest posterior densities (HPD) of population sizes ($\theta$), effective number of migrants ($2Nm$) and time since divergence ($T$) between groups, as inferred with IMa2. Values for migration that are significantly different from zero (LLR test: * p-value <0.05; ** p-value <0.01, Nielsen and Wakeley 2001) are signed with asterisk. A substitution rate of 1% per site per million year and a generation time of one year were assumed to convert the estimates into absolute quantities.

| | Parameter Estimates | | | |
|---|---|---|---|---|
| Group / Species | Ancestral Effective Population Size *Ne* [95%HPD] (millions of individuals) | Current Effective Population Size *Ne* [95%HPD] (millions of individuals) | Effective Number of Migrants *into the population* *2Nm* [95%HPD] (millions of individuals) | Time Since Divergence *T* [95%HPD] (in million years) |
| *P. s. sundbergi +P. s. ladiguesis* | 0.600 | 1.826 | **1.584** | |
| (northern) | [0.104-2.452] | [1.046-3.148] | **[0.3513 – 3.59]**** | 0.536 |
| *P. sundbergi longisulae* | | 3.584 | 0.0075 | [0.266-1.901] |
| (southern) | | [2.228-5.598] | [0 – 1.995] | |
| *P. astriata astriata* | 0.296 | 0.764 | 0.1311 | |
| (northern) | [0.0014 – 0.868] | [0.424-1.280] | [0 – 0.692] | 0.580 |
| *P. astriata semicarinata* | | 0.752 | **0.3910** | [0.251-2.876] |
| (southern) | | [0.390-1.436] | **[0 – 1.503] *** | |
| *P. sundbergi* | 0.002 | 2.880 | 0.0006 | |
| | [0 – 2.408] | [2.143 – 3.737] | [0 – 0.1763] | 6.39 |
| *P. astriata* | | 0.765 | 0.0003 | [4.264 – 8.297] |
| | | [0.564 – 1.035] | [0 – 0.08] | |

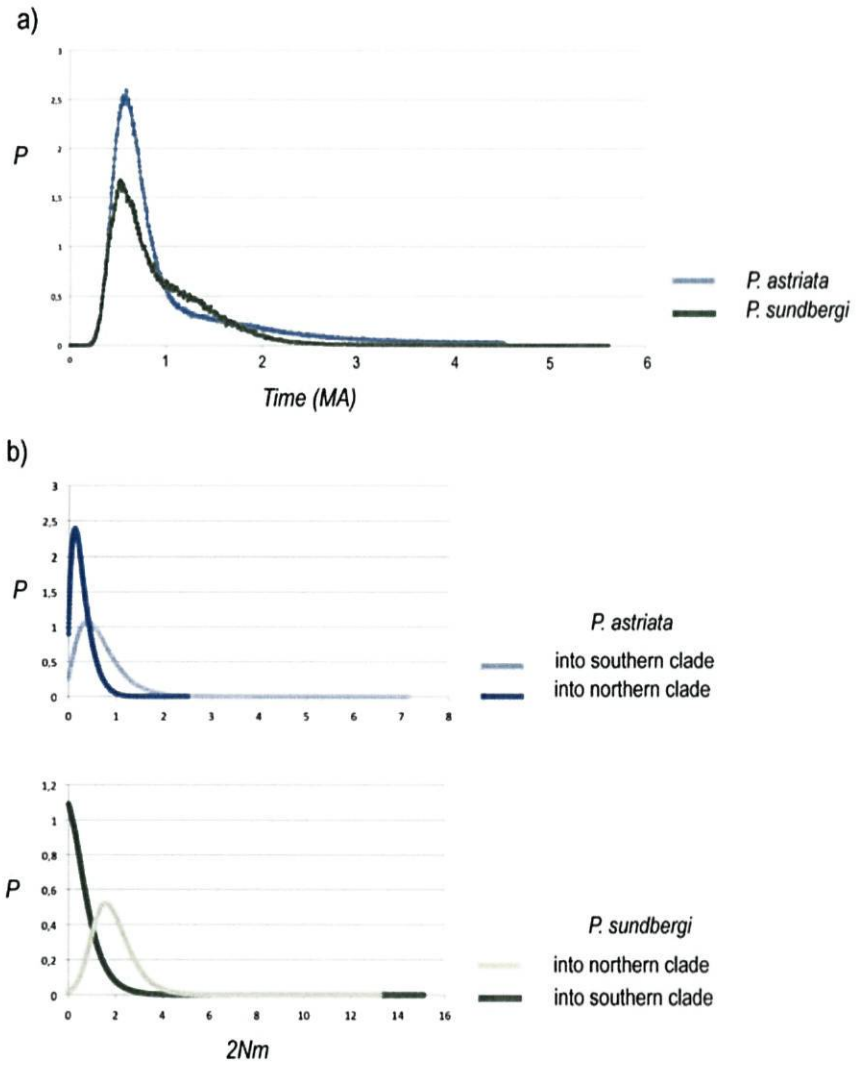



**Figure 4 –** a) Probability distribution for the splitting time between northern and southern clades of *P. astriata* (blue) and *P. sundbergi* (green); b) effective number of migrants (2*Nm*) between those same clades within *P. astriata* (blue) and *P. sundbergi* (green).

*Seychelles' Phelsuma Species Tree*

The *BEAST analysis recovered consistent results across replicates, with high ESS values for all parameters of interest. The maximum clade credibility species-tree (Fig. 5) was in general agreement with inferences made under the isolation-with-migration model in respect to the ages of the branching events. The initial split between the two species (*P. sundbergi* and *P. astriata*) is dated to circa 4Myr ago (MA), although, again, 95% HPD age intervals are too wide for a reliable estimate. Notably, the 95% HPD intervals for the north-south split for both species clearly overlapped. The population of *P. astriata* from Fregate (included here though excluded from the IM analysis) was recovered as sister taxa to *P. astriata semicarinata* (from the northern group), although with relatively low support and node height intervals that could be concordant with an almost simultaneous separation of the three populations. The estimate of the divergence time between the species obtained under the multispecies coalescent was younger than that obtained under the IM model (Table 2) although both are much older than the estimates of diversification within each species.

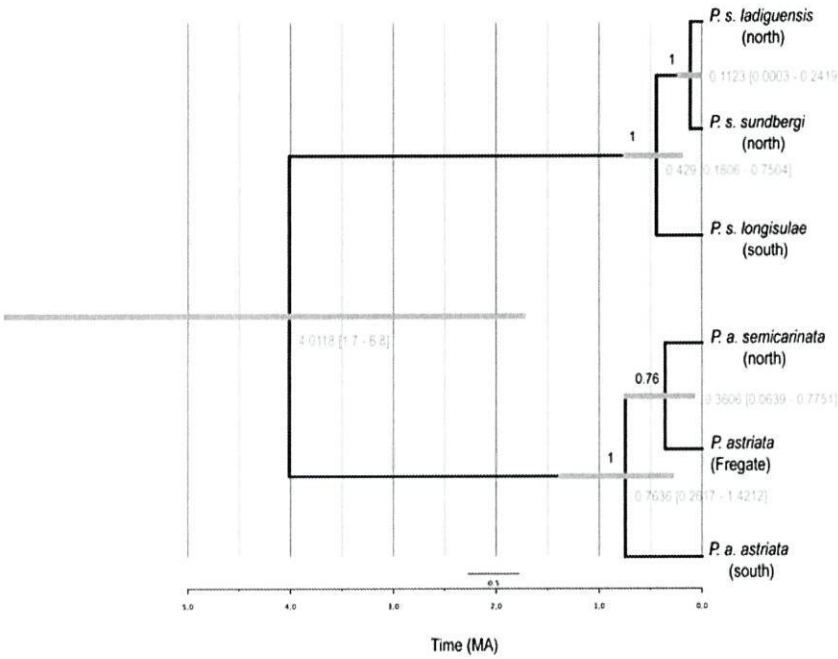



**Figure 5** – "Species" tree of the *Phelsuma* day-geckos endemic from the Seychelles. The tree shown corresponds to the maximum clade credibility tree with mean estimates used for node heights and it is based on the multispecies coalescent analysis of the five molecular markers. Clade posterior probability is indicated above each branch. Node bars correspond to the 95% high posterior credibility intervals for node height (age), that are given below/in front of each node. Horizontal axis corresponds to time in million years before present (MA).

## Discussion

Multilocus approaches and model-based analysis are becoming not only feasible but essential to our understanding of the processes underlying patterns of genetic variation within and between closely related species. In this case they were essential for a better understanding of the time frame and gene flow dynamics of *Phelsuma* within the Seychelles, providing valuable insights to properly address the factors promoting the diversification of this group. Compared to other archipelagos like the Mascarenes or the Comoros, *Phelsuma* radiated much less in the Seychelles, with only two extant species, as opposed to 5 and 10 in the Comoros and Mascarenes, respectively. A determinant factor leading to this low diversification rate must have been the reduced effective geographic isolation between these island populations. As most of the islands can be united with very small reductions of the sea level (Fig. 1), they spent in fact most of the time during the Miocene and Pleistocene connected (see Miller et al., 2005; Warren et al., 2010). Current patterns of genetic structure seem to reflect this predominant role of gene flow at smaller distances, with the biggest differentiation within both species being between the northern and southern groups of islands. Our data (as previous data from Radtkey, 1996) are in agreement with a scenario of allopatric differentiation leading to the formation of the two species, *P. astriata* and *P. sundbergi*. Each species is a monophyletic unit rather than each island. Genetic variation is completely sorted for mtDNA markers and for almost all nuclear markers except for PDC. Finally, there is no evidence of gene flow between the species after the divergence, suggesting complete reproductive isolation when they came into sympatry.

### *Intraspecific Relationships - Phylogeographic Patterns of mtDNA Variation*

The intraspecific patterns of mtDNA variation and geographic structure detected are in agreement with previous arguments from Gardner (1987) and Cheke (1982) with respect to the number of subspecies within each species. Gardner (1986; 1987), using morphological data, did not find any distinction between North and Silhouette populations of *P. sundbergi* and remaining *P. sundbergi longinsulae* populations, and highlighted the fact that Borner and Minuth (1985) made their descriptions based on very few specimens. Accordingly we found two main clades within *P. sundbergi*, corresponding to the northern and southern groups of islands, the latter corresponding to *P. sundbergi longinsulae* and including populations from Silhouette, North and

Fregate. It appears that isolation between Mahé, Silhouette, North and Fregate populations is too recent to be reflected at the mtDNA level. Within the northern clade, a slight differentiation was observed between the western and eastern islands, corresponding to *P. sundbergi sundbergi* and *P. sundbergi ladiguensis*. In Mahé and La Digue we found haplotypes that cluster within different clades/subspecies than they were supposed to (*P. sundbergi sundbergi* in both cases). We did not take detailed morphological measurements necessary to assess the subspecific status of these individuals but considering the genetic distance between these populations/subspecies it seems reasonable to assume these might be cases of occasional migrants rather than ancestral polymorphism. Given the low number of individuals involved; one and two, respectively, we interpreted them as human-mediated introductions excluding them from subsequent analyses. Cheke (1982) already mentions reports of translocated individuals across some islands, which do not appear to form and maintain viable populations. Both species are abundant and attach their eggs to tree trunks and leaves, which can easily be transported along with materials carried between islands and used in human-related activities. Still, these results should be taken into consideration and the possibility of higher levels of gene flow, especially between the two northern subgroups (*P. sundbergi sundbergi* and *P. sundbergi ladiguensis*) should be further investigated.

Within *P. astriata*, the patterns of mtDNA variation also fitted the recognized morphological variation, with the northern and southern groups of islands harbouring two distinct clades that do not share haplotypes, although the mitochondrial genetic distance separating the two groups was substantially lower than in *P. sundbergi*. The individuals from Fregate described by Cheke (1982) as an "intermediate forms" or "stabilized hybrids" between *P. astriata astriata* and *P. astriata semicarinata* actually carry a mtDNA haplotype roughly equally distant from both groups. The nuclear variation assessed is unsorted between these groups and was not useful to assess their origin.

Interestingly, such a north-south main pattern of subdivision, with Fregate populations closely related to remaining southern islands, and also the western-eastern structure within *P. sundbergi* from the northern islands mirror the patterns found within *Urocotyledon inexpectata*, a gecko species co-distributed with *Phelsuma* across the granitic Seychelles (Rocha et al., submitted) and may thus reflect a more generalized biogeographic pattern.

*The origins of outer island populations*

Cheke (1982) discussed the status of the populations from Cosmoledo and Astove, keeping their designation as separate subspecies (*P. longinsulae menaiensis* and *P. astriata astovei*) on the basis of slight colour pattern variations. Gardner (1986; 1987) found no differentiation of *P. sundbergi* populations from the coralline and granitic islands and argued for their probable recent colonization or introductions by man. Accordingly, the fact that all individuals analyzed from each of these populations possess the same mtDNA haplotype, shared with populations from the granitic islands is a clear indication of their very recent origin, most probably as a result of human-related introductions. Specifically, based on mtDNA variation, the population from Cosmoledo could be assigned to *P. sundbergi longinsulae*, Poivre to *P. sundbergi sundbergi*, and the populations from Astove and Alphonse to *P. astriata astriata*.

*Interspecific Relationships and the Diversification of Phelsuma in the Seychelles*

Due to the complete reciprocal monophyly of the main clades within each species at the mtDNA level and the lack of variation and sorting at the nuclear level it seems unrealistic to depict eventual colonization sequence and routes within the Seychelles. Several colonization (and vicariance) scenarios could fit the currently observed geographic patterns of genetic variation. Moreover extinction and demographic changes certainly affected populations differently, further obscuring any historical signal to be inferred from the current patterns of diversity. The Seychelles islands lay on an extremely shallow continental shelf, with eustatic sea-level changes back from the Miocene to present (see Fig. 1 and Supplementary Fig. 1) that resulted in much of the islands spending most of the time connected. Given this scenario it is actually most probable that divergence and diversification within each species in the granitic islands were mostly driven by vicariance through cycles of allopatry and possible contact between populations, rather than through stepping stone colonization events. As also argued by Gardner (1984), current patterns of variation can be explained by the sectioning of pre-existing clinal variation due to rising sea levels. Instead of the asynchronous divergence within each species, argued by Radtkey (1996) to explain the disparate patterns of morphological divergence across island groups, our data strongly suggests the possibility of simultaneous divergence within both species across island groups, most possibly at middle-lower Pleistocene (albeit with large confidence intervals – and a

possible difference of 10X between species). Both IM and multispecies coalescent analysis largely reflect this same pattern. It makes sense that sea level changes affected co-distributed species in similar ways. Detailed data on the time and intensity of sea level changes over the past few Myr (Colonna et al., 1996; Miller et al., 2005; Siddall et al., 2003; Warren et al., 2010) provide us with a framework to understand the diversification of *Phelsuma* in the Seychelles based on allopatry and sea level changes. The first split within the group, the divergence between species, is dated to 4 - 6 MA, with large confidence intervals, depending on the method used – see results – the lowest bound at a confidence interval being circa 2 MA. At this time sea level was mostly oscillating around the current zero level, or higher (Miller et al., 2005 - see supplementary Fig. 1), favouring a scenario of allopatric divergence as soon as both groups of islands were colonized. A similar age range was found for the split between the main clades of *U. inexpectata* (Rocha et al., submitted) further supporting such a scenario. The similar genetic structure patterns in all these species suggest a strong influence of historical factors in shaping diversification patterns across different species. The proposed cooling between 3.3 and 2.5 MA led to a decrease in the sea-level during the Pleistocene, so that during the last 1.5 Myr sea level was for most of the time below its current level, although with a few exceptions that lasted for short periods of time (Miller et al., 2005; Warren et al., 2010). The mostly lower sea level period after 1.5 Mya may have facilitated the dispersal of both species across the two groups of islands while the periods with higher sea levels may have promoted the structure and non-random mating within each species, whose effects started accumulating and leading to slight differentiation between each group, finally culminating in the establishment of at least partially isolated entities whose gene flow between becomes rare, or inexistent.

The application of the IM model to date species divergence time in this case is debatable, given the high levels of within species structure observed. However, Strasburg and Rieseberg (2010) showed that most demographic parameter estimates in IMa are fairly robust to population structure over a wide range of parameter space, down to $Nm$=0.1 among conspecific populations. It is nevertheless possible that estimates of population sizes and divergence times are slightly upward biased in these cases (Strasburg and Rieseberg, 2010).

Given the different levels of genetic divergence between each intraspecific group seen at the mtDNA, very different long-term population sizes have to be assumed for each species to make them compatible with a synchronous north-south split within each. Multiple unlinked loci are extremely useful in addressing this kind of questions, as the expected variance in pairwise

genetic distances from unlinked nuclear loci is directly proportional to the ancestral *Ne*, thereby providing information on coalescent processes in ancestral populations and reducing confidence intervals surrounding estimated divergence times (e.g. Hurt et al., 2009). Accordingly, we obtained higher current and ancestral population sizes within *P. sundbergi* than within *P. astriata*. Interestingly, these estimates oppose to current census sizes for both species, especially evident in the case of *P. sundbergi* populations of the northern islands (Gerlach, 2008; Noble et al., 2010).

Assuming a simultaneous divergence between both northern and southern clades of both species leads us to hypothesize that ecological determinants had a predominant role in the diversification of this group, especially on the different degrees of morphological divergence within each island group. Body size measurements across island groups (Gardner, 1984; Radtkey, 1996) show very clearly that the mean difference in snout-vent length (SVL) between *P. sundbergi* and *P. astriata* within the northern group (Praslin, La Digue, and associated islands) is significantly different from the corresponding difference in the southern group. Gardner (1984) demonstrated the importance of body size with observations of the differential use of palm tree pollen and nectar. Palms from the genera *Lodoicea*, *Deckenia* and *Cocos* all produce male inflorescences that are actively defended for highly nutritious resources, and in all reported cases the largest individual displaced smaller individuals, regardless of the intruding species (Gardner, 1984). This author further suggested that the large body size of *P. sundbergi* in the northern group likely evolved through interference competition associated with the differential distribution of palm trees. Specifically, he argued that because *Lodoicea maldivica* (the palm species with larger size and larger inflorescences) occurs only in the northern islands of Praslin and Curieuse, the selection pressure necessary to drive the evolution of larger body size was absent in the southern group. Radtkey (1996) discarded this hypothesis arguing that competition in *Cocos nucifera* plantations was equally intense, and that this palm, along with *Deckenia*, was distributed throughout all the Seychelles. Nevertheless, *L. maldivica* (native only to the northern islands of Praslin and Curieuse), was the dominant tree on these islands, occurring across a broad range of habitats from the coast to the uplands, prior to human colonization (Fleischer-Dodgley et al., submitted) and recent data highlights the clear association of *P. sundbergi* with adult *L. maldivica* males in Praslin (Noble et al., 2010). Harmon et al. (2007) investigating the competition and community structure within *Phelsuma* from the Mascarene islands also found that one of their unique characteristics compared to other diurnal arboreal

lizards like *Anolis* is the fact that they partition the habitat between palms and non-palm trees. They also reported that interspecific interactions exist and that species rapidly react to other species presence or removal shifting their habitat use. As argued by Gardner (1984) it is therefore likely that in palm forests selection will favour adults with large body-size as they will be able to defend inflorescences successfully, ensuring a constant food supply. It seems no coincidence that all large species of this genus in the Mascarenes are also associated with potentially defensible items, in which large size may be advantageous (Gardner, 1984). In other habitats small size is probably more effective.

All this becomes relevant in the previous context and warrants that further investigation is needed on the ecological determinants of eco-morphological diversification within *Phelsuma* in the Seychelles. According to our results, the intermediate size of *P. sundbergi* in the southern group is not the result of its evolution as a solitary species to use a wider resource distribution; rather ecological factors must be invoked. Certainly species are partitioning their habitat differently within each island, leading to different outcomes in terms of selection and body size evolution. Also in Silhouette habitat segregation is considerable (with *P. astriata* reaching higher sizes than *P. sundbergi* and dominating nectiferous resources) while as in Mahé species seem to co-exist without obvious resource partitioning, although some segregation is possible as *P. sundbergi* is more abundant in hardwoods forest and *P. astriata* in banana and coconut plantations (Gardner, 1984). In Mahé, *P. astriata* seems to be much less abundant at the medium perch height where *P. sundbergi* is most frequently observed; S. Rocha, pers. obs). Further investigation on habitat use and niche partitioning within both species across island groups will be determinant for the understanding of the role of the habitat and interspecific competition in the microevolutionary patterns within this radiation.

In summary, molecular patterns of variation within *Phelsuma* species from the Seychelles largely support current taxonomy, with the status of *P. astriata* population from Fregate remaining undetermined. The anthropogenic origin of all outer islands populations is also supported. Despite extensive sympatric distributions, no gene flow was detected between the two species, whose divergence was most probably pre-Pleistocenic. The genetic structure within each species is well correlated with geography; with main mtDNA clades (subspecies) distributed exclusively across northern and southern islands. Such differentiation was probably caused by isolation due to oscillating sea levels and we detected post-divergence gene flow within both species. The divergence within each species seems to have been simultaneous,

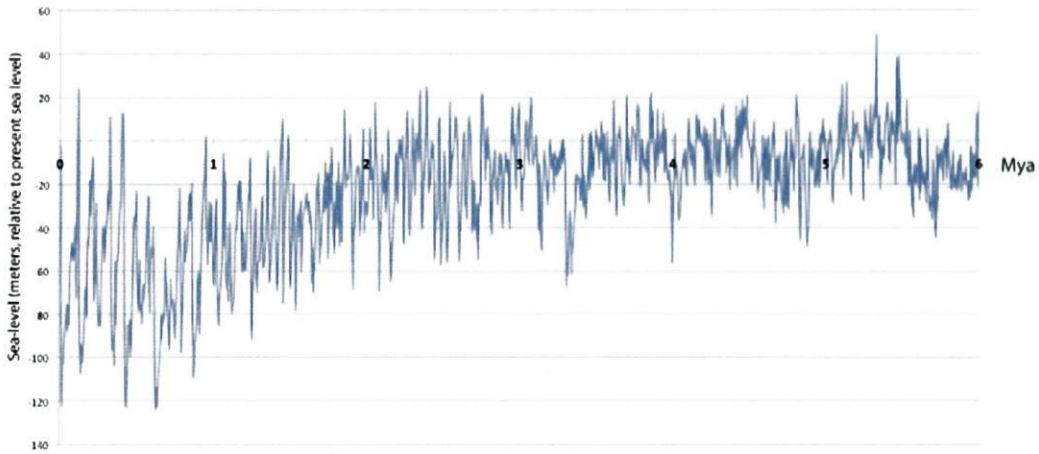
favouring an important role of differential selective pressures, competition, niche evolution, and endemic vegetation distribution in explaining the different degrees of morphological differentiation across island groups.

**Funding**

This research was supported by grants from Fundação para a Ciência e Tecnologia SFRH/BD/1745/2004 (to SR), PTDC/BIA-BDE/65745/2006 and the Gulbenkian Society

**Acknowledgments**

We are grateful to SBS of Seychelles for permits for fieldwork and tissue collection and to the Ministry of Environment and Natural Resources for logistic support during fieldwork. We thank NPTS, ICS Seychelles, SIF and several entities and persons that made fieldwork across all islands possible. An especial acknowledgement is due to Raquel Vasconcelos, Ana Perera and Andreia Silva for sharing the fieldwork and also Justin Gerlach from NPTS for support and information in earlier phases of the study. Thanks to Ana Perera for Figure 1 and to Ricardo Pereira for insightful discussions. We further thank Nancy Bunbury, Anthony Cheke, Miguel Carretero, Luke Harmon and Ricardo Pereira for helpful discussions and comments on previous versions of the manuscript.




**Supplementary Figure 1** – Global sea level estimate derived from $\partial^{18}O$ for the last 6 Myr – from Miller et al. (2005) supplementary Table S1.

**Online Appendix 1** - Locality information for the studied specimens and Genbank accession numbers. For detailed geographical coordinates refer to Location ID in Rocha et al. (2009b). Individuals used for species-tree reconstruction are marked with an *

| | | | Accession Numbers | | | | |
|---|---|---|---|---|---|---|---|
| **Species** | **Individual** | **Locality** | *Cyt-b* | *Rag-2* | Reln*int61* | *MC1R* | *PDC* |
| *P. sundbergi longisulae* | MA1 | 68 Airport, Mahé | # | FJ830318 | | | |
| *P. sundbergi longisulae* | MA7 * | 68 Airport, Mahé | # | # | # | # | # |
| *P. sundbergi longisulae* | MA8 | 68 Airport, Mahé | # | # | # | # | |
| *P. sundbergi longisulae* | MA9 | 25 Beau Vallon (beach), Mahé | # | | | | |
| *P. sundbergi longisulae* | MA10 * | 25 Beau Vallon (beach), Mahé | # | # | # | # | # |
| *P. sundbergi longisulae* | MA20 | 68B Airport, Mahé | # | | | | |
| *P. sundbergi longisulae* | 2MA3 | 22 Glacis, Mahé | # | # | | | |
| *P. sundbergi longisulae* | 2MA4 | 22 Glacis, Mahé | # | | | | |
| *P. sundbergi longisulae* | 2MA13 | 20 North2, Mahé | # | # | | | |
| *P. sundbergi longisulae* | 2MA14a | 20 North2, Mahé | # | | | | |
| *P. sundbergi longisulae* | 2MA14 | 20 North2, Mahé | # | | | | |
| *P. sundbergi longisulae* | 2MA15 | 20 North2, Mahé | # | | | | |
| *P. sundbergi longisulae* | 2MA20 | 18 NorthEast Point, Mahé | # | # | | | |
| *P. sundbergi longisulae* | 2MA26 | 40 Morne Blanc, Mahé | # | # | | | # |
| *P. sundbergi longisulae* | 2MA39 | 29 Mare aux Cochons 2, Mahé | # | # | | | # |
| *P. sundbergi longisulae* | 2MA40 | 29 Mare aux Cochons 2, Mahé | # | # | | | |
| *P. sundbergi longisulae* | 2MA42 | 29 Mare aux Cochons 2, Mahé | # | | | | |
| *P. sundbergi longisulae* | 2MA45 | 26 Beau Vallon (cross), Mahé | # | # | | | |
| *P. sundbergi longisulae* | 2MA46 * | 65 La Reserve 1, Mahé | # | # | # | | # |
| *P. sundbergi longisulae* | 2MA47 | 65 La Reserve 1, Mahé | # | | | | |
| *P. sundbergi longisulae* | 2MA54 | 66 La Reserve 2, Mahé | # | # | | | |
| *P. sundbergi longisulae* | 2MA64 | 55 Anse aux Forbans, Mahé | # | # | | | |
| *P. sundbergi longisulae* | 2MA65 | 55 Anse aux Forbans, Mahé | # | | | | |
| *P. sundbergi longisulae* | 2MA66 | 55 Anse aux Forbans, Mahé | # | | | | |
| *P. sundbergi longisulae* | 2MA67 | 55 Anse aux Forbans, Mahé | # | | | | |
| *P. sundbergi longisulae* | 2MA77 | 67 Grand Anse, Mahé | # | # | # | # | |
| *P. sundbergi longisulae* | 2MA78 | 67 Grand Anse, Mahé | # | | | | |
| *P. sundbergi longisulae* | 2MA81 * | 35 Port Glaud, Mahé | # | # | # | # | # |
| *P. sundbergi longisulae* | 2MA25 | 40 Morne Blanc, Mahé | # | # | | | |
| *P. sundbergi longisulae* | 38721 | 16 Mont Fleuri, Victoria, Mahé | # | # | | | |
| *P. sundbergi longisulae* | 3MA17 | 62 Anse a la Mouche, Mahé | # | # | | # | # |
| *P. sundbergi longisulae* | 3MA18 | 62 Anse a la Mouche, Mahé | # | | | | |
| *P. sundbergi longisulae* | 3MA21 | 62 Anse a la Mouche, Mahé | # | | | | |
| *P. sundbergi longisulae* | 3MA22 | 62 Anse a la Mouche, Mahé | # | | | | |
| *P. sundbergi longisulae* | 3MA23 | 62 Anse a la Mouche, Mahé | # | | | | |
| *P. sundbergi longisulae* | 3MA24 | 62 Anse a la Mouche, Mahé | # | # | | # | # |
| *P. sundbergi longisulae* | 3MA33 | 66 La Reserve 2, Mahé | # | # | | | |
| *P. sundbergi longisulae* | 3MA42 * | 59 Baie Lazare, Mahé | # | # | # | # | # |
| *P. sundbergi longisulae* | 3MA46 | 59 Baie Lazare, Mahé | # | | | | |
| *P. sundbergi longisulae* | 3MA48 | 59 Baie Lazare, Mahé | # | | | | |
| *P. sundbergi longisulae* | 3MA49 | 59 Baie Lazare, Mahé | # | | | | |
| *P. sundbergi longisulae* | 3MA50 | 59 Baie Lazare, Mahé | # | # | | | |
| *P. sundbergi longisulae* | 3MA78 * | 17 Port, Victoria, Mahé | # | # | # | # | # |
| *P. sundbergi longisulae* | 3MA86 | 47 Trois Freres 2, Mahé | # | # | | | |
| *P. sundbergi longisulae* | 3MA94 | 25B Beau Vallon (beach), Mahé | # | # | # | | |
| *P. sundbergi longisulae* | 3MA99 | 25B Beau Vallon (beach), Mahé | # | | | | |
| *P. sundbergi longisulae* | 3MA101 | 24 Mare Anglaise, Mahé | # | # | | | |
| *P. sundbergi longisulae* | 3MA102 | 24 Mare Anglaise, Mahé | # | | | | |
| *P. sundbergi longisulae* | 3MA108 * | 18 NorthEast Point, Mahé | # | # | # | # | # |
| *P. sundbergi longisulae* | 3MA109 | 18 NorthEast Point, Mahé | # | | | | |
| *P. sundbergi longisulae* | 3MA110 | 18 NorthEast Point, Mahé | # | | | | |
| *P. sundbergi longisulae* | 3MA111 | 18 NorthEast Point, Mahé | # | | | | |
| *P. sundbergi longisulae* | 3MA123 | 48 Brilliant, Mahé | # | # | | | |
| *P. sundbergi longisulae* | 3MA128 | 49 Cascade, Mahé | # | | | | |
| *P. sundbergi longisulae* | 3MA129 | 49 Cascade, Mahé | # | | | | |
| *P. sundbergi longisulae* | 3MA130 | 50 Anse aux Pins 1, Mahé | # | # | | | |
| *P. sundbergi longisulae* | 3MA135 | 34 Cap Ternay | # | # | | | |
| *P. sundbergi longisulae* | 3MA137 | 16 Mont Fleuri, Victoria, Mahé | # | # | | | |
| *P. sundbergi longisulae* | 3MA145 | 44 Fairview, Mahé | # | # | | | |
| *P. sundbergi longisulae* | 3MA146 | 44 Fairview, Mahé | # | | | | |
| *P. sundbergi longisulae* | 3MA147 | 17 Port, Victoria, Mahé | # | # | | | |
| *P. sundbergi longisulae* | 3MA151 | 31 Danzil, Mahé | # | # | | | |
| *P. sundbergi longisulae* | 3MA160 | 39 Tea Plantation, Mahé | # | # | | | |
| *P. sundbergi longisulae* | 3MA171 | 45 Copolia, Mahé | # | # | | | |
| *P. sundbergi longisulae* | 3MA178 | 36 Port Glaud 2, Mahé | # | # | | | |
| *P. sundbergi longisulae* | 3MA185 | 35 Port Glaud, Mahé | # | # | | | |
| *P. sundbergi longisulae* | 3MA196 | 64 Anse Boileau, Mahé | | # | | | |
| *P. sundbergi longisulae* | 3MA197 | 64 Anse Boileau, Mahé | # | | | | |
| *P. sundbergi longisulae* | 3MA202 | 67 Grand Anse, Mahé | # | # | | | |
| *P. sundbergi longisulae* | 3MA203 | 67 Grand Anse, Mahé | # | | | | |
| *P. sundbergi longisulae* | 3MA204 | 51 Anse aux Pins 2, Mahé | # | # | | | |
| *P. sundbergi longisulae* | 3MA205 | 51 Anse aux Pins 2, Mahé | # | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *P. sundbergi longisulae* | 3MA206 | 52 Anse Royalle, Mahé | # | # | # | # | |
| *P. sundbergi longisulae* | CF1 * | 69 Cerf Island 1 | # | # | # | # | # |
| *P. sundbergi longisulae* | CF9 * | 70 Cerf Island 2 | # | # | # | # | # |
| *P. sundbergi longisulae* | CF10 * | 70 Cerf Island 2 | # | # | # | # | # |
| *P. sundbergi longisulae* | 7FG * | 114 Fregate | # | # | | # | # |
| *P. sundbergi longisulae* | 9FG * | 114 Fregate | # | # | # | | # |
| *P. sundbergi longisulae* | 17FG | 114 Fregate | | # | # | # | # |
| *P. sundbergi longisulae* | 18FG | 114 Fregate | # | # | # | | |
| *P. sundbergi longisulae* | 22FG | 114 Fregate | # | # | | # | |
| *P. sundbergi longisulae* | 34FG * | 114 Fregate | # | # | # | # | # |
| *P. sundbergi longisulae* | 47FG | 114 Fregate | # | # | | # | # |
| *P. sundbergi longisulae* | 46FG | 114 Fregate | # | # | # | | |
| *P. sundbergi longisulae* | 24FG | 114 Fregate | # | # | | | # |
| *P. sundbergi longisulae* | PhSNorth | 115 North Island | | # | | | |
| *P. sundbergi longisulae* | 7NORTH | 115 North Island | # | # | # | # | |
| *P. sundbergi longisulae* | 8NORTH * | 115 North Island | # | # | # | # | # |
| *P. sundbergi longisulae* | 10NORTH | 115 North Island | # | # | | # | # |
| *P. sundbergi longisulae* | 11NORTH | 115 North Island | # | # | # | # | # |
| *P. sundbergi longisulae* | 1SILH | 71 La Passe 1, Silhouette | # | # | # | # | |
| *P. sundbergi longisulae* | 2SILH * | 71 La Passe 1, Silhouette | # | # | # | # | # |
| *P. sundbergi longisulae* | 3SILH * | 71 La Passe 1, Silhouette | # | # | # | # | # |
| *P. sundbergi longisulae* | 13SILH | 75 Anse Lascars, Silhouette | # | # | | # | |
| *P. sundbergi longisulae* | 16SILH | 75 Anse Lascars, Silhouette | # | # | | # | |
| *P. sundbergi longisulae* | 17SILH * | 75 Anse Lascars, Silhouette | # | # | # | # | # |
| *P. sundbergi longisulae* | 26SILH * | 76 around GB rock, Silhouette | # | # | # | # | # |
| *P. sundbergi longisulae* | 33SILH | 76 around GB rock, Silhouette | # | # | | | # |
| | | | | | | | |
| *P. sundbergi sundbergi* | PL5 * | 99 Anse Volbert, Praslin | # | # | # | # | # |
| *P. sundbergi sundbergi* | PL7 | 99 Anse Volbert, Praslin | # | # | | | |
| *P. sundbergi sundbergi* | PL8 | 99 Anse Volbert, Praslin | # | # | | | |
| *P. sundbergi sundbergi* | PL31 * | 110 Fonde de L'Anse, Praslin | # | # | # | # | # |
| *P. sundbergi sundbergi* | 38727 | 109 Grand Anse, Praslin | # | # | # | # | |
| *P. sundbergi sundbergi* | 5PL * | 99 Anse Volbert, Praslin | # | # | # | # | # |
| *P. sundbergi sundbergi* | 6PL | 99 Anse Volbert, Praslin | # | # | | | |
| *P. sundbergi sundbergi* | 57PL | 102 Valle du Mai, Praslin | # | # | # | | # |
| *P. sundbergi sundbergi* | 73PL * | 99 Anse Volbert, Praslin | # | # | # | # | # |
| *P. sundbergi sundbergi* | 74PL * | 99 Anse Volbert, Praslin | # | # | # | # | # |
| *P. sundbergi sundbergi* | 75PL | 99 Anse Volbert, Praslin | # | # | | | |
| *P. sundbergi sundbergi* | 76PL | 99 Anse Volbert, Praslin | # | # | # | | # |
| *P. sundbergi sundbergi* | 77PL | 99 Anse Volbert, Praslin | # | # | | # | # |
| *P. sundbergi sundbergi* | 78PL | 99 Anse Volbert, Praslin | # | # | | # | |
| *P. sundbergi sundbergi* | 79PL * | 99 Anse Volbert, Praslin | # | # | # | # | # |
| *P. sundbergi sundbergi* | 80PL * | 99 Anse Volbert, Praslin | # | # | # | # | # |
| *P. sundbergi sundbergi* | 13CUR * | 81 Turtle Pond, Curieuse | # | # | # | # | # |
| | | | | | | | |
| *P. sundbergi ladiguensis* | LD1 * | 93 La Veuve Reserve, La Digue | # | # | # | # | # |
| *P. sundbergi ladiguensis* | LD17 * | 95 Belle Vue, La Digue | # | # | # | # | # |
| *P. sundbergi ladiguensis* | LD22 * | 94B To Grand Anse, La Digue | # | # | # | # | # |
| *P. sundbergi ladiguensis* | LD28 | 93 La Veuve Reserve, La Digue | # | # | | | # |
| *P. sundbergi ladiguensis* | 1LD | 93 La Veuve Reserve, La Digue | # | # | # | | # |
| *P. sundbergi ladiguensis* | 17LD * | 93 La Veuve Reserve, La Digue | # | # | # | # | # |
| *P. sundbergi ladiguensis* | 64LD | 96 Anse Source d'Argeant, La Digue | # | # | | # | |
| *P. sundbergi ladiguensis* | 74LD | 96 Anse Source d'Argeant, La Digue | # | # | # | | # |
| *P. sundbergi ladiguensis* | 3BS * | 90 Point 1, Grande Soeur | # | # | | # | # |
| | | | | | | | |
| *P. sundbergi ssp.* | CM5 | 8 Menai Island, Cosmoledo | # | FJ830319 | | | |
| *P. sundbergi ssp.* | CM6 | 8 Menai Island, Cosmoledo | # | | | | |
| *P. sundbergi ssp.* | CM7 | 8 Menai Island, Cosmoledo | # | | | | |
| *P. sundbergi ssp.* | CM8 | 8 Menai Island, Cosmoledo | # | | | | |
| *P. sundbergi ssp.* | CM9 | 8 Menai Island, Cosmoledo | # | # | | | |
| *P. sundbergi ssp.* | PV7 | 14 Poivre | # | # | | | |
| *P. sundbergi ssp.* | PV3 | 14 Poivre | # | FJ830317 | | | |
| | | | | | | | |
| *P. astriata astriata* | 2MA61 * | 55 Anse aux Forbans, Mahé | # | # | # | # | # |
| *P. astriata astriata* | 3MA45 * | 59 Baie Lazare, Mahé | # | # | # | # | # |
| *P. astriata astriata* | 3MA106 * | 18 NorthEast Point, Mahé | # | # | # | # | # |
| *P. astriata astriata* | 3MA158 * | 33 Anse Major, Mahé | # | # | # | # | # |
| *P. astriata astriata* | 3MA161 * | 39 Tea Plantation, Mahé | # | # | # | # | # |
| *P. astriata astriata* | 3MA170 * | 45 Copolia, Mahé | # | # | # | # | # |
| *P. astriata astriata* | 3MA207 * | 53 Anse Louis, Mahé | # | # | # | # | # |
| *P. astriata astriata* | 6MA * | 29 Mare aux Cochons 2, Mahé | # | # | # | # | # |
| *P. astriata astriata* | CF7 * | 70 Cerf Island 2 | # | # | # | # | # |
| *P. astriata astriata* | 38710 * | 71 La Passe 1, Silhouette | # | # | # | # | # |
| *P. astriata astriata* | 38712 | 71 La Passe 1, Silhouette | # | # | | # | |
| *P. astriata astriata* | PHELSUN1 | 75 Anse Lascars, Silhouette | # | # | | # | |
| *P. astriata astriata* | PHELSUN2 | 75 Anse Lascars, Silhouette | # | # | | | |
| *P. astriata astriata* | PHELSUN3 | 75 Anse Lascars, Silhouette | # | # | | | |
| *P. astriata astriata* | 38711 | 75 Anse Lascars, Silhouette | # | # | | | |
| *P. astriata astriata* | 8SILH * | 75 Anse Lascars, Silhouette | # | # | # | | # |
| *P. astriata astriata* | 9SILH * | 75 Anse Lascars, Silhouette | # | # | # | | # |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *P. astriata astriata* | 10SILH * | 75 Anse Lascars, Silhouette | # | # | # | # | # |
| *P. astriata astriata* | 14SILH * | 75 Anse Lascars, Silhouette | # | # | # | # | # |
| *P. astriata astriata* | 15SILH * | 75 Anse Lascars, Silhouette | # | # | # | # | # |
| | | | | | | | |
| *P. astriata semicarinata* | PL6 | 99 Anse Volbert, Praslin | # | # | | | |
| *P. astriata semicarinata* | PL9 * | 99 Anse Volbert, Praslin | # | # | | # | # |
| *P. astriata semicarinata* | PL15 * | 103 Anse Volbert 3, Praslin | # | # | | # | # |
| *P. astriata semicarinata* | PL26 | 102 Valle du Mai, Praslin | # | # | | | |
| *P. astriata semicarinata* | PL27 | 107 Anse Citron, Praslin | # | # | | # | # |
| *P. astriata semicarinata* | PL30 | 110 Fonde de L'Anse, Praslin | # | # | | # | |
| *P. astriata semicarinata* | PL34 | 99 Anse Volbert, Praslin | # | # | | | |
| *P. astriata semicarinata* | PHEASTPRA1 | 109 Grand Anse, Praslin | # | # | | # | |
| *P. astriata semicarinata* | 2PL * | 109 Grand Anse, Praslin | # | # | # | # | # |
| *P. astriata semicarinata* | 24PL | 99 Anse Volbert, Praslin | # | # | # | | |
| *P. astriata semicarinata* | 25PL | 105 Mont Plaisir, Praslin | # | # | # | | |
| *P. astriata semicarinata* | 26PL | 105 Mont Plaisir, Praslin | # | # | | | |
| *P. astriata semicarinata* | 27PL | 105 Mont Plaisir, Praslin | # | # | | # | |
| *P. astriata semicarinata* | 28PL * | 105 Mont Plaisir, Praslin | # | # | # | # | # |
| *P. astriata semicarinata* | 37PL | 110 Fonde de L'Anse, Praslin | # | # | | | |
| *P. astriata semicarinata* | 45PL * | 113 Anse Marie Louise, Praslin | # | # | # | # | # |
| *P. astriata semicarinata* | 65PL | 102 Valle du Mai, Praslin | # | # | # | | |
| *P. astriata semicarinata* | 66PL * | 102 Valle du Mai, Praslin | # | # | # | # | # |
| *P. astriata semicarinata* | 68PL * | 100 Anse Volbert 2, Praslin | # | # | # | # | # |
| *P. astriata semicarinata* | 69PL | 100 Anse Volbert 2, Praslin | # | # | | | |
| *P. astriata semicarinata* | 70PL | 100 Anse Volbert 2, Praslin | # | # | # | # | |
| *P. astriata semicarinata* | 71PL | 100 Anse Volbert 2, Praslin | # | # | # | | |
| *P. astriata semicarinata* | 72PL | 100 Anse Volbert 2, Praslin | # | # | | # | # |
| *P. astriata semicarinata* | 81PL * | 104 Anse La Blague, Praslin | # | # | # | # | # |
| *P. astriata semicarinata* | LD14 * | 95 Belle Vue, La Digue | # | # | # | # | # |
| *P. astriata semicarinata* | LD19 | 95 Belle Vue, La Digue | # | # | # | | # |
| *P. astriata semicarinata* | LD20 | 94 La Veuve Reserve 2, La Digue | # | # | | # | # |
| *P. astriata semicarinata* | 3LD * | 93 La Veuve Reserve, La Digue | # | # | # | # | # |
| *P. astriata semicarinata* | 15LD * | 93 La Veuve Reserve, La Digue | # | # | # | # | # |
| *P. astriata semicarinata* | CUR2 | 82 Trail, Curieuse | # | # | | | |
| *P. astriata semicarinata* | CUR6 | 81 Turtle Pond, Curieuse | # | # | | | |
| *P. astriata semicarinata* | 3CUR * | 80 Point1, Curieuse | # | # | # | # | # |
| *P. astriata semicarinata* | 6CUR | 80 Point1, Curieuse | # | # | # | # | |
| *P. astriata semicarinata* | 7CUR | 81 Turtle Pond, Curieuse | # | # | | # | # |
| *P. astriata semicarinata* | 8CUR * | 81 Turtle Pond, Curieuse | # | # | # | # | # |
| *P. astriata semicarinata* | 15CUR * | 80 Point1, Curieuse | # | # | # | # | # |
| *P. astriata semicarinata* | 40CUR * | 80 Point1, Curieuse | # | # | # | # | # |
| *P. astriata semicarinata* | 17ARD * | 79 Aride Island | # | # | # | # | # |
| *P. astriata semicarinata* | 22ARD | 79 Aride Island | # | # | | # | # |
| *P. astriata semicarinata* | 7CNE * | 89 To Cave, Cousine | # | # | # | # | # |
| *P. astriata semicarinata* | 8CNE * | 89 To Cave, Cousine | # | # | # | # | # |
| *P. astriata semicarinata* | 40CNE * | 87 Office, Cousine | # | # | # | # | # |
| *P. astriata semicarinata* | 41CNE | 87 Office, Cousine | # | # | # | | # |
| *P. astriata semicarinata* | 42CNE * | 87 Office, Cousine | # | # | # | # | # |
| *P. astriata semicarinata* | 43CNE * | 87 Office, Cousine | # | # | # | # | # |
| *P. astriata semicarinata* | 11BS * | 92 north path 2, Grande Soeur | # | # | # | # | # |
| | | | | | | | |
| *P. astriata ssp.* | 23FG * | 114 Fregate | # | # | # | # | # |
| *P. astriata ssp.* | 10FG * | 114 Fregate | # | # | # | # | # |
| *P. astriata ssp.* | 16FG | 114 Fregate | # | # | # | # | |
| | | | | | | | |
| *P. astriata astriata* | AT6 | 7 Astove | # | FJ830248 | | | |
| *P. astriata astriata* | AT15 | 7 Astove | # | FJ830249 | | | |
| *P. astriata semicarinata* | APH1 | 13 Alphonse | # | FJ830250 | | | |
| *P. astriata semicarinata* | APH10 | 13 Alphonse | # | FJ830251 | | | |

## References


Ackerly, D. D., D. W. Schwilk, and C. O. Webb. 2006. Niche evolution and adaptive radiation: testing the order of trait divergence. Ecology 87:S50-61.

