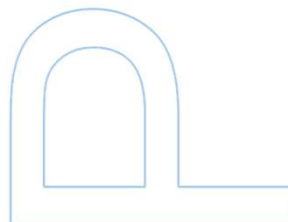
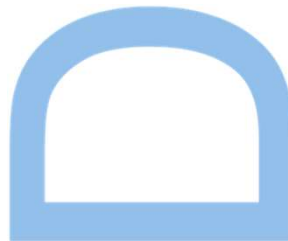
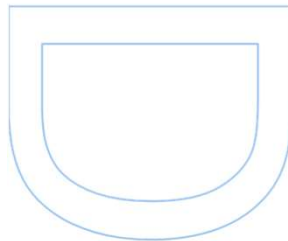
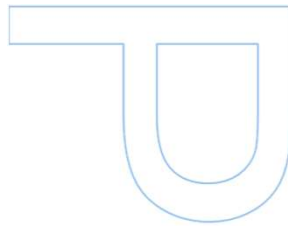
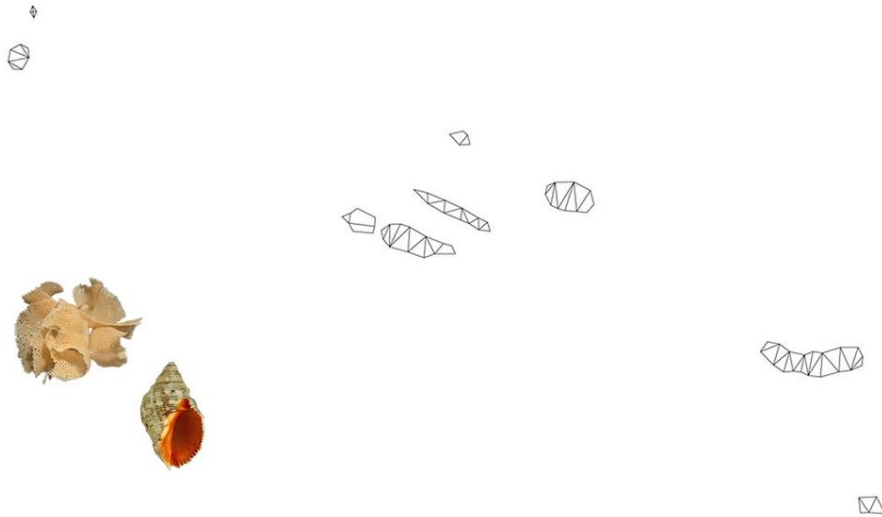


The genetic structure in populations of marine organisms in remote islands: a study of bryozoans and gastropods in the Azores (NE Atlantic)

Lara Valéria Couto Baptista

PhD Thesis presented to the
Faculty of Sciences of the University of Porto, Department of
Biology
Doctoral Program in Biodiversity, Genetics and Evolution
2023





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Doutoramento em Biodiversidade, Genética e Evolução

Departamento de Biologia, Faculdade de Ciências da Universidade do Porto
2023

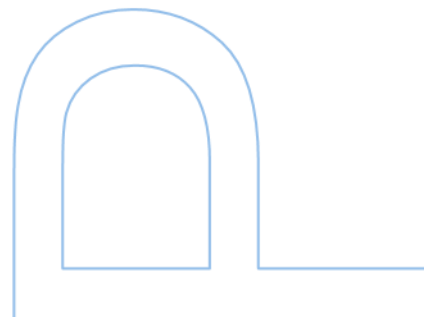
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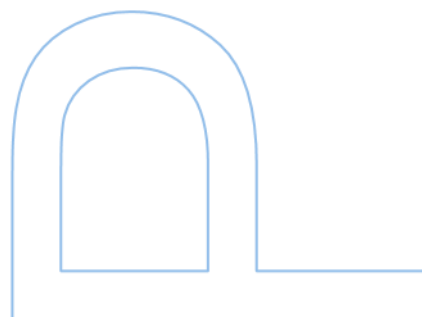
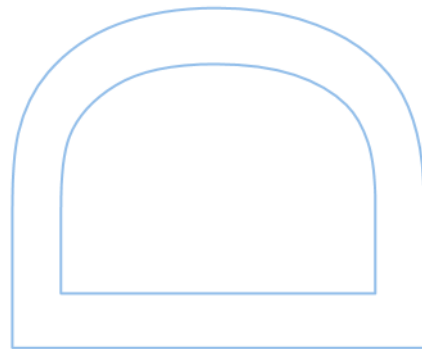


All the corrections determined by the jury, and only those, were made.