Austin, J. J., E. N. Arnold, and C. G. Jones. 2004. Reconstructing an island radiation using ancient and recent DNA: the extinct and living day geckos (*Phelsuma*) of the Mascarene islands. Molecular Phylogenetics and Evolution 31:109-22.

Bandelt, H. J., P. Forster, and A. Rohl. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16:37-48.

Borner, A. R., and W. Minuth. 1985. On the taxonomy of the Indian Ocean lizards of the Phelsuma madagascariensis species group (Reptilia: Geckonidae).. Journal of the Bombay Natural History Society 81:243-281.

Bruen, T. C., H. Philippe, and D. Bryant. 2006. A simple and robust statistical test for detecting the presence of recombination. Genetics 172:2665-81.

Carlquist, S. 1995. A fully fertile intergeneric hybrid derivative from Argiroxipium sandwicence ssp. macrocephalum x Dubautia menziensii (Asteraceae) and its relevance to plant evolution in the Hawaiian Islands. American Journal of Botany 82:1574-1581.

Carranza, S., E. N. Arnold, P. Geniez, J. Roca, and J. A. Mateo. 2008. Radiation, multiple dispersal and parallelism in the skinks, *Chalcides* and *Sphenops* (Squamata: Scincidae), with comments on *Scincus* and *Scincopus* and the age of the Sahara Desert. Molecular Phylogenetics and Evolution 46:1071-94.

Chapple, D. G., P. A. Ritchie, and C. H. Daugherty. 2009. Origin, diversification, and systematics of the New Zealand skink fauna (Reptilia: Scincidae). Molecular Phylogenetics and Evolution 52:470-87.

Cheke, A. S. 1982. *Phelsuma* GRAY 1825 in the Seychelles and neighbouring islands: a re-appraisal of their taxonomy and description of two new forms (Reptilia: Sauria: Gekkonidae). Senckenbergiana biologica 62:181-198.

Colonna, M. J., J. Casanova, W. C. Dullo, and G. Camoin. 1996. Sea-level changes and $\partial$18O record for the past 34,000 yr from Mayotte reef, Indian Ocean. Quaternary Research 46:335-339.

Cox, S. C., S. Carranza, and R. P. Brown. 2010. Divergence times and colonization of the Canary Islands by *Gallotia* lizards. Molecular Phylogenetics and Evolution.

Drummond, A. J., and A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7:214.

Edwards, S. V. 2009. Is a new and general theory of molecular systematics emerging? Evolution 63:1-19.

Edwards, S. V., and P. Beerli. 2000. Perspective: gene divergence, population divergence and the variance in coalescence time in phylogeographic studies. Evolution 54:1839-1854.

Fleischer-Dodgley, F., C. J. Kettle, P. J. Edwards, J. Ghazoul, K. Määttänen, and C. N. Kaiser-Bunbury. submitted. Morphological and genetic differentiation in populations of the dispersal-limited coco de mer (Lodoicea maldivica): implications for management and conservation

Flot, J. F., A. Tillier, S. Samadi, and S. Tillier. 2006. Phase determination from direct sequencing of length-variable DNA regions. Molecular Ecology Notes 6:627-630.

Fu, Y. X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915-25.

Gamble, T., A. M. Bauer, E. Greenbaum, and T. R. Jackman. 2008. Evidence for Gondwanan vicariance in an ancient clade of gecko lizards. Journal of Biogeography 35:88-104.

Gardner, A. S. 1984. The evolutionary ecology and population systematics of day-geckos genus *Phelsuma* in the SeychellesUniversity of Aberdeen, Scotland.

Gardner, A. S. 1986. The biogeography of the Lizards of the Seychelles Islands. Journal of Biogeography 13:237-253.

Gardner, A. S. 1987. The systematics of the *Phelsuma madagascariensis* species group of day geckos (Reptilia: Gekkonidae) in the Seychelles. Zoological Journal of the Linnean Society 91:93-105.

Gerlach, J. 2008. Population and conservation status of the reptiles of the Seychelles islands. Phelsuma 16:30-48.

Glez-Peña, D., D. Gomez-Blanco, M. Reboiro-Jato, F. Fdez-Riverola, and D. Posada. 2010. ALTER: program-oriented conversion of DNA and protein alignments. Nucleic Acids Research:1-5.

Godinho, R., E. G. Crespo, N. Ferrand, and D. J. Harris. 2005. Phylogeny and evolution of the green lizards, *Lacerta spp.* (Squamata: Lacertidae) based on mitochondrial and nuclear DNA sequences. Amphibia-Reptilia 26:271-285.

Grant, B. R., and P. R. Grant. 2008a. Fission and Fusion of Darwin's finches populations. Philosophical Transactions of the Royal Society of London B 363:2821-2829.

Grant, P. R., and B. R. Grant. 1997. Hybridization, sexual imprinting and mate choice. American Naturalist 149:1-28.

Grant, P. R., and B. R. Grant. 2008b. How and why species multiply: the radiation of Darwin's Finches. Princeton University Press, Princeton, NJ.

Grant, P. R., B. R. Grant, and J. C. Deutsch. 1996. Speciation and hybridization. Philosophical Transactions of the Royal Society of London B 351:765-772.

Guindon, S., and O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52:696-704.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95-98.

Harmon, L. J., L. L. Harmon, and C. J. Jones. 2007. Competition and community structure in diurnal arboreal geckos (genus *Phelsuma*) in the Indian Ocean. Oikos 116:1863-1878.

Harmon, L. J., J. Melville, A. Larson, and J. B. Losos. 2008. The role of geography and ecological opportunity in the diversification of day geckos (Phelsuma). Systematic Biology 57:562-73.

Hasegawa, M., H. Kishino, and T. Yano. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22:160-74.

Heled, J., and A. J. Drummond. 2009. Bayesian inference of species trees from multilocus data. Molecular Biology and Evolution 27:570-80.

Hey, J. 2010. Isolation with Migration Models for more than Two Populations. Molecular Biology and Evolution 27:905-920.

Hey, J., and R. Nielsen. 2007. Integration within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. Proceedings of the National Academy of Sciences of the United States of America 104:2785-90.

Hickerson, M. J., G. Dolman, and C. Moritz. 2006. Comparative phylogeographic summary statistics for testing simultaneous vicariance. Molecular Ecology 15:209-223.

Hudson, R. R., M. Kreitman, and M. Aguade. 1987. A test of neutral molecular evolution based on nucleotide data. Genetics 116:153-9.

Hurt, C., A. Anker, and N. Knowlton. 2009. A multilocus test of simultaneous divergence across the Isthmus of Panama using snapping shrimp in the genus Alpheus. Evolution 63:514-30.

Kimura, M. 1969. The number of heterozygous nucleotide sites maintained in a finite population due to steady flux of mutations. Genetics 61:893-903.

Knowles, L. L. 2009. Statistical Phylogeography. Annual Review of Ecology, Evolution, and Systematics 40:593-612.

Kocher, T. D., W. K. Thomas, A. Meyer, S. V. Edwards, S. Paabo, F. X. Villablanca, and A. C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals - amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences of the United States of America 86:6196-6200.

Kozak, K. H., D. W. Weisrock, and A. Larson. 2006. Rapid lineage accumulation in a non-adaptive radiation: phylogenetic analysis of diversification rates in eastern North American woodland salamanders (Plethodontidae: Plethodon). Proceedings of the Royal Society of London B 273:539-46.

Kumar, S., M. Nei, J. Dudley, and K. Tamura. 2008. MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. Briefings in Bioinformatics 9:299-306.

Leache, A. D. 2009. Species trees for spiny lizards (genus Sceloporus): identifying points of concordance and conflict between nuclear and mitochondrial data. Mol Phylogenet Evol 54:162-71.

Librado, P., and J. Rozas. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451-2.

Losos, J. B. 2009. Lizads in an evolutionary tree: ecology and adaptive radiation of anoles. University of California Press, Berkeley.

Losos, J. B. 2010. Adaptive radiation, ecological opportunity, and evolutionary determinism. The American Naturalist 175:623-639.

Mallet, J. 2005. Hybridization as an invasion of the genome. Trends in Ecology and Evolution 20:229-37.

Mertens, R. 1966. Die nichmadagassischen arten and unterarten der geckonengattung Phelsuma. Senckenbergiana biologica 47:85-110.

Miller, K. G., M. A. Kominz, J. V. Browning, J. D. Wright, G. S. Mountain, M. E. Katz, P. J. Sugarman, B. S. Cramer, N. Christie-Blick, and S. F. Pekar. 2005. The Phanerozoic record of global sea-level change. Science 310:1293-8.

Miralles, A., and S. Carranza. 2010. Systematics and biogeography of the Neotropical genus *Mabuya*, with special emphasis on the Amazonian skink *Mabuya nigropunctata* (Reptilia, Scincidae). Molecular Phylogenetics and Evolution 54:857-69.

Nielsen, R., and J. Wakeley. 2001. Distinguishing migration from isolation: a Markov chain Monte Carlo approach. Genetics 158:885-96.

Noble, T., N. Bunbury, C. N. Kaiser-Bunbury, and D. J. Bell. 2010. Ecology and co-existence of two endemic day gecko (Phelsuma) species in Seychelles native palm forest. Journal of Zoology DOI: 10.1111/j.1469-7998.2010.00751.x.

Nylander, J. A., J. C. Wilgenbusch, D. L. Warren, and D. L. Swofford. 2008. AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics 24:581-3.

Palumbi, S. R., A. Martin, S. Romano, W. O. McMillan, L. Stice, and G. Grabowski. 1991. The simple fool's guide to PCR, Version 2.0. Privately published, Hawaii.

Paulo, O. S., J. Pinheiro, A. Miraldo, M. W. Bruford, W. C. Jordan, and R. A. Nichols. 2008. The role of vicariance vs. dispersal in shaping genetic patterns in ocellated lizard species in the western Mediterranean. Molecular Ecology 17:1535-1551.

Pinho, C., S. Rocha, B. M. Carvalho, S. Lopes, S. Mourão, M. Vallinoto, T. O. Brunes, C. F. B. Haddad, H. Gonçalves, F. Sequeira, and N. Ferrand. 2010. New primers for the amplification and sequencing of nuclear loci in a taxonomically wide set of reptiles and amphibians. Conservation Genetics Resources 2:181-185.

Polzin, T., and S. V. Daneschmand. 2003. On Steiner trees and minimum spanning trees in hypergraphs. Operations Research Letters 31:12-20.

Posada, D. 2008. jModelTest: phylogenetic model averaging. Molecular Biology and Evolution 25:1253-6.

Posada, D., and T. R. Buckley. 2004. Model selection and model averaging in phylogenetics: advantages of akaike information criterion and bayesian approaches over likelihood ratio tests. Systematic Biology 53:793-808.

Radtkey, R. R. 1996. Adaptive radiation of day-geckos (*Phelsuma*) in the Seychelles archipelago: a phylogenetic analysis. Evolution 50:604-623.

Rambaut, A. 2009. FigTree v1.3.1available at http://tree.bio.ed.ac.uk/software/figtree/.

Rambaut, A., and A. J. Drummond. 2007. Tracer v1.5, Available from http://beast.bio.ed.ac.uk/Tracer

Ramos-Onsins, S. E., and J. Rozas. 2002. Statistical properties of new neutrality tests against population growth. Molecular Biology and Evolution 19:2092-2100.

Rocha, S., D. J. Harris, and D. Posada. submitted. One or more? Cryptic diversity within the endemic Prehensile-tailed gecko Urocotyledon inexpectata across the Seychelles Islands: patterns of phylogeographic structure and isolation at the multilocus level. Biological Journal of the Linnean Society.

Rocha, S., H. Rosler, P.-S. Gehring, F. Glaw, D. Posada, D. J. Harris, and M. Vences. 2010. Phylogenetic systeatics of day geckos, genus *Phelsuma*, based on molecular and morphological data (Squamata: Gekkonidae). Zootaxa 2429:1-28.

Rocha, S., M. Vences, F. Glaw, D. Posada, and D. J. Harris. 2009. Multigene phylogeny of Malagasy day geckos of the genus *Phelsuma*. Molecular Phylogenetics and Evolution 52:530-537.

Rocha S., D. J. Harris, A. Perera, A. Silva, R. Vasconcelos, M. A. Carretero. 2009b. Recent data on the distribution of Lizards and Snakes of the Seychelles. Herpetological Bulletin 110: 20-32

Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572-4.

Rundell, R. J., and T. Price. 2009. Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. 24:394 - 399.

Rundle, H., and P. Nosil. 2005. Ecological speciation. Ecology Letters 8:336-352.

Sambrook, J., E. F. Fritsh, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual, second edition edition. Cold Spring Harbor Press, Cold Spring Harbor, NY.

Schluter, D. 2000. The ecology of adaptive radiation. Oxford University Press, New York.

Seehausen, O. 2004. Hybridization and adaptive radiation. Trends in Ecology and Evolution 19:198-207.

Seehausen, O. 2006. African cichlid fish: a model system in adaptive radiation research. Proceedings of the Royal Society London B 273:1987-1998.

Seehausen, O., J. J. M. van Alphen, and F. Witte. 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. Science 277:1808-1811.

Siddall, M., E. J. Rohling, A. Almogi-Labin, C. Hemleben, D. Meischner, I. Schmelzer, and D. A. Smeed. 2003. Sea-level fluctuations during the last glacial cycle. Nature 423:853-8.

Simpson, G. G. 1953. The major features of evolution. Columbia University Press, New York, NY.

Stephens, M., N. J. Smith, and P. Donnelly. 2001. A new statistical method for haplotype reconstruction from population data. American Journal of Human Genetics 68:978-989.

Strasburg, J. L., and L. H. Rieseberg. 2010. How robust are "isolation with migration" analyses to violations of the im model? A simulation study. Molecular Biology and Evolution 27:297-310.

Sugiura, N. 1978. Further analysis of the data by Akaike's information criterion and the finite corrections. Communications in Statistics -Theory and Methods:13-26.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585-95.

Tarr, C. L., and R. C. Fleischer. 1995. Evolutionary relationships of the Hawaiian honeycreepers (Aves: Drepanidae). Pages 147-159 *in* Hawaiian biogeography: evolution in a hot-spot archipelago (W. Wagner, and V. Funk, eds.). Smithsonian Institution Press.

Thorpe, R. S., Y. Surget-Groba, and H. Johansson. 2008. The relative importance of ecology and geographic isolation for speciation in anoles. Philosophical Transactions of the Royal Society London B 363:3071-81.

Wagner, M., and V. Funk. 1995. Hawaiian biogeography: evolution on a hot spot archipelago. Smithsonian Institution Press, Washington D.C., USA.

Wakeley, J., and J. Hey. 1997. Estimating ancestral population parameters. Genetics 145:847-855.

Warren, B. H., S. Dominique, J. H. Bruggemann, P. P.-J. Robert, and T. Christophe. 2010. Why does the biota of the Madagascar region have such a strong Asiatic flavour? Cladistics 26:526-538.

Watterson, G. A. 1975. On the number of segregating sites in genetical models without recombination. Theoretical Population Biology 7:256-276.

Whittaker, R. J., and J. M. Fernández-Palacios. 2007. Island Biogeography: Ecology, Evolution and Conservation, 2nd edition. Oxford University Press, Oxford.

# The evolutionary history of *Mabuya* skinks from the Seychelles islands: introgressive hybridization, morphological evolution and patterns of geographic structure.

S. Rocha [1,2,3], A. Perera [1], A. Silva [1,2], D. Posada [3] and D.J. Harris [1,2]

[1]*CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, Rua Padre Armando Quintas, 4485-661 Vairão, Portugal*

[2]*Departamento de Biologia, Faculdade de Ciências, Rua do Campo Alegre FC4 4169-007 Porto, Portugal*

[3]*Departamento de Bioquímica, Genética e Inmunología, Facultad de Biología, Universidad de Vigo, Vigo 36310, Spain*

## Abstract

The Seychelles is a remarkably interesting archipelago for evolutionary studies, but only recently its biogeographic patterns are being explored using molecular markers. Here we used morphological and molecular data to examine diversity and phylogenetic relationships of two endemic skink sister-species from the archipelago: *M. sechellensis* and *M. wrightii*. Mitochondrial DNA genealogy rendered a monophyletic *M. wrightii* nested within a paraphyletic *M. sechellensis*, whereas nuclear DNA sequences from five unlinked markers reflect the accepted taxonomy. Hybridization and massive mtDNA introgression leading to the complete replacement of the native mtDNA lineage of *M. sechellensis* in some of the islands are invoked to explain this result, and morphological variation seems also to reflect this pattern of reticulation. A Mio-Pliocene divergence between both species is suggested. Multilocus molecular data was used to uncover biogeographic patterns within the archipelago, and seemed to reflect shared patterns with other co-distributed lizard taxa; specifically a north-south marked structure, a close relationship between populations from Fregate island and southern islands populations, and also a detectable isolation within the southern group, between Mahé and Silhouette+North islands. Gene flow from these latter islands towards the northern group was also suggested. These results add to the growing body of evidence of the influence of geographic distance and sea-level oscillations in shaping the genetic structure of Seychellois taxa and of the existence of common biogeographic patterns across the archipelago.

Keywords: Seychelles, hybridization, introgression, *Mabuya*, phylogeography, islands.

## Introduction

The Seychelles are a particular archipelago because, lying on a shallow continental shelf, sea level decreases of roughly just 60 m, are enough to unite all the granitic islands, which have thus been connected and isolated several times in the past (Figure 1A). The cyclic eustatic sea level variations along and before the Pleistocene (Figure 1B) led therefore to multiple opportunities for vicariance, inter-island dispersal and mixing, which must have shaped the diversification and genetic structure of their inhabitants. Specifically, periods of higher sea level should promote vicariance and divergence in allopatry between populations from the main groups of islands, while periods of lower sea levels might allow for migration between the islands that could erase any putative differentiation meanwhile generated, and facilitate contact between recently differentiated species. However, the habitat connecting the islands at lower sea-level times can hardly be assumed to have been ideal for terrestrial vertebrate taxa, and the assumption of high connectivity and dispersal is not straightforward. This is relevant because isolated and fragmented habitats like this, with islands of different sizes, landscapes and ecological characteristics, provide optimal conditions for speciation and for local adaptation, which need to be to be recognized, from both an evolutionary and conservation point of view.

*Mabuya sechellensis* and *M. wrightii* are two sister-species of skinks, endemic to this archipelago and most probably with an African origin (Carranza *et al.* 2001a, Rocha *et al.* 2010). While *M. sechellensis* is abundant and widespread across all granitic islands in this group, *M. wrightii* has currently a very restricted range of less than 4 $km^2$, being considered Vulnerable according to IUCN criteria (Gerlach & Ineich 2006). *M. wrightii* inhabits well-preserved small islands and offshore islets with seabird colonies and without introduced rat populations (Figure 1A). Records from additional islands exist, some of confirmed established populations from where the species has gone extinct (Isle aux Vache Marines, off Mahé, and Marianne, in the northern group), and others of isolated individual records, such as the one from Mahé (Gerlach 2007; Rendhal 1939). In some islands both species can be found in sympatry. The most evident distinction between these two species is their adult body size. *Mabuya wrightii* is a "gigantic" form; larger and heavy-bodied, reaching 138 mm while *M. sechellensis* only reaches 107 mm (Gerlach 2007). They also differ in several other morphometric and scalation characters (Gerlach 2007), and coloration is also distinct. The two species are thus very easily distinguished in the field, even as juveniles. Gerlach (2005) noted, however, cases of morphological convergence

between these species, and populations that appeared to be "intermediate", highlighting the possible dynamism of these insular taxa in terms of both distribution range changes and morphological plasticity. The almost exclusive presence of *M. wrightii* in islands with seabird colonies and its frequent predation of tern eggs (Honegger 1966; Vesey-Fitzgerald 1947) led to early suggestions that they evolved as specialized exploiters of seabird colonies, taking advantage of the huge food availability associated with this kind of habitat. However, *M. sechellensis* exploits seabird resources just as efficiently (Brooke & Houston 1983; Cheke 1984; Houston 1978), and the current distribution of *M. wrightii* may simply reflect a negative association with rats.

Recent results on mtDNA variation confirmed Seychellois *Mabuya* spp. as a monophyletic clade but indicated that *M. sechellensis* is paraphyletic relative to *M. wrightii* (Rocha *et al.* 2010). Gigantism is a common phenomenon that has evolved many times in other oceanic island taxa (Meiri *et al.* 2010). In fact molecular studies have shown that the two giant skink species from the Cape Verde islands; *Mabuya (Macroscincus) coctei* and *Mabuya vaillanti*, evolved independently (Carranza *et al.* 2001b; Miralles *et al.* 2010), as also did some of the extant giant *Gallotia* species from the Canary Islands (Cox *et al.* 2010). The hypothesis of multiple evolution of gigantism has not been excluded within the Seychellois clade since only one individual of *M. wrightii* was included in the previous analysis. Alternative explanations for the mtDNA-level paraphyly of *M. sechellensis* involve incomplete lineage sorting or the occurrence of hybridization and introgression. Therefore the evaluation of intraspecific (nuclear and mtDNA) diversity across both species becomes essential to answer this question.

Surprisingly, very few studies have examined patterns of variation and diversification within the Seychelles. Historically Mahé and surrounding islands, plus Silhouette and North (herein called southern- or Mahé-group) have been considered a biogeographic unit separate from Praslin-La Digue group (herein called northern- or Praslin-group), while Fregate is generally viewed as an isolated or intermediate unit (Cheke 1984; Scott 1933). This biogeographic pattern is apparent in some faunal lists for insects (Scott 1933) and molluscs (Gerlach & van Bruggen 1999) as also in studies of intraspecific variability within amphibians (Nussbaum & Wu 1995) and reptiles (Gardner 1987; Radtkey 1996), but does not appear to stand for other groups (Gerlach 1999; Nussbaum 1984). Recent phylogeographic studies using molecular markers (Rocha 2010) are showing that genetic variability within some lizard taxa fits to this main north-south biogeographic division, with pre-Pleistocenic divergences between

main lineages. Further structure within each group is also sometimes detected. The combined evidence from multiple taxa will allow us to distinguish taxa-specific patterns from general factors explaining diversity and diversification across the archipelago.

The combination of different sets of markers, with different inheritance modes, and an understanding of the recent demography and putative range shifts of a species are essential to provide complete and reliable answers when analyzing instances of potential hybridization and introgression (Excoffier *et al.* 2009). Here we explored several lines of evidence, using molecular markers and morphological data, to further investigate and explain the result of mtDNA paraphyly of the Seychelles skink. We provide some biogeographic hypothesis for the evolution of the two congeneric *Mabuya* species from the Seychelles islands. Additionally we explore patterns of geographic structure and gene flow that contribute to our understanding of the biogeography of the archipelago.

## Material and Methods

### *DNA extraction, amplification and sequencing*

Tissue samples were collected and stored in 100% ethanol and DNA extraction followed standard salt or phenol-chloroform protocols (Kocher *et al.* 1989; Sambrook *et al.* 1989). A total of 144 individuals (117 *M. sechellensis* and 27 *M. wrightii*), distributed across 11 islands – three of them harbouring sympatric populations - and covering almost the complete distribution of both species were sequenced for a mitochondrial marker, cytochrome-*b* (Figure 1A). A subset of these (between three and ten per island) were sequenced for four nuclear protein-coding genes: phosducin (PDC), oocyte-maturation factor MOS (C-mos), recombination-activating gene 2 (RAG2), and melanocortin-1 receptor (MC1R); and one intron of the reelin gene (RELN*int*61). Detailed information on the individuals genotyped per species and gene and precise locality data is given on Appendix 1.

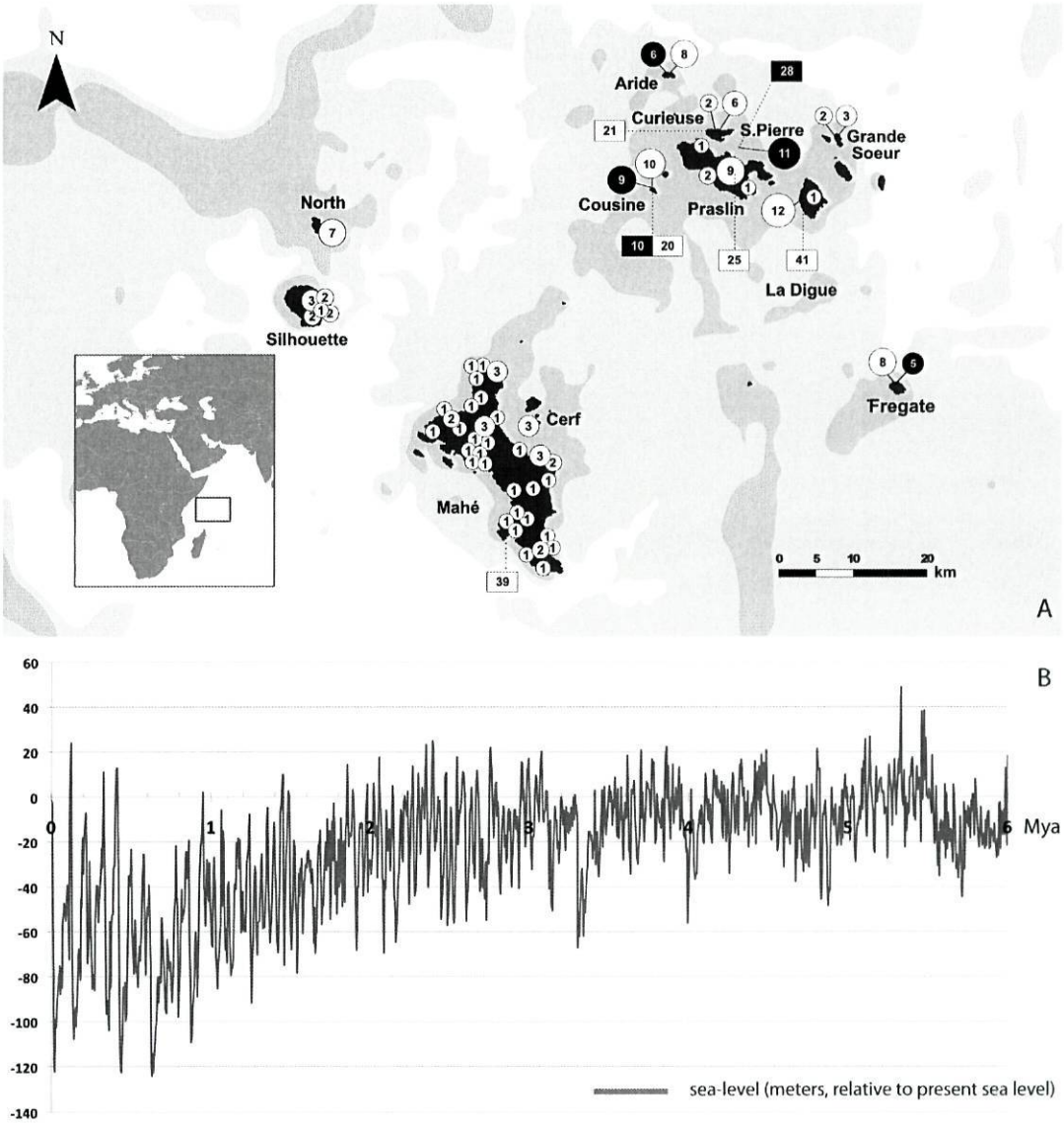



**Figure 1** – Map with sampling localities and last 6 My sea-level data. A) Map of the granitic Seychelles (Western Indian Ocean) showing individuals sampled at each island/locality. White labels indicate *M. sechellensis* individuals and black labels *M. wrightii*. Boxes indicate numbers and localities of individuals included in morphological analyses. Different shadings show areas that would be emerged at -30 m (dark grey) and -50 m (light grey) below present sea level (bpsl) stands. With a sea level of -60 m all the islands in the group would be connected. Data on Indian Ocean sea level changes support low-stands of up to 145 ± 5 m bpsl at six episodes in the last 0.5 Ma, some of which persisted for up to 50,000 years at a time (Camoin *et al.* 2004; Colonna *et al.* 1996; Rohling *et al.* 1998; Siddall *et al.* 2003). Geological records going back further suggest low-stands of 80 - 120 m bpsl at 0.64, 0.88, 1.04, 1.26 and 1.54 Ma, and eleven low-stand episodes of

50 m or more bpsl during the last 5 My (Miller *et al.* 2005). B) Sea level estimate derived from $\partial^{18}O$ for the last 6 My (from Miller et al., 2005; supplementary Table S1). Overall, data shows that previously to circa of two million years ago (Mya) the sea level was mostly oscillating around the current zero level, after which a decrease in sea-level occurred during all the Pleistocene, associated with the formation of ice sheets in the northern hemisphere, leading to a sea level below its current level for most of the time in the last 1.5 My, with a few exceptions lasting for short periods of time (Miller et al. 2005; see also Warren et al. 2010). By contrast, above sea-level stands were much less dramatic (ca. 11 and 17 m in the Pleistocene according to Siddall et al. 2003 with the highest value inferred during the last 5 My being 25 m – Miller at al. 2005), indicating that a high percentage of most granitic islands maintained almost their entire area.

A fragment of 711 bp of the cyt-b mtDNA gene was initially amplified for some individuals with forward primers CB1 (5'-CCATCCAACATCTCAGCATGATGAAA-3') or CBL14841 (5'-GCTTCCATCCAACATCTCAGCATGATGAAA-3', as in Austin et al. 2004); both modified from original CBL14841 (Kocher *et al.* 1989) and reverse primer CB3H (Palumbi *et al.* 1991). Specific primers for these species were then designed; CBMabPLF3 (5'-AGCATGATGAAATTTTGGTTCC-3') and CBMabR (5'-GGGTTGGCTGGTGTGAAG-3'), forward and reverse respectively, which amplify an internal region of 658 bp. Amplifications were conducted in 15 - 25 µl reactions using standard conditions with annealing temperatures of 50 - 52ºC and magnesium concentration of 1 - 1.5mM. For PDC we used primers and conditions as in Gamble et al. (2008), for C-mos we used primers Mos-F and Mos-R and conditions as in Godinho et al. (2005) and Rag-2 fragment amplification was as in Rocha et al. (2009). Primers and conditions for MC1R and RELN*int*61 are given in Pinho et al. (2010). Annealing temperature adjustments were made when necessary. PCR products were purified using ExoSAP-IT and sequenced either in a local automated DNA sequencing apparatus or by a commercial facility (Macrogen, Seoul, Korea). Sequences obtained are deposited on GenBank under accession numbers #####-##### (to be added upon manuscript acceptance). For most individuals nuclear fragments were sequenced for both strands to assure double peaks were real and not basecalling errors. Sequences were aligned manually using BioEdit (Hall 1999).

*Haplotype determination (nuclear genes)*

For the individuals that were heterozygous for insertions or deletions (some individuals at the RELN*int*61 fragment) we resolved the haplotype phases using the method described by Flot et al. (2006). After, we applied the Bayesian algorithm implemented in PHASE (Stephens *et al.* 2001) to the complete dataset, using the previously determined genotypes as known phases. For remaining fragments PHASE was applied directly. We used all positions for which phase was resolved with a posterior probability higher than 0.8 (though only in very few cases phase was resolved with less than 1 or 0.9 probability). The small percentage of cases where phase calls were resolved with less than 0.8 probability (2 cases for Rag-2, 4 for RELN*int*61 and 5 for MC1R) were coded as missing data (N) for the haplotype networks reconstruction and IM analyses and with ambiguity codes for the remaining sequence analyses, but individuals were never excluded. Multiple runs of PHASE were carried out for each dataset, and phase calls checked for consistency.

*Gene genealogies, age and demographic estimates under coalescent models*

The cyt-*b* alignment was unambiguous. We did not observe double peaks or stop codons that could suggest the presence of pseudogenes. This dataset was collapsed to single haplotypes using ALTER (Glez-Peña *et al.* 2010), and the best fitting model estimated separately for each codon position using jModeltest (Posada 2008) and Phyml (Guindon & Gascuel 2003) and the corrected Akaike information criteria (Posada & Buckley 2004; Sugiura 1978). We then used MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003) to reconstruct the haplotypes phylogeny (BI) under a partitioned model (dataset divided into codon positions for which model parameters are independently optimized), thought appropriate for protein-coding regions (Brandley *et al.* 2005; Brown & Lemmon 2007). Two runs of five million generations were performed with default heating parameters, sampled each 1000$^{th}$ sample. Convergence and congruence across runs were assessed using AWTY (Nylander *et al.* 2008).

Median-joining (MJ) networks (Bandelt *et al.* 1999) with maximum parsimony (MP) posterior optimization (Polzin & Daneschmand 2003) were constructed using NETWORK v4.510 (http://fluxus-engineering.com) to depict the relationships within nuclear and mitochondrial haplotypes. For the mitochondrial dataset, MJ networks were constructed for each main clade separately. In the nuclear intron RELN*int*61 seven indels (six of them longer than one base pair)

were needed to align the data. As multiple-base insertions or deletions are likely to have resulted from a single evolutionary step, we pruned the data leaving only the first base of the indel, as there was no variation across the remaining taxa within the indel. Within one of the indels present in some *M. sechellensis* individuals, *M. wrightii* exhibited variation that would be removed by this pruning method, and we decided to conserve that position, coding it homogeneously (with the same base present in all individuals without the indel) within *M. sechellensis*, to avoid discarding that information.

We used BEAST v1.4.8 (Drummond & Rambaut 2007) to obtain age estimates of the nodes of interest. To estimate the time of divergence between *M. sechellensis* and *M. wrightii* we selected a random subset of two individuals of each species and intraspecific mtDNA lineage, 6 in total, and estimated the age of the root of the tree (mtDNA) under an uncorrelated lognormal relaxed clock model, using a Yule tree prior and the best fit model of nucleotide substitution. The substitution rate used was 1% with a 0.27% standard deviation (normal distribution) as in Paulo et al. (2008), which seems to be the most appropriate rate for this fragment (cyt-b) for reptiles. Similar rates have been consistently recovered for mtDNA fragments of other lizards and skinks at the intrageneric level (Carranza *et al.* 2008; Chapple *et al.* 2009; Cox *et al.* 2010). For comparison we repeated the analysis under a strict clock model. Multiple runs of 30-90 million steps were performed, sampled at appropriate intervals to obtain 10,000 final samples (trees) and the initial 10% of the trees discarded as burnin. Tracer v1.4 was used to visualize the results and to check the effective sample size of each parameter (Rambaut & Drummond 2007) to ensure convergence.

The time to the most recent common ancestor (TMRCA) and past population size dynamics (demographic changes through time) of *M. wrightii* and of each mtDNA clade within *M. sechellensis* were co-estimated in BEAST using the Bayesian skyline plot (Drummond *et al.* 2005). We used the Bayesian skyline tree prior, given it fits a wide range of demographic scenarios, estimating the effective breeding population size through time. To allow continuous variation, we used the linear model, where population size is allowed to grow or decline between change points. The appropriate model of nucleotide substitution was estimated for each codon position of the cyt-b dataset, using jModeltest. Because the same model was selected for the 1st and 2nd positions, the SRD06 model (Shapiro *et al.* 2006) was used, except in the *M. wrightii* dataset, for which a single unpartitioned model provided a better fit. To determine if the demographic signatures observed in the mtDNA were also evident in the nuclear genome we analysed both

species nuclear datasets using the Extended Bayesian Skyline Plot model (Heled & Drummond 2008) implemented in BEAST v1.5.2, which extends the previous coalescent based method mainly in the ability to analyze multiple loci. A strict clock model was used in all cases for the BSP and EBSP analyses as we were dealing with intraspecific datasets with low variability. Runs of 30-50 million steps were performed and results visualized using Tracer, as before.

*Gene variability and neutrality tests*

To characterize variability levels of each marker and molecular diversity within each species, we used DNAsp v5.00.7 (Librado & Rozas 2009) to calculate summary diversity statistics for each species/lineage: haplotype (Hd) and nucleotide ($\pi$) diversity, number of segregating sites (S) and the population mutation parameter $\theta w$ (Watterson 1975). We also estimated Tajima's D (Tajima 1989), Fu's (1997) Fs and Ramos-Onsins and Rozas (Ramos-Onsins & Rozas 2002) $R_2$ statistics and determined their significance using 10,000 coalescent simulations.

*Morphological Variability*

In order to assess the level of morphological differentiation between species and populations, 184 individuals from five populations of *M. sechellensis* (Mahé, La Digue, Praslin, Curieuse and Cousine) and two populations of *M. wrightii* (Cousine and Saint Pierre) were analyzed (Figure 1A and Online Appendix 1 for more details regarding sample size per island). All individuals were captured by hand and released at the same site after data collection. The study only included adult individuals, establishing 66.5 mm and 106 mm snout vent length (SVL) as minimum adult size for *M. sechellensis* and *M. wrightii* respectively, based on field observations (presence of mating bites and pregnancy). Specimens were sexed by the presence of hemipenis.

For the morphological analyses, we measured 27 characters grouped in three sets of variables: 15 linear measurements, 5 pholidotic variables and 7 colour pattern characters (Online Appendix 2). All linear measurements were recorded in the field by the same person (AP) in order to minimize measurement error. Snout-vent length (SVL) was taken to the closest 0.1 mm with a ruler while the other characters were taken to the closest 0.01 mm using a digital calliper. Meristic and colour pattern variables were obtained in the laboratory from digital pictures taken in the field. To warrant the highest objectivity in the pholidotic counting and colour pattern variables data was recorded by two observers (AP and AS) independently and the

mean value (for pholidotic characters) or consensus (for colour pattern variables) were used in the analyses. However, in 52 cases, pholidotic data or body measurements were not recorded due to poor picture quality or limb amputation (1.38% of the data), and thus they were substituted by group means. All bilateral variables were taken from the right side of the animal. Prior to analyses, linear measurements were log-transformed and checked for normality (Lilliefors test, $p>0.05$) and homoscedasticity (Levene test, $p>0.05$). Since body measurements were highly correlated to SVL ($p<0.05$ in all cases) and simultaneously body size is the most distinctive character between *M. wrightii* and *M. sechellensis*, we used an isometric correction (Somers 1986) to isolate the relative contribution of size and shape to the differentiation of the populations. For that we created an isometric vector where all linear measurements (log-transformed) were projected to obtain a multivariate representation of the isometric size of each individual (mSIZE). After that we regressed each variable on it to obtain residuals for each that were then used as size-corrected variables (Kaliontzopolou *et al.* 2010). We then used the isometric body size (mSIZE) as multivariate representation of size, and the combination of the remaining isometric-size corrected variables as representation of shape. In order to have a general idea of the effect of sex, species and population on the size, shape and pholidotic variables, we performed nested (M)ANOVAs using SPECIES, SEX, POPULATION nested in SPECIES, and their interaction separately on size (represented by mSIZE), shape (represented by the remaining isometric-size corrected variables) and pholidotic variables (Online Appendix 2). Since both species live in sympatry in Cousine island, individuals were identified based on morphological features (colour pattern and body size) and *a-priori* assigned to each species. They are named hereafter as "Cousine *M. sechellensis*" and "Cousine *M. wrightii*" respectively.

We performed a Canonical Discriminant Function Analysis (CDFA) on shape (isometric-corrected variables) and pholidosis variables to investigate which ones maximize the differentiation between islands. The distance between CDFA population centroids was measured using Mahalanobis formulation. This metric was in turn used to represent the morphological relationships between populations, through an unweighted pair-group method (UPGMA). Finally, generalized variation in colour pattern in all populations was analyzed using a Multiple Correspondence Analysis (MCA), which allowed the simultaneous visualization of the relationships between the colouration characters and the populations analyzed (McGarigal *et al.* 2000). Finally, a nested MANOVA on MCA individual scores was then used to test for significant differences in colour pattern among SPECIES, SEX, POPULATION nested in SPECIES, and their

interaction. All analyses were performed using Statistica (StatSoft Inc., 2005) v7.1 and R (R Development Core Team, 2009).

*Inter-island migration dynamics*

We applied the isolation-with-migration (IM) model of Nielsen and Wakeley (2001), as implemented in the program IMa (Hey & Nielsen 2007) to obtain estimates of migration for *M. sechellensis* between pairs and groups of islands, under a two-population model. We performed these analyses only within *M. sechellensis* because the current distribution of *M. wrightii* is most probably greatly shaped by extinction, as evidenced by the available historical records, and its current restricted distribution was not adequate to test biogeographic hypotheses across the archipelago. The IM model takes into account population divergence and gene flow in the same framework, and is therefore appropriate to disentangle between the relative effects of recent isolation and migration between closely related groups. Our goal was to test if the relationships between *M. sechellensis* island populations could be simply explained by the recent isolation of a wide-ranging panmictic population or if migration was relevant in shaping current genetic structure, and how. In this regard, the model-testing approach implemented in IMa (Hey & Nielsen 2007) was used to compare the likelihood of models with and without migration. Note that not all possible comparisons between islands were made, but only the most relevant/plausible ones taking into account the geography of the archipelago. We obtained gene flow estimates based on both mtDNA+nuclear data as well as using nuclear data alone.

However, applying the IM model to a multiple-population scenario we are violating one of its main assumptions, i.e., the inexistence of other unsampled populations exchanging genes with the sampled populations or their ancestor. Strasburg and Rieseberg (2010) assessed the effect of this and other violations of the IM model assumptions and found that not only population sizes estimates were overestimated (in populations that receive migrants from a third population) but also that migration rates estimates were biased (third population gene flow causes an overestimation of the amount of gene flow to the "receiver" population and an underestimation in the opposite direction). These biases were small for low levels of gene flow but for higher levels they became substantial. Nevertheless, our goal was not to estimate accurate rates of migration between all specific populations but to test for the existence of migration within and across groups, by accepting or rejecting models involving zero-migration in

one or both directions. Further IM model assumptions include lack of (substantial) population structure, neutrality, no recombination within loci and demographic stability. Therefore, to further investigate the genetic structure within the *M. sechellensis* dataset we also performed a hierarchical analysis of molecular variance (AMOVA, Excoffier *et al.* 1992), using Arlequinv3.1.1 (Excoffier *et al.* 2005). The significance of the AMOVA was tested with 10,000 permutations. Selection was tested for with the Tajima's (1989) and Fu and Li (1993) D statistics (by-gene and multilocus), as well as a multilocus HKA test (Hudson *et al.* 1987) for the nuclear fragments using *M. wrightii* as "outgroup", using the SITES and HKA programs (http://genfaculty.rutgers.edu/hey/software). Additionally, the heterogeneity test as implemented in Hahn et al. (2002), was performed for all coding markers. The latter is based on the assumption that selection and demography have different effects on synonymous and non-synonymous substitutions and it is a simple modification of the standard D tests to allow the distinction between demographic and selective events by looking for processes producing different patterns on the different categories of mutations. Finally, the software PhiPack (http://www.math.auckland.ac.nz/~bryant/software/) was used to test for recombination with the pairwise homoplasy index ($\Phi w$ statistic) of Bruen *et al.* (2006), which is a powerful detector of recombination, little affected by the effects of recurrent mutations and exponential growth.

## Results

*Genetic variability and neutrality tests*

As expected, nuclear fragments were much less polymorphic than cyt-*b* (Table 1). Among the nuclear markers, MC1R seemed to be the most variable, with the highest overall nucleotide diversity. Watterson's estimator, $\theta w$, was constantly smaller in *M. wrightii*. Most markers exhibited significant negative values of Tajima's D for *M. sechellensis* and positive values for *M. wrightii*. Accordingly, Fu's $F_s$ was usually negative and the $R_2$ statistics was lower, and for most markers they depart significantly from the values expected under demographic stability for *M. sechellensis*. They thus suggest that a recent population expansion might have occurred in *M. sechellensis*, but not in *M. wrightii*. No evidence for selection or recombination was detected (Supporting information).

*Gene genealogies and geographic structure of genetic variation*

The phylogenetic analysis (mtDNA) unveiled two divergent lineages (ca. 9% divergent; uncorrected p-distance) (Figure 2). The first lineage corresponds to the *M. sechellensis* individuals from the northern islands (Praslin-group), while the second is formed by the *M. sechellensis* from the southern islands (Mahé-group) plus the Fregate island population, two individuals from Aride (Praslin-group) and *M. wrightii*.

In the northern lineage, *M. sechellensis* forms a star-shaped phylogeny, with the majority of haplotypes differing by one to two substitutions from the central haplotype. In the southern group, the *M. sechellensis* haplotypes are more scattered, and differing by a higher number of mutations, though without any obvious inter- or intra-island geographic structure. The most distant haplotypes (16 substitutions apart) are both found in the same island (Mahé). Both Mahé and Silhouette haplotypes are widespread within the network. Haplotypes from Cerf (off Mahé) are closely related to Mahé. Haplotypes from Fregate have a central position within the network, and are the closest to *M. wrightii*. Haplotypes of *M. wrightii* define a well-supported clade of closely related haplotypes, only five substitutions apart from the closest *M. sechellensis* haplotype. Despite the low variability observed within *M. wrightii* (six haplotypes) only one haplotype is shared across islands (Cousine and Aride). Haplotype diversity within *M. sechellensis* is higher in both clades, but haplotype sharing across localities is also limited, with only two instances in the southern group (between Mahé and Silhouette) as well as in the northern group (between Praslin and Curieuse and between Praslin and La Digue and Curieuse; the latter involving the central haplotype).

In contrast, *M. sechellensis* and *M. wrightii* define exclusive groups for the nuclear markers (Figure 3). Here, the diversity levels are reduced in comparison with the mitochondrial data, and there is no clear phylogenetic structure within each species. Genealogies of *M. sechellensis* usually conform to star-shaped phylogenies whereas *M. wrightii* exhibits a lower number of alleles at all loci and, for polymorphic loci, alleles have more balanced frequencies. The minimum number of mutations that separate each species' alleles varies between one (in PDC) and 11 (in MC1R).

**Table 1** – Summary statistics, neutrality and population growth tests for the different species based on mitochondrial and nuclear DNA variation. Within *M. sechellensis* values are presented for the two mtDNA lineages (see results) separately. MAg stands for Mahé-group (the southern lineage) and PLg for Praslin-group (or the northern lineage).

| Locus | Taxa | Length (bp) | N | Polymorphism | | | | | Neutrality | Population Growth | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | H | Hd | S | π | θw | Tajima's D | Fs | $R_2$ |
| Cyt-b | All | 632 | 144 | 63 | 0.969 | 112 | 0.04892 | | | | |
| | *M. sechellensis* (MAg) | | 70 | 33 | 0.920 | 48 | 0.00756 | 0.01586 | -1.72717* | -19.107** | 0.0447* |
| | *M. sechellensis* (PLg) | | 47 | 23 | 0.958 | 30 | 0.00611 | 0.01078 | -1.45627* | -13.528** | 0.0577* |
| | *M. wrightii* | | 27 | 6 | 0.755 | 8 | 0.004 | 0.0033 | 0.66483 | 0.9444 | 0.1517 |
| C-mos | All | 510 | 176 | 5 | 0.432 | 5 | 0.00161 | | | | |
| | *M. sechellensis* (MAg) | | 64 | 3 | 0.062 | 2 | 0.00012 | 0.00083 | -1.4358* | -3.3791* | 0.0870 |
| | *M. sechellensis* (PLg) | | 70 | 2 | 0.159 | 1 | 0.00031 | 0.00041 | -0.28941 | 0.1050 | 0.0795 |
| | *M. wrightii* | | 42 | 1 | - | 0 | - | - | - | - | - |
| Rag-2 | All | 796 | 176 | 15 | 0.730 | 15 | 0.00274 | | | | |
| | *M. sechellensis* (MAg) | | 64 | 9 | 0.410 | 5 | 0.00057 | 0.00213 | -1.9058* | -8.066** | 0.0366* |
| | *M. sechellensis* (PLg) | | 72 | 5 | 0.660 | 4 | 0.00102 | 0.00104 | -0.03032 | -0.369 | 0.1025 |
| | *M. wrightii* | | 40 | 4 | 0.631 | 3 | 0.00105 | 0.00089 | 0.40365 | 0.220 | 0.1393 |
| Reln*int*61 | All | 912 | 156 | 20 | 0.733 | 22 | 0.00224 | | | | |
| | *M. sechellensis* (MAg) | | 56 | 7 | 0.531 | 6 | 0.00068 | 0.00145 | -1.32232 | -3.673* | 0.0581 |
| | *M. sechellensis* (PLg) | | 64 | 9 | 0.586 | 9 | 0.00092 | 0.00211 | -1.51098* | -4.614* | 0.0514 |
| | *M. wrightii* | | 36 | 8 | 0.789 | 9 | 0.00282 | 0.00239 | 0.53440 | -0.112 | 0.1403 |
| MC1R | All | 633 | 152 | 7 | 0.617 | 14 | 0.00601 | | | | |
| | *M. sechellensis* (MAg) | | 60 | 6 | 0.580 | 4 | 0.00256 | 0.00136 | 1.97662 | 0.685 | 0.2043 |
| | *M. sechellensis* (PLg) | | 66 | 4 | 0.397 | 4 | 0.00082 | 0.00133 | -0.8206 | -0.563 | 0.0648 |
| | *M. wrightii* | | 26 | 2 | 0.517 | 1 | 0.00082 | 0.00041 | 1.59084 | 1.619 | 0.2585 |
| PDC | All | 375 | 182 | 6 | 0.466 | 5 | 0.00133 | | | | |
| | *M. sechellensis* (MAg) | | 62 | 3 | 0.211 | 4 | 0.00058 | 0.00170 | -1.29675 | -2.638* | 0.0522 |
| | *M. sechellensis* (PLg) | | 74 | 3 | 0.105 | 2 | 0.00028 | 0.00109 | -1.2114* | -2.348* | 0.0610 |
| | *M. wrightii* | | 46 | 1 | - | 0 | - | - | - | - | - |

N, number of individuals (mtDNA) or chromosomes (nuclear DNA); H, number of haplotypes; Hd, haplotype diversity; S, number of segregating sites; π, nucleotide diversity; θw, population mutation parameter, calculated according to Watterson (1975). Fs, Fu's (1997) Fs; $R_2$, Ramos-Onsins and Rozas's (2002) $R_2$. Summary statistics were calculated excluding indels and positions coded as missing data. * significant at P<0.05; ** significant at P<0.01

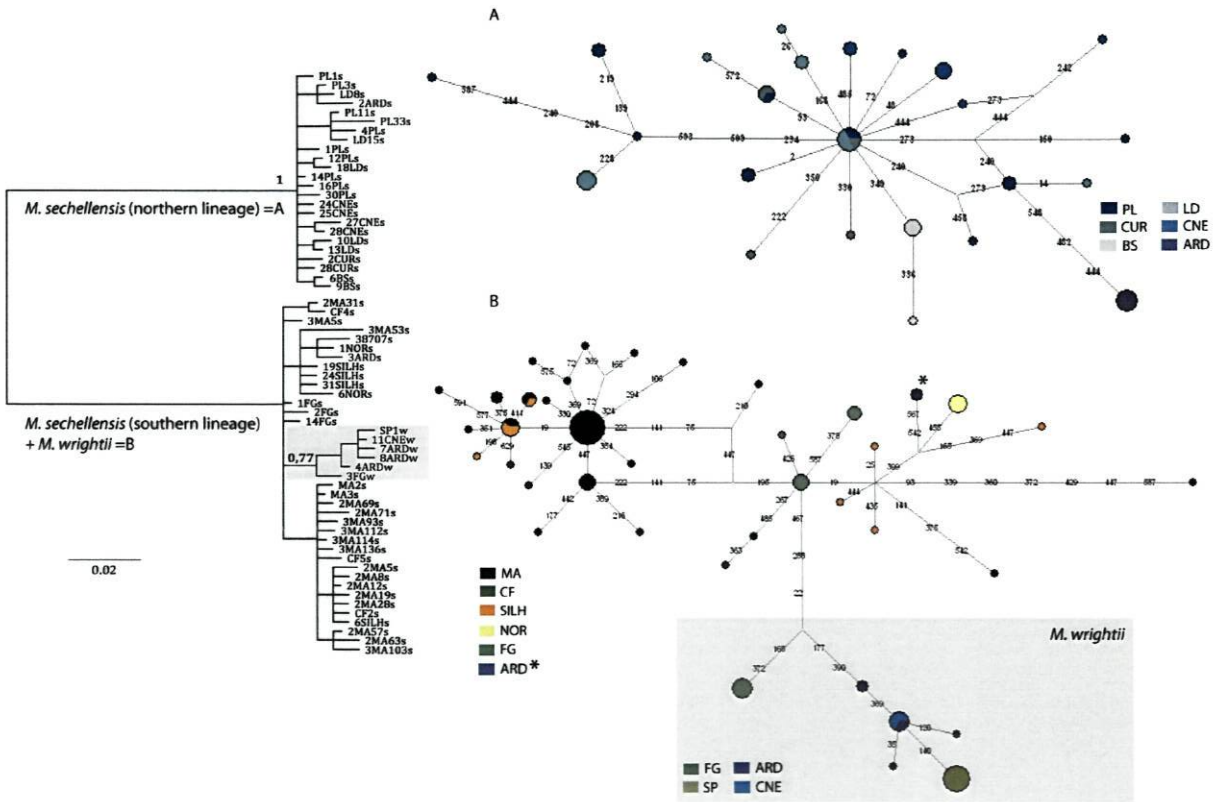



**Figure 2 -** Midpoint rooted BI tree and MJ networks for mtDNA data. Left: mitochondrial DNA tree. *Mabuya wrightii* haplotypes are highlighted with grey shading. Upper right (A): mitochondrial DNA haplotype network for the *M. sechellensis* "northern lineage" (Praslin-group). Bottom right (B): mitochondrial DNA haplotype network for the *M. sechellensis* "southern lineage" (Mahé-group) + *M. wrightii*. An haplotype harboured by *M. sechellensis* individuals from Aride Island (2) clustering within the "southern lineage" is marked with an asterisk. *M. wrightii* haplotypes are highlighted in grey shading. Islands are colour coded (MA: Mahé; CF: Cerf; SILH: Silhouette; NOR: North; FG: Fregate; SP: Saint Pierre; ARD; Aride; CNE: Cousine; PL: Praslin; CUR: Curieuse; LD: La Digue; BS: Grande Soeur).

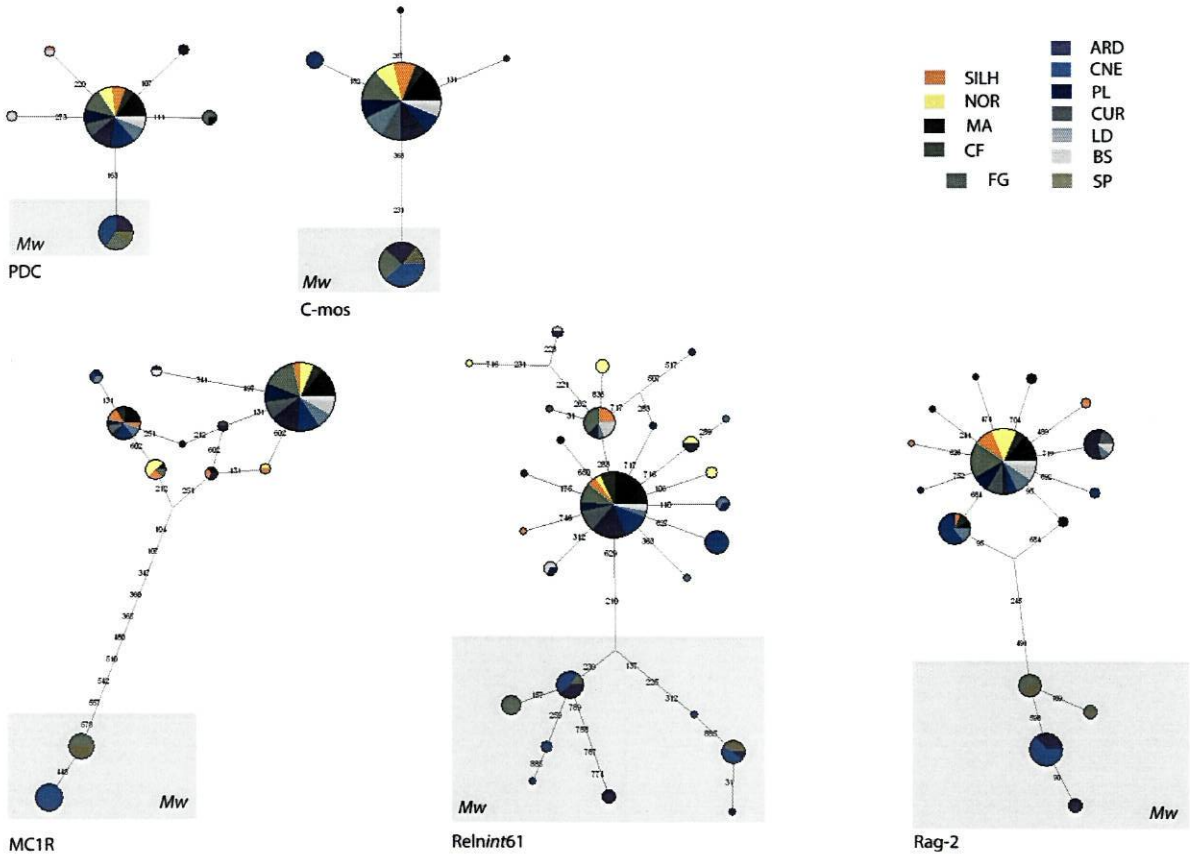



**Figure 3 -** MJ networks for the nuclear data. Islands are colour coded (see legend from Figure 2) and haplotypes belonging to *M. wrightii* populations (*Mw*) highlighted in grey.

In summary, the resulting geographical distribution of mitochondrial and nuclear lineages within *M. sechellensis* is clearly different (Figure 4). Whereas the nuclear variation can be sorted into two groups corresponding to the recognized species, *M. sechellensis* from the southern islands (plus Fregate) exhibit mtDNA haplotypes phylogenetically closer to the ones harboured by *M. wrightii* (represented in different tones of purple) than to the remaining *M. sechellensis* mtDNA haplotypes. The exceptions are two individuals from Aride (northern group), which harbour haplotypes that cluster within the southern group.

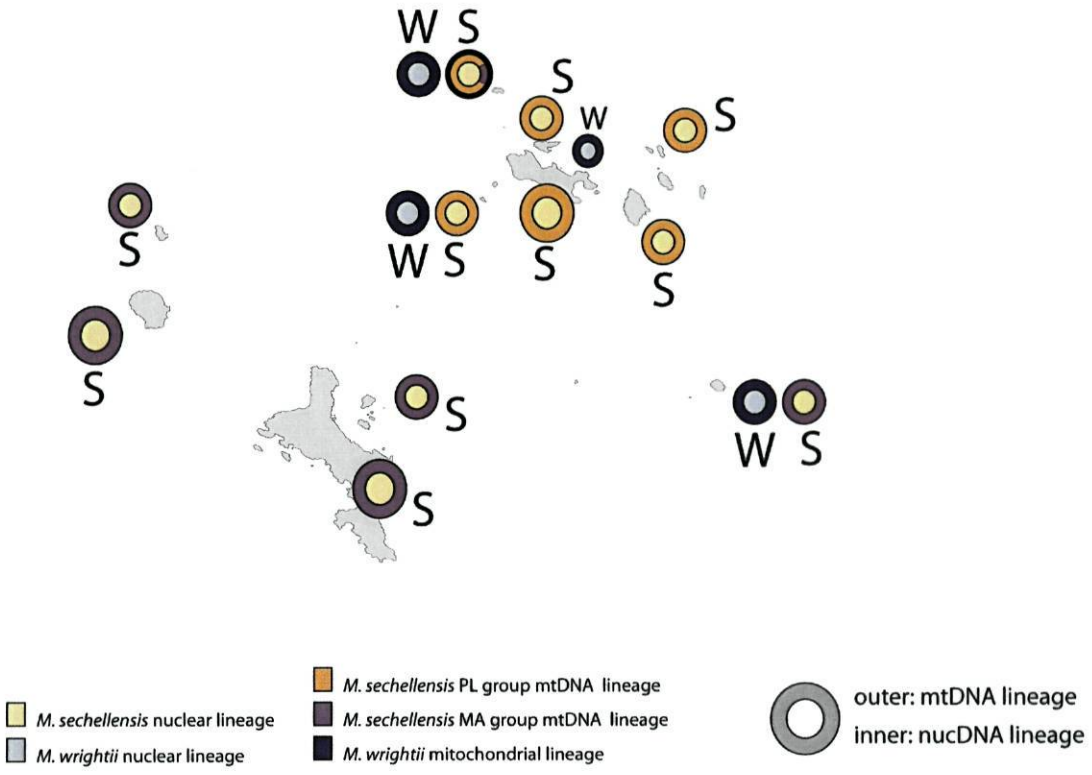



**Figure 4 -** Geographic distribution of mitochondrial and nuclear lineages. S: *M. sechellensis* populations; W: *M. wrightii* populations. Aride Island population of *M. sechellensis*, where some individuals harbouring mtDNA haplotypes from the southern lineage were found, is highlighted (dark ring). "PL group" stands for Praslin-group (the northern group of islands) and "MA group" for Mahé-group (the southern group of islands, including Fregate).

*Age and demographic estimates*

Despite the very large credibility intervals around the estimated dates (Table 2), according to the mtDNA data the age of the deepest node of the tree seems to be pre-Pleistocenic, possibly mid to late Miocenic. The estimates obtained under a strict clock model resulted in narrower posterior credibility intervals, but support an equally ancient root. The estimated ages for the most recent common ancestors of the two *M. sechellensis* lineages and of *M. wrightii* seem considerably more recent.

**Table 2 –** Time to the most recent common ancestor (TMRCA) estimates (mean and 95% credibility interval) obtained from the mitochondrial DNA dataset for 1) both species/lineages (corresponding to the root of the tree from figure 2, left); 2) *M. sechellensis* from Mahé-group (southern clade); 3) *M. sechellensis* from Praslin-group (northern clade) and 4) *M. wrightii*. Values are in million years.

| | Clock Model and Tree Prior | | |
|---|---|---|---|
| Clade | Relaxed clock (Uncorrelated lognormal) Yule Tree Prior | Strict Clock Yule Tree Prior (1) / Bayesian Skyline (2-4) | |
| 1. rootheigh | 6.13 (2.32-11.7) | 6.00 (4.14-8.47) | |
| 2. *M. sechellensis* (MAg) | | 0.78 (0.25-1.44) | |
| 3. *M. sechellensis* (PLg) | | 0.45(0.14-0.86) | |
| 4. *M. wrightii* | | | 0.36 (0.068-0.74) |

The Bayesian skyline plots seem to indicate a recent population expansion for *M. sechellensis* but a continuous decrease for *M. wrightii* (Figure 5, A-C) and suggest that the population sizes for *M. sechellensis* is orders of magnitude larger than for *M. wrightii*. This difference is probably slightly lower as the estimated population size is scaled by the generation time, which may be higher in *M. wrightii*, as usual for larger-sized lacertids (Bauwens 1999; Carretero 2006). For the nuclear data, the Extended Bayesian skyline analyses reinforce these results (Figure 5, D-E).

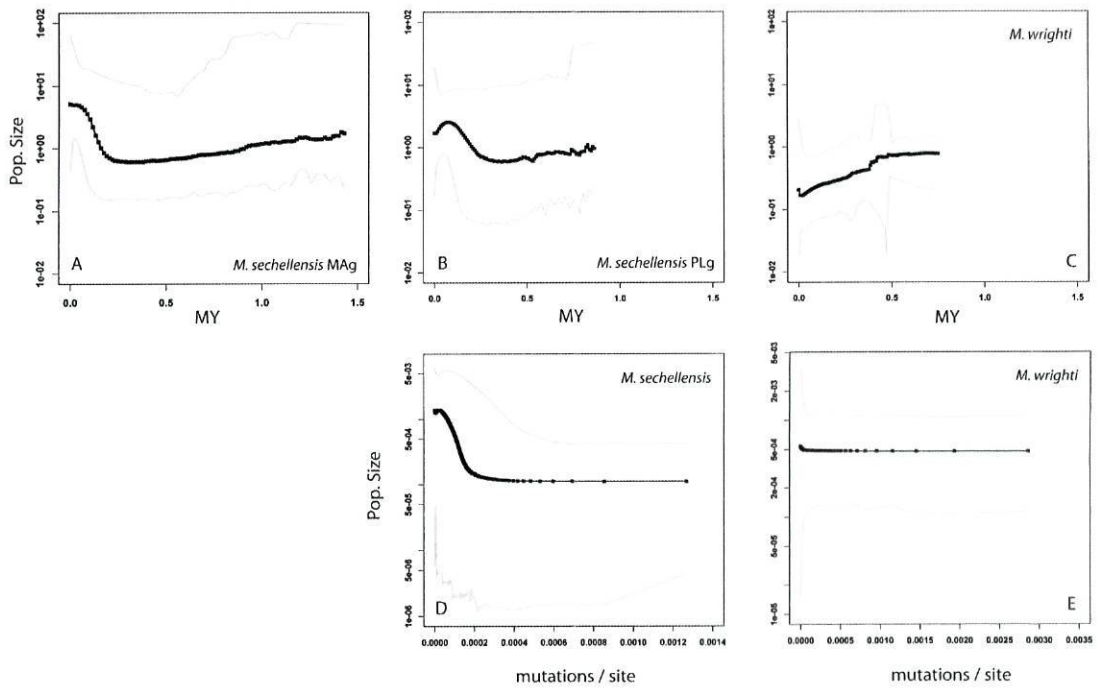



**Figure 5** - Results from Bayesian Skyline Plot for mtDNA for *M. sechellensis* southern lineage (A) and northern lineage (B) and *M. wrightii* (C) and from the multilocus Extended Bayesian Skyline Plot (nuclear loci) for *M. sechellensis* (D) and *M. wrightii* (E). Axis are in million years (X) and Ne*t (the product of the effective population size and the generation length in million years) (Y) for the BSP plots (A-C) and in mutations-per-site (X) and Ne*μ (the product of the effective population size and the mutation rate) (Y) for the EBSP plots (D-E). Note that while BSP population size estimates are comparable across species, EBSP estimates for the different species are not, because the two loci without variation within *M. wrightii* (PDC and C-mos) were not included in the analysis. Black dotted lines represent mean estimated and grey lines the 95% upper and lower intervals.

*Morphological Variation*

*Analysis of morphometric and scalation characters*

Males and females differed both in size and shape (Table 3), but not in pholidosis. Regarding general differences between the two species, they differed in pholidosis and size, but not in shape. Populations (considered as a factor nested within species) differed in all variables (in all cases $p<0.001$; Table 3). Finally, although the degree of sexual dimorphism (interaction sex*species) was similar in both species (Table 3), we found local differences in pholidosis but not in the rest of the variables analyzed.

**Table 3** - Summary of the (M)ANOVA results on the effect of the species, sex and population (as factor nested in species) on size (mSIZE), shape (isometric-corrected variables), pholidosis (raw variables) and colour pattern (individual MCA factor scores). For each factor analyzed, degrees of freedom (d.f.) and results of the F value and level of significance (p) for each dataset are provided. See materials and methods for more details.

| | | species | sex | pop(species) | species*sex | pop*sex |
|---|---|---|---|---|---|---|
| | d.f. | 1 | 1 | 5 | 1 | 5 |
| size (mSIZE) | F | 2713,79 | 11,4728 | 16,3961 | 0,0666 | 1,359 |
| | p | **<0.0001** *** | **<0.001** ** | **<0.001** ** | 0.797 n.s. | 0.242 n.s. |
| shape | F | 0,325 | 7,796 | 4,568 | 1,583 | 1,081 |
| (isometric-corrected variables) | p | 0.99 n.s. | **<0.0001** *** | **<0.0001** *** | 0.089 n.s. | 0.310 n.s. |
| pholidosis | F | 18,477 | 0,201 | 3,256 | 1,352 | 1,832 |
| (raw variables) | p | **<0.0001** *** | 0.961 n.s. | **<0.0001** *** | 0.245 n.s. | **0.008** * |
| Colour pattern | F | 118,660 | 0,276 | 10,937 | 2,896 | 2,121 |
| (MCA individual scores) | p | **<0.0001** *** | 0.893 n.s. | **<0.0001** *** | **0.024** * | **0.003** * |

Since males and females differed in some of the variables analyzed or in their interaction, both sexes were considered separately in the multivariate analyses. Regarding the CDFA, variables that contributed most to the CDF1 (45% in males and 52.1% in females) were head measurements (HH, NED and SED) in males and head measurements (HH, EL, NED, SED) and number of supraciliar scales (SPC) in females (Table 4). Trunk length (TrL), tail width (TW) and number of lamelas (LM) in males and head length (HL) and lamellae (LM) and number of scales

between nostril and eye (SNE) in females contributed most to CDF2 (20.9% in males and 27% in females; Table 4) while limb length (CFL) and LM in males and LM in females represented most of the variation across CDF3 (13.7% in males and 11.2% in females; Table 4). The UPGMA clustering (Figure 6 a and b) showed that two populations of *M. sechellensis* (Mahé and Cousine) were the most distinctive ones, while *M. wrightii* populations appeared mixed with other *M. sechellensis*. This pattern was generally similar in males and females. The populations best discriminated by shape and pholidosis (Figure 6 a and b) were the ones from Cousine (100% of individuals correctly classified in both sexes) followed by Mahé (95%) in males and Curieuse in females (91%).

**Table 4** – Summary results of the canonical discriminant function analysis on the shape and pholidosis for males and females. For each sex, pooled within-groups correlations of each variable, eigenvalues and cumulative percentage (% cumulative) of the variance explained for the first three discriminant functions is given. The total percentage of individuals correctly classified is also provided (partial percentages per locality are detailed in Figure 6).

| | CDFA | | | | | |
|---|---|---|---|---|---|---|
| | Males | | | Females | | |
| | CDF1 | CDF2 | CDF3 | CDF1 | CDF2 | CDF3 |
| LogTrL | -0,039 | **0,426** | 0,093 | 0,088 | 0,262 | 0,210 |
| LogTW | 0,066 | **0,424** | 0,224 | -0,169 | 0,032 | 0,017 |
| LogFLL | -0,230 | -0,277 | 0,067 | 0,176 | -0,125 | 0,032 |
| LogFFL | -0,063 | -0,063 | 0,120 | 0,224 | -0,094 | -0,056 |
| LogHLL | -0,150 | -0,152 | -0,066 | 0,233 | 0,001 | -0,295 |
| LogCFL | -0,033 | -0,144 | **0,309** | 0,169 | -0,034 | 0,074 |
| LogHL1 | -0,185 | -0,075 | -0,014 | 0,208 | **-0,303** | -0,044 |
| LogHW | 0,052 | 0,151 | 0,071 | 0,092 | -0,079 | 0,085 |
| LogHH | **0,355** | 0,083 | -0,075 | **-0,412** | 0,108 | 0,201 |
| LogOD | -0,012 | -0,140 | -0,201 | -0,166 | -0,035 | -0,201 |
| LogEL | 0,282 | -0,146 | -0,248 | **-0,496** | -0,005 | -0,117 |
| LogNED | **-0,309** | 0,023 | 0,120 | **0,451** | 0,046 | 0,084 |
| LogSED | **-0,349** | -0,055 | 0,028 | **0,393** | -0,086 | 0,030 |
| LogEED | -0,004 | -0,191 | -0,229 | 0,110 | -0,008 | -0,040 |
| LM | -0,159 | **0,354** | **-0,590** | 0,024 | **-0,458** | **0,505** |
| SPC | 0,221 | -0,273 | 0,110 | **-0,338** | 0,274 | 0,073 |
| SLS | 0,200 | -0,230 | 0,048 | -0,069 | 0,000 | -0,029 |
| SEE | 0,113 | -0,004 | 0,009 | -0,054 | -0,238 | -0,111 |
| SNE | 0,075 | -0,037 | -0,092 | -0,194 | **-0,354** | -0,222 |
| % classification | 86,4 | | | 81,3 | | |
| Eigenvalues | 3,116 | 1,446 | 0,945 | 3,000 | 1,555 | 0,646 |
| % cumulative | 45,00 | 65,89 | 79,55 | 52,10 | 79,10 | 90,33 |

*Analysis of Colour Patterns*

The first three axes of the MCA (Figure 6 c and d) explained a total of 57% and 56% of the male and female colour variation, respectively. In males, the existence of dorsolateral lines and the body ventral colouration were the characters that contributed most to the first axis (25%), while the head ventral colouration and the existence of dorsal black spots mostly explained the variation in the second axis (18%; Figure 6 c). In females, ventral pigmentation, lack of dorsolateral lines and the existence of dorsal black spots contributed most to the first axis (22.6%), while the presence of dorsolateral lines and the ventral head colouration were the most important characters explaining the variation in the second axis (18.1%, Figure 6 d). In both males and females, the plot of the first two axis shows that Cousine island has the most distinct pattern among all *M. sechellensis* due to the presence of white dorsal spots and lack of ventral orange pigmentation, while populations from Praslin, Curieuse and la Digue have similar colour pattern (no dorsal spots, lack of head ventral colouration and presence of continuous dorsal lines). Interestingly, the population from Mahé shows intermediate colour pattern between *M. seychellensis* and *M. wrightii* populations (Figure 6 c and d). Such differences in colour pattern were significant between species, populations, and their interaction with sex (species*sex, population*sex), but not between sexes (Table 3).

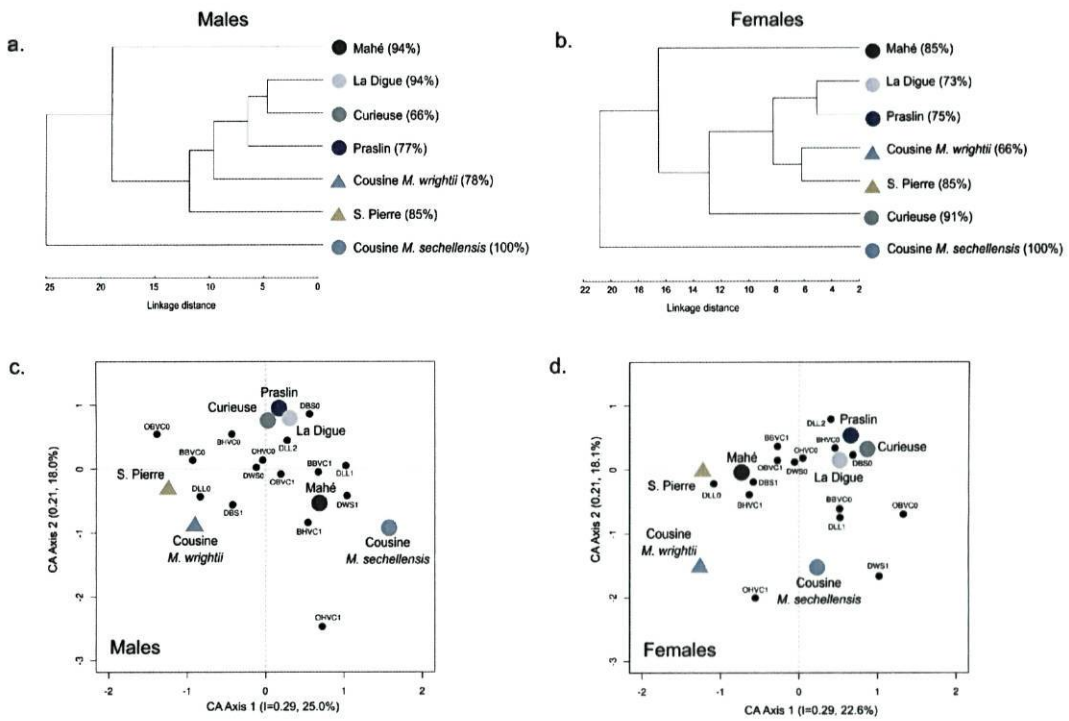



**Figure 6** - Graphic representation of the multivariate analyses. Upper graphs show the dendrogram obtained from clustering the CDFA for males (a) and females (b). Numbers within brackets after the locality indicate the percentage of correct classification of individuals per locality obtained on the CDFA. Lower graphs represent the relative position of the populations and coloration characters analysed against the first two MCA axes (% inertia, % variance) for males (c) and females (d). Populations are colour coded (as in Figures 2 and 3), with solid circles representing *M. sechellensis* and triangles *M. wrightii*. Meanings of characters abbreviations are detailed in Online Appendix 2.

### *Migration dynamics in M. sechellensis*

The analysis of molecular variance reflected the lack of structure at the levels of interest (Supplementary Table S1). Variation was higher within populations than among populations or groups for all markers except for cyt-b. This is clearly due to the fixation of the introgressed lineage in one of the groups. However, variation attributable to structure among populations is still very low and justifies the application of the IM model at pairwise populations.