The Supervisor,

Sérgio Ávila

Porto, 30/05/2023



*À minha família, aos que cá estão e os que já não,
que sempre me apoiaram neste sonho*

Declaração de Honra

Eu, Lara Valéria Couto Baptista, natural de Vila do Porto, residente em Almagreira com o telefone 910740287, nacional de Portugal, portadora do Cartão de Cidadão nº 14619841, inscrita no Programa Doutoral em Biodiversidade, Genética e Evolução da Faculdade de Ciências da Universidade do Porto declaro, nos termos do disposto na alínea a) do artigo 14.º do Código Ético de Conduta Académica da U.Porto, que o conteúdo da presente tese reflete as perspetivas, o trabalho de investigação e as minhas interpretações no momento da sua entrega.

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Foram publicados quatro artigos científicos em revistas internacionais indexadas e com arbitragem científica, como resultado dos trabalhos desenvolvidos no âmbito desta tese. Segue-se a listagem dos mesmos, nos quais a candidata consta como primeira autora em colaboração com outros autores, esclarecendo ter participado ativamente na sua conceção, obtenção e análise de dados, discussão de resultados, e elaboração da sua forma publicada.

1. Baptista, L., Santos, A. M., Cabezas, M. P., Cordeiro, R., Melo, C., & Ávila, S. P. (2019). Intertidal or subtidal/circalittoral species: which appeared first? A phylogenetic approach to the evolution of non-planktotrophic species in Atlantic Archipelagos. *Marine Biology*, 166, 1–16. <https://doi.org/10.1007/s00227-019-3536-y>
2. Baptista, L., Meimberg, H., Ávila, S. P., Santos, A. M., & Curto, M. (2021). Dispersal ability, habitat characteristics, and sea-surface circulation shape population structure of *Cingula trifasciata* (Gastropoda: Rissoidae) in the remote Azores Archipelago. *BMC Ecology and Evolution*, 21(1), 128. <https://doi.org/10.1186/s12862-021-01862-1>
3. Baptista, L., Santos, A. M., Melo, C. S., Rebelo, A. C., Madeira, P., Cordeiro, R., Botelho, A. Z., Hipólito, A., Pombo, J., Voelker, A. H. L., & Ávila, S. P. (2021). Untangling the origin of the newcomer *Phorcus sauciatus* (Mollusca: Gastropoda) in a remote Atlantic archipelago. *Marine Biology*, 168(1), Article number: 9. <https://doi.org/10.1007/s00227-020-03808-5>
4. Baptista, L., Berning, B., Waeschenbach, A., Curto, M., Meimberg, H., Santos, A. M., & Ávila, S. P. (2022) Morphospecies and molecular diversity of ‘lace corals’: the genus *Reteporella* (Bryozoa: Cheilostomatida) in the central North Atlantic Azores Archipelago. *BMC Ecology and Evolution*, 22, 128. <https://doi.org/10.1186/s12862-022-02080-z>

Esta tese foi desenvolvida tendo como instituição de acolhimento principal BIOPOLIS-CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Vairão Portugal, e secundária INF-BOKU, Institut für Integrative Naturschutzforschung, Universität für Bodenkultur Wien, Viena, Áustria. A Faculdade de Ciências da Universidade do Porto foi a instituição conferente de grau. O trabalho foi orientado pelo Doutor Sérgio P. Ávila e co-orientado pelos Doutores António Múrias dos Santos e Manuel Curto.