Overall, the IM analyses recovered signs of migration across islands. In most comparisons, models with zero migration were rejected (Supplementary Table S3), both when

comparing close (e.g. Silhouette and North; Praslin and Aride) and distant islands (e.g. Silhouette with La Digue; Fregate with La Digue and Praslin; Mahé and Fregate). In the pairwise comparisons of individual islands, failure to reject models without gene flow happened only in three instances, always involving the island of Mahé (with North, Silhouette and La Digue). When islands were collapsed into groups, the estimated *Nm* values from the northern islands (Praslin-group) to others (based only on nuclear data) were usually low or not significantly different from zero (Table 5). On the other hand, the estimated *Nm* values from Silhouette+North into the Praslin-group population were significantly different from zero, even after excluding the two Aride individuals whose mtDNA haplotypes were nested within the haplotypes from the Mahé-group. Their exclusion seemed justified given their low number and that they could be recent migrants instead of reflecting historical processes – Aride is a highly-visited nature reserve and *M. sechellensis* can be easily translocated with boats or material. Furthermore we did not collect morphological data from Aride population which would allow us to eliminate or confirm this hypothesis. Migration between Mahé (+Cerf) and Silhouette+North was not significantly different from zero, corroborating previous results (pairwise comparisons between islands based on mtDNA and nuclear data; Supplementary Table S3). Contrastingly, *Nm* estimates from Fregate island to both Mahé+Cerf and Silhouette+North islands were large. Estimates made clustering all the islands into two populations representing the main mtDNA lineages, and using only the nuclear markers returned consistent positive estimates of migration (2*Nm* into the southern clade = 1.8975; 2*Nm* into northern clade = 1.3617). However, migration from the northern islands (Praslin-group) into the southern ones (Mahé-group) was not significant (Supplementary Table S4).

**Table 5 –** Population migration rates (*2Nm*) estimated between groups of islands under the isolation-with-migration model for two populations, using only the nuclear data. *2Nm1* corresponds to the population migration rate from population 2 to population 1 (genes migrating INTO population one) and *2Nm2* to the population migration rate from population 1 into population 2. Values higher that 1 are highlighted. Non rejected models involving zero-migration estimates are reported (highlighted for zero reciprocal estimates).

| | | **Population 2** | | | |
|---|---|---|---|---|---|
| | | **Silhouette + North** | **Mahé+Cerf** | **Fregate** | **Praslin-group** [a] |
| **Population 1** | **Silhouette + North** | | | | *2Nm1*= 0,05592<br>***2Nm2*=1,91752**<br><br>$\theta_1\ \theta_2\ \theta_A\ m_1{=}0\ m_2$ |
| | **Mahé+Cerf** | ***2Nm1*=1,3912**<br>*2Nm2*=0,0056<br><br>$\theta_1\ \theta_2\ \theta_A\ m_1\ m_2{=}0$<br>$\theta_1\ \theta_2\ \theta_A\ m_1{=}0\ m_2$<br>$\boldsymbol{\theta_1\ \theta_2\ \theta_A\ m_1{=}\ m_2{=}0}$ | | ***2Nm1*= 21,2531**<br>*2Nm2*=0,00043<br><br>All zero-m models rejected | *2Nm1*= 0,006788<br>*2Nm2*= 0,283477<br><br>$\theta_1\ \theta_2\ \theta_A\ m_1\ m_2{=}0$<br>$\theta_1\ \theta_2\ \theta_A\ m_1{=}0\ m_2$<br>$\boldsymbol{\theta_1\ \theta_2\ \theta_A\ m_1{=}\ m_2{=}0}$<br>$\boldsymbol{\theta_1{=}\ \theta_2\ \theta_A\ m_1{=}\ m_2{=}0}$ |
| | **Fregate** | *2Nm1*=0,0002<br>***2Nm2*= 15,7605**<br><br>$\theta_1\ \theta_2\ \theta_A\ m_1{=}0\ m_2$ | | | *2Nm1*= 0,13851<br>*2Nm2*=0,00853<br><br>$\theta_1\ \theta_2\ \theta_A\ m_1\ m_2{=}0$ |
| | **Praslin-group** [a] | | | | |

[a] Praslin-group consists of Praslin, La Digue, Cousine, Aride, Curieuse and Big Sister populations. Individuals from Aride that exhibited haplotypes clustering with Mahé-group haplotypes were not included in the analysis.

## Discussion

*Divergence and introgressive hybridization between M. sechellensis and M. wrightii*

Population level sampling within both species confirmed previous results of mtDNA paraphyly of *M. sechellensis* (Rocha *et al.* 2010) which exhibits a genetic structure highly correlated with geography. Its two mtDNA lineages are harboured by individuals respectively from northern (Praslin-group) and southern (Mahé-group) islands, these latter within which a monophyletic *M. wrightii* is nested. Given the contrasting nuclear genealogies, where each species harbours distinct haplotype groups, introgressive hybridization seems to best explain current data, while incomplete lineage sorting or multiple evolution of gigantism seem less probable.

According to this interpretation, the age of the deepest split within the mtDNA tree reflects the separation of the two species (*M. wrightii* and *M. sechellensis*), roughly around 6 Mya, in the Mio-Pliocene. The high credibility intervals reflect the limited power to estimate this age (less than 1kb data and no calibration within the phylogeny itself; Brown & Yang 2010), which must be seen as a rough approximation. Nevertheless, both estimates under a relaxed or a global clock model point to a pre-Pleistocenic age, at the border between Mio- and Pliocene, and are at the moment our best possible guess for the age of these species.

At least one, most possibly ancient, instance of hybridization, leading to the introgression of *M. wrightii*'s mtDNA into the *M. sechellensis* populations of the southern islands needs to be invoked to explain the resulting phylogeny. The direction of the hybridization and the geographic pattern of the introgression are hard to interpret, as *M. wrightii* is currently much larger than *M. sechellensis,* and females usually resist copula, selecting for larger and stronger males (eg. Cooper & Vitt 1997). Also, *M. wrightii* is almost absent from the southern group of islands (except for Fregate), though it might have been present in the past (Cheke 1984; Gerlach 2007). Nevertheless, similar levels of divergence to the ones observed for the nuclear markers between the two *Mabuya* species were observed between species (or clades) of other groups, where mtDNA divergences are comparable to the values reported here between the two *Mabuya* lineages (Rocha 2010). Furthermore, there is no differentiation within *M. sechellensis* at any of the nuclear markers. We thus suggest that the mtDNA lineage currently harboured by all *M. sechellensis* from the southern islands represents the ancestral *M. wrightii* mtDNA lineage, currently fixed in *M. sechellensis* from the southern islands, after introgression.

The main differentiation between both species is size. However, the analyses of other variables independent to size, i.e., shape, pholidosis and colour pattern also revealed differences between populations and species. Remarkably, Mahé and Cousine island populations were the most distinct within *M. sechellensis,* with the Mahé population revealing an intermediate colour pattern between *M. sechellensis* and *M. wrightii,* and the Cousine population (where both species occur in sympatry) presenting the most distinct colour pattern. Whether or not these results directly reflect hybridization (Mahé population) and reinforcement (Cousine population) is worth investigating. Since other "monospecific", "hybrid" and sympatric populations exist in the Seychelles, and their distribution is limited and easy to characterize for relevant ecological factors, this group can be an interesting model for studies on the effects of these evolutionary forces and on the possibility and importance of reinforcement within this group (see Servedio & Noor 2003).

We did not detect ongoing hybridization, although the number of individuals genotyped from sympatric populations was not high. The minimum age of the mtDNA introgression event is dated at the Pleistocene (0.78 Mya [0.25-1.44]), using the TMRCA of the *M. sechellensis* southern clade as its estimate. The fact that this estimate is older than the estimated age of the MRCA of *M. wrightii* itself (0.36 Mya [0.068-0.74]) suggests the probable extinction of lineages within *M. wrightii.* It agrees with its long-term reduction in population size reflected by the BSP and past records of its extended distribution (Cheke 1984; Gerlach 2007). In fact, although the current pattern of introgression may suggest asymmetric hybridization, primarily between *M. wrightii* females and *M. sechellensis* males in the southern group, it may be that the dynamics of the hybridization was very different from what can be currently inferred due to the extinction of a considerable proportion of *M. wrightii* populations. However, this kind of asymmetric introgression pattern is abundant in nature and, although we cannot exclude the existence of an adaptive advantage (Ballard & Melvin 2010), it can be explained without the need to invoke selection acting upon mtDNA (Currat *et al.* 2008). Many cases of substantial female specific mtDNA introgression have been reported, with variable levels of mitochondrial replacement, often without, or with little evidence of, nuclear introgression (Berthier *et al.* 2006; Melo-Ferreira *et al.* 2005; Petit & Excoffier 2009; Roca *et al.* 2005). Furthermore, simulation studies suggest that very small amounts of gene flow are enough to explain situations of mitochondrial replacement, whereas the higher effective population size and higher intraspecific migration

rates of the nuclear genome may counteract genetic drift preventing extensive introgression (Petit & Excoffier 2009; Takahata & Slatkin 1984).

The factors leading to the observed introgression remain largely speculative, as the Seychelles seem to have been a very dynamic scenario in terms of habitat availability and island connectivity and these two species could have experienced large range shifts, especially during the Pleistocene. It may be for example that the divergence between the two species occurred in the northern group, or in Fregate, where the introgression event could also have taken place, and that the spread and colonization of *M. sechellensis* through the southern group happened only afterwards, by individuals already carrying the introgressed mitochondria. Such a scenario could explain the central position of mtDNA haplotypes from Fregate Island in the introgressed *M. sechellensis* lineage. However, in that case the *M. sechellensis* populations from the Praslin group should be more diverse than those from the southern group, reflecting their older age, which was not the case, although posterior demographic fluctuations could obscure such pattern. Also, historical records exist for the presence of *M. wrightii* in Mahé and Ile aux Vaches Marines, off Mahé (Cheke 1984; Gerlach 2005; Gerlach 2007), suggesting its previous presence in the southern group. Another possibility thus, is that both species co-existed in the southern group for some time and that strong demographic imbalances shaped the direction and pattern of introgression.

*Frequency-dependent assortative mating or invasion of a new area as drivers of extensive mtDNA introgression*

Population size dynamics estimates (Figure 5) suggest positive growth for *M. sechellensis* and negative growth for *M. wrightii*. Long-term effective population sizes and relative densities of both species may have played a significant role on the pattern and direction of hybridization and introgression, through a mechanism known as Hubb's principle (Hubbs 1955; Mayr 1963). The rationale is that, in species where females exert male choice through prezygotic isolation, hybridization rate will increase when species relative abundance becomes sharply unbalanced, because females belonging to the rare species, surrounded by too many heterospecific males, are more likely to make mate-recognition errors and heterospecific matings (Chan *et al.* 2006; Wirtz 1999). Brooke and Houston (1983) report total population sizes of 37,600 and 8,600 individuals for *M. sechellensis* and *M. wrightii*, respectively, at Cousin Island (roughly, a 4 to 1 ratio). If we

assume a generally lower density of the larger species, a decline of population size of *M. wrightii* from an already unbalanced density, due to competition or other external factors, such as predation, may help explain the asymmetric direction of hybridization and mitochondrial DNA introgression from *M. wrightii* into *M. sechellensis*. Posterior fluctuations in population sizes and demographic expansions of *M. sechellensis* within the southern islands may have led to the fixation of this "alien" mtDNA lineage, and additionally contributed to the decline of *M. wrightii* populations, by competition. In fact, hybridization can lead to the extinction of one of the involved species (Rhymer & Simberloff 1996), if a stable hybrid zone is not formed (Buggs 2007). Frequency-dependent assortative mating models (Chan 2003; Chan & Levin 2005) show that in cases where the abundance of species is unbalanced and female choice is relevant, the rate of introgression is high and strongly dependant on the proportion of immigrants. Furthermore, introgression is higher for maternally inherited markers than for biparental ones. When immigrants are rare the per-capita flow of maternally inherited markers is virtually uninhibited (Chan & Levin 2005). These predictions from first generation dynamics are exacerbated by subsequent generations, as mostly individuals from the more abundant species will surround F1 hybrids, and backcrosses with them will be favoured, a situation that will easily result in loss of differentiation at neutral loci. This may ultimately lead to the rare species "extinction" and to the dilution of its genome, although its genes will persist in hybrid individuals.

The spread of a species in a new area already occupied by a closely related species is another situation where species proportions can be highly unbalanced. Simulations and empirical data (Currat *et al.* 2008; Excoffier *et al.* 2009) show that if interbreeding is not severely prevented, introgression occurs almost exclusively from the local to the invading species, and at higher levels for uniparental markers. The invasion and demographic expansion of *M. sechellensis* to the southern group of islands already occupied by *M. wrightii* could also have lead to the currently observed patterns of introgression.

It would be interesting to further explore these hypotheses by genotyping a larger sample of the genome of each species and use more variable markers, field-studies on density and mating behaviour, together with and an extensive sampling of several allopatric, sympatric and "hybrid" populations of these species to further test the possible role of each species' relative abundance and interspecific recognition in the hybridization and introgression.

The spread of these introgressed mitochondria throughout all the islands in the southern group is remarkable and can be more easily understood in the context of Pleistocenic sea level fluctuations and the connectivity of these islands in times of lower sea depths (see Figure 1). Though largely shaped by introgression, there is a clear north-south division in the genetic structure of both species within the archipelago, a pattern that matches other co-distributed species, like *Phelsuma sp.* (Radtkey 1996; Rocha *et al.* submitted-b) and *Urocotyledon* (Rocha *et al.* submitted-a). Fregate population of *M. sechellesis* is clearly more related to populations from the Mahé-group and is the most differentiated within *M. wrightii*, which is extinct in the remaining southern islands, thus possibly reflecting a shared biogeographic pattern. Nevertheless, some gene flow is detected between these groups (of *M. sechellensis*), especially into the northern group. More specifically, gene flow was detected from the islands of Silhouette and North (grouped) into the northern group and from this into Fregate, even using only nuclear data. This result is corroborated by the mtDNA data, with two individuals of *M. sechellensis* from Aride (northern group) carrying haplotypes that clustered within the southern mtDNA lineage, closely related to North and Silhouette haplotypes. Reasoning that these could be due to recent human introductions and not reflect historical patterns, we excluded these individuals in all estimates of gene flow, and still recovered concordant patterns. In fact with only small (-30m) sea-level low-stands the plateau where Silhouette and North Islands lay would emerge as an extremely large area, rendering migration into the northern group possible. This is an interesting pattern that warrants further investigation in this and across other groups.

The lack of structure observed within each *M. sechellensis* mtDNA clade, together with the lack of differentiation at the nuclear markers could be explained by a very recent partitioning of a wide ranging and homogeneous distribution due to the recent submergence of the Seychelles plateau (i.e. mainly by ancestral polymorphism), or by frequent inter-island migrations, especially within each island group (i.e. mainly high levels of gene flow). Estimates of migration within and across groups do not reject the existence of gene flow both within and between main geographic groups. Accordingly, migration in one or both directions was always detected between Praslin and the remaining islands of the northern group and within the southern group for most of the combinations tested.

In our "pairwise" population analyses, if migration is detected, even if it is influenced by, or the result of, gene flow with a third population, we are in fact detecting overall gene flow, which is what we intended to test for. Furthermore, analyses were performed in several ways, including clustering populations (islands) in bigger groups using geographical criteria (Table 5), ultimately involving only two groups, where this specific assumption is thus not violated. The IM model, as incorporated in the IMa method (Hey & Nielsen 2007) further assumes demographic stability of both descendant populations, which was also violated in our analysis at least for some of the comparisons, given that an overall demographic expansion for *M. sechellensis* was inferred. Under a scenario of exponential population growth since initial divergence, current population size estimates should be biased downwards. However, the accuracy of other parameter estimates should be comparable to the simple demographic scenario (Strasburg & Rieseberg 2010). As we were mainly interested in comparing migration estimates, we do not consider that this specific violation is relevant to our analyses.

Two interesting patterns were further observed within the southern group. The possible absence of gene flow between Mahé and Silhouette and North Islands, and the apparent high levels of migration from Fregate to Mahé and also Silhouette and North Islands. The first might reflect the greater isolation of Silhouette and North, separated from remaining islands by greater sea-depths, and thus likely isolated for longer periods. This isolation may have affected other organisms in similar ways (a concordant pattern is found within the co-distributed gecko *Urocotyledon inexpectata* – Rocha *et al.* submitted-a) and it is thus worth investigating. The second pattern, a higher migration from Fregate southwards, is especially remarkable as it is the expected direction of migration to explain the geography of the introgression both if an already introgressed lineage of *M. sechellensis* spread across the southern group after hybridization but also if the scenario was one of invasion of the southern islands by *M. sechellensis* leading to hybridization and introgression with local *M. wrightii*.

## Conclusion

Hybridization and mtDNA introgression are put forward as best explaining mitochondrial and nuclear DNA variability patterns in these species, constituting a remarkable example of asymmetric mtDNA introgression and massive mtDNA replacement across a large parte of a species range. Both species, whose divergence is apparently Mio-Pliocenic, currently exhibit contrary demographic trends and *M. sechellensis* demographic (and maybe range) expansion most possibly facilitated the fixation of the introgressed *M. wrightii* mtDNA lineage across part of its distribution.

Gene flow inference points also to a considerable influence of migration across islands in shaping the genetic structure of *M. sechellensis*. However, morphological data suggest that despite migration, island populations have considerable cohesion. The fact that all *M. sechellensis* populations studied are well discriminated, with generally high classification success, including populations that show little to no differentiation at the molecular level, may reflect some degree of phenotypic structure or local adaptation. The molecular data further revealed some relevant biogeographic patterns: isolation and restricted gene flow between the northern and southern islands; a close relationship of Fregate populations with the southern ones; the higher isolation of Silhouette and North within the southern group and the occurrence of gene flow between North and Silhouette towards the northern islands. These patterns coincide in several aspects with others inferred for other lizard groups (Rocha *et al.* submitted-a; Rocha *et al.* submitted-b) and proportionate a better understanding of the generation and distribution of biodiversity within the Seychelles archipelago.

**Acknowledgments**

We are grateful to SBS of Seychelles for permits for fieldwork and tissue collection and to the Ministry of Environment and Natural Resources for logistic support during fieldwork. We thank NPTS, ICS Seychelles, SIF and several entities and persons that made fieldwork across all islands possible. This research was supported by grants from Fundação para a Ciência e Tecnologia SFRH/BD/1745/2004 (to SR), PTDC/BIA-BDE/65745/2006 and the Gulbenkian Society. An especial acknowledgement is due to Raquel Vasconcelos for her contribution to the fieldwork and to Catarina Pinho, Miguel Carneiro and Rui Faria for help taming IM analyses. Further thanks to Alexandra Sá Pinto, Angela Ribeiro, Antigoni Kaliontzopolou, Anthony Cheke, Catarina Pinho, Graciela Sotelo, José Melo-Ferreira, Miguel Carretero and Ricardo Pereira for helpful discussions and comments on previous versions of the manuscript.

## Supplementary Information

**Supplementary Table S1** – AMOVA. Percentage of variation accounted for across partitions for *M. sechellensis*.

| Locus | Source of Variation % | | |
|---|---|---|---|
| | Among groups | Among Populations (within groups) | Within Populations |
| Cyt-b | 91,87* | 3,68*** | 4,44*** |
| PDC | 1, 07* | 8,96* | 89,98*** |
| C-mos | 0,97 | 23,42** | 75,61*** |
| Mc1R | 1,61* | 5,62* | 92,77* |
| RELNint61 | 0,64* | 17,82*** | 81,53*** |
| Rag-2 | 4,75*** | 26,79*** | 68,46* |

*P<0,05; **P<0,001; ***P<0,0001

Groups refer to Mahé-group (Mahé, Cerf, Silhouette, North and Fregate) and Praslin-group (Praslin, La Digue, Aride, Cousine, Curieuse, Marianne and Big Sister). Populations refer to individual islands. Results clearly describe the lack of structure at the levels of interest. The pattern observed at the mitochondrial DNA level (of higher structure among groups) is due to the fixation of the introgressed mtDNA lineage in one of the groups but variation attributable to structure among populations is very low and thus justifies the application of the IM model at pairwise populations. At the nuclear markers is clear that the majority of variation at all loci is distributed within populations and not among populations and not even among groups.

**Supplementary Table S2** – Heterogeneity tests, HKA test and recombination

| | Heterogeneity Test DSyn/DNonSyn ΔD (P-value) | HKA SD (P-value) | Recombination Φw P-value |
|---|---|---|---|
| Cyt-b | 1,45646 -0,49137 1.95 (P=0,09) | | |
| PDC | 0,22245 -1,31937 1,54 (P=0,12) | | 1 (1/-) |
| C-mos | -0,24760 0,11477 -0,14 (P=0,6) | | 1 (-/-) |
| Mc1R | 1,24295 0,85324 0,39 (P=0,67) | | 0,834 (0,17/-) |
| RELN*int*61 | | | 0,013* (0,264/0,0633) |
| Rag-2 | 0,18394 -0,86535 1,05 (P=0,39) | | 0,3129 (1/1) |
| | | 6.6371 (0,15635) | |

*P<0,05*

The heterogeneity test addresses differences between values of *D* for non-synonymous and synonymous mutations. Significant values (1000 iterations) indicate that different processes appear to be acting on non-synonymous and synonymous mutations (Hahn et al., 2002). It was not performed for RELN*int*61 as this is a non-coding region. Neither heterogeneity tests nor HKA, applied to the full dataset, were significant at any marker. Φ*w* P-value represents the p-value for observing the tested sequences under the null hypothesis of no recombination, calculated using the Φ statistic (Bruen et al., 2006). Values are given for the complete dataset as well as for *M. sechellensis* and *M. wrightii* separately. Significant values were obtained for the RELN*int*61 dataset, but not when each species dataset is tested separately. Values not given (-) are impossible to calculate due to too few informative sites.

**Supplementary Table S3** – Results of the tests of nested models for the paiwise comparisons between island populations within *M. sechellensis*. Non-rejected models involving zero-migration estimates (zero-m) are indicated. Reciprocal comparisons in which both zero-migration models were not rejected are highlighted.

**Population 2**

| Population 1 | North | Silhouette | Mahé | Fregate | Praslin | La Digue | Cousine | Aride | Curieuse | Big Sister |
|---|---|---|---|---|---|---|---|---|---|---|
| **North** | | | | $\theta_1\ \theta_2\ \theta_A\ m_1{=}0\ m_2$ | $\theta_1\ \theta_2\ \theta_A\ m_1\ m_2{=}0$ | $\theta_1\ \theta_2\ \theta_A\ m_1{=}0\ m_2$ | $\theta_1\ \theta_2\ \theta_A\ m_1{=}0\ m_2$ | $\theta_1\ \theta_2\ \theta_A\ m_1{=}0\ m_2$ | *Not performed* | *Not performed* |
| **Silhouette** | All zero-m models rejected | | | All zero-m models rejected | $\theta_1\ \theta_2\ \theta_A\ m_1{=}0\ m_2$ | All zero-m models rejected | *Not performed* | *Not performed* | *Not performed* | *Not performed* |
| **Mahé** | $\theta_1\ \theta_2\ \theta_A\ m_1{=}\ m_2{=}0$ **$\theta_1\ \theta_2{=}\ \theta_A\ m_1{=}\ m_2{=}0$** (*1) | $\theta_1\ \theta_2\ \theta_A\ m_1\ m_2{=}0$ $\theta_1\ \theta_2\ \theta_A\ m_1{=}0\ m_2$ **$\theta_1\ \theta_2\ \theta_A\ m_1{=}\ m_2{=}0$** **$\theta_1{=}\ \theta_2\ \theta_A\ m_1{=}\ m_2{=}0$** **$\theta_1\ \theta_2{=}\ \theta_A\ m_1{=}\ m_2{=}0$** | | All zero-m models rejected | $\theta_1\ \theta_2\ \theta_A\ m_1{=}0\ m_2$ | $\theta_1\ \theta_2\ \theta_A\ m_1{=}0\ m_2$ **$\theta_1{=}\ \theta_A\ \theta_2\ m_1{=}\ m_2{=}0$** | *Not performed* | *Not performed* | *Not performed* | *Not performed* |
| **Fregate** | | | | | All zero-m models rejected | All zero-m models rejected | *Not performed* | *Not performed* | *Not performed* | *Not performed* |
| **Praslin** | | | | | | $\theta_1\ \theta_2\ \theta_A\ m_1{=}0\ m_2$ | $\theta_1\ \theta_2\ \theta_A\ m_1{=}0\ m_2$ | All zero-m models rejected (*2) | $\theta_1\ \theta_2\ \theta_A\ m_1\ m_2{=}0$ | $\theta_1\ \theta_2\ \theta_A\ m_1{=}0\ m_2$ |
| **La Digue** | | | | | | | *Not performed* | *Not performed* | *Not performed* | *Not performed* |
| **Cousine** | | | | | | | | *Not performed* | *Not performed* | *Not performed* |
| **Aride** | | | | | | | | | *Not performed* | *Not performed* |
| **Curieuse** | | | | | | | | | | *Not performed* |
| **Big Sister** | | | | | | | | | | |

*1 – bimodal distributions for $\theta_1$ *and* $m_1$

*2 – Individuals with mtDNA haplotypes clustering with the "Mahé-group" removed for this comparison.

The likelihood of alternative models was calculated from the LLR estimates provided by IMa. Tested models were the seven incorporated in IMa: $\theta_1\ \theta_2\ \theta_A\ m_1\ m_2{=}0$; $\theta_1\ \theta_2\ \theta_A\ m_1{=}0\ m_2$; $\theta_1\ \theta_2\ \theta_A\ m_1{=}\ m_2{=}0$; $\theta_1{=}\ \theta_2\ \theta_A\ m_1{=}\ m_2{=}0$; $\theta_1{=}\ \theta_2{=}\ \theta_A\ m_1{=}\ m_2{=}0$; $\theta_1{=}\ \theta_A\ \theta_2\ m_1{=}\ m_2{=}0$; $\theta_1\ \theta_2{=}\ \theta_A\ m_1{=}\ m_2{=}0$. Note that when the null model is true and has a parameter fixed at the boundary of the parameter space (zero), LLR should be assymptotically distributed as a random variable that takes the value 0 with a probability 0.5 and taken on a value with a $\chi^2_1$ distribution with probability 0.5 (Hey and Nielsen, 2007). The critical values for that distribution are *p<0.05 LLR >= 2.704; **p<0.01 LLR >= 5.412 and ***p<0.001 LLR >= 9.550 (Jody Hey, pers. comm.). When both parameters are bounded at zero ($m_1{=}\ m_2{=}0$), the test statistic for these models is best calculated from the relative likelihood of the "$m_1{=}\ m_2{=}0$" model compared to respective "$m_1{=}\ m_2{\neq}0$" model following the previous described distribution (Jody Hey, pers. comm.) Only when neither test was significant "$m_1{=}\ m_2{=}0$" was accepted.

**Supplementary Table S4** – Results of tests of nested models for Mahé- and Praslin-groups of *M. sechellensis* and estimated population migration rates between the two groups.

Population 1=Mahé-group (+Fregate); Population 2=Praslin group.

$2Nm1 = 1{,}8975$; $2Nm2 = 1{,}3617$

| Model | Log (P) | 2LLR | df | P-value |
|---|---|---|---|---|
| $\theta_1\ \theta_2\ \theta_A\ m_1 = m_2$ | -2.8528 | 4.4088 | 1 | 0.0357 |
| $\theta_1\ \theta_2\ \theta_A\ m_1\ m_2{=}0$ | -2.4477 | 3.5986 | 1* | rejected ($p<0.05$) |
| $\theta_1\ \theta_2\ \theta_A\ m_1{=}0\ m_2$ | -0.6538 | 0.0108 | 1* | *not rejected* |
| $\theta_1\ \theta_2\ \theta_A\ m_1{=}\ m_2{=}0$ | -67.2009 | 133.1050 | 2* | rejected ($p<0.001$) |
| $\theta_1{=}\ \theta_2\ \theta_A\ m_1\ m_2$ | -3.7983 | 6.2998 | 1 | 0.0120 |
| $\theta_1{=}\ \theta_2 = \theta_A\ m_1\ m_2$ | -7.2996 | 13.3023 | 2 | 0.0012 |
| $\theta_1{=}\ \theta_2\ \theta_A\ m_1{=}\ m_2$ | -4.4955 | 7.6942 | 2 | 0.0213 |
| $\theta_1{=}\ \theta_2\ \theta_A\ m_1{=}\ m_2{=}0$ | -74.1700 | 147.0431 | 3* | rejected ($p<0.001$) |
| $\theta_1{=}\ \theta_2{=}\ \theta_A\ m_1{=}\ m_2$ | -8.2766 | 13.2563 | 3 | 0.0041 |
| $\theta_1{=}\ \theta_2{=}\ \theta_A\ m_1{=}\ m_2{=}0$ | -114.3521 | 227.4073 | 4* | rejected ($p<0.001$) |
| $\theta_1{=}\ \theta_A\ \theta_2\ m_1\ m_2$ | -5.5759 | 9.8550 | 1 | 0.0016 |
| $\theta_1{=}\ \theta_A\ \theta_2\ m_1 = m_2$ | -7.2037 | 13.1106 | 2 | 0.0014 |
| $\theta_1{=}\ \theta_A\ \theta_2\ m_1{=}\ m_2{=}0$ | -113.8057 | 226.3146 | 3* | rejected ($p<0.001$) |
| $\theta_1\ \theta_2{=}\ \theta_A\ m_1\ m_2$ | -4.5044 | 7.7119 | 1 | 0.0054 |
| $\theta_1\ \theta_2{=}\ \theta_A\ m_1{=}\ m_2$ | -6.6627 | 12.0285 | 2 | 0.0024 |
| $\theta_1\ \theta_2{=}\ \theta_A\ m_1{=}\ m_2{=}0$ | -77.2838 | 153.2707 | 3* | rejected ($p<0.001$) |

**Appendix 1** - Locality information for the studied specimens and Genbank accession numbers. For detailed geographical coordinates refer to Location ID in Rocha et al. (2009b).