Este trabalho foi apoiado pela Fundação para a Ciência e Tecnologia (FCT) através da atribuição da bolsa de doutoramento (SFRH/BD/135918/2018). As saídas de campo, visando a recolha de amostras para este projeto, foram apoiadas por fundos do Fundo Regional de Desenvolvimento Europeu (FEDER) e Governo Regional dos Açores, através do Programa Operacional Açores 2020, no âmbito dos projetos AZORESBIOPORTAL—PORBIOTA (ACORES-01-0145-FEDER-000072), VRPROTO: Virtual Reality PROTOtype: the geological history of “Pedra-que-pica” (ACORES-01-0145-FEDER-000078), e “Azores Portal da Biodiversidade dos Açores.”: M1.1.A/INFRAEST CIENT/001/2022, bem como através das reuniões científicas “Ciência nas Zonas Costeiras” (2020-2022; M3.3.B/ORG.R.C./016/2020, M3.3.B/ORG.R.C./002/2021, M3.3.B/ORG.R.C./010/2022/EDIÇÃO 1), International Workshop “Palaeontology in Atlantic Islands” em Santa Maria (2019-2022; M3.3.B/ORG.R.C./034/2019, M3.3.B/ORG.R.C./018/2020, M3.3.B/ORG.R.C./005/2021, M3.3.B/ORG.R.C./008/2022/EDIÇÃO 1) e Cabo Verde (M3.3.G/EXPEDIÇÕES CIENTÍFICAS/005/2022), financiadas pelo Governo Regional dos Açores. Mergulho científico em várias ilhas dos Açores foi possível através dos projetos PRIBS - Estratégia Regional para a Prevenção e Controlo de Espécies Exóticas Invasoras no âmbito do projeto LIFE IP AZORES NATURA (LIFE17 ipe/pt/000010) e PAPS - “ELABORAÇÃO DE PLANO DE AÇÃO DO PALEOPARQUE DE SANTA MARIA”, contratualizada pela Direção Regional do Ambiente (Secretaria Regional da Energia, Ambiente e Turismo), com cabimento orçamental na CE 010107. A amostragem biológica foi conduzida sob autorização oficial de recolha para fins científicos (CCIP 24/2019/DRCT, CCIP 29/2019/DRCT, CCIP 35/2019/DRCT + AMP/2019/016-DRAM, e AMP/2018/014-DRAM pela Direção Regional da Ciência e Tecnologia do Governo Regional dos Açores; licença 01/ICFN/2019-FAU MAD pelo Governo Regional da Madeira). A visita às coleções biológicas do Natural History Museum, Londres, Reino Unido foi suportada pelo Projeto SYNTHESYS+ através do Programa de Actividades Integrativas H2020. O trabalho laboratorial foi suportado pelo Programa Operacional Norte 2020, sob o Acordo de Parceria PORTUGAL 2020 através do FEDER com o projeto MarInfo (NORTE-01-0145-FEDER-000031), e pela Bolsa de Investigação Roger Bamber atribuída pela Porcupine Marine Natural History Society.

Preferred Citation

Baptista L. 2023. The genetic structure in populations of marine organisms in remote islands: a study of bryozoans and gastropods in the Azores (NE Atlantic). Ph.D. Thesis, Faculdade de Ciências da Universidade do Porto, Portugal.



Acknowledgements

Quatro anos que passaram num abrir e fechar de olhos, numa roda viva de viver aventuras de uma vida a bordo do RV Meteor durante 32 dias, de ter o privilégio de conhecer pessoas incríveis em congressos e reuniões científicas em várias cidades, de mergulhar em todos os arquipélagos do Nordeste Atlântico, aprender mais em visitas a laboratórios e instituições, de discutir ideias com colegas a trabalhar em áreas completamente distintas, de ter inspiração súbita para dar resposta a problemas ou surgir com novas perguntas em momentos totalmente aleatórios. Todos estes momentos e superação de desafios permitiram-me crescer enquanto pessoa e cientista. Mas nada disto teria acontecido sem uma rede de pessoas incríveis à minha volta, que passo agora a agradecer:

Ao meu orientador Sérgio P. Ávila, por ter dado uma oportunidade a uma miúda curiosa de 14 anos e indubitavelmente marcar o percurso académico que me trouxe até este momento. Obrigada por acreditar em mim desde então, de me colocar questões tão interessantes e desafiantes de testar com genética, e pela confiança que me deu ao integrar-me na sua equipa desde 2016, a par da organização de eventos científicos e inclusão em vários projetos que me deram “estaleca” para lidar com várias situações burocráticas.

Ao meu co-orientador António Múrias dos Santos, pelo apoio nas análises filogenéticas e pelas discussões científicas que se tornaram cada vez mais interessantes e desafiantes desde 2016, quando comecei o mestrado consigo. Obrigada por confiar no meu julgamento científico e despender longas horas a discutir as melhores abordagens, mais robustas, para as questões que vão surgindo.

Ao meu co-orientador Manuel Curto, pela sua disponibilidade desde o primeiro dia quer para a tese como para projetos paralelos, pela partilha de tanto conhecimento, por desmistificar as abordagens bioinformáticas, e por todas as discussões tão produtivas para fazer sentido dos (por vezes estranhos) padrões biogeográficos nos Açores. Obrigada pela tua humanidade e por compreenderes que, por vezes, a pressão do doutoramento leva a melhor.

To Björn Berning, unofficial supervisor (for bureaucratic reasons) but official advisor of this Ph.D. project, for introducing me to bryozoans ~12 years ago and for now dragging me into their dazzling world, for challenging me to accomplish more and think further. Especially, thank you for your friendship.

To Harald Meimberg, head of the INF-BOKU (my second hosting institution) and official advisor, thank you for receiving me in Vienna, for providing all the tools needed

to address the questions of my Ph.D. project, for showing me different perspectives of the results, and for the informal meetings.

Às inúmeras amigas que nasceram no CIBIO durante estes anos, obrigada por todas as pausas para café, jantaradas, “rants”, cervejas, e conversas sem nexos. E uma obrigada especial à Inês e à Alessia, que se tornaram tão próximas durante os últimos tempos.

To the bryozoologists Basti, Julian, and Thomas in Vienna, for sharing passionate discussions about these weird organisms and becoming good friends, always ready for a chill evening.

Aos meus colegas e amigos no Marine Palaeontology and Biogeography Working Group – Ana Cristina, Carlos e Patrícia desde 2016, e, mais recentemente, Cátia, Frederik e Livia – obrigada pelo vosso apoio nesta jornada e por tornarem o trabalho divertido. A todos os colegas que participaram nas campanhas nos Açores, Canárias e Cabo Verde (2018-2022), bem como no cruzeiro científico M150 – BIODIAZ (2018), obrigada por todos os risos e memórias criadas depois de dias de trabalho duro!

To Andrea Waeschenbach, for hosting me in the Natural History Museum of London for two weeks, granting me access to her Phidoloporidae collection, sharing her expertise and knowledge in the bryozoans, as well as invaluable tips for me to grow as a scientist.

To the collaborators that provided samples and shared observations from areas I could not visit in person: Ana Cristina Rebelo, André Freiwald, Andreia Braga-Henriques, Edoardo Casoli, Esther Martín-González, Eva Cacabelos, Federico Betti, Giulia Fassio, Guðmundur Guðmundsson, Isadora Moniz, João Brum, João Faria, João Gonçalves, Kai Horst George and the M150’s scientific and ship crews, Marco Oliverio, Marina Carreiro-Silva, Marlene Nória, Max Wisshak, Marzia Bo, Sebastian Decker, Serge Gofas, Teresa Silva, and Titouan Seimandi.

To the collaborators that provided technical support in several methodologies: André Freiwald, Benjamim Sames, Eva Dornstauder-Schramler, Max Wisshak, Nicol Mahnken, Paulo D. Taylor, and Roberto Resendes.

À Teresa, pelos cafés e conversa de balcão, e ao Sr. Bernardino pela paciência, boleias fora das horas do shuttle e por desenrascar o que é preciso.

I also take this opportunity to acknowledge the associations from which I received grants or awards during my Ph.D.: UNESCO/IOC Project Office for IODE (2019; OTGA Training Course, Oostende, Belgium); Governo Regional dos Açores (2019; 18th IBA conference, Liberéc, Czech Republic); Synthesys+ (2021; two weeks visit to the Natural History Museum of London); Porcupine Marine Natural History Society (2022; Roger

Bamber Award); Fundação Luso-Americana para o Desenvolvimento (2022; Azores Summer School; Santa Maria, Azores); International Bryozoology Association for the Travel Award and Best Oral Presentation (2022; 19th IBA Conference, Dublin, Ireland).

Chegar ao final desta caminhada desafiante teria sido impossível sem uma “Aldeia”, que sempre me espicaçou a voar mais alto mas estando lá para amparar as quedas.

Aos meus amigos mais próximos:

Beatriz e Bruna, pela distância diminuída pelas conversas diárias por Whatsapp, pela partilha de momentos bons e maus, por acreditarem em mim e por me fazerem “acordar para a vida” há 10 anos. Sofia, por seguirem esta minha aventura com tanto interesse e preocupação desde a licenciatura.

Ana, Sophia, and Berenice for breaking the routine in Porto with a glass of wine and some snacks to accompany deep as well as crazy conversations, for sharing the victories and failures in work and life.

Sara, a irmã de sangue diferente, por estares presente mesmo longe, por me acompanhares tão perto enquanto tu própria lutas com o teu louco percurso.