| Species | Code | Location | Island | Accession Numbers | | | | | | Morph. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Cyt-*b* | C-mos | Rag2 | Reln*int*61 | Mc1R | PDC | |
| *M. sechellensis* | PL35 | 106 Anse Boudin | Praslin | # | | | | | | |
| *M. sechellensis* | PL33 | 109 Grand Anse | Praslin | # | | | | | | |
| *M. sechellensis* | 1PL | 109 Grand Anse | Praslin | # | # | # | | # | # | |
| *M. sechellensis* | 1FG | 114 Fregate | Fregate | # | # | # | # | # | # | |
| *M. sechellensis* | 2FG | 114 Fregate | Fregate | # | # | # | # | # | # | |
| *M. sechellensis* | 4FG | 114 Fregate | Fregate | # | # | # | # | # | # | |
| *M. sechellensis* | 5FG | 114 Fregate | Fregate | # | # | # | # | # | # | |
| *M. sechellensis* | 11FG | 114 Fregate | Fregate | # | # | # | | # | # | |
| *M. sechellensis* | 14FG | 114 Fregate | Fregate | # | # | # | # | # | # | |
| *M. sechellensis* | 50FG | 114 Fregate | Fregate | # | # | # | # | # | # | |
| *M. sechellensis* | 51FG | 114 Fregate | Fregate | # | # | # | # | | # | |
| *M. sechellensis* | 1NORTH | 115 North Island | North | # | # | # | # | # | # | |
| *M. sechellensis* | 2NORTH | 115 North Island | North | # | # | # | # | # | # | |
| *M. sechellensis* | 3NORTH | 115 North Island | North | | # | # | # | # | # | |
| *M. sechellensis* | 4NORTH | 115 North Island | North | # | | | | | | |
| *M. sechellensis* | 5NORTH | 115 North Island | North | # | # | # | # | # | # | |
| *M. sechellensis* | 6NORTH | 115 North Island | North | # | | | | | | |
| *M. sechellensis* | 9NORTH | 115 North Island | North | # | # | # | # | # | # | |
| *M. sechellensis* | 2MA1 | 16 Mont Fleuri, | Mahé | # | | | | | | |
| *M. sechellensis* | 2MA19 | 18 NorthEast Point | Mahé | # | # | | | | | |
| *M. sechellensis* | 3MA112 | 18 NorthEast Point | Mahé | # | | | | | | |
| *M. sechellensis* | 3MA114 | 18 NorthEast Point | Mahé | # | | | | | | |
| *M. sechellensis* | 2MA12 | 20 North2 | Mahé | # | # | # | # | # | # | |
| *M. sechellensis* | 2MA8 | 21 Glacis2 | Mahé | # | | | | | | |
| *M. sechellensis* | 2MA5 | 22 Glacis | Mahé | # | # | | # | | | |
| *M. sechellensis* | 3MA103 | 24 Mare Anglaise | Mahé | # | # | # | # | # | # | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *M. sechellensis* | 3MA93 | 25B Beau Vallon | Mahé | # | # | # | # | # | # | |
| *M. sechellensis* | 2MA34 | 27 Danzil 3 | Mahé | # | | | | | | |
| *M. sechellensis* | 2MA35 | 27 Danzil 3 | Mahé | # | | | | | | |
| *M. sechellensis* | 2MA41 | 29 Mare aux Cochons | Mahé | # | | | | | | |
| *M. sechellensis* | 3MA148 | 31 Danzil | Mahé | # | | | | | | |
| *M. sechellensis* | 3MA136 | 34 Cap Ternay | Mahé | # | # | | | # | # | |
| *M. sechellensis* | 2MA28 | 38 Tea Factory | Mahé | # | | | | | | |
| *M. sechellensis* | 3MA159 | 39 Tea Plantation | Mahé | # | | | | | | |
| *M. sechellensis* | 2MA22 | 40 Morne Blanc | Mahé | # | # | # | | # | # | |
| *M. sechellensis* | 3MA164 | 41 Casse Dent | Mahé | # | | | | | | |
| *M. sechellensis* | 3MA138 | 43 Salazie | Mahé | # | | | | | | |
| *M. sechellensis* | 2MA31 | 46 Trois Freres 1 | Mahé | # | | # | # | # | # | |
| *M. sechellensis* | 3MA79 | 46 Trois Freres 1 | Mahé | # | | | | | | |
| *M. sechellensis* | 3MA84 | 46 Trois Freres 1 | Mahé | # | | | | | | |
| *M. sechellensis* | 3MA124 | 49 Cascade | Mahé | # | | | | | | |
| *M. sechellensis* | 3MA131 | 50 Anse aux Pins 1 | Mahé | # | | | | | | |
| *M. sechellensis* | 2MA69 | 54 Anse aux Frobans | Mahé | # | | | | | | |
| *M. sechellensis* | 2MA63 | 55 Anse aux Forbans | Mahé | # | | # | | | | |
| *M. sechellensis* | 3MA59 | 56 Ptit Police | Mahé | # | | | | | | |
| *M. sechellensis* | 2MA57 | 57 Quatre Bornes | Mahé | # | | | | | | |
| *M. sechellensis* | 3MA53 | 57 Quatre Bornes | Mahé | # | # | # | # | # | # | |
| *M. sechellensis* | 3MA65 | 58 Anse Intendance | Mahé | # | | | | | | |
| *M. sechellensis* | 3MA37 | 59 Baie Lazare | Mahé | # | | | | | | |
| *M. sechellensis* | 3MA10 | 60 To Anse Soleil | Mahé | # | | | | | | |
| *M. sechellensis* | 3MA5 | 62 Anse a la Mouche | Mahé | # | # | # | # | # | # | yes |
| *M. sechellensis* | M03 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M04 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M10 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M11 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M12 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M13 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M14 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M15 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M16 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M17 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M18 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M19 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M20 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M21 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M22 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M24 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M25 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M26 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M27 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M28 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M29 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M30 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M31 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M32 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M33 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M34 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M35 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M36 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M37 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M38 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M39 | 62 Anse a la Mouche | Mahé | | | | | | | yes |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *M. sechellensis* | M40 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M41 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M42 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M6 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M7 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M8 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | M9 | 62 Anse a la Mouche | Mahé | | | | | | | yes |
| *M. sechellensis* | 3MA70 | 63 Anse a la Mouche | Mahé | # | | | | | | |
| *M. sechellensis* | 3MA27 | 64 Anse Boileau | Mahé | # | | | | | | |
| *M. sechellensis* | 2MA48 | 66 La Reserve 2 | Mahé | # | | | | | | |
| *M. sechellensis* | 2MA71 | 67 Grand Anse | Mahé | # | | | | | | |
| *M. sechellensis* | MA2 | 68 Airport | Mahé | # | # | # | # | # | # | |
| *M. sechellensis* | MA3 | 68 Airport | Mahé | # | | # | # | # | # | |
| *M. sechellensis* | MA6 | 68 Airport | Mahé | # | | | | | | |
| *M. sechellensis* | MA17 | 68B Airport | Mahé | # | | | | | | |
| *M. sechellensis* | MA19 | 68B Airport | Mahé | # | | | | | | |
| *M. sechellensis* | CF2 | 69 Cerf Island 1 | Cerf | # | # | # | # | # | # | |
| *M. sechellensis* | CF4 | 69 Cerf Island 1 | Cerf | # | # | # | # | # | # | |
| *M. sechellensis* | CF5 | 69 Cerf Island 1 | Cerf | # | # | # | # | # | # | |
| *M. sechellensis* | 38707 | 71 La Passe 1 | Silhouette | # | | | | | | |
| *M. sechellensis* | 38708 | 71 La Passe 1 | Silhouette | | # | # | # | # | # | |
| *M. sechellensis* | 6SILH | 73 La Passe 3 | Silhouette | # | | | | | | |
| *M. sechellensis* | 38715 | 75 Anse Lascars | Silhouette | | # | # | # | # | # | |
| *M. sechellensis* | 19SILH | 75 Anse Lascars | Silhouette | # | | | | | | |
| *M. sechellensis* | 24SILH | 76 around GB rock | Silhouette | # | # | # | # | # | # | |
| *M. sechellensis* | 27SILH | 76 around GB rock | Silhouette | # | | | | | | |
| *M. sechellensis* | 42SILH | 76 around GB rock | Silhouette | # | # | # | | # | # | |
| *M. sechellensis* | 30SILH | 77 Gratte Fesse | Silhouette | # | # | # | # | # | # | |
| *M. sechellensis* | 31SILH | 77 Gratte Fesse | Silhouette | # | # | # | | | | |
| *M. sechellensis* | 1ARD | 79 Aride Island | Aride | | # | # | # | | # | |
| *M. sechellensis* | 2ARD | 79 Aride Island | Aride | # | # | # | # | # | # | |
| *M. sechellensis* | 3ARD | 79 Aride Island | Aride | # | # | # | # | # | # | |
| *M. sechellensis* | 5ARD | 79 Aride Island | Aride | # | # | # | # | # | # | |
| *M. sechellensis* | 6ARD | 79 Aride Island | Aride | # | | # | # | # | # | |
| *M. sechellensis* | 19ARD | 79 Aride Island | Aride | # | # | # | # | # | # | |
| *M. sechellensis* | 21ARD | 79 Aride Island | Aride | # | # | # | # | # | # | |
| *M. sechellensis* | 23ARD | 79 Aride Island | Aride | # | # | | # | # | # | |
| *M. sechellensis* | 2CUR | 80 Point1 | Curieuse | # | # | # | # | # | # | yes |
| *M. sechellensis* | 23CUR | 80 Point1 | Curieuse | # | | | | | | yes |
| *M. sechellensis* | 24CUR | 80 Point1 | Curieuse | # | | | | | | yes |
| *M. sechellensis* | 27CUR | 80 Point1 | Curieuse | # | # | # | # | # | # | yes |
| *M. sechellensis* | 28CUR | 80 Point1 | Curieuse | # | | | | | | yes |
| *M. sechellensis* | 43CUR | 80 Point1 | Curieuse | # | # | # | | # | # | yes |
| *M. sechellensis* | 1-CUR | 80 Point1 | Curieuse | | | | | | | yes |
| *M. sechellensis* | 21-CUR | 80 Point1 | Curieuse | | | | | | | yes |
| *M. sechellensis* | 22-CUR | 80 Point1 | Curieuse | | | | | | | yes |
| *M. sechellensis* | 25-CUR | 80 Point1 | Curieuse | | | | | | | yes |
| *M. sechellensis* | 26-CUR | 80 Point1 | Curieuse | | | | | | | yes |
| *M. sechellensis* | 30-CUR | 80 Point1 | Curieuse | | | | | | | yes |
| *M. sechellensis* | 31-CUR | 80 Point1 | Curieuse | | | | | | | yes |
| *M. sechellensis* | 32-CUR | 80 Point1 | Curieuse | | | | | | | yes |
| *M. sechellensis* | 33-CUR | 80 Point1 | Curieuse | | | | | | | yes |
| *M. sechellensis* | 34-CUR | 80 Point1 | Curieuse | | | | | | | yes |
| *M. sechellensis* | 41-CUR | 80 Point1 | Curieuse | | | | | | | yes |
| *M. sechellensis* | 42-CUR | 80 Point1 | Curieuse | | | | | | | yes |
| *M. sechellensis* | 44-CUR | 80 Point1 | Curieuse | | | | | | | yes |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *M. sechellensis* | 45-CUR | 80 Point1 | Curieuse | | | | | | | yes |
| *M. sechellensis* | 5-CUR | 80 Point1 | Curieuse | | | | | | | yes |
| *M. sechellensis* | CUR1 | 82 Trail | Curieuse | | # | # | # | # | # | |
| *M. sechellensis* | CUR3 | 82 Trail | Curieuse | | # | # | # | # | # | |
| *M. sechellensis* | 24CNE | 87 Office | Cousine | # | | # | | | | |
| *M. sechellensis* | 25CNE | 87 Office | Cousine | # | # | # | # | # | # | yes |
| *M. sechellensis* | 26CNE | 87 Office | Cousine | # | # | # | # | | # | yes |
| *M. sechellensis* | 27CNE | 87 Office | Cousine | # | # | # | # | # | # | yes |
| *M. sechellensis* | 28CNE | 87 Office | Cousine | # | # | # | # | # | # | yes |
| *M. sechellensis* | 30CNE | 87 Office | Cousine | | | # | # | # | # | yes |
| *M. sechellensis* | 31CNE | 87 Office | Cousine | | # | | # | # | # | yes |
| *M. sechellensis* | 36CNE | 87 Office | Cousine | # | # | # | # | # | # | yes |
| *M. sechellensis* | 37CNE | 87 Office | Cousine | | # | # | # | # | # | yes |
| *M. sechellensis* | 38CNE | 87 Office | Cousine | # | # | # | # | | # | yes |
| *M. sechellensis* | 29-CNE | 87 Office | Cousine | | | | | | | yes |
| *M. sechellensis* | 6BS | 91 north path | Grande Soeur | # | # | # | # | # | # | |
| *M. sechellensis* | 7BS | 91 north path | Grande Soeur | # | | # | | # | # | |
| *M. sechellensis* | 8BS | 91 north path | Grande Soeur | # | # | # | # | | # | |
| *M. sechellensis* | 9BS | 91 north path | Grande Soeur | # | # | # | # | # | # | |
| *M. sechellensis* | 10BS | 92 north path 2 | Grande Soeur | | # | # | # | # | # | |
| *M. sechellensis* | LD3 | 93 La Veuve Reserve | La Digue | # | # | # | | | # | |
| *M. sechellensis* | LD8 | 93 La Veuve Reserve | La Digue | # | | | | | | |
| *M. sechellensis* | LD11 | 93 La Veuve Reserve | La Digue | # | | | | | | |
| *M. sechellensis* | 10LD | 93 La Veuve Reserve | La Digue | # | | # | | # | | |
| *M. sechellensis* | 13LD | 93 La Veuve Reserve | La Digue | # | | | | | | |
| *M. sechellensis* | 16LD | 93 La Veuve Reserve | La Digue | # | | # | | # | # | |
| *M. sechellensis* | 18LD | 93 La Veuve Reserve | La Digue | # | | | | | | |
| *M. sechellensis* | 27LD | 93 La Veuve Reserve | La Digue | # | # | | | | | yes |
| *M. sechellensis* | 28LD | 93 La Veuve Reserve | La Digue | # | # | # | # | # | # | yes |
| *M. sechellensis* | 29LD | 93 La Veuve Reserve | La Digue | # | | | | | | yes |
| *M. sechellensis* | 31LD | 93 La Veuve Reserve | La Digue | | # | | | | | yes |
| *M. sechellensis* | 36LD | 93 La Veuve Reserve | La Digue | # | # | # | # | # | # | yes |
| *M. sechellensis* | 12-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 19-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 20-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 21-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 22-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 23-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 24-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 25-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 26-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 30-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 32-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 33-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 34-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 35-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 37-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 38-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 39-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 40-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 41-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 42-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 43-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 44-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 45-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 46-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *M. sechellensis* | 47-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 48-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 49-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 50-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 51-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 52-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 53-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 60-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 70-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 71-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 76-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | 77-LD | 93 La Veuve Reserve | La Digue | | | | | | | yes |
| *M. sechellensis* | LD15 | 95 Belle Vue | La Digue | # | # | # | # | # | # | |
| *M. sechellensis* | PL1 | 98 Baie Ste Anne | Praslin | # | # | | # | # | # | |
| *M. sechellensis* | PL3 | 99 Anse Volbert | Praslin | # | | | | | | |
| *M. sechellensis* | PL11 | 99 Anse Volbert | Praslin | # | # | # | # | # | # | |
| *M. sechellensis* | 4PL | 99 Anse Volbert | Praslin | # | | | | | | |
| *M. sechellensis* | 12PL | 99 Anse Volbert | Praslin | # | | | | | | yes |
| *M. sechellensis* | 14PL | 99 Anse Volbert | Praslin | # | | | | | | yes |
| *M. sechellensis* | 16PL | 99 Anse Volbert | Praslin | # | | | | | | yes |
| *M. sechellensis* | 18PL | 99 Anse Volbert | Praslin | # | | | | | | yes |
| *M. sechellensis* | 30PL | 99 Anse Volbert | Praslin | # | # | # | # | # | # | yes |
| *M. sechellensis* | 33PL | 99 Anse Volbert | Praslin | # | # | # | # | # | # | yes |
| *M. sechellensis* | 10-PL | 99 Anse Volbert | Praslin | | | | | | | yes |
| *M. sechellensis* | 11-PL | 99 Anse Volbert | Praslin | | | | | | | yes |
| *M. sechellensis* | 13-PL | 99 Anse Volbert | Praslin | | | | | | | yes |
| *M. sechellensis* | 15-PL | 99 Anse Volbert | Praslin | | | | | | | yes |
| *M. sechellensis* | 17-PL | 99 Anse Volbert | Praslin | | | | | | | yes |
| *M. sechellensis* | 19-PL | 99 Anse Volbert | Praslin | | | | | | | yes |
| *M. sechellensis* | 20-PL | 99 Anse Volbert | Praslin | | | | | | | yes |
| *M. sechellensis* | 21-PL | 99 Anse Volbert | Praslin | | | | | | | yes |
| *M. sechellensis* | 22-PL | 99 Anse Volbert | Praslin | | | | | | | yes |
| *M. sechellensis* | 31-PL | 99 Anse Volbert | Praslin | | | | | | | yes |
| *M. sechellensis* | 32-PL | 99 Anse Volbert | Praslin | | | | | | | yes |
| *M. sechellensis* | 34-PL | 99 Anse Volbert | Praslin | | | | | | | yes |
| *M. sechellensis* | 35-PL | 99 Anse Volbert | Praslin | | | | | | | yes |
| *M. sechellensis* | 36-PL | 99 Anse Volbert | Praslin | | | | | | | yes |
| *M. sechellensis* | 38-PL | 99 Anse Volbert | Praslin | | | | | | | yes |
| *M. sechellensis* | 4-PL | 99 Anse Volbert | Praslin | | | | | | | yes |
| *M. sechellensis* | 7-PL | 99 Anse Volbert | Praslin | | | | | | | yes |
| *M. sechellensis* | 8-PL | 99 Anse Volbert | Praslin | | | | | | | yes |
| *M. sechellensis* | 9-PL | 99 Anse Volbert | Praslin | | | | | | | yes |
| *M. sechellensis* | | | Mahé | AF280134 | | | | | | |
| *M. wrightii* | 3FG | 114 Fregate | Fregate | # | # | # | | # | # | |
| *M. wrightii* | 8FG | 114 Fregate | Fregate | # | # | # | # | # | # | |
| *M. wrightii* | 21FG | 114 Fregate | Fregate | # | # | # | # | | # | |
| *M. wrightii* | 49FG | 114 Fregate | Fregate | # | # | # | # | | # | |
| *M. wrightii* | 52FG | 114 Fregate | Fregate | # | # | | # | # | # | |
| *M. wrightii* | 4ARD | 79 Aride Island | Aride | # | | # | | | # | |
| *M. wrightii* | 7ARD | 79 Aride Island | Aride | # | # | # | # | | # | |
| *M. wrightii* | 8ARD | 79 Aride Island | Aride | # | # | # | # | | # | |
| *M. wrightii* | 9ARD | 79 Aride Island | Aride | # | # | # | # | | # | |
| *M. wrightii* | 10ARD | 79 Aride Island | Aride | # | # | # | # | | # | |
| *M. wrightii* | 18ARD | 79 Aride Island | Aride | # | # | # | # | | # | |
| *M. wrightii* | SP1 | 86 Saint Pierre | Saint Pierre | # | | | | | # | |
| *M. wrightii* | SP2 | 86 Saint Pierre | Saint Pierre | # | # | # | # | # | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *M. wrightii* | SP3 | 86 Saint Pierre | Saint Pierre | # | # | # | | # | # | |
| *M. wrightii* | SP4 | 86 Saint Pierre | Saint Pierre | # | # | # | # | # | # | |
| *M. wrightii* | 3SP | 86 Saint Pierre | Saint Pierre | # | | | | | | yes |
| *M. wrightii* | 6SP | 86 Saint Pierre | Saint Pierre | # | | | | | | yes |
| *M. wrightii* | 8SP | 86 Saint Pierre | Saint Pierre | # | | | | | | yes |
| *M. wrightii* | 9SP | 86 Saint Pierre | Saint Pierre | # | | | | | | yes |
| *M. wrightii* | 10SP | 86 Saint Pierre | Saint Pierre | # | | | | | | |
| *M. wrightii* | 15SP | 86 Saint Pierre | Saint Pierre | # | | | | | | yes |
| *M. wrightii* | 17SP | 86 Saint Pierre | Saint Pierre | # | | | | | | yes |
| *M. wrightii* | 1-SP | 86 Saint Pierre | Saint Pierre | | | | | | | yes |
| *M. wrightii* | 10-SP | 86 Saint Pierre | Saint Pierre | | | | | | | yes |
| *M. wrightii* | 11-SP | 86 Saint Pierre | Saint Pierre | | | | | | | yes |
| *M. wrightii* | 12-SP | 86 Saint Pierre | Saint Pierre | | | | | | | yes |
| *M. wrightii* | 13-SP | 86 Saint Pierre | Saint Pierre | | | | | | | yes |
| *M. wrightii* | 14-SP | 86 Saint Pierre | Saint Pierre | | | | | | | yes |
| *M. wrightii* | 16-SP | 86 Saint Pierre | Saint Pierre | | | | | | | yes |
| *M. wrightii* | 18-SP | 86 Saint Pierre | Saint Pierre | | | | | | | yes |
| *M. wrightii* | 19-SP | 86 Saint Pierre | Saint Pierre | | | | | | | yes |
| *M. wrightii* | 2-SP | 86 Saint Pierre | Saint Pierre | | | | | | | yes |
| *M. wrightii* | 20-SP | 86 Saint Pierre | Saint Pierre | | | | | | | yes |
| *M. wrightii* | 21-SP | 86 Saint Pierre | Saint Pierre | | | | | | | yes |
| *M. wrightii* | 22-SP | 86 Saint Pierre | Saint Pierre | | | | | | | yes |
| *M. wrightii* | 23-SP | 86 Saint Pierre | Saint Pierre | | | | | | | yes |
| *M. wrightii* | 24-SP | 86 Saint Pierre | Saint Pierre | | | | | | | yes |
| *M. wrightii* | 25-SP | 86 Saint Pierre | Saint Pierre | | | | | | | yes |
| *M. wrightii* | 26-SP | 86 Saint Pierre | Saint Pierre | | | | | | | yes |
| *M. wrightii* | 27-SP | 86 Saint Pierre | Saint Pierre | | | | | | | yes |
| *M. wrightii* | 28-SP | 86 Saint Pierre | Saint Pierre | | | | | | | yes |
| *M. wrightii* | 4-SP | 86 Saint Pierre | Saint Pierre | | | | | | | yes |
| *M. wrightii* | 5-SP | 86 Saint Pierre | Saint Pierre | | | | | | | yes |
| *M. wrightii* | 7-SP | 86 Saint Pierre | Saint Pierre | | | | | | | yes |
| *M. wrightii* | 1CNE | 87 Office | Cousine | | # | # | # | # | # | yes |
| *M. wrightii* | 11CNE | 87 Office | Cousine | | # | # | | # | # | yes |
| *M. wrightii* | 12CNE | 87 Office | Cousine | | # | # | # | # | # | yes |
| *M. wrightii* | 13CNE | 87 Office | Cousine | | | | # | # | # | yes |
| *M. wrightii* | 17CNE | 87 Office | Cousine | # | # | # | # | # | # | yes |
| *M. wrightii* | 19CNE | 87 Office | Cousine | # | # | # | # | | # | yes |
| *M. wrightii* | 20CNE | 87 Office | Cousine | | # | | | # | # | yes |
| *M. wrightii* | 22CNE | 87 Office | Cousine | # | # | # | # | | # | yes |
| *M. wrightii* | 32CNE | 87 Office | Cousine | # | # | # | # | # | # | yes |
| *M. wrightii* | 10-CNE | 87 Office | Cousine | | | | | | | yes |
| *M. wrightii* | 14-CNE | 87 Office | Cousine | | | | | | | yes |
| *M. wrightii* | 15-CNE | 87 Office | Cousine | | | | | | | yes |
| *M. wrightii* | 16-CNE | 87 Office | Cousine | | | | | | | yes |
| *M. wrightii* | 18-CNE | 87 Office | Cousine | | | | | | | yes |
| *M. wrightii* | 21-CNE | 87 Office | Cousine | | | | | | | yes |
| *M. wrightii* | 23-CNE | 87 Office | Cousine | | | | | | | yes |
| *M. wrightii* | 33-CNE | 87 Office | Cousine | | | | | | | yes |
| *M. wrightii* | 34-CNE | 87 Office | Cousine | | | | | | | yes |
| *M. wrightii* | 35-CNE | 87 Office | Cousine | | | | | | | yes |
| *M. wrightii* | 9-CNE | 87 Office | Cousine | | | | | | | yes |
| *M. wrightii* | | | Fregate | AF280135 | | | | | | |

## References


Ballard JWO, Melvin RG (2010) Linking the mitochondrial genotype to the organismal phenotype. *Molecular Ecology* **19**, 1523-1539.

Bandelt HJ, Forster P, Rohl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16**, 37-48.

Bauwens D (1999) Life-history variation in lacertid lizards. *Natura Croatica* **8**, 239-252.

Berthier P, Excoffier L, Ruedi M (2006) Recurrent replacement of mtDNA and cryptic hybridization between two sibling bat species Myotis myotis and Myotis blythii. *Proceedings of the Royal Society London B* **273**, 3101-3109.

Brandley MC, Schmitz A, Reeder TW (2005) Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. *Systematic Biology* **54**, 373-390.

Brooke MdL, Houston DC (1983) The biology and biomass of the skinks Mabuya sechellensis and Mabuya wrightii on Cousin Island, Seychelles (Reptilia: Scincidae). *Journal of Zoology, London.* **200**, 179-195.

Brown JM, Lemmon AR (2007) The importance of data partitioning and the utility of Bayes factors in Bayesian phylogenetics. *Systematic Biology* **56**, 643-655.

Brown RP, Yang Z (2010) Bayesian Dating of Shallow Phylogenies with a Relaxed Clock. *Systematic Biology* **59**, 119-131.

Bruen TC, Philippe H, Bryant D (2006) A simple and robust statistical test for detecting the presence of recombination. *Genetics* **172**, 2665-2681.

Buggs RJA (2007) Empirical study of hybrid zone movement. *Heredity* **99**, 301-312.

Camoin GF, Montaggioni LF, Braithwaite CJR (2004) Late glacial to post glacial sea levels in the Western Indian Ocean. *Marine Geology* **206**, 119-146.

Carranza S, Arnold EN, Geniez P, Roca J, Mateo JA (2008) Radiation, multiple dispersal and parallelism in the skinks, *Chalcides* and *Sphenops* (Squamata: Scincidae), with comments on *Scincus* and *Scincopus* and the age of the Sahara Desert. *Molecular Phylogenetics and Evolution* **46**, 1071-1094.

Carranza S, Arnold EN, Mateo JA, Lopez-Jurado LF (2001a) Parallel gigantism and complex colonization patterns in the Cape Verde scincid lizards Mabuya and Macroscincus (Reptilia: Scincidae) revealed by mitochondrial DNA sequences. *Proc Biol Sci* **268**, 1595-1603.

Carranza S, Arnold EN, Mateo JA, Lopez-Jurado LF (2001b) Parallel gigantism and complex colonization patterns in the Cape Verde scincid lizards Mabuya and Macroscincus (Reptilia: Scincidae) revealed by mitochondrial DNA sequences. *Proceedings of the Royal Society London B* **268**, 1595-1603.

Carretero MA (2006) Reproductive cycles in Mediterranean lacertids: Plasticity and constraints. In: *Mainland and Insular lizards. A mediterranean perspective.* (eds. Corti C, Lo Cascio P, Biaggini M). Firenze University Press, Florence.

Chan C, Ballantyne KN, Aikman H, *et al.* (2006) Genetic analysis of interspecific hybridisation in the world's only Forbes' parakeet (Cyanoramphus forbesi) natural population. *Conservation Genetics* **7**, 493–506.

Chan KMA (2003) *The effects of slightly leaky prezygotic isolating barriers and the use of phylogenetic tree shape to study diversification*, Princeton University.

Chan KMA, Levin SA (2005) Leaky prezigotic isolation and porous genomes: rapid introgression of maternally inherited DNA. *Evolution* **59**, 720-729.

Chapple DG, Ritchie PA, Daugherty CH (2009) Origin, diversification, and systematics of the New Zealand skink fauna (Reptilia: Scincidae). *Molecular Phylogenetics and Evolution* **52**, 470-487.

Cheke AS (1984) Lizards of the Seychelles. In: *Biogeography and ecology of the Seychelles Islands* (ed. Stoddart DR), pp. 245–258. Dr. W. Junk, The Hague.

Colonna MJ, Casanova J, Dullo WC, Camoin G (1996) Sea-level changes and $\partial$18O record for the past 34,000 yr from Mayotte reef, Indian Ocean. *Quaternary Research* **46**, 335-339.

Cooper WEJ, Vitt LJ (1997) Maximizing male reproductive success in the broad-headed skink (Eumeces laticeps) by mate-guarding, size-assortative pairing, and opportunistic extra-pair mating. *Amphibia-Reptilia* **18**, 59-73.

Cox SC, Carranza S, Brown RP (2010) Divergence times and colonization of the Canary Islands by *Gallotia* lizards. *Molecular Phylogenetics and Evolution*.

Currat M, Ruedi M, Petit RJ, Excoffier L (2008) The hidden side of invasions: massive introgression by local genes. *Evolution* **62**, 1908-1920.

Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *Bmc Evolutionary Biology* **7**, 214.

Drummond AJ, Rambaut A, Shapiro B, Pybus OG (2005) Bayesian Coalescent Inference of Past Population Dynamics from Molecular Sequences. *Molecular Biology and Evolution* **22**, 1185-1192.

Excoffier L, Foll M, Petit RJ (2009) Genetic Consequences of Range Expansions. *Annual Review of Ecology, Evolution, and Systematics* **40**, 481-501.

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evol Bioinform Online* **1**, 47-50.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**, 479-491.

Flot JF, Tillier A, Samadi S, Tillier S (2006) Phase determination from direct sequencing of length-variable DNA regions. *Molecular Ecology Notes* **6**, 627-630.

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* **147**, 915-925.

Fu YX, Li WH (1993) Statistical Tests of Neutrality of Mutations. *Genetics* **133**, 693-709.

Gamble T, Bauer AM, Greenbaum E, Jackman TR (2008) Evidence for Gondwanan vicariance in an ancient clade of gecko lizards. *Journal of Biogeography* **35**, 88-104.

Gardner AS (1987) The systematics of the Phelsuma madagascariensis species group of day geckos (Reptilia: Gekkonidae) in the Seychelles. *Zoological Journal of the Linnean Society* **91**, 93-105.

Gerlach J (1999) Snails of the genus Pachnodus (Mollusca; Gastropoda; Enidae): their origins and evolution. *Journal of Biogeography* **26**, 251-255.

Gerlach J (2005) Inter-island variation and taxonomy of Seychelles Trachylepis. *African Journal of Herpetology* **54**, 31-42.

Gerlach J (2007) *Terrestrial and freshwater vertebrates of the Seychelles islands* Backhuys Publishers, Leiden, The Netherlands.

Gerlach J, Ineich I (2006) Trachylepis wrightii. In: *IUCN Red List of Threatened Species*.

Gerlach J, van Bruggen AC (1999) Streptaxidae (Mollusca: Gastropoda: Pulmonata) of the Seychelles Islands, Western Indian Ocean. *Zool. Verh. Leiden* **328**, 1-60.

Glez-Peña D, Gomez-Blanco D, Reboiro-Jato M, Fdez-Riverola F, Posada D (2010) ALTER: program-oriented conversion of DNA and protein alignments. *Nucleic Acids Research*, 1-5.

Godinho R, Crespo EG, Ferrand N, Harris DJ (2005) Phylogeny and evolution of the green lizards, *Lacerta spp.* (Squamata: Lacertidae) based on mitochondrial and nuclear DNA sequences. *Amphibia-Reptilia* **26**, 271-285.

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52**, 696-704.

Hahn MW, Rausher MD, Cunningham CW (2002) Distinguishing between selection and population expansion in an experimental lineage of bacteriophage T7. *Genetics* **161**, 11-20.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95-98.

Heled J, Drummond AJ (2008) Bayesian inference of population size history from multiple loci. *Bmc Evolutionary Biology* **8**, 289.

Hey J, Nielsen R (2007) Integration within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 2785-2790.

Honegger RE ( 1966) Beobachtungen an der Herpetofauna der Seychellen. *Salamandra* **2**, 21-36.

Houston D (1978) The influence of sea birds on the terrestrial fauna of Cousin island. In: *Glasgow university expedition to Seychelles - 1977. Preliminary Report.*, p. 32 pp. Cyclostyled.

Hubbs CL (1955) Hybridization between fish species in nature. *Systematic Zoology* **4**, 1-20.

Hudson RR, Kreitman M, Aguade M (1987) A test of neutral molecular evolution based on nucleotide data. *Genetics* **116**, 153-159.

Kaliontzopolou A, Carretero MA, Llorente GA (2010) Intraspecific ecomorphological variation: linear and geometric morphometrics reveal habitat-related patterns within Podarcis bocagei wall lizards. *Journal of Evolutionary Biology* **23**, 1234-1244.

Kocher TD, Thomas WK, Meyer A, *et al.* (1989) Dynamics of mitochondrial DNA evolution in animals - amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences of the United States of America* **86**, 6196-6200.

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**, 1451-1452.

Mayr E (1963) *Animal Species and Evolution* Belknapp Press of Harvard University, Cambridge, MA.

McGarigal K, Cushman S, Stafford S (2000) *Multivariate statistics for wildlife and ecology research* Springer-Verlag, New York.

Meiri S, Raia P, Phillimore AB (2010) Slaying dragons: limited evidence for unusual body size evolution on islands. *Journal of Biogeography* **in press doi:10.1111/j.1365-2699.2010.02390.x.**

Melo-Ferreira J, Boursot P, Suchentrunk F, Ferrand N, Alves PC (2005) Invasion from the cold past: extensive introgression of mountain hare (Lepus timidus) mitochondrial DNA into three other hare species in northern Iberia. *Molecular Ecology* **14**, 2459-2464.

Miller KG, Kominz MA, Browning JV, *et al.* (2005) The Phanerozoic record of global sea-level change. *Science* **310**, 1293-1298.

Miralles A, Vasconcelos R, Harris DJ, Perera A, Carranza S (2010) An integrative taxonomic revision of the Cape Verde skinks (Squamata, Scincidae). *Zoologica Scripta* **in press.**

Nielsen R, Wakeley J (2001) Distinguishing migration from isolation: a Markov chain Monte Carlo approach. *Genetics* **158**, 885-896.

Nussbaum RA (1984) The amphibians of the Seychelles. In: *Biogeography invertebrate and vertebrate taxonomy, ecology and and ecology of the Seychelles Islands* (ed. Stoddart DR), pp. 378-415. Dr. W. Junk, The Hague.

Nussbaum RA, Wu SH (1995) Distribution, variation, and systematics of the Seychelles treefrog, *Tachycnemis seychellensis* (Amphibia: Anura: Hyperoliidae). *Journal of Zoology* **236**, 383-406.

Nylander JA, Wilgenbusch JC, Warren DL, Swofford DL (2008) AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics* **24**, 581-583.

Palumbi SR, Martin A, Romano S, *et al.* (1991) *The simple fool's guide to PCR, Version 2.0.* Privately published, Hawaii.

Paulo OS, Pinheiro J, Miraldo A, *et al.* (2008) The role of vicariance vs. dispersal in shaping genetic patterns in ocellated lizard species in the western Mediterranean. *Molecular Ecology* **17**, 1535-1551.

Petit RJ, Excoffier L (2009) Gene flow and species delimitation. *Trends in Ecology and Evolution* **24**, 386-389.

Pinho C, Rocha S, Carvalho BM, *et al.* (2010) New primers for the amplification and sequencing of nuclear loci in a taxonomically wide set of reptiles and amphibians. *Conservation Genetics Resources* **2**, 181-185.

Polzin T, Daneschmand SV (2003) On Steiner trees and minimum spanning trees in hypergraphs. *Operations Research Letters* **31**, 12-20.

Posada D (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* **25**, 1253-1256.

Posada D, Buckley TR (2004) Model selection and model averaging in phylogenetics: advantages of akaike information criterion and bayesian approaches over likelihood ratio tests. *Systematic Biology* **53**, 793-808.

Radtkey RR (1996) Adaptive radiation of day-geckos (*Phelsuma*) in the Seychelles archipelago: a phylogenetic analysis. *Evolution* **50**, 604-623.

Rambaut A, Drummond AJ (2007) Tracer v1.5, Available from http://beast.bio.ed.ac.uk/Tracer

Ramos-Onsins SE, Rozas J (2002) Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution* **19**, 2092-2100.

Rendhal H (1939) Zur Herpetologie der Seychellen. I. Reptilien. *Zoologishces. Jahrbuche (Sytematischen)* **72**, 255-328.

Rhymer JM, Simberloff D (1996) Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics* **27**, 83-109.

Roca AL, Giorgiadis N, O'Brien SJ (2005) Cytonuclear genomic dissociation in African elephant species. *Nature Genetics* **37**, 96-100.

Rocha S (2010) *Phylogeny, Phylogeography and Colonization Patterns of Reptiles in Indian Ocean Islands*, University of Porto.

Rocha S, Carretero MA, Harris DJ (2010) Genetic diversity and phylogenetic relationships of Mabuya spp. (Squamata: Scincidae) from western Indian Ocean islands. *Amphibia-Reptilia* **31**, 375-385.

Rocha S, Harris DJ, Posada D (submitted-a) One or more? Cryptic diversity within the endemic Prehensile-tailed gecko Urocotyledon inexpectata across the Seychelles Islands: patterns of phylogeographic structure and isolation at the multilocus level. *Biological Journal of the Linnean Society.*

Rocha S, Posada D, Harris DJ (submitted-b) The time frame of diversification of day-gecko genus Phelsuma on the Seychelles and its driving mechanisms. *Systematic Biology.*

Rocha S, Vences M, Glaw F, Posada D, Harris DJ (2009) Multigene phylogeny of Malagasy day geckos of the genus *Phelsuma*. *Molecular Phylogenetics and Evolution* **52**, 530-537.

Rocha S., Harris, D.J., Perera, A., Silva, A., Vasconcelos, R., Carretero, M.A. (2009b) Recent data on the distribution of Lizards and Snakes of the Seychelles. Herpetological Bulletin, 110, 20-32.

Rohling EJ, Fenton M, Jorissen FJ, *et al.* (1998) Magnitudes of sea-level lowstands of the past 500,000 years. *Nature* **394**, 162-165.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572-1574.

Sambrook J, Fritsh EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*, second edition edn. Cold Spring Harbor Press, Cold Spring Harbor, NY.

Scott H (1933) General conclusions regarding the insect fauna of the Seychelles and adjacent islands. *Transactions of the Linnean Society of London 2nd series (Zoology)* **19**, 307-391.

Servedio M, Noor MA (2003) The Role of Reinforcement in Speciation: theory and data. *Annual Review of Ecology, Evolution, and Systematics* **34**, 339-364.

Shapiro B, Rambaut A, Drummond AJ (2006) Choosing appropriate substitution models for the phylogenetic analysis of protein-coding sequences. *Mol Biol Evol* **23**, 7-9.

Siddall M, Rohling EJ, Almogi-Labin A, *et al.* (2003) Sea-level fluctuations during the last glacial cycle. *Nature* **423**, 853-858.

Somers K (1986) Multivariate allometry and removal of size with Principal Component Analysis. *Systematic Biology* **35**, 359-368.

Stephens M, Smith NJ, Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. *American Journal of Human Genetics* **68**, 978-989.

Strasburg JL, Rieseberg LH (2010) How robust are "isolation with migration" analyses to violations of the im model? A simulation study. *Molecular Biology and Evolution* **27**, 297-310.

Sugiura N (1978) Further analysis of the data by Akaike's information criterion and the finite corrections. *Communications in Statistics -Theory and Methods*, 13-26.

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**, 585-595.

Takahata N, Slatkin M (1984) Mitochondrial gene flow. *Proceedings of the National Academy of Sciences of the United States of America* **81**, 1764-1767.

Vesey-Fitzgerald D (1947) Reptiles and Amphibians from the Seychelles Archipelago. *Ann. Mag. Nat. Hist. (series 11)* **14**, 577-583.

Warren BH, Dominique S, Bruggemann JH, Robert PP-J, Christophe T (2010) Why does the biota of the Madagascar region have such a strong Asiatic flavour? *Cladistics* **26**, 526-538.

Watterson GA (1975) On the number of segregating sites in genetical models without recombination. *Theoretical Population Biology* **7**, 256-276.

Wirtz P (1999) Mother species - father species: unidirectional hybridization in animals with female choice. *Animal Behaviour* **58**, 1-12.

# CHAPTER 5

## GENERAL DISCUSSION

This work aimed at contributing to the knowledge of the diversity within several reptile groups from the Western Indian Ocean region and to a better understanding of its genesis (including oceanic dispersals and intra-islands or intra-archipelago diversifications) and of the time frame it occurred.

Altogether, the Western Indian Ocean archipelagos comprise an extraordinary diverse array of islands, with ages from up to 120 My of isolation (Madagascar), to 65 My (the granitic Seychelles), through 1-10 My old volcanos (the Comoros and the Mascarenes), to coral atolls just a few tens or hundreds of thousands of years of age. They show considerable similarities in their terrestrial biotas, but their understanding is complicated given the astounding levels of diversity – new taxa are constantly being reported and described, even for groups though to be reasonably well-known like vertebrates – and also by substantial recent extinctions and changes in island areas due to fluctuating sea-levels. Considerable advances over the last years, especially due to the application of molecular techniques, have led to a comprehensive understanding of the time and extent of colonization of these archipelagos from potential sources in Africa, Asia or Australia, at least for some major groups, mainly vertebrates and higher plant taxa. On the other hand, the nature of speciation events that have occurred on them and the degree to which their indigenous biotas have been impacted in the recent past by natural and human-mediated processes is still far from achieved. For many groups, even knowledge on alpha-diversity and the degree of distinctiveness of different island populations or species is scarce. In this thesis we explore some of these questions for selected groups of reptiles. Reptiles, possibly the non-flying vertebrate group with better dispersing abilities, tend to be highly abundant on islands (e.g. Rodda and Dean-Bradley, 1992), where they exhibit high levels of endemism (Kier et al., 2009). Lizards in particular, represent a highly abundant and diverse group of island reptiles (Whitaker and Fernández-Palacios, 2007), many times undergoing niche broadening and conducting vital ecosystem functions on islands, like pollination and seed dispersal (Hansen et al., 2007; Kaiser-Bunbury et al., 2009; Oleson and Valido, 2003; Whitaker, 1987), which are usually fulfilled by mammals, birds and insects in mainland areas. They are thus one of the most relevant groups to be studied since understanding historical patterns will help towards a better understanding of ecosystem functioning and conservation, especially in island settings.

## 5.1 Phylogenetic Relationships, Colonization Patterns and Systematics of day-geckos of the genus *Phelsuma*

Day-geckos of the genus *Phelsuma* are certainly one of the most – if not the most – conspicuous and representative groups of lizards from the Western Indian Ocean region. Widespread across all archipelagos, where they generally represent island or archipelago endemisms, this highly diversified group (more than 40 species are currently recognized) represents an excellent model for exploring historical patterns of structure and diversification in the region, and especially within Madagascar, where they are found in a variety of habitats across the entire island, from primary forest regions, dry southern seasonal forests and scrublands, western coastal forests, eastern low and mid-altitude humid forests and even in some high altitude humid regions. Furthermore, understanding the diversification of the genus itself, and why it has it done so to such a greater extent than others in the same region it's a relevant endeavour, where both geography and ecological opportunity may have played a determinant role (Harmon et al., 2008).

### *5.1.1 Phylogenetic Relationships and Systematics Revision*

In the first chapter of this thesis we proposed that the genus *Phelsuma* diversified in Madagascar, from where remaining archipelagos were colonized, and that diversification appears to have been fast to the point that with current data basal relationships are mostly unresolved (*Paper II*). We further suggested that while the Seychelles' and Mascarenes' species assemblages are a result of single colonizations and *in situ* diversifications, the Comoros (although also harbouring a small endemic radiation) were colonized several times, were more affected by human-mediated introductions (*Paper I*) and that independent colonizations to Aldabra and Pemba (East Africa) also occurred. Although species-level diversity and relationships are not yet fully characterized and are clearly in the need of revision at least for some groups (*Paper III*), eight well supported clades were defined, and the inferred phylogeny constitutes a valuable framework for further research in evolutionary biology and systematics. To further ease this task and make this entire clade more manageable, we proposed to divide it into 11 species-groups reflecting the evolutionary relationships within the genus, which we further characterized for relevant phenetic characters, evidencing the plasticity and multiple evolution of some of them but also the phylogenetic utility of others. The continuous description of new species within this

genus (e.g. Glaw et al., 2010), that can now more easily be assigned to a major clade highlight the usefulness of the developed work and the need for further assessment within this group.

*5.1.2 Time Estimates and the Age of Phelsuma: a Recent or Not-So Recent Group?*

One question that remains open is the time frame of *Phelsuma* diversification and island colonization. Recent estimates of both *Phelsuma* separation from other gekkonids (~7 Mya) and intrageneric diversification (5Mya) were first inferred (*see Paper I*) in concordance with estimates by other authors (Austin et al., 2004). These estimates were obtained through a relaxed clock framework and calibrations on recent nodes of the tree (2.1 Mya; age of Reunion for *P. cepediana* - *P. borbonica* split and 0.5 Mya (age of Grand Comoro for *P. comorensis* - *P. lineata* split). However, as correctly noted by Harmon et al. (2008), such a recent estimate implies high rates of molecular evolution and might not be as accurate as expected. Porter et al. (2005) describe very clearly the effect of the method used and of the number and distribution of calibrations on the divergence age estimates. In particular they show that when older fossil calibrations are removed the estimates of the entire backbone of the phylogeny are pulled towards younger dates (in their case with differences as high as 152 My), while the opposite trend is observed when removing younger calibrations (as also highlighted by Hugall et al., 2007).

In order to try to assess potential biases in our estimates due to this confounding factors we carried out additional analyses. We gathered large datasets from comprehensive multilocus phylogenies from the whole genus (datasets from *paper II*) and applied the same relaxed clock framework as in paper I; i.e. the Bayesian molecular dating procedure implemented in PAML/Multidivtime (Thorne et al., 1998; Thorne and Kishino, 2002; Yang, 1997) for obtaining estimates across the *Phelsuma* tree. In addition to estimates using the ML combined tree (as from *paper II*) and two shallow calibration points (2.1 Mya as in paper I and 0.125 Mya, corresponding to the age of last submergence of Aldabra, for the *P. abbotti checkei* and *P. abbotti abbotti*), extra datasets were assembled and different analyses were performed using either mitochondrial plus nuclear or only nuclear markers and different sets of calibration points (see appendix). In addition to the main lineages within *Phelsuma* we included representatives of main groups of *Squamata* (*Teiioidea, Rhineuridae, Bipedidae, Iguanidae, Xantusidae, Scincidae* and *Pygopodinae*) and also several other Tetrapods (*Sphenodon, Gallus, Mus, Xenopus*). These allowed us to use

deep fossil calibrations (from 58 to 330 Mya – see Table 5.1.1 in appendix) and obtain independent estimates for the age of the root of *Phelsuma* radiation and the divergence between *Phelsuma* and *Rhoptropella/Lygodactylus* – herein the "*old calibrations*" dataset.

Depending on the dataset used, the estimates for the age of crown diversification within *Phelsuma* and its split from *Rhoptropella/Lygodactylus* were substantially different (Tables 5.1.3-5.1.5; Figures 5.1.1 and 5.1.2), ranging from ~6 Mya for the divergence between *Phelsuma* and *Rhoptropella* and ~4Mya for diversification within *Phelsuma* (using mitochondrial plus nuclear dataset and only intrageneric calibrations), to ~84 Mya for the *Phelsuma* vs. other gekkonids split and 45 Mya for the *Phelsuma* crown diversification (using both deep and shallow calibrations on the mitochondrial plus nuclear "*old calibrations*" dataset). Using the *combined* (mitochondrial plus nuclear) dataset the pattern observed when estimating ages using different sets of calibrations was always one of severe underestimation of the deeper nodes when only shallow calibrations were used (compared to the accepted ages for those same nodes based on fossil data), and of some ages inferred for the shallower nodes being older than respective island ages when only deeper calibrations were used. This revealed a major influence of the calibration scheme used and highlighted the lack of reliability we should be aware of for these estimates. As nuclear genes are less affected by saturation than mitochondrial genes, and after exploring in detail the effects of different calibration schemes on a nuclear dataset (which revealed less bias in age estimation respect to the set of calibrations – shallow or deep – used; see Table 5.1.5); we finally obtained estimates for *Phelsuma* separation from outgroup and crown diversification based solely on the nuclear dataset and using all available calibration points, of 79.3 (53.2-107.8) Mya and 52.5 (30.6-79.8) Mya, respectively (Table 5.1.5).

Porter et al. (2005) showed that older calibrations can produce more consistent backbone estimates, and hence more stable estimates across the tree, and, perhaps more importantly in this case, how *Multidivtime* (soft-bounded node calibrations) estimates remained stable as younger calibrations were removed and even when only a single deep fossil calibration was incorporated. Thus, it seems now very likely from our data that previous estimates (Austin et al., 2004; *paper I*) were severely biased downwards and that the age of this group is probably up to several times older. Factors that may have led to spurious, younger estimates include saturation of the mitochondrial markers and the exclusive use of shallow calibration points. Saturation will cause compression of the deeper branches and, depending on the calibrations used (deep/shallow) will lead to over- or underestimates.

Acknowledging the huge differences in the estimates respect to dataset and calibrations used, any current estimate of age for this clade must be considered with care. If previous values seem evident underestimates, estimates using deep and shallow calibrations and nuclear data alone may also be overestimates, possibly due to underparametrization of the model and to the lack of calibration points of moderate depth inside the Gekkonidae, since all fossil calibrations used were located outside geckos and pygopods. Although additional calibration points were used (island ages) they were very shallow at this scale (located at the tips of the *Phelsuma* clade) and most possibly insufficient to ensure accurate rate modelling and node age estimation across the main part of the tree.

A thorough determination of diversification and island colonizations time frame within this clade remains to be achieved. The collection of more data, the inclusion of more Gekkonid taxa and possible calibrations within them, and the use of more accurate "species-trees" estimation methods with the implementation of calibrations in the species phylogeny itself (rather than in the gene genealogies) could lead to the achievement of a more precise estimate of the age of the genus *Phelsuma*, allowing for more precise biogeographic conclusions.