E, por fim, à minha Família. Aqueles que me apoiam incondicionalmente ainda que seja difícil perceber porque segui este caminho, aos que me dão a força para continuar a seguir os meus sonhos, e aos que serão sempre porto seguro quando o vento não for de feição. Obrigada por me terem dado a força de começar esta caminhada quando o pior aconteceu e eu não sabia se conseguiria avançar.

Aos meus Avós, agora sim já tenho o “canudo” mas é improvável que deixe de queimar as pestanas em breve. Obrigada por perceberem que estudar estes bichinhos estranhos me faz feliz.

Ao meu Pai.

Obrigada, Mãe, pelo exemplo que és! Por me mostrares que, mesmo quando tudo parece mal, há sempre um lado bom para nos agarrarmos. Por me conheceres melhor do que ninguém. Por estares sempre lá nos momentos em que me vou abaixo. Por mereceres um doutoramento honorário por tanto me ouvires divagar sobre as minhas perguntas e problemas de investigação, pelas sessões de amostragem, e por teres aprendido a identificar microgastrópodes. E, obrigada, Joana, por saberes o que são mitocôndrias e briozoários, por aliviares tão facilmente o ambiente com piadas totós, por me apoiares nesta vida de croma! Obrigada pelo vosso amor incondicional e por acreditarem em mim mais do que alguma vez eu acreditarei em mim própria, sem vocês não teria chegado aqui!

Resumo

O Arquipélago do Açores, de origem vulcânica, é um laboratório natural privilegiado para o estudo de processos e padrões evolutivos que atuam em ilhas remotas, especialmente para compreender a distribuição de espécies marinhas moldada pela sua capacidade de dispersão. Nos invertebrados marinhos, a capacidade de atravessar barreiras geográficas, como por exemplo massas de água profundas ou correntes oceânicas superficiais, está intrinsecamente associada ao seu desenvolvimento larvar: espécies cujas larvas com um longo período de desenvolvimento larvar conseguem facilmente manter a conectividade em grandes áreas geográficas, enquanto espécies com larvas não-planctotróficas geralmente têm uma distribuição mais restrita e estrutura populacional marcada. Isto tem importantes implicações biogeográficas e evolutivas, tendo resultado em várias hipóteses no sentido de explicar como os invertebrados marinhos colonizaram e atualmente mantêm a conectividade em áreas remotas como os Açores, especialmente as espécies com uma fase adulta sésil ou com capacidade de dispersão limitada. Assumindo o processo de “rafting” (dispersão em jangada) como o mecanismo de dispersão predominante em espécies sem fase larvar ou com larvas não-planctotróficas em águas temperadas, esta tese procurou entender a biodiversidade marinha e mecanismos biogeográficos no Arquipélago do Açores através do estudo de espécies de gastrópodes e briozoários com diferentes tipos de desenvolvimento larvar. Para responder às perguntas colocadas, foi usada uma combinação de marcadores mitocondriais e nucleares (incluindo microssatélites) distribuídos no genoma, permitindo uma perspetiva mais abrangente das dinâmicas de herdabilidade e padrões filogeográficos a diferentes escalas temporais e espaciais.

Os padrões esperados de acordo com os modelos teóricos que relacionam o tipo de larva e estrutura genética populacional foram testadas com gastrópodes das espécies com larva não-planctotrófica da família Rissoidae Gray, 1847 e da espécie planctotrófica *Stramonita haemastoma* (Linnaeus, 1767). No caso de *S. haemastoma*, foi detectada panmixia nos arquipélagos do Atlântico Nordeste e no Mar Mediterrâneo, já que a capacidade de sobrevivência das suas larvas na coluna de água (~3 meses) favorece a conectividade em grandes áreas. Pelo contrário, a inferência de forte estrutura populacional em espécies com larvas não-planctotróficas sugere e reforça a sua utilidade como objetos de estudo no campo da (paleo)biogeografia. Duas espécies de rissóideos – *Alvania mediolittoralis* Gofas, 1989 e *A. sleursi* (Amati, 1987) – foram usadas para testar uma hipótese biogeográfica proposta por Ávila (2006, 2013), que explica como duas espécies de gastrópodes congéneres, ambas com larva não-

planctotrófica mas diferente zonação batimétrica, ocorrem simultaneamente em dois arquipélagos remotos. Deste estudo, deduziu-se que a espécie intertidal *A. mediolittoralis* fosse evolutivamente mais recente, dando suporte molecular à hipótese testada que realça a importância da zonação batimétrica na capacidade de dispersão. Os fatores determinantes de estrutura populacional de espécies intertidais em ilhas remotas foi explorado mais detalhadamente com estudos incidindo no rissóideo *Cingula trifasciata* (J. Adams, 1800).

A dinâmica populacional de invertebrados marinhos com larva não-planctotrófica foi inferida para os gastrópodes *C. trifasciata* e *Phorcus sauciatus* (Koch, 1845), e para o género de briozoários sésseis *Reteporella* Busk, 1884, cada um permitindo diferentes perspectivas na análise dos padrões biogeográficos em ilhas oceânicas vulcânicas. Os estudos nos gastrópodes realçaram o efeito da fase da vida durante a qual o rafting ocorre, o tipo de habitat, correntes oceanográficas de pequena dimensão, e eventos meteorológicos extremos nos processos de dispersão nos Açores, enquanto eventos aleatórios podem resultar em assinaturas genéticas de eventos de dispersão a longa distância. Estudos no género de briozoário *Reteporella* permitiram explorar os processos de diversificação e especiação num arquipélago remoto. Abordagens taxonómicas integrativas revelaram a existência de pelo menos sete espécies de *Reteporella* nos Açores, quatro das quais novas para a ciência. A diferenciação ecológica associada com batimetria parece moldar a dinâmica populacional de espécies com larva não-planctotrófica e zonação batimétrica alargada, já que uma linhagem divergente habitando águas superficiais foi detetada com marcadores moleculares. Assinaturas de eventos de hibridização interespecífica foram também detetados com dados de microsatélites, provavelmente derivados da troca de material nuclear não-codificante entre congéneres em simpatria com barreiras semipermeáveis. O teste desta hipótese de hibridização como processo não-neutral depende de estudos filogenómicos futuros.

Os resultados desta tese suportam a ideia de que a capacidade de dispersão de invertebrados marinhos durante o seu tempo de vida influencia os níveis de diferenciação e, conseqüentemente, eventos de especiação. Para espécies com larva não-planctotrófica, níveis de diferenciação mais elevados são mais facilmente atingidos devido a limitações na sua dispersão, contribuindo para a singularidade biogeográfica da eco-região dos Açores. Vários fatores bióticos e abióticos interagem diferentemente para moldar a diversidade e evolução de espécies de invertebrados marinhos com larva não-planctotrófica dentro e fora dos Açores, originando padrões de estrutura genética únicos, alguns dos quais ainda por descobrir. A expansão de abordagens filogeográficas

e de genética populacional a mais taxa irá certamente providenciar uma história mais completa da evolução marinha em arquipélagos remotos, desvendando os mecanismos de colonização, bem como o papel destas ilhas como centros de diversificação no meio do oceano.

Palavras-chave

Biogeografia Marinha Insular; ilhas oceânicas vulcânicas; arquipélagos Atlânticos; modos de desenvolvimento larvar; “rafting”; filogenética; genética de populações; taxonomia integrativa; biodiversidade; introgressão interespecífica; diferenciação ecológica; espécies crípticas; SSR-GBAS; marcadores mitocondriais e nucleares.

Abstract

The volcanic oceanic Azores Archipelago is a privileged natural laboratory to study fascinating evolutionary processes and patterns that operate in remote areas, especially to understand the distribution of marine species that is shaped by their dispersal ability. For marine invertebrates, the ability to cross geographical barriers posed by deep-waters and sea-surface circulation is intrinsically linked to their larval development: species with long-lived planktotrophic larvae can easily maintain connectivity across wide geographical areas, whilst those with non-planktotrophic larvae usually have restricted ranges and strong genetic structure. This has important biogeographical and evolutionary implications, with many biogeographical hypotheses trying to explain how marine invertebrate species, especially those with sessile adults or limited dispersal ability, colonize and maintain connectivity in remote areas such as the Azores Archipelago. Assuming rafting as the predominant dispersal mechanism in temperate waters for organisms without a larval stage or with non-planktotrophic larvae, this thesis aimed to address the marine biodiversity and biogeographic mechanisms in the Azores by studying gastropod and bryozoan species with different types of larval development. A combination of mitochondrial and nuclear (including microsatellite) markers, scattered across the genome, were used to address the questions posed, granting a broader perspective of the inheritance dynamics and phylogeographic patterns at different temporal and spatial scales.