### *5.1.3 Are "Old" (Paleocene-Miocene) Estimates Compatible with Island Ages and the Geological History of the Region?*

The consideration of the possibility that the true divergence and diversification times within the genus are older than previous estimates (Austin et al., 2004; *paper II*) requires also new assumptions on the colonization history of the region. For the Mascarene *Phelsuma* it may be hypothesized that they differentiated earlier than the emergence of the current islands, colonizing what are nowadays submerged banks like Nazareth or Saya de Malha (Figure 1.8), and later moving south to their extant distribution. A colonization of these banks from southeast Asia has been invoked for the ancestor of the emblematic Dodo and Solitaire birds, both extinct now but inhabiting the Mascarenes until the 17$^{th}$ century. Using mtDNA data (1.4 kb) and two fossil calibrations (the penguin - albatross divergence at 58 Mya and the chicken – guinea fowl at 40 Mya; Cooper and Penny, 1997), Shapiro et al (2002) found that the Dodo and Solitaire separated from their Asian sister-group *Caloenas* during mid/late Eocene, around 42 Mya, and subsequently diverged at around 25.6 (17.6 - 35.9) Mya. The similarity between the timing of the Dodo/Solitaire divergence and the first geological evidence of land in the Mascarene island chain

led Shapiro et al. (2002) to suggest that island stepping stones could have been used before the two species eventually found their way to Mauritius and Rodrigues. A similar example may be found in another Mascarene lineage, the extinct *Cylindraspis* tortoises (Cheke and Hume, 2008). Austin and Arnold (2001) report p-distances of 11.9 - 16.9% between the different Mascarene endemic species for the 428 bp of the cytochrome-b fragment sequences. Not mentioning ages, the authors infer the colonization sequence from the obtained phylogeny, only based on the nowadays-exposed islands of Mauritius, Reunion and Rodrigues, but, taking into account the slow rate of mtDNA evolution in turtles (Avise et al., 1992) and the rate commonly applied to this fragment in turtles, of 0.4 - 0.6% divergence per lineage per My (Caccone et al., 1999), a colonization and initial diversification predating the initial diversification of the currently emerged islands of Mauritius, Reunion and Rodrigues must be invoked. Assuming an initial diversification as old as the actual islands (10 Mya) would imply rates of evolution as fast as the ones assumed for mammals (~1.6%), which seems unlikely (Rand, 1994). Similar cases of island hopping are argued for several representatives of the Mascarene reptile fauna (Cheke and Hume, 2008)

As for other colonization ages within *Phelsuma*, assuming the estimates obtained using the nuclear data solely (appendix; Table 5.1.5), the Andamanes and Seychelles, together with the Mascarenes, colonizations seem to be the older events (mean estimates around 30 Mya but credibility intervals up to 60 Mya), followed by the colonization originating *P. nigristriata* (9 - 42 Mya), then the Comoros endemic clade, and finally *P. parkeri* (0.42 - 22.5 Mya), with the origin of *P. comorensis* and *P. abbotti* considerably more recent. The fact that all these estimates are compatible with respective island ages (not used as calibration points) is worth highlighting, as also that the age of the divergence between the two species from the Seychelles estimated under the IM model (paper VIII) is compatible with the values here obtained. Nevertheless, most age estimates have too wide credibility intervals, and are thus of very little informative value. Yet, the examination of the available stem and crown ages for the island groups suggests that the colonization of the different island groups did not occurred in a concerted manner, and that dispersal was not concentrated in any particular time interval. Instead, putative colonizations are spread throughout the *Phelsuma* phylogeny, with the Comoros archipelago having received three asynchronous colonizations, two relatively old and one considerably more recent. Also there is apparently no correlation between the geological age of subaerial emergence of island and the estimated age of its colonization, or between the age of colonization and species

diversity. For example, the Seychelles are the oldest island group (~65 My) but its colonization does not appear to be older that of the Andamanes or the Mascarenes. Even accounting for the ages of the banks that the Mascarenes' clade could have first colonized (they could have first emerged ~40 Mya), they are still much younger than the Seychelles. The Comoros have a maximum of 15 My and may well have been colonized fast after their subaerial emergence, with a more recent colonization of the youngest island (*P. comorensis* in Grand Comore) probably also soon after its estimated initial emergence.

It is intriguing that despite the very early colonization of the Andamanes by *Phelsuma*, these geckos do not appear to have radiated there, contrary to most other *Phelsuma* lineages that colonized archipelagos surrounding Madagascar. The area of Andaman and Nicobar islands, which they inhabit, is over 8,000 km$^2$, and the habitat is typically tropical rainforest, similar to eastern Madagascar rainforests where *Phelsuma* apparently diversified extensively. Nevertheless, they apparently did not undergo extensive radiations in the Andamanes, indicating either cryptic, still unrecognized, diversity (morphologically indistinguishable but highly differentiated lineages were also recently unveiled within *P. cepediana*, Austin et al., 2004) or a young colonization of major parts of their current distribution range. Considering the old age estimated for the split between *P. andamanense* and remaining species, some island hopping in currently submerged areas may also be hypothesized.

### *5.1.4 Using Islands as Calibration Points*

Previous considerations highlight the potential problems arising when using islands as calibration points. Besides the methodological problems of using only shallow calibrations, important assumptions are made when using island ages as calibrations, and the possibility of alternative explanations must be carefully considered. The assumption that an endemic island lineage colonized an isolated oceanic island soon after its emergence seems justified in some cases (especially when islands are recent and/or harbour endemic and/or highly diversified groups). However, in the complex geological history of the Western Indian Ocean islands, where volcanism and eustatic sea-level changes likely led to a dynamic system concerning sub-aerial exposed areas through time, this assumption may not always be correct. Furthermore, the age of islands may be known imprecisely, and lineage extinction or the possibility of insufficient sampling of extant lineages must be considered. Dispersal abilities of the organisms, comparison

of the obtained with the supposed rates, and the existence of alternative plausible scenarios must be carefully taken into consideration when calibrating with island ages (see Thorpe et al., 2005 for an example of similar problems in the Lesser Antilles).

In the Western Indian Ocean area, several studies have been carried out using islands as calibration points. Vences et al. (2003) assumed for frogs, which certainly are a group with very limited overseas dispersal capacities, that the earliest possible age of the Comoran island of Mayotte was a conservative estimate of the colonization of this island from Madagascar. This same calibration was later used in a deep phylogeny of amphibians (San Mauro et al., 2005) and turned out to be highly compatible with much deeper fossil calibrations. In contrast, Warren et al. (2003; 2005) used ages of Grand Comoro, Aldabra and Reunion to calibrate phylogenetic trees of birds and obtained very young estimates for the colonization of Madagascar, or of the Malagasy region. This required invoking very high rates of evolution and ages up to seven times higher could be obtained using the commonly used passerine molecular clock of 2% divergence per my (Warren et al., 2003).

It is clear that dating phylogenies is an essential step to understand the biogeography of a group of organisms and to infer general patterns within a region and that the generalization of the use of relaxed clock methods was a huge advance in this respect, that is leading to continuous refinements of the estimated dates at several levels (e.g. Hugall et al., 2007; Poux et al., 2005; Vidal and Hedges, 2005; Yoder and Yang, 2004). For Madagascar the emerging picture is one of the predominance of Cenozoic dispersers, with very recent age estimates for some clades of reptiles and birds (reviewed in Yoder and Nowak, 2006), but it is most important that these estimates keep being considered as hypotheses and that new data keeps being collected and alternative explanations tested. For example, both Cheke and Hume (2008) and Warren et al. (2010) highlight the fact that the estimate dates for the Dodo and Solitaire divergence (Shapiro et al., 2002; above) implies an unprecedentedly low rate of avian cytochrome-b evolution and that ages of 10 and 6 Mya could be obtained from applying more usually common values (~2% per million year) for avian cytochrome-b evolution.

New methodologies will certainly have a preponderant role: divergence time estimations available so far are made in the context of gene trees or concatenation approaches, where divergence times are estimated under the assumption that all gene trees share the same topology. It is now clear that these can differ, and substantially differ from the underlying species tree, especially for cases of recent and fast diversification (Degnan and Rosenberg, 2009), as is

most possibly the case of several Western Indian Ocean clades. Moreover, because the timing of gene divergences necessarily predates the actual speciation event (unless gene flow accompanies species divergence), conclusions based on gene trees will most probably overestimate divergence times, in contrast to multilocus coalescent approaches (Carstens and Knowles, 2007; Edwards and Beerli, 2000). New multilocus coalescent models for estimating species phylogenies (reviewed in Knowles, 2009), coupled with relaxed phylogenetics with calibrations directly implemented in the estimated species phylogeny (e.g. McCormack et al., 2010) are promising tools to this continuous refinement of age estimates in the biogeography context and a more precise and accurate understanding of the timing of species diversification.

This is an essential knowledge to accurately tackle the processes that influenced taxa diversification. Nevertheless, huge and biodiverse areas such as Madagascar will always represent considerable challenges because its taxa diversity is far from being accurately known. This is evident even in this group of day-geckos, thought to be one of the best-known vertebrate clades due to its conspicuousness, but where in fact the number of known species almost doubled along the last 10 years, and new species are continuously described (Berghof and Trautmann, 2009; Glaw et al., 2010; Glaw et al., 2009; Lerner, 2004; Nussbaum et al., 2000; Raxworthy et al., 2007; Rösler et al., 2001; Schönecker et al., 2004). In this regard, surrounding archipelagos are easier to explore.

## 5.2 Phylogenetic Relationships, Genetic Diversity and Islands Population Status of Three Widespread Western Indian Ocean Groups

An essential step in understanding the biogeographic history of a region, and before diversification processes can be tackled, is to distinguish natural from human-mediated patterns and to assess the relative influence of these in shaping species distributions. This is not always a straightforward task. For some species cryptic differentiation may exist, and apparently large and homogeneous ranges may harbour in fact multiple lineages. Morphological plasticity may also be high, and many species can be morphologically similar, confusing distribution and taxonomic data, which hampers a proper understanding of the history of that group. This was the case for a few lizard taxa in the Western Indian Ocean region, namely *Gehyra* and *Hemidactylus* geckos, but also partially for *Mabuya comorensis – maculilabris* "complex". For these groups it was not clear how many - if any - island populations represented endemic taxa or how much of their distribution was due to human-mediated dispersals. We have now clarified their phylogenetic relationships and the origin of several islands populations (*papers IV-VI*).

### *5.2.1 Antropogenically shaped Patterns of Distribution*

Introduced reptile species can have various negative impacts on native species, including predation, competition for food, basking sites and other resources, hybridization and other genetic effects, spread of diseases and parasites, and poisoning through toxic skin glands or venomous bites (e.g. Cole et al., 2005; Petren and Case, 1996). They may also alter the habitat of native species and disrupt ecosystem dynamics. These processes are especially dangerous if they happen on islands (Butterfield et al., 1997), where the number of endemic species is higher and ecosystems are more vulnerable to introductions (Shine et al., 2000). Unfortunately, it is on islands that this phenomenon is occurring 110 times more frequently and with a higher probability of successful establishment relative to mainland systems (Kraus, 2003). Given that many of the Western Indian Ocean islands have impoverished reptile communities, it becomes most important that potential non-natural patterns are tackled.

Geckos are a family of lizards that are particularly well suited to overseas dispersal (Kluge, 1969) and many are also commensal. Therefore they are also likely to be translocated by humans, and its not surprising that most of the species whose distributions seem to be most

influenced by man belong to this group. In the Indian Ocean region this is especially evident for several species of house geckos genus *Hemidactylus* (*paper V*), and for *Gehyra mutilata* (*paper IV*), but also for some species of the genus *Phelsuma*, that appear to have been introduced both to the Comoros and the Mascarenes (see *paper I and III*). Also, cases of most possibly human-mediated introductions were depicted within *Mabuya striata* and *Mabuya comorensis* (*paper VI*) Possibly human-related patterns of dispersal across islands were also found within the Seychelles (chapter 3), although most of these are less clear and deserve future investigation. Additionally, it may be that the two extant terrapin species in the Seychelles (*Pelusios castanoides* and *P. subniger*; that are considered the only Crytically Endangered reptile taxa in the Seychelles and target of specific conservation actions) are in fact introduced (Silva et al., 2010). All these cases highlight the importance of this kind of assessment, especially in island environments, where introduced taxa can more easily establish and spread.

The case of *Hemidactylus* provides us a remarkable example of the problems of dealing with widespread taxa with unclear distributions and partial sampling. Despite previous studies (Rocha et al., 2005; Vences et al., 2004) it was difficult to draw conclusions about taxonomic status and evolutionary history of these taxa, and their interpretation was controversial in a few aspects. It is most important that diversity and relationships between populations are clearly assessed both in their insular and continental areas of putative origin. The improvement of the coverage of the species distribution makes us now more confident in having reached a more accurate picture of these species biogeographic history, but even so current results must still be taken with care. Cryptic variation was uncovered in all species in their native range, but remains largely uncharacterized.

### *5.2.2 The Endemic Lineages of the Aldabra Archipelago*

The genetic diversity and geographic structure of the Aldabra populations of *H. mabouia* was surprising. Dealing with a widespread, commensal species, whose insular distribution seems mostly shaped by human mediated introductions, a very recently submerged atoll was a largely unexpected location for apparently native, endemic lineages to be found, but that was exactly the case (*paper V*). Coalescent–based calculations confirm the possibility that all haplotypes from *H. mabouia* population from Aldabra shared a common ancestor only 0.125 Mya, the time estimated since the last Aldabra submergence. Distinct lineages inhabiting the Aldabra archipelago were

recovered also for the coastal skink *Cryptoblepharus boutonii* (unpublished data). Western Indian Ocean populations of Madagascar, the Comoros and the Mascarenes of this widespread Indo-Pacific genus had already been shown to represent an endemic lineage resulting from natural transoceanic dispersal from Australia or Indonesia (Rocha et al., 2006) and new data from Aldabra populations shows that yet another lineage is present (Figure 5.2.1). If resulting from another independent colonization from Australasia, this would be an impressive case of double long transmarine dispersal within a lizard clade. However, eight of the nine extinct and living Pleistocene reptile fauna of Aldabra have their closest relatives in Madagascar, which is their most likely source of origin (Arnold, 1979). The origin of these populations of *Cryptoblepharus* from Aldabra must thus be investigated through a comprehensive phylogenetic study of the whole genus. It may be that further diversity remains to be characterized from Malagasy populations, from where these may have originated. This constitutes an example of how the study of diversity within (recent) island populations may give us hints on important aspects of the evolutionary history of a species in their "continental" areas of origin, where they may be more difficult to tackle.

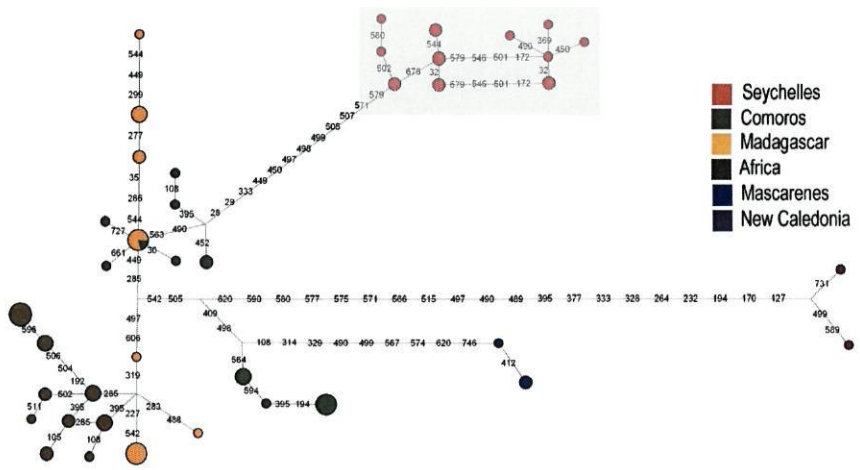



**Figure 5.2.1** – Median Joining network for partial 12s and 16s rRNA genes (~760 bp) for *Cryptoblepharus boutonii*. Numbers along branches represent mutational steps. Haplotypes from Aldabra and Cosmoledo islands (Seychelles; in red, grey box) constitute a lineage quite differentiated from remaining Western Indian Ocean populations.

*5.2.3.The Mabuya maculilabris Species-Complex in the Western Indian Ocean islands.*

Mitochondrial DNA data was also used to describe genetic variation and explore the phylogenetic relationships of insular populations of some species of the skink genus *Mabuya* for which both taxonomic status and origin across Western Indian Ocean islands were unclear. Specifically, the distinction and relationship between *M. maculilabris* and *M. comorensis* was controversial, as well as the identity and phylogenetic relationships of several island populations assigned to these species. Further, the status and possible distinction of several island populations of *M. striata* were also unknown (see *paper VI*).

These were clarified in paper VI, where the distinctiveness of *M. comorensis* relative to *M. maculilabris* was confirmed (albeit their close relationship), with *M. comorensis* making *M. maculilabris* paraphyletic. The population level sampling of the study allowed the clarification of the identity and status of some island populations such as *M. maculilabris casuarinae* (from Mozambique Island) and *M. comorensis* from Madagascar (Nosy Tanikely) and further showed that *Mabuya maculilabris* harbours at least four highly divergent lineages (and probably more as its continental range remains largely unexplored).

The inclusion of all available African *Mabuya* in this study was particularly useful as it unveiled a common deep differentiation between west and east African lineages within *M. maculilabris* and *M. striata*, a pattern that is also evident in the disjoint distribution of some other reptile species (Broadley, 2001; Ineich and Chirio, 2004; Ineich et al., 2004; Rasmussen, 2005) and that may be reflective of a once broader, more contiguous past distribution, when more mesic conditions may have linked the Eastern Arc Mountains to the forests of west Africa (Diamond and Hamilton, 1980; Gasse, 2006; Lovett and Wasser, 1993; Maley, 1996). These may thus constitute good model species where to explore phylogeographic patterns related to Pleistocene climatic oscillations in central Africa. Further, three instances of mtDNA paraphyly were depicted; one involving the Seychellois species, *M. sechellensis* and *M. wrightii* and the other two involving *M. margaritifera* with both *M. striata* and *M. quinquetaeniata*. Respect to the Seychellois species we now are able to explain the pattern (see chapter 3, *paper IX*), which involves the existence of hybridization and mtDNA introgression between these two species. Possibly similar situations of hybridization can explain these other paraphyly observations, and should be taken into account when further studies involving these species are conducted.

A long-standing unsolved question concerns the colonization of Madagascar by *Mabuya*

spp. Our results seem to support the existence of two colonization events, but considering that more than 70 species are estimated to belong to this clade (and only 17 are included in our study), the question of a single or multiple colonizations of Madagascar must remain open.

### *5.2.4 The Use of Molecular Data to Distinguish Human-Related from Natural Patterns*

The analysis of the phylogeographic structuring of populations can be useful to unweave old natural island colonizations from more recent human-mediated dispersal (e.g.Austin, 1999; Jesus, 2002; Jesus et al., 2001; Vences et al., 2004) but mtDNA data alone is not always conclusive. The dispersal of *Gehyra* populations across the Pacific islands is one example where molecular data is clearly insufficient: human colonization of Pacific islands is also extremely recent and the hypothesis that this species was already present on the islands before human arrival cannot be rejected with the current data. In contrast to the Indian Ocean islands, across most Pacific islands *Gehyra mutilata* occupies a wide range of habitats, and not only anthropogenic ones. Within the Western Indian Ocean region, a similar case can be argued for the Seychelles terrapins genus *Pelusios*: molecular data revealed a complete lack of variation at the mtDNA level for the two extant species across the Seychelles archipelago (Silva et al., 2010) but both a recent arrival to the archipelago by human action or strong founder effect allied to low evolution rate described for turtle mtDNA may explain the observed pattern. In both cases the use of highly variable markers such as microsatellites and the comparison with proposed human migration routes (especially for the Pacific islands) is needed to address dispersal mechanisms of these taxa.

The three papers that compose the third chapter of this thesis and whose implications were just discussed all are based on single mtDNA markers. This has to be taken into account and the results must be subject to careful interpretation. Although we think that all the results presented are meaningful and deserve consideration, multilocus approaches are always advisable, and any study wishing to complement or further explore the patterns here unveiled should take that into consideration.

## 5.3 Diversity and Diversification on the Seychelles Archipelago

The Seychelles is, surprisingly, perhaps the least studied of the Western Indian Ocean archipelagos in terms of the genetic diversity and structure of its extant taxa, lacking previous studies using molecular markers to explore phylogeographic patterns across the archipelago. Using reptiles as model organisms we aimed to test previous hypotheses concerning biogeographic structure across the granitic islands and tried to unveil general patterns and factors promoting diversification within the archipelago.

### *5.3.1 Fieldwork Surveys and Improving Distribution Data Across the Seychelles.*

Despite systematic studies on the vertebrate fauna of the Seychelles dating back to the early 80's and even after publications dedicated exclusively to reptile taxa (Gardner, 1986) and two very recent publications covering species accounts and tentative distributions (Bowler, 2006; Gerlach, 2007), precise distribution records are largely lacking and many distributions are clearly incomplete. Thus, a fundamental step to understand which groups could be used to address questions on the biogeography of the archipelago was the improvement of records on the distribution of species. This was achieved at the same time that sampling was performed and a compilation of data gathered by us over roughly 12 weeks of fieldwork between 2005 and 2008 (Rocha et al., 2009) provided an updated distribution record of lizards and snakes across the Seychelles (granitics and coralline) islands with over 120 sampled localities resulting in approximately 900 observations and a reasonable coverage of main islands from the different groups. Despite many other groups being equally distributed across at least some of the islands, due to the quantity of available samples, distribution data, and best coverage across species distribution, we ultimately focused on three groups for our questions of structure and diversification within the Seychelles: *Urocotyledon, Phelsuma* and *Mabuya* genera.

In each of the manuscripts that compose the third chapter, we described the genetic structure of each group and explored the evolutionary history of each clade. Along this discussion we will thus only revisit some of the common patterns inferred and that can prompt general biogeographic hypotheses that could be tested in other organisms.

### *5.3.2 Common Phylogeographic Patterns Across the Granitic Seychelles*

*The major influence of distance in the genetic structure of organisms: the northern islands-southern islands phylogeographic break*

The most evident pattern shared by all species here analyzed is the northern-southern structure of the genetic variation. The northern and southern groups of islands (Praslin, La Digue and surrounding islands vs. Mahé, Silhouette and North – and Fregate, see below) generally exhibited exclusive mtDNA lineages, which furthermore were the deepest lineages within each species. This pattern was very clear in *Urocotyledon inexpectata* (for which some nuclear markers also exhibited compatible patterns; *paper VII*) and also in both *Phelsuma* species (although the affinities of Fregate population of *P. astriata* are still unclear, see below) as well as, although less clearly, within *Mabuya sechellensis* and *M. wrightii*. Although also shaped by introgression, mtDNA lineages of *M. sechellensis* were structured across northern and southern (introgressed) islands. The higher differentiation of the population of *M. wrightii* from Fregate (currently extinct in the southern group) relative to northern populations lead to the possibility of the same north-south biogeographic pattern being the case for this species also. This pattern was directly correlated with distance (and not with sea-levels, which would make Silhouette and North the most isolated islands relative to all others), indicating its major influence in shaping the distribution of species and genetic diversity across these islands.

*The southern affinities of Fregate populations*

In previous assessments of Seychelles biogeography, Fregate was generally viewed as an isolated or intermediate unit (Cheke, 1984; Radtkey, 1996; Scott, 1933). Nevertheless, for the majority of species we studied, Fregate populations were closer to the southern islands populations of Mahé, Silhouette and North, within which they were clearly nested. The only case where this pattern was not observed was within *Phelsuma astriata*, which seemed more related with populations from the northern islands. However, support values for their closer relationship to the northern clade were low, and their phylogenetic affinities remain unclear. There is no explanation for this pattern; the distance from Fregate to both groups is roughly the same and sea levels seem to have affected connectivity to both groups similarly (see chapter 1).

*Additional structure within both northern and southern clades*

Additional differentiation was depicted for some of the species both within the northern and southern groups. Again it was very marked within *Urocotyledon inexpectata*, where in fact no haplotype sharing across islands was observed at mtDNA level, and higher within the northern group. Two slightly divergent lineages were defined by mtDNA haplotypes from the northwestern (Praslin+Curieuse+Aride) and northeastern islands (La Digue and Big Sister), respectively. This higher level of differentiation within the northern group in a west-east axis was paralleled by *P. sundbergi*, where two slightly differentiated lineages also defined the two northern recognized subspecies.

With clearly less marked differentiation (not evidenced in divergent lineages), possible signs of substructure were depicted within the southern group both in *U. inexpectata*, *P. astriata* and *M. sechellensis*. Although differentiation was minimal, within *U. inexpectata* no haplotype sharing exists between Mahé and Silhouette islands, between which gene flow estimates were not significantly different from zero (*paper VII*). Similarly, within *M. sechellensis*, although haplotypes are shared between Mahé and Silhouette, gene flow estimates between Mahé and Silhouette (and North) were not different from zero (*paper IX*). For *P. astriata* no estimates of gene flow between these islands were obtained, but the almost complete sorting of mtDNA haplotypes across these islands indicates that gene flow has been (at most) restricted. At least within *Urocotyledon* (for which time estimates were obtained) this "isolation" within the northern clade seems very recent (~0.5 [0.18-1.22] Mya). This was probably the case for other species also (given the low degree of differentiation and the estimated age of remaining divergence events within those groups), thus most probably reflecting patterns of isolation generated during the late Pleistocene, and related to the latest high sea level stands. Again, this evidences the predominant role of distance in shaping the genetic structure of archipelago organisms. Closer islands, even that separated by higher sea depths and thus possibly isolated during more time, were apparently able to maintain gene flow between them and show only recent signs of isolation.

*Main divergences within each group are pre-Pleistocenic*

Also paralleled across all groups are the ages of their deepest divergences. Both the differentiation between the two *Mabuya* and *Phelsuma* species and between the northern and southern clades of *Urocotyledon inexpectata* seem pre-Pleistocenic and most probably date back to the Miocene. The mean estimates for all groups of ~6 Mya but upper bounds of confidence intervals went up to 8 – 15 Mya. This was not surprising given the old age of the islands and the cyclic influence that oscillating sea levels must have had along the last few million years. Basically, the scenario has all the necessary requirements to promote diversification of taxa once they have colonized the Seychelles plateau.

### *5.3.3 Similar Patterns and Divergence Ages in Other Taxa*

The patterns now described for these taxa seem to be paralleled across other lizard taxa from the Seychelles, while others exhibit even more complicated ones. Unpublished data for the endemic chameleon, *Archaius tigris* (after Townsend et al., 2010) and the skink genus *Pamelaescincus* show similar northern-southern patterns of genetic structure, of apparently similar degrees (7-8% mtDNA uncorrected distances, respectively). Other groups, like the skink genus *Janetaescincus* or the gecko genus *Ailuronyx*, seem to exhibit more complicated patterns with multiple highly divergent lineages (up to 17%, uncorrected p-distances at cytochrome-*b*) without obvious relationships with geography. Possibly older than the ones studied in this thesis, these groups had more time for interisland dispersals and where also more affected by local extinctions. Further data will be needed to properly understand their diversity and evolutionary history.

*5.3.4 Seychelles Reptile Fauna: a stronger African or Asian "flavour"?*

Although we did not specifically address the origins of Seychellois reptile fauna in this thesis, the information gathered here, together with further unpublished data by us, and data published in recent years, now provide the opportunity for a reassessment of the affinities of extant reptile fauna from the Seychelles, which seem to be mostly with the African continent.

After the recent discovery of the southern African affinities and reassignment of the chameleon species *Callumna tigris* to its own genus *Archaius* (Townsend et al., 2010), direct relationships with Madagascar stand only for the day-geckos genus *Phelsuma* and the giant tortoises genus *Aldabrachelys* (Austin et al., 2003). On the other hand, the African component is represented by the two species of the genus *Mabuya* and by the house snake species *Lamprophis geometricus*, which interestingly shows strong affinities to *Lamprophis virgatus* from Gabon (unpublished data). Of older, but also African origin, seem to be species of the genus *Ailuronyx* (an endemic genus from the Seychelles, which is basal to a big Afro-Malagasy gecko clade; A. Bauer, *personal communication*) and the sucker-tailed gecko *Urocotyledon inexpectata*. The remaining four species of this genus are both from West and East Africa and attempts to assess its phylogenetic position placed it as part of an ancient broad Afro-Malagasy radiation (Bauer, 1990; Kluge and Nussbaum, 1995; Rittenhouse et al., 2000). Clear Asian affinities are restricted to the endemic wolf-snake *Lycognatophis seychellensis*, which is closely related to other Asian natricids (Vidal et al., 2008). Of special interest are the endemic and sister-genera *Janetaescincus* and *Pamelaescincus*, whose closest affinities are yet unclear. They are sister taxa to all the remaining African and Malagasy "scincines", (Brandley et al., 2005) and a thorough evaluation of their origin requires their study together with all Indian and Sri Lankan "scincines" as well.

A study by Warren et al. (2010) recently highlighted the strong Asiatic "flavour" of Malagasy region biota. Considering all the archipelagos of the western Indian Ocean region as a whole, the authors highlight the disproportionate contribution of Asian (especially Indian) lineages to the region, especially considering that the divergence ages for many of those clades were not compatible with vicariant scenarios, and the much higher distance of Madagascar from India (3796 km) than from Africa (413 km). For Madagascar only, the Asian contribution across plants, vertebrate and invertebrate groups (31%) is almost as high as the African one (38%), while in vertebrates the Asian component (32%) exceeds the African one (29%) (Yoder and Nowak, 2006). This trend is largely verified for the Mascarenes also (Cheke and Hume, 2008).

Warren et al. (2010) considered the role of times of lower sea level in the recent geological past and the resulting chain of islands between the granitic Seychelles, Mascarenes and India and the Madagascar region and argued for their important role in facilitating the colonization of the Mascarenes and Madagascar by Oriental taxa. Should thus be expected that the Seychelles showed an even stronger bias towards an Asian ancestry of its taxa. Despite the lack of molecular studies that can demonstrate it, this seems to be the case (see chapter 1). Plants (Christensen, 1912; Summerhayes, 1931), insects (Dijkstra, 2007; Holdhaus, 1928; Scott, 1933), terrestrial invertebrates and land snails (Gardiner, 1936; Garth, 1984; Nevill, 1869), molluscs (Gerlach, 2006), land birds (Benson, 1985) all seem to have a major component of Asian ancestors. Amphibians are also of Asian ancestry in majority (Cecilians and Sooglosids vs Hyperolids), though in this case most certainly Gondwanan vicariants (Biju and Bossuyt, 2003; van der Meijden et al., 2007; Zhang and Wake, 2009). Especially considering that this is the pattern observed for Mascarenes reptiles also, it is actually surprising that the majority of reptile taxa from the Seychelles islands seem to have their closest relatives in Africa and Madagascar. As suggested by Townsend et al. (2010) it may certainly be that the key to this incongruence lies in the changing position of Madagascar and other landmasses relative to the large Indian Ocean gyre (rotating current) over time. Thus, throughout the Paleogene, the prevailing current was actually eastward towards Madagascar and the Seychelles and could have facilitated the colonization of the islands from Africa (see chapter 1 and Figure 5.3.1). Large freshwater outflows, able to create large reduced-salinity plumes that can extend for considerable distances may have also created opportunities for colonization of islands via rafting (Cherubin and Richardson, 2007; Measey et al., 2007; Townsend et al., 2010). In the light of these new hypotheses, it will certainly be interesting to thoroughly test the origins of Seychellois taxa using molecular phylogenies and to better understand the evolutionary history of Seychelles islands biota.

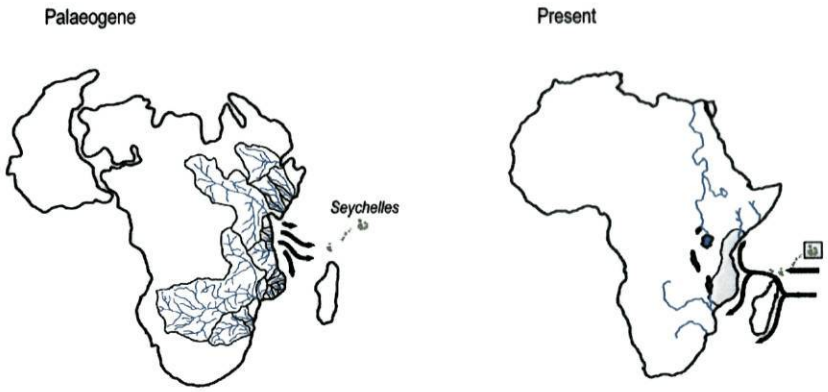



**Figure 5.3.1** Relevant Paleogene (~65 – 23 Mya) oceanic currents (bold arrows) and major eastwards drainages from the Late Cretaceous – Paleogene of Africa (left) and present day oceanic currents and eastwards flowing African rivers. *From Townsend et al. (2010).*

### *5.3.5 The usefulness of multilocus approaches*

It is now very clear, both in the field of phylogenetics and phylogeography, the usefulness of multilocus datasets and how they can dramatically improve the performance of analytical methods and parameter estimation (Carling and Brumfield, 2007; Hickerson et al., 2006). Not only laboratorial and computational performance issues led to this change, but also the comprehension that a single gene genealogy is but one realization of a stochastic process and that its direct equation with the species history may be highly misleading (Edwards, 2009), especially under certain conditions (Degnan and Rosenberg, 2009). The impressive degree of introgression observed within *Mabuya sechellensis* highlights how processes like hybridization can impact island populations (complete replacement of mtDNA in some populations) and distort inferences based on single markers and highlights the need of multilocus approaches to properly unveil phylogeographic patterns and speciation history, especially in closely related taxa. The utility and power of multilocus approaches for parameter estimation was also evident when comparing ages of differentiation within both Seychellois *Phelsuma*, and allowed us to question a long-standing belief about the ages of diversification within each species, providing age estimates that seem very counter-intuitive from those that would be estimated from mtDNA data alone.

The work here presented constitutes in many ways a first approach to questions about diversity,

diversification and structure of Seychelles island populations explicitly making use of molecular markers. Nevertheless, it is again most important that inferences regarding phylogenies, gene flow estimates and ages of divergence continue to be explored and updated as data and resources allow more powerful approaches to these questions. It is clear that much improvement in our understanding of the details of biogeography and evolutionary history within this region is yet to be achieved. Further exploration of patterns of other co-distributed taxa and comparative model-based explorations that try to integrate and infer demographic parameters across taxa, (Hickerson et al., 2010), are promising lines of research for a deeper understanding of factors shaping biodiversity and tempo and mode of evolution of the Seychelles biota.

## 5.4 Concluding Remarks

This work aimed at contributing to the knowledge of the biogeography and evolutionary history of Western Indian Ocean reptile taxa. For obvious reasons, Madagascar has been a privileged target amongst the Western Indian Ocean islands, and archipelagos such as the Comoros and the Seychelles remain strikingly understudied, despite their long recognized importance as biodiversity hotspots.

### *5.4.1 Final Considerations and Major Conclusions*

Focusing on a few particular groups, we contributed to the knowledge of their evolutionary history, unveiling natural and human-driven patterns of distribution within this region, but also highlighted challenges yet to be overcome to properly understand to biogeographic history of the region, especially concerning its temporal axis. Furthermore, we provided some of the first comprehensive assessments of genetic diversity and genetic structure patterns within the Seychelles, demonstrating how isolation, connectivity, and possibly ecological factors shaped the diversification of these islands endemics.

More specifically, the major conclusions of this work were:

i) the genus *Phelsuma* is a remarkably diverse clade that diversified in Madagascar, from where it colonized the surrounding archipelagos. While the Mascarenes and the Seychelles species assemblages are apparently the result of single colonizations and *in situ* diversification, the Comoros archipelago seem to have undergone several natural colonizations. Several of the hypothesized relationships within this genus seem unlikely, largely due to the plasticity of many of the phenetic characters used. In fact, the morphological distinctness and diagnosability of the lineages identified here was rather low, rendering limited value to morphological characters for establishing the phylogenetic relationships within this group.

ii) the young ages previously reported for the genus *Phelsuma* are most probably erroneous, although the time-frame of its diversification was impossible to ascertain with confidence. The use of nuclear data and fossil calibrations revealed the possibility of a much older age for this clade than previously estimated, and highlighted the problems of using only mtDNA data and shallow island calibrations in phylogenetic dating.

iii) the distributions of *Gehyra mutilata* and of all species of the genus *Hemidactylus* in the Western Indian Ocean islands have been probably shaped by anthropogenic factors. Most likely, all *Hemidactylus* species occurring in the Comoros and the granitic Seychelles archipelagos are not native, although Madagascar and the coralline archipelago of Aldabra seem to have been naturally colonized. Cryptic variation was observed in all species of *Hemidactylus* in their native range.

iv) the distinctiveness between *Mabuya maculilabris* and *M. comorensis* was confirmed with molecular data. *M. comorensis* was found to harbour considerable differentiation within the Comoros, with exclusive mtDNA lineages across the four islands, and to reflect a West to East colonization pathway from Africa. The Malagasy populations of this species seem to have resulted from human-mediated introductions from one of the Comoro islands (Moheli). A similar introduction from Moheli was also inferred for "*M. maculilabris casuarinae*", from the Mozambique island.

v) cryptic variation was reported within *M. maculilabris*, which clearly harbours a high number of lineages distributed across the Western Indian Ocean region and also West Africa. Cryptic differentiation (West-East Africa) was also observed within *M. striata*, although in this case its Western Indian Ocean insular range harbours little differentiation relative to the continental populations, possibly reflecting non-natural patterns.

vi) cryptic diversity was unveiled within *Urocotyledon inexpectata*, an endemic gecko species from the Seychelles, which harbours two highly differentiated lineages, exclusively distributed across the northern and the southern groups of islands. The main split was dated back to the Miocene/Late Pliocene. Gene flow between these groups of islands is apparently non-existent. Further structure within each group, higher in the northern one, was depicted.

vii) the divergence between the two species of the genus *Phelsuma* endemic to the Seychelles was also most probably Miocenic and no evidences of gene flow between them were detected. Time estimates suggest also a possibly synchronous divergence between subspecies within each species, renovating the interest in the role of ecology and differential selective pressures in the diversification of these species across the archipelago.

viii) *Mabuya sechellensis* was found to be paraphyletic relative to its sister species *M. wrightii*, at the mtDNA level. Ancient hybridization between these species leading to massive mtDNA introgression seems to best explain the current results, although the determinants of the hybridization process and of the observed introgression pattern remain largely speculative. Again, the age of the divergence between the two species in question is most probably pre-Pleistocenic.

ix) common patterns were found in the genetic structure and evolutionary history of the Seychellois taxa, such as a north-south phylogeographic structure; a close relationship of Fregate island populations to populations from the southern islands, additional substructure across both northern and southern groups, and similar pre-Pleistocenic divergences between main clades/species within each group.

*5.4.2 Future Perspectives*

Despite the contribution of this work to a general better understanding of lizard taxa biogeography in the Western Indian Ocean islands, and in particular to a better knowledge of Seychelles islands biogeography, many relevant questions remain yet to be answered and others were generated along this work.

The distribution of the diversity within Madagascar and the mechanisms underlying the diversification of their taxa remain poorly understood with a poor fit of all the proposed models of diversification across taxa (Vences et al., 2009; Weyeneth et al., 2010). In fact, Madagascar is rapidly standing out as a model region in the study of species diversification (Vences et al., 2009) and, among other groups, the day geckos of the genus *Phelsuma* stand out as one of the ideal groups where these mechanisms can further be explored, now that a better knowledge on their diversity and phylogenetic relationships was largely achieved. A main challenge of this endeavour, as highlighted within this thesis, will be to obtain better estimates of the temporal component of diversification mechanisms.

Further research will also be needed, especially by extensively sampling continental areas, to ascertain with confidence the suggested natural and non-natural colonization patterns of the widespread commensal species of the genus *Hemidactylus* across the Western Indian Ocean archipelagos. The presence of endemic lineages on the archipelago of Aldabra is relevant from a biogeographic point of view and should be further clarified.

As for the Seychelles archipelago, the works presented in this thesis are, to our knowledge, the first extensive studies on the phylogeographic structure of their taxa using molecular markers. It will be most interesting to test for these patterns across other taxa and to try to distinguish between general and taxon-specific patterns amongst its endemic groups. Apparently older groups, in particular, should further provide us valuable insights into deeper portions of the biogeographic history of the archipelago. Particularly interesting were the results obtained concerning the time frame of the diversification of the *Phelsuma* species within this archipelago. Further studies on their ecology and niche partitioning across the entire archipelago will be needed to further test the role of habitat, selective pressures and ecological factors on their diversification and may in turn have important implications concerning the conservation of native habitats for the maintenance of their diversity (Noble et al., 2010).

## 5.5 References


Arnold, E.N., 1979. Indian Ocean giant tortoises: their systematics and island adaptations. Philosophycal Transactions of the Royal Society of London Series-B Biological Sciences 286, 127-145.

Austin, C.C., 1999. Lizards took express train to Polineisa. Science 397, 113-114.

Austin, J., Arnold, E.N., 2001. Ancient mitochondrial DNA and morphology elucidate an extinct island radiation of Indian Ocean giant tortoises (Cylindraspis). Proceedings of the Royal Society of London Series- B 268, 2515-2523.

Austin, J.J., Arnold, E.N., Bour, R., 2003. Was there a second adaptive radiation of giant tortoises in the Indian Ocean? Using mitochondrial DNA to investigate speciation and biogeography of Aldabrachelys (Reptilia, Testudinidae). Molecular Ecology 12, 1415-1424.

Austin, J.J., Arnold, E.N., Jones, C.G., 2004. Reconstructing an island radiation using ancient and recent DNA: the extinct and living day geckos (*Phelsuma*) of the Mascarene islands. Molecular Phylogenetics and Evolution 31, 109-122.

Avise, J.C., Bowen, B.W., Lamb, T., Meylan, A.B., Bermingham, E., 1992. Mitochondrial DNA Evolution at a Turtle's Pace: Evidence for Low Genetic Variability and Reduced Microevolutionary Rate in the Testudines. Molecular Biology and Evolution 9, 457-473.

Bauer, A.M., 1990. Phylogeny and biogeography of the geckos of southern Africa and the islands of the western Indian Ocean islands: a preliminary analysis. In: Peters, G., Hutterer, R. (Eds.), Vertebrates in the Tropics. Zoologishes Forschungsinstitut und Museum A. Koening, Bonn, Germany, pp. 275-284.

Benson, C.W., 1985. Origins of Seychelles land birds. In: Stoddart, D.R. (Ed.), Biogeography and ecology of the Seychelles Islands. Dr. W. Junk, The Hague, pp. 469-486.