Theoretical expectations related to larvae type and consequent population genetic structure were tested with gastropod species, either with non-planktotrophic larvae, such as those from the family Rissoidae Gray, 1847 and the planktotrophic *Stramonita haemastoma* (Linnaeus, 1767). For *S. haemastoma*, panmixia was detected across the NE Atlantic Archipelagos and Mediterranean Sea, as the survival capacity of its larvae in the water column (~3 months) favours connectivity across wide areas. Contrariwise, the success of these studies and inference of strong population structure in marine invertebrates with non-planktotrophic larvae reinforce their usefulness as ideal subjects in the field of (palaeo)biogeography. Two rissoid species – *Alvania mediolittoralis* Gofas, 1989 and *A. sleursi* (Amati, 1987) – were used to test a biogeographical hypothesis proposed by Ávila (2006, 2013), which explains the rare simultaneous existence of two congeneric non-planktotrophic gastropod species, with different bathymetric specific ranges, in two remote archipelagos. The intertidal species *A. mediolittoralis* was inferred as evolutionarily younger, providing molecular support to the hypothesis tested, which states the importance of bathymetric zonation in the dispersal ability. The factors

underlying the population structure of intertidal species in remote islands were further explored with studies focused in the rissoid *Cingula trifasciata* (J. Adams, 1800).

Population dynamics of Azorean marine invertebrates with non-planktotrophic larvae were inferred for the gastropods *C. trifasciata* and *Phorcus sauciatus* (Koch, 1845), and the sessile bryozoan genus *Reteporella* Busk, 1884, each providing different insights on biogeographical patterns in volcanic oceanic islands. Studies in the gastropods highlighted life stages at which rafting occurs, habitat type, small-scale oceanographic currents, and extreme weather events as the major forces shaping the frequency and timing of dispersal events and gene flow in the Azores, whilst random chance events can result in unexpected signatures of occasional long-distance dispersal. Studies in the bryozoan genus *Reteporella* provided insights into the processes of diversification and speciation in a remote archipelago. Integrative taxonomic approaches revealed the existence of at least seven *Reteporella* species in the Azores, four of which new to science. Ecological differentiation associated with bathymetry seems to shape the population dynamics of species with non-planktotrophic modes of larval development and large bathymetrical ranges, as a divergent lineage inhabiting shallow-waters was detected with molecular markers. Signatures of potential interspecific hybridization events were also detected with microsatellite dataset, likely due to the exchange of non-coding nuclear material between sympatric congeners with semipermeable boundaries. Testing of the hybridization hypothesis as a non-neutral process awaits future phylogenomic studies.

The results of this thesis support the idea that the dispersal capacity of marine invertebrates in early or late-life stages influences the differentiation levels and, consequently, speciation events. For species with non-planktotrophic larvae, higher differentiation levels are more easily achieved due to limitations in their dispersal, contributing to the biogeographic uniqueness of the Azores ecoregion. Several biotic and abiotic factors interact differently to shape the diversity and evolution of marine invertebrate species with non-planktotrophic larvae within and outside the Azores, giving rise to new, unique patterns of genetic structure, many of which still unknown. Expanding phylogeographic and population genetics to more taxa will certainly provide a more complete history of marine evolution in remote archipelagos, uncovering the mechanisms of colonization and understanding the role of these islands as centres of diversification in the middle of the ocean.

Keywords

Marine Island Biogeography; volcanic oceanic islands; Atlantic archipelagos; modes of larval development; rafting; phylogenetics; population genetics; integrative taxonomy; biodiversity; interspecific introgression; ecological differentiation; cryptic species; SSR-GBAS; mitochondrial and nuclear markers.

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Figure 5.11. Directional gradient of the frequency of each genetic cluster per geographical locality, estimated in the STRUCTURE reconstruction for: **a.** the optimal K=4; **b.** the maximum informative K=8. Number of species (n) known to occur in each locality is indicated under the locality designation. Abbreviations are depicted in black for islands (SMA – Santa Maria, SMG – São Miguel, TER – Terceira, FAI – Faial, FLW – Flores) and in green for seamounts (FOR – Formigas, PAB – Princess Alice Bank, AB –

Azores Bank, ChB – Chino Bank, CB – Condor Bank, GB – Gigante Bank). Coastline shape retrieved from the Portuguese Hydrographic Institute (<https://www.hidrografico.pt/op/33>) and bathymetry, indicated at the right margin, derived from GEBCO 2020 (https://www.gebco.net/data_and_products/gridded_bathymetry_data/). 178

List of Abbreviations

The following list contemplates all the abbreviations used throughout this dissertation, intending to facilitate its reading. Units and symbols from the metric system and following the International System of Units will not be included in the list, since publications from recognized authorities can be consulted regarding these topics.