Berghof, H.P., Trautmann, G., 2009. Eine neue Art der Gattung Phelsuma Gray, 1825 (Sauria: Gekkonidae) von der Ostküste Madagaskars. Sauria 31, 5-14.

Biju, S.D., Bossuyt, F., 2003. New frog family from India reveals an ancient biogeographical link with the Seychelles. Nature 425, 711-714.

Bowler, J., 2006. Wildlife of Seychelles. WildGuides, Hampshire.

Brandley, M.C., Schmitz, A., Reeder, T.W., 2005. Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. Systematic Biology 54, 373-390.

Broadley, D.G., 2001. A review of the genus Thelotornis A. Smith in eastern Africa, with description of a new species from Usambara Mountains (Serpentes: Colubridae: Dipsolidhini). African Journal of Herpetology 50, 53-70.

Butterfield, B.P., Meshaka, W.E.J., Guyer, C., 1997. Non-indigenous amphibians and reptiles; pp 123-137. In: Simberloff, D., Schmitz, D.C., Brown, T.C. (Eds.), Strangers in Paradise: impact and management of non-indigenous species in Florida. Island Press, Washington, D.C.

Caccone, A., Gibbs, J.P., Ketmaier, V., Suatoni, E., Powell, J.R., 1999. Origin and evolutionary relationships of giant Galápagos tortoises. Proceedings of the National Academy of Sciences of the United States of America 96, 13223-13228.

Carling, M.D., Brumfield, R.T., 2007. Gene sampling strategies for multi-locus opulation estimates of genetic diversity (theta). PLos One 2, e160.

Carstens, B.C., Knowles, L.L., 2007. Estimating species phylogeny from gene-tree probabilities despite incomplete lineage sorting: an example from Melanoplus grasshoppers. Systematic Biology 56, 400-411.

Cheke, A.S., 1984. Lizards of the Seychelles. In: Stoddart, D.R. (Ed.), Biogeography and ecology of the Seychelles Islands. Dr. W. Junk, The Hague, pp. 245–258.

Cheke, A.S., Hume, J.P., 2008. Lost Land of the Dodo. An Ecological History of Mauritius, Réunion & Rodrigues. A. & C. Black and New Haven Conn.: Yale University Press, London.

Cherubin, L.M., Richardson, P.L., 2007. Carribean current variability and the influence of the Amazon and Orinoco freshwater plumes. Deep Sea Res. (I Oceanogr. Res. Pap.) 54, 1451-1473.

Christensen, C., 1912. On the ferns of the Seychelles and the Aldabra Group. Transactions of the Linnean Society of

London 1, 409-425.

Cole, N.C., Jones, C.G., Harris, S., 2005. The need for enemy-free space_ the impact of invasive gecko on island endemics. Biological Conservation 125, 467-474.

Cooper, A., Penny, D., 1997. Mass Survival of Birds Across the Cretaceous-Terciary Boundary: Molecular Evidence. Science 275, 1109 - 1113.

Degnan, J.H., Rosenberg, N.A., 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. Trends in Ecology and Evolution 24, 332-340.

Diamond, A.W., Hamilton, A.C., 1980. The distribution of forest passerine birds and Quaternary climatic change in tropical Africa. Journal of Zoology 191, 379-402.

Dijkstra, K.-D.B., 2007. Gone with the wind: westward dispersal across the Indian Ocean and island speciation in Hemicordulia dragonflies (Odonata: Corduliidae). Zootaxa 1493, 27-48.

Edwards, S.V., 2009. Is a new and general theory of molecular systematics emerging? Evolution 63, 1-19.

Edwards, S.V., Beerli, P., 2000. Perspective: gene divergence, population divergence and the variance in coalescence time in phylogeographic studies. Evolution 54, 1839-1854.

Gardiner, J.S., 1936. Concluding remarks on the distribution of the land and marine fauna, with a list of the water temperature observations. Transactions of the Linnean Society of London 19, 447-464.

Gardner, A.S., 1986. Herpetofauna of the Seychelles. British Herpetological Society Bulletin 16, 25-27.

Garth, J.S., 1984. Brachyuran decapod crustaceans of coral reef communities of the Seychelles and Amirante islands. In: Stoddart, D.R. (Ed.), Biogeography and ecology of the Seychelles Islands. Dr. W. Junk, The Hague, pp. 103-122.

Gasse, F., 2006. Climate and hydrological changes in tropical Africa during the past million years. Comptes Rendus Palevolution 5, 35-43.

Gerlach, J., 2006. Terrestrial and freshwater Mollusca of the Seychelles islands. Backhuys Publishers, Leiden, The Netherlands.

Gerlach, J., 2007. Terrestrial and freshwater vertebrates of the Seychelles islands. Backhuys Publishers, Leiden, The Netherlands.

Glaw, F., Gehring, P.-S., Köhler, J., Franzen, M., Vences, M., 2010. A new dwarf species of day gecko, genus Phelsuma, from the Ankarana pinnacle karst in northern Madagascar. Salamandra 46, 83-92.

Glaw, F., J., K., Vences, M., 2009. A new species of cryptically coloured day gecko (Phelsuma) from the Tsingy de Bemaraha National Park in western Madagascar. Zootaxa 2195, 61-68.

Hansen, D.M., Kiesbuy, H.C., Jones, C.G., Muller, C.B., 2007. Positive indirect interactions between neighboring plant species via a lizard pollinator. American Naturalist 169, 534-542.

Harmon, L.J., Melville, J., Larson, A., Losos, J.B., 2008. The role of geography and ecological opportunity in the diversification of day geckos (Phelsuma). Systematic Biology 57, 562-573.

Hickerson, M.J., Carstens, B.C., Cavender-Bares, J., Crandall, K.A., Graham, C.H., Johnson, J.B., Rissler, L., Victoriano, P.F., Yoder, A.D., 2010. Phylogeography's past, present, and future: 10 years after. Molecular Phylogenetics and Evolution 54, 291-301.

Hickerson, M.J., Stahl, E.A., Lessios, H.A., 2006. Test for simultaneous divergence using approximate Bayesian computation. Evolution 60, 2435-2453.

Holdhaus, K., 1928. Die geographishe verbreitung der Insekten. In: Schroder, C. (Ed.), Handbuch der Entomologie, pp. 592-1057.

Hugall, A.F., Foster, R., Lee, M.S.Y., 2007. Calibration Choice, Rate Smoothing, and the Pattern of Tetrapod Diversification According to the Long Nuclear Gene RAG-1. Systematic Biology 56, 543-563.

Ineich, I., Chirio, L., 2004. L'archipel afro-montagnard et les afinités de son herpetofaune: description d'une espèce nouvelle indiquant des relations phylétiques entre le Cameroun et l'Afrique de l'est (Lacertilia, Scincidae, genre Trachylepis). Bull. Soc. Zool. France 129, 317-331.

Ineich, I., Schmitz, A., Chirio, L., LeBreton, M., 2004. New species of Leptosiaphos (Scincidae) from Adamaoua Massif, Central-Northern Province, Cameroon. Journal of Herpetology 38, 546-550.

Jesus, J., 2002. An introduced population of Hemidactylus mabouia (MOREAU DE JONNÉS, 1818) on Madeira Island. Herpetozoa 15, 179-180.

Jesus, J., Brehm, A., Pinheiro, M., Harris, D.J., 2001. Relationships of Hemidactylus (Reptilia: Gekkonidae) from the Cape Verde Islands: What mitochondrial DNA data indicate. Journal of Herpetology 35, 672-675.

Kaiser-Bunbury, C.N., Memmot, J., Müller, C.B., 2009. Community structure of pollination webs of Mauritian heathland habitats. Perspectives in Plant Ecology, Evolution and Systematics 11, 241-254.

Kier, G., Kreft, H., Lee, T.M., Jetz, W., Ibisch, P.L., Nowicki, C., Mutke, J., Barthlott, W., 2009. A global assessment of endemism and species richness across island and mainland regions. Proceedings of the National Academy of Sciences of the United States of America 106, 9322-9327.

Kluge, A.G., 1969. The evolution and geographic origin of the New World Hemidactylus mabouia-brookii complex (Gekkonidae, Sauria). Miscelaneous Publications of the Museum of Zoology University of Michigan, 1-78.

Kluge, A.G., Nussbaum, R.A., 1995. A review of African-Madagascan gekkonid lizard phylogeny and biogeography (Squamata). Miscelaneous Publications of the Museum of Zoology University of Michigan 183, 1-20.

Knowles, L.L., 2009. Statistical Phylogeography. Annual Review of Ecology, Evolution, and Systematics 40, 593-612.

Kraus, F., 2003. Invasion pathways for terrestrial vertebrates. In: Carlton, J., Ruiz, G., Mack, R. (Eds.), Invasive species: vectors and management strategies. Island Press, Washington, DC.

Lerner, A., 2004. A new taxonomically isolated species of the genus Phelsuma Gray, 1825 from the Ampasindava Peninsula, Madagascar. Phelsuma 12, 91-98.

Lovett, J.C., Wasser, S.K., 1993. Biogeography and Ecology of the rainforests of Eastern Africa. Cambridge University Press, Cambridge, UK.

Maley, J., 1996. The African rain forest - main characteristics of changes in vegetation and climate from the Upper Cretaceous to the Quaternary. Roy. Soc. Edimburgh Proc. Sect. B (Biol. Sci.) 104, 31-74.

McCormack, J.E., Heled, J., Delaney, K.S., Peterson, A.T., Knowles, L.L., 2010. Calibrating Divergence Times on Species Trees Versus Gene Trees: Implications for Speciation History of Aphelocoma Jays. Evolution (accepted article) doi: 10.1111/j.1558-5646.2010.01097.x.

Measey, G.J., Vences, M., Drewes, R., Chiari, Y., Melo, M., Bourles, B., 2007. Freshwater paths across the ocean: molecular phylogeny of the frog Ptychadena newtoni gives insights into amphibian colonization of oceanic islands. Journal of Biogeography 34, 7-20.

Nevill, G., 1869. Aditional notes on the land-shells of the Seychelles islands. Proceedings of the Zoological Society of London 4, 61-66.

Noble, T., Bunbury, N., Kaiser-Bunbury, C.N., Bell, D.J., 2010. Ecology and co-existence of two endemic day gecko (Phelsuma) species in Seychelles native palm forest. Journal of Zoology in press, DOI: 10.1111/j.1469-7998.2010.00751.x.

Nussbaum, R.A., Raxworthy, C.J., Raselimanana, A.P., Ramanamanjato, J.B., 2000. New species of Day Gecko, Phelsuma Gray (Reptilia: Squamata: Gekkonidae), from the Réserve Naturelle Intégrale d'Andohahela, southern Madagascar. Copeia 2000, 763-770.

Oleson, J., Valido, A., 2003. Lizards as pollinators and seed dispersers: an island phenomenon. Trends in Ecology and Evolution 18, 177-181.

Petren, K., Case, T.J., 1996. An experimental demonstration of exploitation competition in an ongoing invasion. Ecology 77, 118-132.

Porter, M.L., PÈrez-Losada, M., Crandall, K.A., 2005. Model-based multi-locus estimation of decapod phylogeny and divergence times. Molecular Phylogenetics and Evolution 37, 355-369.

Poux, C., Madsen, O., Marquard, E., Vieites, D.R., de Jong, W.W., Vences, M., 2005. Asynchronous colonization of Madagascar by the four endemic clades of primates, tenrecs, carnivores, and rodents as inferred from nuclear genes. Systematic Biology 54, 719-730.

Radtkey, R.R., 1996. Adaptive radiation of day-geckos (*Phelsuma*) in the Seychelles archipelago: a phylogenetic analysis. Evolution 50, 604-623.

Rand, D., 1994. Thermal habit, metabolic rate and the evolution of mitochondrial DNA. Trends in Ecology and Evolution 9, 125-131.

Rasmussen, J.B., 2005. A review of the slender stilettosnake, Atractaspis aterrima Günther 1863 (Serpentes: Atractaspidae). Tropical Zoology 18, 137-148.

Raxworthy, C.J., Ingram, C.M., Rabibisoa, N., Pearson, R.G., 2007. Applications of ecological niche modeling for species delimitation: A review and empirical evaluation using day geckos (Phelsuma) from Madagascar. Systematic Biology 56, 907-923.

Rittenhouse, D.R., Russell, A.P., Bauer, A.M., 2000. Laryngotracheal morphology of Afro-Madagascan geckos - a comparative survey. Journal of Morphology 245, 241-268.

Rocha, S., Carretero, M.A., Harris, D.J., 2005. Diversity and phylogenetic relationships of Hemidactylus geckos from the Comoro islands. Molecular Phylogenetics and Evolution 35, 292-299.

Rocha, S., Carretero, M.A., Vences, M., Glaw, F., Harris, D.J., 2006. Deciphering patterns of transoceanic dispersal: the evolutionary origin and biogeography of coastal lizards Cryptoblepharus in the Western Indian Ocean region. Journal of Biogeography 33, 13-22.

Rocha, S., Harris, D.J., Perera, A., Silva, A., Vasconcelos, R., Carretero, M.A., 2009. Recent data on the distribution of lizards and snakes of the Seychelles. Herpetological Bulletin 110, 20-32.

Rodda, G.H., Dean-Bradley, K., 1992. Excess density compensation of island herpetofaunal assemblages. Journal of Biogeography 29, 623-632.

Rösler, H., Obst, F.J., Seipp, R., 2001. Eine neue Taggecko-Art von Westmadagaskar: Phelsuma hielscheri sp.n. (Reptilia: Sauria: Gekkonidae). Zoologische Abhandlungen Staatliches Museum für Tierkunde in Dresden 51, 51-60.

San Mauro, D., Vences, M., Alcobendas, M., Zardoya, R., Meyer, A., 2005. Initial diversification of living amphibians predated the breakup of Pangea. The American Naturalist 165, 590-599.

Schönecker, P., Bach, S., Glaw, F., 2004. Eine neue Taggecko-Art der Gattung Phelsuma aus Ost-Madagaskar (Reptilia: Squamata: Gekkonidae). Salamandra 40, 105-112.

Scott, H., 1933. General conclusions regarding the insect fauna of the Seychelles and adjacent islands. Transactions of the Linnean Society of London 2nd series (Zoology) 19, 307-391.

Shapiro, B., Sibthorpe, D., Rambaut, A., Austin, J., Wragg, G.M., Bininda-Emonds, O.R.P., Lee, P.L.M., Cooper, A., 2002. Flight of the Dodo. Science 295, 1683-.

Shine, S., Williams, N., Gündling, L., 2000. A guide to designing legal institutional frameworks on alien invasive species. IUCN, Cambridge and Bonn.

Silva, A., Rocha, S., Gerlach, J., Rocamora, G., Dufrenne, A., Harris, D.J., 2010. Assessment of mtDNA genetic diversity within the terrapins Pelusios subniger and Pelusios castanoides across the Seychelles islands. Amphibia-Reptilia 31, 583-588.

Summerhayes, V.S., 1931. An enumeration of the angiosperms of the Seychelles archipelago. Transactions of the Linnean Society of London 19, 261-299.

Thorne, J., Kishino, H., Painter, I., 1998. Estimating the rate of evolution of the rate of molecular evolution. Molecular Biology and Evolution 15, 1647-1657.

Thorne, J.L., Kishino, H., 2002. Divergence time and evolutionary rate estimation with multilocus data. Systematic Biology 51, 689-702.

Thorpe, R.S., LeadBeater, D.L., Pook, C.E., 2005. Molecular Clocks and geological dates: cytochrome b of Anolis extremus substantially contradicts dating of Barbados emergence. Molecular Ecology 14, 2087-2096.

Townsend, T.M., Tolley, K.A., Glaw, F., Böhme, W., Vences, M., 2010. Eastwards from Africa: palaeocurrents-mediated chameleon dispersal to the Seychelles islands. Biology Letters (accepted paper) doi: 10.1098/rsbl.2010.0701.

van der Meijden, A., Boistel, R., Gerlach, J., Ohler, A., Vences, M., Meyer, A., 2007. Molecular phylogenetic evidence for paraphyly of the genus Sooglossus, with the description of a new genus of Seychellean frogs. Biological Journal of the

Linnean Society 91, 347-359.

Vences, M., Vieites, D.R., Glaw, F., Brinkmann, H., Kosuch, J., Veith, M., Meyer, A., 2003. Multiple overseas dispersal in amphibians. Proceedings of the Royal Society London Series-B 270, 2435-2442.

Vences, M., Wanke, S., Vieites, D.R., Branch, W.R., Glaw, F., Meyer, A., 2004. Natural colonization or introduction? Phylogeographical relationships and morphological differentiation of house geckos Hemidactylus from Madagascar. Biological Journal of the Linnean Society 83, 115-130.

Vences, M., Wollenberg, K.C., Vieites, D.R., Lees, D.C., 2009. Madagascar as a model region of species diversification. Trends in Ecology and Evolution 24, 456-465.

Vidal, N., Branch, W.R., Pauwels, O.S.G., Hedges, S.B., Broadley, D.G., Wink, M., Cruaud, C., Joger, U., Nagy, Z.T., 2008. Dissecting the major African snake radiation: a molecular phylogeny of the Lamprophiidae Fitzinger (Serpentes, Caenophidia). Zootaxa 1945, 51-66.

Vidal, N., Hedges, S.B., 2005. The phylogeny of squamate reptiles (lizards, snakes, and amphisbaenians) inferred from nine nuclear protein-coding genes. Comptus Rendus Biologie 328, 1000-1008.

Warren, B.H., Bermingham, E., Bowie, R.C., Prys-Jones, R.P., Thebaud, C., 2003. Molecular phylogeography reveals island colonization history and diversification of western Indian Ocean sunbirds (Nectarinia: Nectariniidae). Molecular Phylogenetics and Evolution 29, 67-85.

Warren, B.H., Bermingham, E., Prys-Jones, R.P., Thebaud, C., 2005. Tracking island colonization history and phenotypic shifts in Indian Ocean bulbuls Hypsipetes: Pycnonotidae). Biological Journal of the Linnean Society 85, 271-287.

Warren, B.H., Dominique, S., Bruggemann, J.H., Robert, P.P.-J., Christophe, T., 2010. Why does the biota of the Madagascar region have such a strong Asiatic flavour? Cladistics 26, 526-538.

Weyeneth, N., Goodman, S.M., Ruedi, M., 2010. Do diversification models of Madagascar's biota explain the population structure of the endemic bat Myotis goudoti (Chiroptera: Vespertilionidae)? Journal of Biogeography in press, DOI: 10.1111/j.1365-2699.2010.02393.x.

Whitaker, A.H., 1987. The roles of lizards in New Zealand plant reproductive strategies. New Zealand Journal of Botany 25, 315-328.

Whitaker, R.J., Fernández-Palacios, J.M., 2007. Island Biogeography: Ecology, Evolution and Conservation. Oxford University Press, Oxford.

Yang, Z., 1997. PAML: a program for package for phylogenetic analysis by maximum likelihood. CABIOS, 555-556.

Yoder, A.D., Nowak, M.D., 2006. Has Vicariance or Dispersal Been the Predominant Biogeographic Force in Madagascar? Only Time Will Tell. Annual Review of Ecology, Evolution, and Systematics 37, 405-431.

Yoder, A.D., Yang, Z., 2004. Divergence dates for Malagasy lemurs estimated from multiple gene loci: geological and evolutionary context. Molecular Ecology 13, 757-773.

Zhang, P., Wake, M.H., 2009. A mitogenomic perspective on the phylogeny and biogeography of living caecilians (Amphibia: Gymnophiona). Molecular Phylogenetics and Evolution 53, 479-491.

## 6. Appendix

### Supplementary data, methodology and results of age estimates of the genus *Phelsuma*.

### (see Discussion - Section 5.1.1)

Using the Maximum likelihood (ML) tree of the *combined* dataset (mitochondrial + nuclear data) from paper II we tested the molecular clock hypothesis through a likelihood ratio test (Felsenstein, 1988). Because the molecular clock hypothesis was clearly rejected, we used the Bayesian molecular dating procedure implemented in PAML/*Multidivtime* (Yang, 1997; Thorne and Kishino, 2002; Thorne *et al.*, 1998) for obtaining time estimates across the *Phelsuma* tree. The *combined* dataset and its ML tree were used as input data. In this case we did not use each individual gene trees because the number of tips is much smaller for the nuclear partitions due to their lower substitution rate, and Multidivtime does not deal well with zero or very small branch lengths (J. Thorne, *personal communication*). Alternatively, and because the accommodation of multiple genes with their specific rates of variation has been shown to provide improved estimates of both times and rates of evolution (Yang and Yoder, 2003), a reduced dataset was analyzed including only the 29 individuals that showed distinct sequences for all genes. In both cases, ML estimates of the transition/transversion ratio and nucleotide frequencies were obtained in PAML 3.14 (Yang, 1997) under the F84 + G model of DNA substitution. Next, these parameters were used to re-estimate branch lengths and their approximate variance-covariance matrix, and to derive estimates of divergence times and 95% credibility intervals (CI). These analyses were carried out using *Multidistribute* (Thorne and Kishino, 2002). The rate at the root node (*rtrate*), the mean of the prior distribution for the rate of molecular evolution (*brownmean*) and standard deviations were calculated as recommended in Rutshman (2005). In all runs the burnin period was of 100,000 cycles, sample frequency = 100 and number of samples 20,000. Convergence was assessed by running multiple analyses (starting from different random initial states), by comparing prior and posterior distributions of divergence times, and by assessing the proportion of successful changes of parameters along the Markov chain. In every case posterior estimates were considerably different from the priors and

posterior credibility intervals much smaller, indicating data contained useful information. For the prior of the ingroup root node age (*rttm*; including *Rhoptropella ocellata*) we used 7 mya (following Austin *et al.*, 2004), but also 14 mya and 21 Mya, to test the effect of this prior in the analysis. We used the two calibration points available: 2.1 Mya for the split *P. cepediana / P. borbonica*, assuming that the ancestor of *P. borbonica* colonized Reunion soon after its emergence, the same strategy used before by Austin et al. (2004), and, following the same rationale, 0.125 Mya for the split between *P. abbotti abbotti* (Aldabra) and *P. abbotti chekei* (Madagascar), that corresponds to the age of the current emergence of Aldabra. A further possible calibration point, the *P. lineata / P. comorensis* split and the age of Grand Comoro, was not used because we knew from the previous study (Rocha et al., 2007, *paper I*), that there are *P. lineata* individuals (the one sequenced by Austin *et al.*, 2004, for example) that are more closely related to *P. comorensis* than the ones we sampled (4.8% vs. 6.4% p-distance at Cyt-*b*). Under these assumptions, island ages represent upper bounds (i.e., maximum limits) on a node's age, but as the use of solely upper (or lower) bounds that furthermore are not uniformly distributed across the tree (and especially if located on shallow nodes) can induce considerable bias in time estimates (Porter *et al.*, 2005), we also accommodated one lower interval in the analysis by restricting the variation in the age of the *P. borbonica* split (2.1-1.6 Mya).

A second set of analyses were performed using either the *combined* or only the *nuclear* dataset, adding representatives of main groups of Squamata (Teiioidea, Rhineuridae, Bipedidae, Amphisbaenidae, Trogonophidae, Varanidae, Helodermatidae, Anguidae, Serpentes, Iguanidae, Xantusidae, Scincidae and Pygopodinae) and also several other tetrapods (*Sphenodon*, *Gallus*, *Mus* and *Xenopus;* in the following referred to as *"old calibrations"* dataset). This allowed us to use deep fossil calibrations and obtain independent estimates for the age of the root of the *Phelsuma* radiation and the divergence between *Phelsuma* and *Rhoptropella/Lygodactylus.* In Porter et al. (2005), estimates were shown to be more consistent when using deeper calibrations. Again, the alignment of Cyt-*b* was unambiguous and for 16s we first used MAFFT (Katoh et al., 2005), more efficient at high divergence levels (Wilm et al., 2006) and the L-INS-i strategy and afterwards imported the obtained alignment into ProAlign (Löytynoja and Milinkovitch, 2003) and realigned it from that point excluding again columns with posterior probabilities (PP) lower that 90. Then, we obtained a ML tree for this set of taxa using GARLI (Zwickl, 2006) (information on the taxa used and accession numbers are given below in Table 5.1.1) and ran Multidivtime with the following fossil calibrations: the Synapsida-Diapsida

(mammal-bird) split, at 312-330 Mya and the Lepidosauria-Archosauria (bird-lizard) split, at 259-299 Mya, both following Benton and Donaghue (2007). The Squamata fossil calibrations used included oldest teiid (*Ptilodon*, Aptian-Albian, 112 Mya; Nydam et al., 2002) and oldest rhineurid (*Plesiorhineura*, Danian, 64 Mya; Sullivan, 1985), both following Vidal and Hedges (2005), and also the scincomorph-anguimorph split (168 Mya; Evans, 2003), following Hugall et al. (2007). We also included the Rhynchocephalia (*Sphenodon*)-Squamata split at 227 Mya (Sues and Olsen, 1990) and constrained the split *Heloderma*-Anguidae to be older than 58 Mya (following Hugall et al., 2007) (see Table 5.1.2 for a more comprehensive rationale explaining the fossil calibrations used).

**Table 5.1.1** - List of non-gekkonid taxa used for divergence time estimation and accession numbers.

| LABEL | SPECIES / gene fragment | ACESSION NUMBER | SOURCE |
|---|---|---|---|
| Xenopus | Xenopus laevis / 16s, Cyt-b | NC_001573 | Roe et al., 1985 |
| | Xenopus laevis / Rag-1 | L19324 | Greenhalgh et al., 1993 |
| | Xenopus laevis / Rag-2 | L19325 | Greenhalgh et al., 1993 |
| | Xenopus laevis / C-mos | M25366 | Freeman et al., 1989 |
| Mus | Mus musculus / 16s, Cyt-b | NC_006914 | Mori et al., unpublished |
| | Mus musculus / Rag-1 | NM_009019 | Bergstrom et al., 2008 |
| | Mus musculus / Rag-2 | M64796 | Oettinger et al., 1990 |
| | Mus musculus / C-mos | J00372 | Van Beveren et al., 1981 |
| Gallus | Gallus gallus / 16s, Cyt-b | NC_001323 | Valverde et al., 1994 |
| | Gallus gallus / Rag-1 | M58530 | Carlson et al., 1991 |
| | Gallus gallus / Rag-2 | M58531 | Carlson et al., 1991 |
| | Gallus gallus / C-mos | AY056925 | Barker et al, 2002 |
| Sphenodon | Sphenodon punctatus / 16s, Cyt-b | NC_004815 | Rest et al., 2003 |
| | Sphenodon punctatus / Rag-1 | AY662576 | Townsend et al., 2004 |
| | Sphenodon punctatus / Rag-2 | UNPUBLISHED | Vidal and Hedges, 2005 |
| | Sphenodon punctatus / C-mos | AF039483 | Saint et al., 1998 |
| Teiioidea | Cnemidophorus deppei / 16s | AY046473 | Reeder et al., 2001 |
| | Cnemidophorus deppei / Cyt-b | AF006303 | Radtkey et al., 1997 |
| | Aspidoscelis tigris / Rag-1 | AY662620 | Townsend et al., 2004 |
| | Ameiva auberi / Rag-2 | DQ119629 | Fry et al., 2006 |
| | Kentropyx calcarata / C-mos | AF420864 | Pellegrino et al., 2001 |
| Rhineuridae | Rhineura floridana / 16s, Cyt-b | NC_006282 | Macey et al., 2004 |
| | Rhineura floridana / Rag-1 | AY662618 | Townsend et al., 2004 |
| | Rhineura floridana / Rag-2 | DQ119631 | Fry et al., 2006 |
| | Rhineura floridana / C-mos | AY487347 | Vidal and Hedges, 2004 |
| Bipedidae | Bipes biporus / 16s, Cyt-b | NC_006287 | Macey et al., 2004 |
| | Bipes biporus / Rag-1 | AY662616 | Townsend et al., 2004 |
| | Bipes biporus / Rag-2 | DQ119632 | Fry et al., 2006 |
| | Bipes biporus / C-mos | AF038482 | Saint et al., 1998 |
| Amphisbaenidae | Amphisbaena schmidti / 16s, Cyt-b | AY605475 | Macey et al., 2004 |
| | Amphisbaena xera / Rag-1 | AY662619 | Townsend et al., 2004 |
| | Amphisbaena cubana / Rag-2 | DQ119633 | Fry et al., 2006 |
| | Amphisbaena cubana / C-mos | AY487346 | Vidal and Hedges, 2004 |
| Trogonophidae | Trogonophis wiegmanni / 16s | EF545721 | Harris and Mendonça, 2007 |
| | Diplometopon zarudnyi / Cyt-b | NC_006283 | Macey et al., 2004 |
| | Trogonophis wiegmanni / Rag-1 | AY662617 | Townsend et al., 2004 |
| | Trogonophis wiegmanni / Rag-2 | DQ119634 | Fry et al., 2006 |
| | Trogonophis wiegmanni / C-mos | AY444025 | Kearney et al., 2004 |
| Varanidae | Varanus niloticus / 16s, Cyt-b | NC_008778 | Kumazawa, 2007 |
| | Varanus griseus / Rag-1 | AY662608 | Townsend et al., 2004 |
| | Varanus dumerilii / Rag-2 | DQ119637 | Fry et al., 2006 |
| | Varanus rosenbergui / C-mos | AF039480 | Saint et al., 1998 |
| Helodermatidae | Heloderma suspectum / 16s, Cyt-b | NC_008776 | Kumazawa, 2007 |
| | Heloderma suspectum / Rag-1 | AY662606 | Townsend et al., 2004 |

| | | | |
|---|---|---|---|
| | Heloderma suspectum / Rag-2 | DQ119635 | Fry et al., 2006 |
| | Heloderma suspectum / C-mos | AY662566 | Townsend et al., 2004 |
| Anguidae | Abronia graminea / 16s | AB080273 | Kumazawa and Nishida, 2007 |
| | Anniella pulchra / Cyt-b | AF195090 | Pearse and Pogson, 2000 |
| | Anniella pulchra / Rag-1 | AY662605 | Townsend et al., 2004 |
| | Anniella pulchra / Rag-2 | DQ119636 | Fry et al., 2006 |
| | Anniella pulchra / C-mos | AY487350 | Vidal and Hedges, 2004 |
| Serpentes | Liasis savuensis / 16s | AF544820 | Vidal and Hedges, 2002 |
| | Liasis fuscus / Cyt-b | AF241400 | Harvey et al., 2000 |
| | Cylindrophis ruffus / Rag-1 | AY662613 | Townsend et al., 2004 |
| | Liasis savuensis / Rag-2 | DQ119639 | Fry et al., 2006 |
| | Liasis savuensis / C-mos | AF544726 | Vidal and Hedges, 2002 |
| Leiocephalus | Leiocephalus sp. / 16s | L41446 | Reeder, 1995 |
| | Leiocephalus carinatus / Rag-1 | AY662613 | Townsend et al., 2004 |
| | Leiocephalus barahonensis / Rag-2 | DQ119640 | Fry et al., 2006 |
| | Leiocephalus barahonensis / C-mos | DQ119594 | Fry et al., 2006 |
| Iguaninae | Iguana iguana / 16s | AB028756 | Ota et al., 1999 |
| | Cyclura nubila / Cyt-b | AF020255 | Petren and Case, 1997 |
| | Sauromalus ater / Rag-1 | AY662591 | Townsend et al., 2004 |
| | Cyclura nubila / Rag-2 | DQ119641 | Fry et al., 2006 |
| | Cyclura nubila / C-mos | DQ119595 | Fry et al., 2006 |
| Xantusidae | Xantusia henshawi / 16s | AY140992 | Vicario et al., 2002 |
| | Xantusia henshawi / Cyt-b | DQ090885 | Yoder et al., unpublished |
| | Xantusia vigilis /Rag-1 | AY662642 | Townsend et al., 2004 |
| | Xantusia vigilis /Rag-2 | DQ119626 | Fry et al., 2006 |
| | Xantusia vigilis /C-mos | AF148703 | Harris et al., 2001 |
| Scincidae | Eumeces inexpectatus / 16s | AY308204 | Schmitz, 2003 |
| | Eumeces fasciatus / Cyt-b | DQ241673 | Howes et al., 2006 |
| | Eumeces inexpectatus / Rag-1 | AY662632 | Townsend et al., 2004 |
| | Eumeces inexpectatus / Rag-2 | DQ119628 | Fry et al., 2006 |
| | Eumeces skiltonianus / C-mos | AF315396 | Harris et al., 2001 |
| Pygopodinae | Lialis jicari / 16s | AY134528 | Jennings et al., 2003 |
| | Lialis jicari / Rag-1 | AY662628 | Townsend et al., 2004 |
| | Lialis burtonis / Rag-2 | EF534948 | Gamble et al., 2008 |
| | Lialis jicari / C-mos | AF090850 | Donnellan et al, 1999 |

**Table 5.1.2** – Fossil calibrations. Age in Mya.

| Calibration Point (node) | Age | Reference | Observation |
|---|---|---|---|
| Synapsid-Diapsid (mammal – bird) | 312-330 | Benton and Donaghue (2007) | Full justification in www.fossilrecord.net |
| Lepidossaurian - Archossaurian (bird – lizard) | 259-299 | Benton and Donaghue (2007) | Full justification in www.fossilrecord.net |
| Oldest teiid (Ptilodon) | 112 | Nydam and Cifelli (2002) (following Vidal and Hedges, 2005) | |
| Oldest rhineurid (Plesiorhineura) | 64 | Sullivan (1985) (following Vidal and Hedges, 2005) | |
| Scincomorpha - Anguimorpha | 168 | Evans (2003) (following Hugall et al., 2007) | This calibration was shown by Hugall et al. (2007) to be one of the most consistent within squamata, and thus prefered to others like Parviraptor used by Vidal and Hedges (2005) and Wiens et al. (2006). |
| Rhyncocephalia (Sphenodon)-Squamata | 227 | Sues and Olsen (1990) | Although ages obtained for this node are constantly older (Vidal and Hedges, 2005; Hugall et al., 2007; Sanders and Lee, 2007), fossil calibrations here were incorporated as lower bounds and thus even a calibration that would otherwise (fixing the node age) possibly lead to underestimates, like this one, can be informative in this context. |
| Heloderma-Anguidae | > 58 | Vidal and Hedges (2005) calibrated this node at 106 mya using Primaderma fossil (Nydam, 2000) and later, Wiens et al. (2006) and Hugall et al. (2007), based on the same fossil, used instead 99 mya to calibrate the split Heloderma-Elgaria (= *Heloderma*-Anguidae). Both Wiens et al. (2006) and Hugall et al. (2007) obtained younger dates for these nodes (when not used as calibrations), as we did when using only other calibrations and thus we tentatively constrained this node to be ≥ 58 my, which is the lower value (70 my) less SD (12) obtained by Hugall et al. (2007). A possible explanation to this discrepancy is that this fossil (Primaderma) has been overinterpreted as an anguid when all that can be said is that it is an anguimorph. However, as noted by Hugall et al. (2007), without numerous adjacent calibrations it cannot be ascertained if the calibration is an overestimate, or the reconstructed ages are underestimates. Our approach is a reasonable conservative compromise with the goal of providing some informative prior on that part of the tree. | |

Depending on the dataset used, the obtained estimates for the age of *Phelsuma* crown diversification and for the stem age of *Phelsuma* (*Phelsuma/Rhoptropella/Lygodactylus* split) were substantially different. Significant differences were encountered among results based on the three datasets analyzed: dataset 1, the combined alignment, including only *Phelsuma* plus *Rhoptropella*; dataset 2, the combined alignment, including *Phelsuma*, *Rhoptropella*, and other Squamata and Tetrapoda (the *"old calibrations"* taxon set) and dataset 3, the alignment of nuclear genes only, including the *"old calibrations"* taxon set as in dataset 2. Age estimates obtained under different calibrations schemes are given in tables 5.1.3 – 5.1.5. An overlap of several chronograms for the first and third dataset under different calibration schemes are shown in figures 5.1.1 – 5.1.2.

Using only *Phelsuma* plus *Rhoptropella*, mitochondrial and nuclear genes, and the available intra-generic calibrations (dataset 1), the age estimates obtained (given as mean, and lower and upper CI in parentheses) were notably young, 6.56 (4.58, 9.31) Mya for the divergence between *Rhoptropella* and *Phelsuma* and 4.03 (3.05, 5.37) Mya for the crown diversification within the genus (Table 5.1.3, third column). Mean ages of island colonization ranged from 3.89 Mya for the Mascarenes, 3.74 Mya for the Andamans, and 2.84 Mya for the Seychelles, to below 1 Mya for the dispersals originating *P. parkeri*, *P. comorensis*, and *P. abbotti*. Modifying the prior for the ingroup root node resulted only in a slight increase of the estimates in every case, suggesting no major influence. Also, separate analyses of a reduced dataset with independent gene's rate priors resulted in similar age estimates, although with considerably smaller credibility intervals (not shown).

Acknowledging that even slight saturation at the mtDNA level together with the use of solely shallow calibration points could be leading to severe underestimation of the deeper nodes in the tree, we also tried a set of deep calibrations (datasets 2 and 3; Tables 5.1.4 and 5.1.5). The age estimates thus obtained were much older, both when using only deep calibration points, or combinations of deep and shallow points.

Using dataset 2, i.e., mitochondrial plus nuclear sequences in concert with the *"old calibrations"* taxon set, the estimates obtained when only the shallow calibrations were used (e.g. Table 5.1.4, last column), resulted in severe underestimates of all the deeper nodes. When only deeper calibrations were used (Table 5.1.4, first and second columns), some of the ages inferred for shallower nodes (e.g. C and G) were higher that respective island ages. Using all available calibrations this dataset leads to average estimates of 84 Mya for the *Phelsuma* vs. other

gekkonids split and 45 Mya for *Phelsuma* crown diversification.

Because nuclear genes are less affected by saturation effects than mitochondrial genes, we are prone to consider the time estimates based on the nuclear gene sequences only (dataset 3) as the most reliable of these. We thus explored more in detail the effects of the various calibration schemes on this dataset. Figure 5.1.2 and Table 5.1.5 show the massive effect on the deeper nodes estimations of using only shallow calibrations (red chronogram), only deep calibrations (purple tree), deep calibrations plus internal fossils (orange tree) and all calibrations (blue tree). Even using only the shallow 2.1 Mya calibration, the expected values for the two deepest nodes (the Synapsida-Diapsida and Archosauria-Lepidosauria splits) were contained in the estimated 95% CI, although the estimated mean age was clearly an underestimate relative to it. The age of the other splits within Squamata were in almost all cases lower than the age from fossil data.

Using the calibrations in the other extreme of the tree (mammal-bird and bird-lizard splits: Table 5.1.5, first column) resulted in estimated ages for the remaining taxa congruent with the fossil record, except for the scincomorph-anguimorph split for which the upper CI was slightly lower than the fossil estimate. Adding the Squamata fossil calibrations (orange tree) resulted in a considerable increase of all estimates across the tree providing average estimates of 87.7 Mya and 61.7 Mya for the splits between *Rhoptropella/Lygodactylus/Phelsuma* and the initial *Phelsuma* diversification, respectively. The estimates obtained for some of the nodes within *Phelsuma* using these calibrations (for the *P. borbonica – P. cepediana* and the *P. abbotti abbotti – P. abbotti chekei* splits) were too high, at least in these two cases to be considered good aproximations, i.e., the minimum value of the obtained 95% CI exceeds the maximum age of respective islands emergence, indicating some overestimation in this part of the tree.

Adding the two available shallow calibrations made the estimates slightly younger in this part of the tree, and can be considered as our current best possible estimate of the *Phelsuma* stem and crown ages: 79.3 Mya (53.2–107.8) for the separation of *Phelsuma* from other gekkonids and 52.5 Mya (30.6–79.8) for the *Phelsuma* crown diversification (Table 5.1.5, fourth column; blue chronogram in Figure 5.1.2). Island colonization events seem to have occurred widespread along *Phelsuma* evolutionary history, but CI intervals are too wide for reliable estimates.

**Table 5.1.3 -** Mean divergence dates and 95% credibility intervals (CI) obtained by *Multidivtime* bayesian approach, under several calibration schemes and priors, using the combined *Phelsuma* dataset and *Rhoptropella ocellata* (dataset I). Calibrations, if applicable, are shown on the left, with enforced calibrations indicated by shading. Estimates for some of the main supported nodes are given. Node labelling is the same as in Figure 2, paper II. Three of the chronograms (*) obtained under these calibration schemes are represented in Figure 5.1.1

| Calibration (Mya) | Node | Calibration Scheme | | | | |
|---|---|---|---|---|---|---|
| | | 7MY rttm prior *P. borbonica* < 2.1 *(*yellow)* | 7MY rttm prior *P. borbonica* 1.6- 2.1 *(*orange)* | 7MY rttm prior *P. borbonica* 1.6- 2.1 *P. abbotti abbotti* < 0.125 | 14MY rttm prior *P. borbonica* 1.6- 2.1 | 21MY rttm prior *P. borbonica* 1.6- 2.1 *(*purple)* |
| | | Estimated Age (95% CI) | | | | |
| | *Rhoptropella-Phelsuma* | 4.79 (1.39, 10.71) | 9.24 (5.96, 13.88) | 6.56 (4.58, 9.31) | 9.94 (6.35, 15.11) | 10.4 (6.69, 15.76) |
| | *Phelsuma* (A) | 2.92 (0.83, 6.52) | 5.72 (3.90, 8.27) | 4.03 (3.05, 5.37) | 6.05 (4.12, 8.77) | 6.28 (4.26, 9.08) |
| | Mascarenes stem-split | 2.79 (0.79, 6.22) | 5.48 (3.75, 7.91) | 3.89 (2.95, 5.17) | 5.79 (3.95, 8.35) | 6.02 (4.01, 8.68) |
| | Mascarenes (B) | 2.32 (0.65, 5.16) | 4.56 (3.18, 6.42) | 3.36 (2.58 , 4.44) | 4.80 (3.34, 6.84) | 4.95 (3.44, 7.06) |
| < 2.1 / 2.1-1.6 | *P. cepediana - P. borbonica* (C) | 0.89 (0.28, 1.94) | 1.82 (1.61, 2.08) | 1.73 (1.60, 2.01) | 1.83 (1.61, 2.08) | 1.85 (1.61, 2.09) |
| | *P. mutabilis – P. breviceps* (D) | 2.42 (0.68, 5.43) | 4.73 (3.16, 6.92) | 3.32 (2.44, 4.51) | 5.00 (3.34, 7.37) | 5.18 (3.45, 7.63) |
| | *P. guttata and P. madagascariensis* group (E) | 2.39 (0.67, 5.35) | 4.69 (3.14, 6.89) | 3.16 (2.36, 4.27) | 4.95 (3.30, 7.27) | 5.14 (3.42, 7.58) |
| | *P. madagascariensis* (F) | 1.75 (0.49, 3.98) | 3.44 (2.24, 5.16) | 2.15 (1.54, 2.97) | 3.63 (2.34, 5.47) | 3.77 (2.43, 5.71) |
| < 0.125 | *P. abbotti chekei – P. abbotti abbotti* (G) | 0.21 (0.05, 0.54) | 0.41 (0.20, 0.77) | 0.11 (0.09, 0.12) | 0.44 (0.21, 0.81) | 0.45 (0.22, 0.84) |
| | *P. vanheygeni* – Seychelles group | 2.13 (0.59, 4.80) | 4.18 (2.75, 6.19) | 2.84 (2.06, 3.87) | 4.42 (2.90, 6.57) | 4.58 (3.02, 6.82) |
| | *P. astriata – P. sundbergi* (H) | 1.03 (0.27, 2.43) | 2.03 (1.19, 3.24) | 1.39 (0.87, 2.09) | 2.13 (1.24, 3.45) | 2.21 (1.29, 3.57) |
| | *P. parkeri* (I) | 0.57 (0.15, 1.40) | 1.13 (0.61, 1.91) | 0.65 (0.39, 1.03) | 1.18 (0.64, 2.01) | 1.23 (0.66, 2.07) |
| | *P. quadriocellata – P. lineata* (J) | 1.45 (0.40, 3.34) | 2.84 (1.81, 4.31) | 2.02 (1.39, 2.85) | 2.99 (1.90, 4.57) | 3.11 (1.99, 4.72) |
| | *P. lineata – P. comorensis* | 0.54 (0.13, 1.32) | 1.07 (0.59, 1.76) | 0.76 (0.45, 1.21) | 1.12 (0.63, 1.87) | 1.16 (0.65, 1.95) |
| | *P. laticauda* (K) (=Clade L stem) | 1.67 (0.46, 3.83) | 3.28 (2.12, 4.91) | 2.33 (1.65, 3.24) | 3.46 (2.24, 5.22) | 3.59 (2.32, 5.40) |
| | Comoros (L) | 1.41 (0.38, 3.23) | 2.77 (1.74, 4.19) | 1.96 (1.35, 2.79) | 2.92 (1.84, 4.46) | 3.02 (1.91, 4.61) |
| | *P. dubia – P. modesta* (M) | 1.93 (0.54, 4.36) | 3.79 (2.50, 5.59) | 2.70 (1.95, 3.71) | 3.99 (2.62, 5.92) | 4.14 (2.72, 6.14) |
| | *P. dubia – P. flavigularis* (N) | 1.52 (0.42, 3.49) | 2.98 (1.92, 4.48) | 2.14 (1.49, 2.99) | 3.15 (2.02, 4.76) | 3.26 (2.09, 4.92) |
| | *P. hielscheri–P. malamakibo–P. berghofi* (O) | 0.85 (0.22, 1.99) | 1.66 (0.98, 2.64) | 1.19 (0.76, 1.78) | 1.76 (1.04, 2.79) | 1.81 (1.08, 2.92) |
| | *P. nigristriata – P. modesta* (P) | 1.62 (0.44, 3.69) | 3.18 (2.04, 4.77) | 2.27 (1.58, 3.18) | 3.35 (2.13, 5.05) | 3.47 (2.21, 5.27) |
| | *P. barbouri – P. pronki* (Q) | 0.34 (0.08, 0.88) | 0.67 (0.34, 1.21) | 0.49 (0.25, 0.85) | 0.71 (0.35, 1.26) | 0.73 (0.37, 1.31) |
| | *P. andamanense* | 2.72 (0.77, 6.08) | 5.33 (3.62, 7.73) | 3.74 (2.80, 5.03) | 5.64 (3.80, 8.19) | 5.85 (3.94, 8.52) |

**Table 5.1.4 -** Mean divergence dates and 95% credibility intervals (CI) obtained by *Multidivtime* bayesian approach for a mitochondrial + nuclear dataset (including mammal, bird, *Sphenodon* and major Squamata representatives – dataset 2), under several calibration schemes and priors. Calibrations, if applicable, are shown on the left, with enforced calibrations indicated by shading. Values for some of the nodes are given. Nodes are from the respective ML tree (identical to the one in figure 5.1.3) and define the same splits as those identically labelled in Figure 2, paper II.

| Calibration (Mya) | Node | Calibration Scheme | | | | |
|---|---|---|---|---|---|---|
| | | [312.3 – 330.4] [259.7 – 299.8] | [312.3 – 330.4] [259.7 – 299.8] Fossils | [312.3 – 330.4] [259.7 – 299.8] Fossils *P. borbonica* < 2.1 | [312.3 – 330.4] [259.7 – 299.8] Fossils *P. borbonica* < 2.1 *P. abbotti abbotti* < 0.125 | *P. borbonica* < 2.1 |
| | | Estimated Age (95% CI) | | | | |
| 312.3 – 330.4 | Mammal - Bird+Lizard | 321.8 (312.9, 329.6) | 322 (312.9, 329.5) | 321.7 (312.9, 329.5) | 321.6 (312.9, 329.5) | 78.7 (44.6, 129.2) |
| 259.7 – 299.8 | Bird-Lizard | 273.1 (260.2, 295.4) | 285 (263.8, 299.1) | 284.3 (263.3, 299) | 284.1 (262.8, 299.1) | 58.4 (33.4, 94.4) |
| >227 | *Sphenodon* | 218.2 (184.9, 253.4) | 258.9 (234.4, 284.6) | 257.9 (232.9, 284.5) | 257.6 (232.7, 284.2) | 45.0 (26.2, 71.9) |
| > 112 | Teiid | 99.8 (72.1, 130.9) | 156 (140.8, 173) | 154.8 (139.9, 170.6) | 154.5 (139.4, 170.3) | 19.1 (11.2, 29.7) |
| > 64 | Rhineurid | 79.3 (55.6, 106.4) | 125 (105, 144.7) | 124.2 (103.7, 143.9) | 123.9 (103.4, 143.7) | 15.1 (8.7, 23.8) |
| > 168 | Scincomorph-Anguimorph | 102.7 (74.7, 134.4) | 160.6 (148.3, 176.4) | 159.3 (146.9, 173.8) | 159.1 (146.7, 173.6) | 19.6 (11.6, 30.6) |
| > 58 | Heloderma-Anguidae | 68.6 (46.7, 94.7) | 108.8 (86.5, 130.7) | 108 (85, 130) | 108 (85.2, 130.2) | 13 (7.3, 20.8) |
| | *Rhoptropella-Lygodactylus-Phelsuma* | 68.7 (47.1, 94.5) | 104.9 (82.5, 128.6) | 89.2 (59.4, 117.7) | 84.4 (52.9, 114) | 12.3 (7.4, 18.5) |
| | *Phelsuma* (A) | 41.5 (27.2, 59.3) | 63.4 (46.3, 82.6) | 49.2 (28.2, 71.6) | 45.4 (24.4, 68) | 7.17 (4.4, 10.5) |
| | Mascarenes stem | 36.1 (23.5, 52.1) | 55 (39.6, 72.7) | 41.5 (23, 61.5) | 38 (19.7, 58,1) | 6.1 (3.8, 8.9) |
| [ < 10 ] | Mascarenes (B) | 29.5 (18.7, 43.5) | 45.1 (31.5, 61) | 31.9 (16.5, 49.7) | 29.1 (14.1, 46.4) | 4.9 (3.03, 7.1) |
| < 2.1 / 2.1-1.6 | *P. cepediana - P. borbonica* (C) | 12.4 (7.0, 19.8) | 18.8 (11.5, 28.7) | 1.8 (1.2, 2.09) | 1.9 (1.2, 2.09) | 1.78 (1.17, 2.09) |
| | *P. madagascariensis* (F) | 22.5 (13.9, 33.7) | 34.3 (23.1, 47.9) | 26.4 (14.1, 40.6) | 23.6 (11.8,37.5) | 3.89 (2.3, 5.9) |
| < 0.125 | *P. abb. chekei – P. abbotti abbotti* (G) | 2.7 (1.2, 5.1) | 4.2 (2.1, 7.5) | 3.3 (1.4, 6.2) | 0.073 ( 0.005, 0.123) | 0.483 (0.227, 0.87) |
| | Seychelles (H) | 13.3 (7.7, 21.2) | 20.5 (12.8, 30.8) | 15.6 (8.08, 25.5) | 14.2 (6.8, 23.6) | 2.26 (1.27, 3.6) |
| | *P. parkeri* (I) | 7.5 (4, 12.7) | 11.6 (6.6, 18.9) | 8.9 (4.25, 15.5) | 7.8 (3.5, 13.9) | 1.29 (0.69, 2.18) |
| | *P. nigristriata – P. modesta* (P) | 22.9 (14.1, 34.6) | 34.8 (23.5, 48.8) | 26.9 (14.6, 41.5) | 24.8 (12.6, 39.05) | 3.9 (2.3, 6.1) |
| | Comoros stem (K) | 22.6 (13.9, 34) | 55 (39.6, 72.7) | 41.5 (23, 61.5) | 38 (19.7, 58.1) | 6.1 (3.8, 8.9) |
| | Comoros (L) | 17.9 (10.8, 27.59) | 27.3 (17.8, 39.5) | 21.3 (11.3, 33.5) | 19.6 (9.9, 31.7) | 3.1 (1.8, 4.9) |

**Table 5.1.5** - Mean divergence dates and 95% credibility intervals (CI) obtained by *Multidivtime* bayesian approach, for a nuclear (3 genes) dataset (including mammal, bird, *Sphenodon* and major Squamata representatives), under several calibration schemes and priors. Calibrations, if applicable, are shown on the left, with enforced calibrations indicated by shading. Values for some of the nodes are given. Nodes are from the respective ML tree (figure 5.1.3) for that dataset and define the same splits as those identically labelled in Figure 2, paper II. Three of the chronograms (*) obtained under these calibration schemes are represented in Figure 5.1.2.

| Calibration (Mya) | Node | Calibration Scheme | | | | |
|---|---|---|---|---|---|---|
| | | [312.3 – 330.4]<br>[259.7 – 299.8]<br>*(*purple)* | [312.3 – 330.4]<br>[259.7 – 299.8]<br>Fossils<br>*(*orange)* | [312.3 – 330.4]<br>[259.7 – 299.8]<br>Fossils<br>*P. borbonica* < 2.1 | [312.3 – 330.4]<br>[259.7 – 299.8]<br>Fossils<br>*P. borbonica* < 2.1<br>*P. abbotti abbotti* < 0.125<br>*(*blue)* | *P. borbonica* < 2.1<br>*(*red)* |
| | | Estimated Age (95% CI) | | | | |
| 312.3 – 330.4 | Mammal - Bird+Lizard | 321.3 (312.8, 329.5) | 321.4 (312.8, 329.5) | 321.4 (312.8, 329.5) | 321.4 (312.8, 329.5) | 153.4 (64.5, 329.6) |
| 259.7 – 299.8 | Bird-Lizard | 277.2 (260.6, 297.7) | 287.7 (266.2, 299.3) | 287.6 (266.1, 299.3) | 287.5 (265.9, 299.3) | 123.3 (51.7, 266) |
| >227 | *Sphenodon* | 217.7 (179, 257) | 259.9 (235, 285.2) | 260 (234.9, 285.6) | 260.04 (235.2, 285.3) | 93.6 (39.5, 203.4) |
| > 112 | Teiid | 94.4 (63.9, 129.6) | 151.2 (130.6, 171.5) | 150.4 (130, 169.9) | 150.1 (129.9, 169.6) | 36.9 (14.8, 85.2) |
| > 64 | Rhineurid | 71.1 (45.72, 102.9) | 116.8 (89.6, 142.6) | 115.9 (88.7, 141.2) | 115.3 (88.1, 140.1) | 27.5 (10.6, 64.6) |
| > 168 | Scincomorph-Anguimorph | 98.4 (67.78, 133.9) | 156.7 (139.9, 174.9) | 155.9 (139.5, 173.5) | 155.7 (139.2, 173.2) | 38.6 (15.6, 88.5) |
| > 58 | Heloderma-Anguidae | 61 (37.29, 91.5) | 101.7 (71.6, 129.7) | 100.6 (71.2, 128.6) | 100.2 (71.8, 127.8) | 23.3 (8.7, 55.2) |
| | *Rhoptropella- Phelsuma* | 54.1 (32.63, 81.7) | 87.7 (61.5, 115.8) | 82.2 (56.2, 110.9) | 79.3 (53.2, 107.8) | 18.9 (7.4, 45.8) |
| | *Phelsuma* (A) | 36.4 (19.7, 59.9) | 61.7 (38.4, 89.3) | 55.5 (33.2, 82.6) | 52.47 (30.6, 79.8) | 11.7 (4.38, 29.7) |
| | Mascarenes stem | 22.5 (10.1, 40.8) | 39.3 (19.9, 64.7) | 30.1 (12.6, 54.3) | 28.1 (11.8, 51.4) | 6.16 (2.18, 16.4) |
| [ < 10 ] | Mascarenes (B) | 17.1 (6.71, 33.6) | 30.1 (12.9, 54.2) | 15.7 (2.93, 37.3) | 14.75 (2.8, 34.9) | 3.65 (1.4, 9.7) |
| < 2.1 / 2.1-1.6 | *P. cepediana - P. borbonica* (C) | 13.1 (4.22, 27.41) | 23.0 (7.9, 45.1) | 1.46 (0.40, 2.07) | 1.47 (0.40, 2.08) | 1.59 (0.64, 2.08) |
| | *P. madagascariensis* (F) | 12.8 (4.051, 27.63) | 22.8 (7.77, 46.3) | 20.2 (6.6, 42.16) | 15.5 (3.84, 35.3) | 3.85 (0.96, 11.3) |
| < 0.125 | *P. a. chekei – P. a. abbotti* (G) | 4.6 (0.24, 13.62) | 8.2 (0.39, 23.7) | 7.27 (0.351, 21.8) | 0.063 (0.003, 0.12) | 1.38 (0.060, 5.1) |
| | *P.vanheygeni* – Seychelles | 24.25 (11.24, 43.10) | 41.06 (21.15, 66.57) | 36.25 (17.93, 60.78) | 33.4 (16.1, 57.06) | 7.58 (2.51, 20.15) |
| | *P. astriata – P. sundbergi* (H) | 7.9 (1.039, 20.21) | 13.9 (1.78, 34.2) | 12.2 (1.48, 30.3) | 11.2 (1.28, 28.05) | 2.48 (0.29, 7.89) |
| | *P. parkeri* (I) | 6.3 (0.47, 17.39) | 11.3 (0.81, 30.4) | 10.02 (0.69, 27.3) | 7.83 (0.42, 22.5) | 1.85 (0.12, 6.43) |
| | *P. nigristriata – P. modesta* (P) | 15.6 (6.04, 30.27) | 26.1 (10.8, 47.6) | 23.8 (9.6, 44.1) | 22.5 (9, 42.02) | 5.06 (1.45, 13.7) |
| | *P. laticauda – P.vnigra* | 13.4 (3.8, 28) | 22.7 (6.7, 44.5) | 20.6 (5, 40.8) | 19.5 (5.6, 39.7) | 4.3 (0.9, 12.3) |
| | *P. lineata – P.pasteuri* | 18.4 (8, 34) | 31 (14.7, 53.3) | 28.2 (13.1, 49.2) | 26.6 (12.2, 47) | 5.9 (1.8, 15.8) |
| | *P. lineata – P. pusila* | 3.5 (0.18, 10.43) | 6 (0.31, 17.6) | 5.4 (0.28, 16.12) | 5.2 (0.27, 15.37) | 1.15 (0.052, 4,08) |
| | *P. andamanense* | 28.26 (13.91, 48.36) | 48.4 (27.09-, 4.54) | 41.11 (21.03, 66.89) | 38.46 (19.2, 63.6) | 8.47 (3.03, 22.2) |

**Figure 5.1.1** Chronograms obtained by *Multidivtime* for some of the calibration schemes and data shown in Table 5.1.3. Chronograms for the different calibration schemes are overlapped. Yellow: 7 My *rttm* prior and *P. cepediana – P. borbonica* split calibrated at < 2.1 Mya. Orange: 7 My *rttm* prior and *P. cepediana – P. borbonica* split calibrated at 1.6 - 2.1 Mya. Purple: 21 My prior and *P. cepediana – P. borbonica* split calibrated at < 2.1 Mya. The tree giving origin to chronograms is the ML tree of *Phelsuma* dataset (all outgroups except *Rhoptropella* removed), and is basically identical to the Bayesian tree presented in Figure 2, paper II. The spread of the mean estimates for the root node and for node A (*Phesuma* ingroup root, as in Table 5.1.3) across calibration schemes is represented by the grey bar.

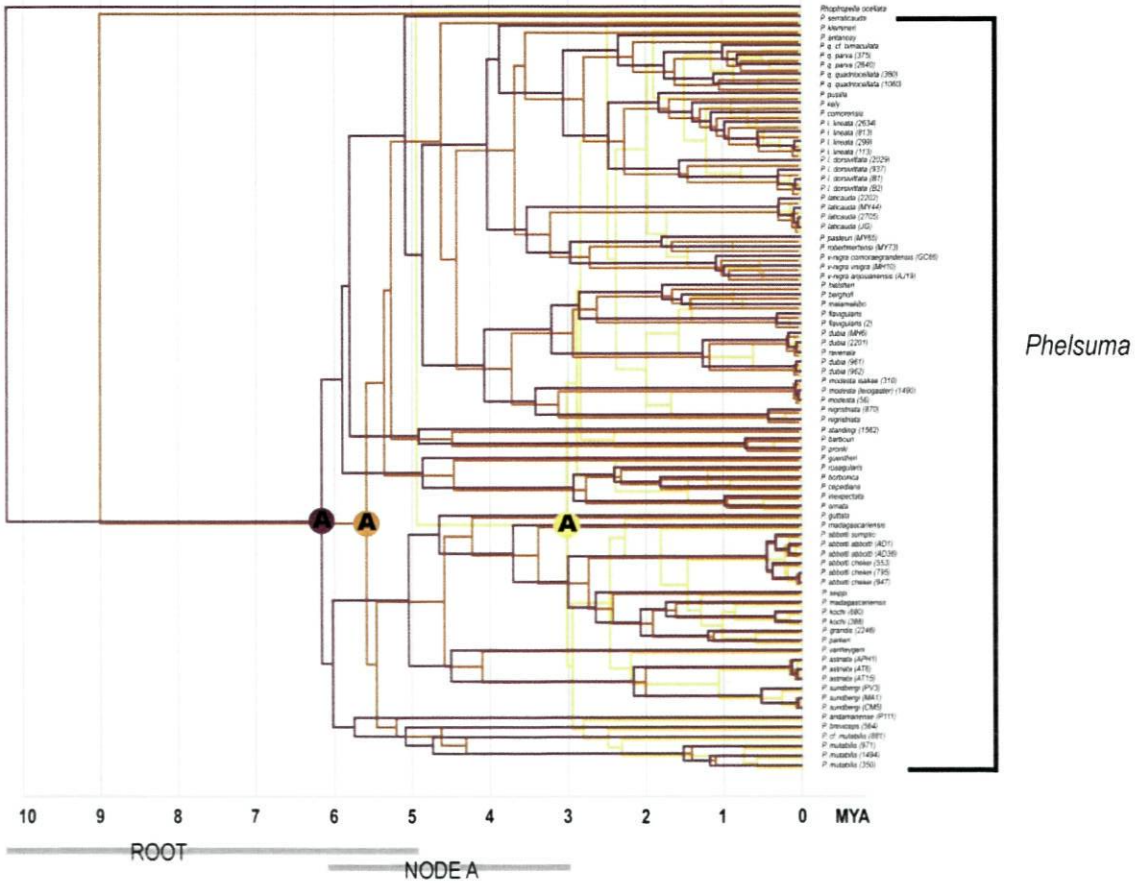


**Figure 5.1.2** Chronograms obtained by *Multidivtime* for some of the calibration schemes and data shown in Table 5.1.5. Chronograms for the different calibration schemes are overlapped. Purple: Synapsida-Diapsida and Archosauria-Lepidosauria calibration points (two deeper nodes). Orange: previous and 5 additional fossil calibrations (left column). Blue: all fossil and island age calibrations available (9 nodes). Red: only Reunion age and *P. borbonica-P. cepediana* split as calibrations. The tree giving origin to chronograms is the ML tree in Figure 5.1.3, with a deep branching pattern identical to the one recovered by Vidal and Hegdes (2005). The spread of the mean estimates for the split between *Phelsuma* and its outgroup (R) and for node A (*Phesuma* ingroup root, as in Table 5.1.5) across calibration schemes are represented by the grey bar.

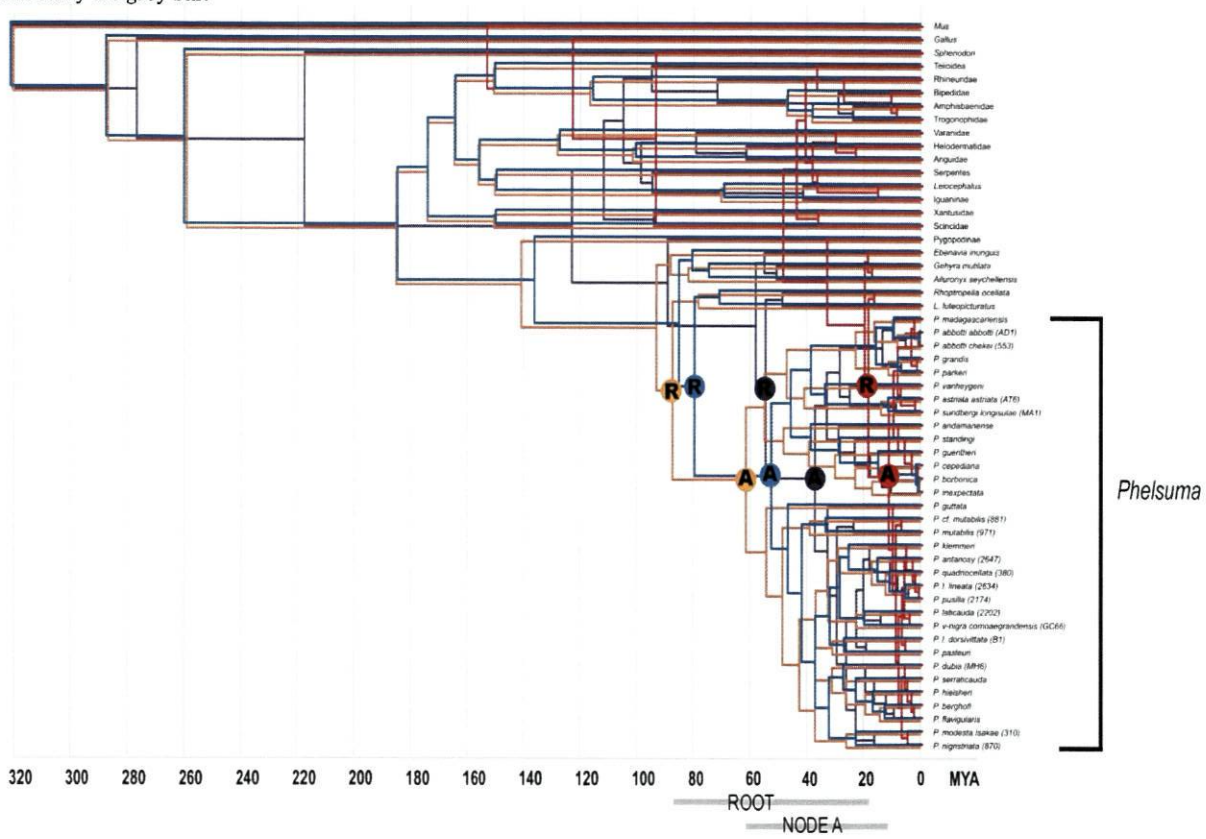
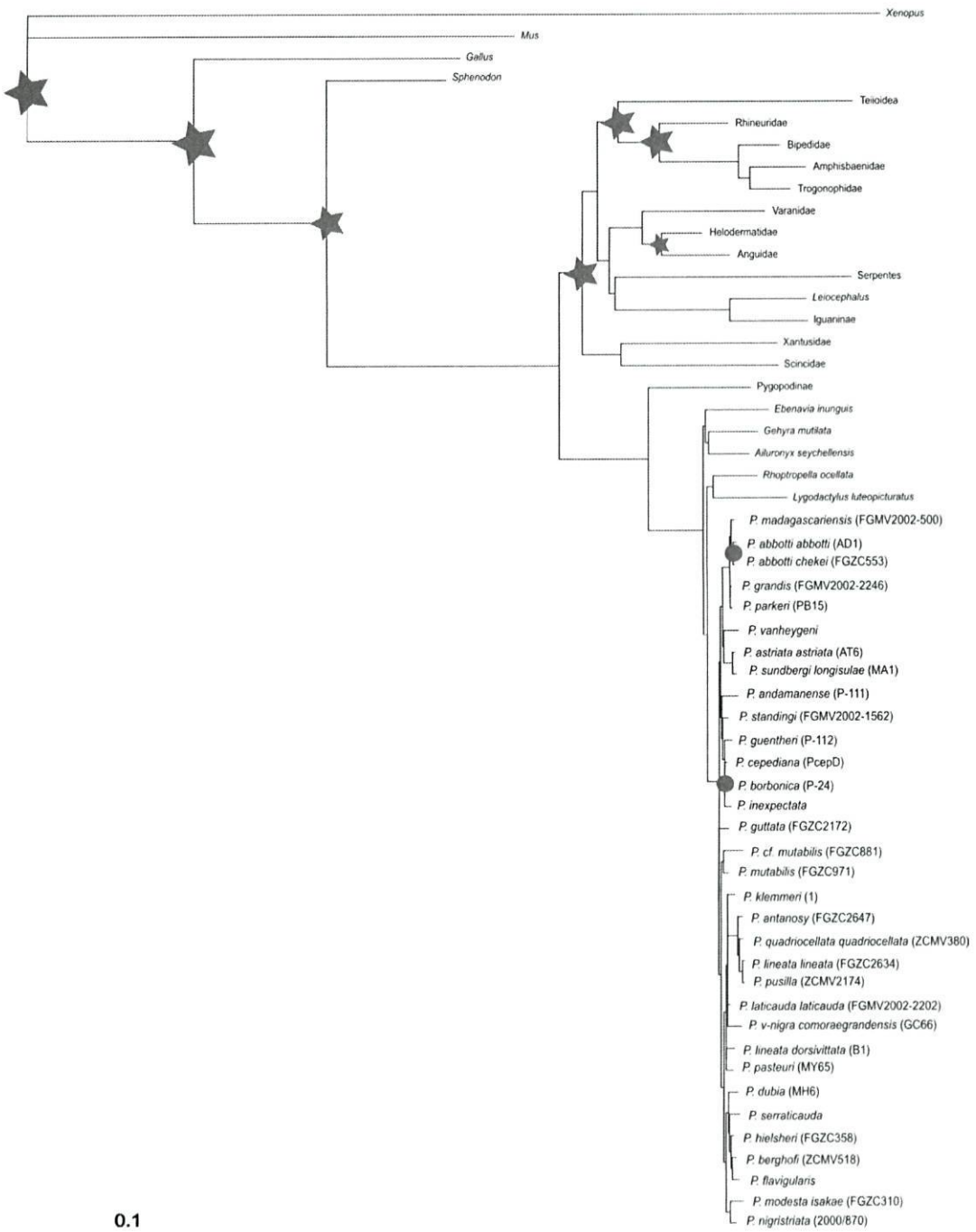


**Figure 5.1.3 –** Maximum Likelihood tree topology for the "old calibrations" taxonset using the 3 nuclear genes (dataset 3 – Table 5.1.5 and Figure 5.1.2) obtained with GARLI (Zwickl, 2006) and 1X106 generations. The tree shows the distributions of the fossil (stars) and island (circles) calibrations used for age estimates.



## References


Austin, J.J., Arnold, E.N., Jones, C.G., 2004. Reconstructing an island radiation using ancient and recent DNA: the extinct and living day geckos (*Phelsuma*) of the Mascarene islands. Molecular Phylogenetics and. Evolution 31, 102–109.

Barker, F.K., Barrowclough, G.F., Groth, J.G., 2002. A phylogenetic hypothesis for passerine birds: taxonomic and biogeographic implications of an analysis of nuclear DNA sequence data. Proceedings of the Royal Society of London B 269, 295-308.

Benton, M.J., Donoghue, P.C. J., 2007. Paleontological evidence to date the Tree of Life. Molecular Biology and Evolution 24, 26-53.

Bergstrom, K.S., Guttman, J.A., Rumi, M., Ma, C., Bouzari, S., Khan, M.A., Gibson, D.L., Vogl, A.W., Vallance, B.A., 2008. Modulation of intestinal goblet cell function during infection by an attaching and effacing bacterial pathogen. Infectious Immunology 76, 796-811.

Carlson, L.M., Oettinger, M.A., Schatz, D.G., Masteller, E.L., Hurley, E.A., McCormack, W.T., Baltimore, D., Thompson, C.B., 1991. Selective expression of RAG-2 in chicken B cells undergoing immunoglobulin gene conversion. Cell 64, 201-208.

Donnellan, S.C., Hutchinson, M.N. Saint, K.M., 1999. Molecular evidence for the phylogeny of Australian gekkonoid lizards. Biol. J. Linn. Soc. Lond. B 67, 97-118.

Evans, S., 2003. At the feet of the dinosaurs: the early history and radiation of lizards. Biological Reviews 78, 513-551.

Felsenstein, J. 1988. Phylogenies from molecular sequences: inference and reliability. Annual Reviews in Genetics 22, 521-65.

Freeman, R.S., Pickham, K.M., Kanki, J.P., Lee, B.A., Pena, S.V., Donoghue, D.J., 1989. *Xenopus* homolog of the mos protooncogene transforms mammalian fibroblasts and induces maturation of *Xenopus* oocytes. Proceedings of the National Academy of Sciences os the United States of America 86, 5805-5809.

Fry, B.G., Vidal, N., Norman, J.A., Vonk, F.J., Scheib, H., Ramjan, S.F., Kuruppu, S., Fung, K., Hedges, S.B., Richardson, M.K., Hodgson, W.C., Ignjatovic, V., Summerhayes, R., Kochva, E., 2006. Early evolution of the venom system in lizards and snakes. Nature 439, 584-588.

Gamble,T., Bauer, A.M., Greenbaum, E., Jackman, T.R., 2008. Evidence for Gondwanan vicariance in an ancient clade of gecko lizards. Journal of Biogeography 35, 88-104.

Greenhalgh, P., Olesen, C.E., Steiner, L.A., 1993. Characterization and expression of recombination activating genes (RAG-1 and RAG-2) in *Xenopus laevis* Journal of Immunology 151, 3100-3110.

Harris, D.J., Marshall, J.C., Crandall, K.A., 2001. Squamate relationships based on C-mos nuclear DNA sequences. Amphibia-Reptilia 22, 235-242.

Harris, D.J., Mendonça, B., 2007. Genetic variation within *Trogonophis wiegmanni* Kaup 1830. Belgian Journal of Zoology 137, 239-242.

Harvey, M.B., Ammerman, L.K., Barker, D.G., Chippindale, P.T., 2000. Systematics of pythons of the *Morelia amethistina* complex with the description of three new species. Herpetological Monographs 14, 139-185.

Howes, B.J., Lindsay, B., Lougheed, S.C., 2006. Range-wide phylogeography of a temperate lizard, the five-lined skink (*Eumeces fasciatus*). Molecular Phylogenetics and Evolution 40, 183-194.

Hugall, A.F., Foster, R., Lee, M.S.Y., 2007. Calibration choice, rate smoothing, and the pattern of tetrapod diversification according to the long nuclear gene RAG-1. Systematic Biology 56, 543–563.

Jennings, W.B., Pianka, E.R., Donnellan, S., 2003. Systematics of the lizard family Pygopodidae with implications for the diversification of Australian temperate biotas. Systematic Biology 52, 757-780.

Katoh, K., Kuma, K., Toh, H., Miyata, T., 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Research 33, 511-518.

Kearney, M., Stuart, B.L., 2004. Repeated evolution of limblessness and digging heads in worm lizards revealed by DNA from old bones. Proceedings of the Royal Society of London, B 271, 1677-1683.

Kumazawa,Y., Nishida, M., 1995. Variations in mitochondrial tRNA gene organization of reptiles as phylogenetic markers. Molecular Biology and Evolution 12, 759-772.

Kumazawa,Y., 2007. Mitochondrial genomes from major lizard families suggest their phylogenetic relationships and ancient radiations. Gene 388, 19-26.

Löytynoja, A., Milinkovitch, M., 2003. A hidden Markov model for progressive alignment. Bioinformatics 19, 1505-1513.

Macey, J.R., Papenfuss, T.J., Kuehl, J.V., Fourcade, H.M., Boore, J.L., 2004. Phylogenetic relationships among amphisbaenian reptiles based on complete mitochondrial genomic sequences. Molecular Phylogenetics and Evolution 33, 22-31.

Mori, M., Higuchi, K., Tanaka, M., unpublished. Unique transitional nucleotide mutations in the mitochondrial DNA of Senescence-accelerated Mouse (SAM) strains.

Nydam, R.L., Cifelli, R.L., 2002 Lizards from the Lower Cretaceous (Aptian-Albian) Antlers and Cloverly formations. Journal of Vertebrate Paleontology 22, 286–298.

Oettinger, M.A., Schatz, D.G., Gorka, C., Baltimore, D., 1990. RAG-1 and RAG-2, adjacent genes that synergistically activate V(D)J recombination. Science 248, 1517-1523.

Ota, H., Honda, M., Kobayashi, M., Sengoku, S. Hikida, T., 1999. Phylogenetic relationships of the eublepharid geckos (Reptilia: Squamata): a molecular approach. Zoological Sciences 16, 659-666.

Pearse, D.E., Pogson, G.H., 2000. Parallel evolution of the melanic form of the California legless lizard, *Anniella pulchra*, inferred from mitochondrial DNA sequence variation. Evolution 54, 1041-1046.

Pellegrino, K.C.M., Rodrigues, M.T., Yonenaga-Yassuda,Y., Sites, J.W. Jr., 2001. A molecular perspective on the evolution of microteiid lizards (Squamata, Gymnophthalmidae), and a new classification for the family. Biological Journal of the Linnean Society ,London B 74, 315-338.

Petren, K., Case, T.J., 1997. A phylogenetic analysis of body size evolution and biogeography in chuckwallas (*Sauromalus*) and other iguanines. Evolution 51, 206-219.

Porter, M., Pérez-Losada, M., Crandall, K.A., 2005. Model based multilocus estimation of decapod phylogeny and divergence times. Molecular Phylogenetics and Evolution 37, 355–369.

Reeder, T.W., 1995. Phylogenetic relationships among phrynosomatid lizards as inferred from mitochondrial ribosomal DNA sequences: substitutional bias and information content of transitions relative to transversions. Molecular Phylogenetics and Evolution 4, 203-222.

Rest, J.S., Ast, J.C., Austin, C.C., Waddell, P.J., Tibbetts, E.A., Hay, J.M. and Mindell, D.P., 2003. Molecular systematics of primary reptilian lineages and the tuatara mitochondrial genome. Molecular Phylogenetics and Evolution 29, 289-297.

Roe, B.A., Ma, D.P., Wilson, R.K., Wong, J.F.,1985. The complete nucleotide sequence of the *Xenopus laevis* mitochondrial genome. Journal of Biological Chemistry 260, 9759-9774.

Rutschmann, F., 2005. Bayesian molecular dating using PAML/multidivtime. A step-by-step manual. University of Zürich, Switzerland. Available at http://www.plant.ch.

Saint, K.M., Austin, C.C., Donnellan, S.C., Hutchinson, M.N., 1998. C-mos, a nuclear marker useful for squamate phylogenetic analysis Molecular Phylogenetics and Evolution 10, 259-263.

Sanders, K.L., Lee, M. S.Y, 2007. Evaluating molecular clock calibrations using Bayesian analyses with soft and hard bounds. Biology Letters 3, 275-279.

Schmitz, A., 2003 Taxonomic and phylogenetic studies on scincid lizards (Reptilia: Scincidae). Unpublished thesis University Bonn, Germany.

Sues, H.-D., Olsen, P.E., 1990. Triassic vertebrates of Gondwanan aspect from the Richmond Basin of Virginia. Science 249, 1020–1023.

Thorne, J.L., Kishino, H, Painter, I.S., 1998. Estimating the rate of evolution of the rate of molecular evolution. Molecular Biology and Evolution 15, 1647-1657.

Thorne, J.L., Kishino, H., 2002. Divergence time and evolutionary rate estimation with multilocus data. Systematic Biology 51, 689-702.

Townsend, T., Larson, A., Louis, E., Macey, J.R. Molecular phylogenetics of Squamata: the position of snakes, amphisbaenians, and dibamids, and the root of the squamate tree. Systematic Biology 53, 735-757.

Valverde, J.R., Marco, R., Garesse, R., 1994. A conserved heptamer motif for ribosomal RNA transcription termination in animal mitochondria. Proceedings of the National Academy of Sciences os the United States of America 91, 5368-5371.

Van Beveren, C., van Straaten, F., Galleshaw, J.A., Verma, I.M., 1981. Nucleotide sequence of the genome of a murine sarcoma virus. Cell 27, 97-108.

Vicario, S., Caccone, A. Gauthier, J., 2003. Xantusiid night lizards: a puzzling phylogenetic problem revisited using likelihood-based Bayesian methods on mtDNA sequences. Molecular Phylogenetics and Evolution 26, 243-261.

Vidal, N., Hedges, S.B., 2002. Higher-level relationships of snakes inferred from four nuclear and mitochondrial genes. Comptes Rendus Biologies 325, 977-985.

Vidal, N., Hedges, S.B., 2004. Molecular evidence for a terrestrial origin of snakes. Proceedings of the Royal Society of London B 271, 226-229.

Vidal, N., Hedges, S.B., 2005. The phylogeny of squamate reptiles (lizards, snakes, and amphisbaenians) inferred from nine nuclear protein coding genes. Compus Rendus Biologies 328, 1000-1008.

Wiens, J. J., M. C. Brandley, and T. W. Reeder. 2006. Why does a trait evolve multiple times within a clade? Repeated evolution of snake-like body form in squamate reptiles. Evolution 60:123–141.

Wilm, A., Mainz, I., Steger, G., 2006. An enhanced RNA alignment benchmark for sequence alignment programs. Algorithms in Molecular Biology 1, 1-19.

Yang, Z., 1997. PAML: a program package for phylogenetic analysis by maximum likelihood. CABIOS 13, 555-556 (http://abacus.gene.ucl.ac.uk/software/paml.html).

Yang, Z., Yoder, A.D., 2003. Comparison of likelihood and Bayesian methods for estimating divergence times using multiple gene loci and calibration points, with application to a radiation of cute looking mouse lemur species. Systematic Biology 52, 705-716.

Raselimanana, A., Noonan, B., Karanth, K.P., Gauthier, J., Yoder, A.D., 2009. Phylogenetics and evolution of Malagasy plated lizards. Molecular Phylogenetics and Evolution 50, 336-344.

Zwickl, D.J., 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D.dissertation, The University of Texas at Austin.

www.bio.utexas.edu/faculty/antisense/garli/Garli.html

PHYLOGENY, PHILOGEOGRAPHY AND COLONIZATION PATTERNS OF REPTILES IN INDIAN OCEAN ISLANDS
Sara Rodrigues Passos Rocha
Orç. 32 A | Ano: 2010 | 11 Exemplares (1ª Edição) | Produção Gráfica: www.ajnet.net | tel./fax: 252 681 975


FC
Biblioteca
Faculdade de Ciências
Universidade do Porto
D0000139033