16S	Small ribosomal subunit gene
28S	Large ribosomal subunit gene
AB	Azores Bank
AIC	Akaike Information Criterion
AL	Allele Length
AnC	Antilles Current
AMOVA	Hierarchical analysis of Molecular Variance
AzC	Azores Current
AzCC	Azores Counter Current
AZO	Azores Archipelago
BI	Bayesian Inference
bPTP	Bayesian implementation of the Poisson Tree Process model
BS	Bootstrap Support
BSA	Bovine Serum Albumine
CAN	Canaries Archipelago
ca.	circa
CB	Condor Bank
CC	Canary Current
cf.	<i>confer</i>
ChB	Chino Bank
CIBIO	Centro de Investigação em Biodiversidade e Recursos Genéticos
COI	Cytochrome C oxidase subunit 1
CR	Croatia
CUC	Canary Upwelling Current
CVU	Corvo Island
DBUA	Department of Biology of the University of the Azores
DD	Decimal degrees
DNA	Deoxyribonucleic acid
dNTP	Deoxyribose Nucleotide Triphosphate
DOP	Department of Oceanography and Fisheries of the University of the
e.g.	<i>exempli gratia</i> (for example)
EMBL-EBI	European Bioinformatics Institute
EML	Estimated Marginal Likelihood
ESS	Effective Sample Size
ESP	Spain
ETIB	Equilibrium Theory of Island Biogeography
FAI	Faial Island
FCT	Fundação para a Ciência e Tecnologia
FCUP	Faculdade de Ciências da Universidade do Porto
FLW	Flores Island

FOR	Formigas Seamount
F_{ST} , F_{SC} , F_{CT} ; ϕ_{ST}	Fixation index
GAL	Galicia
GB	Gigante Bank
GBAS	Genotyping by Amplicon Sequencing
GC	Guinea Current
GDM	General Dynamic Model
gDNA	Genomic DNA
GRW	Graciosa Island
GS	Gulf Stream
GSM	Glacial Sensitive Model
GTR	General Time Reversible
Hd	Haplotype diversity
He	Expected heterozygosity
Ho	Observed heterozygosity
HWE	Hardy-Weinberg Equilibrium
IBT	Island Biogeography Theory
ICE	Iceland
i.e.	<i>id est</i> (that is)
IPC	Iberian Poleward Current
ITS2	internal transcribed spacer 2
Hap	haplotype
HKY	Hasegawa-Kishino-Yano
HPD	Highest Posterior Density
KAT	Kattegat
ky	Thousand years
Ma	Million years ago
MAD	Madeira Archipelago
MAR	Mid-Atlantic Ridge
MC	Mauritania Current
MCMC	Markov Chain Monte Carlo
MED	Mediterranean Sea
MgCl ₂	Magnesium Chloride
ML	Maximum Likelihood
MNHN	Muséum National d'Histoire Naturelle; Paris, France
MNHUK	Natural History Museum London, UK
MOR	Morocco
mPTP	Multi-rate Poisson Tree Process model
mtDNA	Mitochondrial DNA
myr	Million years
NAC	North Atlantic Current
NCBI	National Centre for Biotechnology Information
nDNA	Nuclear DNA
NEC	North Equatorial Current
NECC	North Equatorial Counter Current
NGS	Next-generation sequencing
np	Non-planktotrophic
OLL	Biologiezentrum of the Oberösterreichische Landes-Kultur GmbH,

PAB	Princess Alice Bank
PC	Portugal Current
PCoA	Principal Coordinates Analysis
PCR	Polymerase Chain Reaction
<i>pers. comm.</i>	Personal communication
<i>pers. obs.</i>	Personal observation
PIX	Pico Island
POR	Portugal
PP	Posterior probability
RAD	Restriction site Associated DNA
RAD	Restriction site Associated DNA Sequencing
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
SAB	Sabrina Seamount
SaM	Senckenberg am Meer in Wilhelmshaven; Hamburg, Germany
SEL	Selvagens Archipelago
SEM	Scanning Electron Microscopy
SH-aLTR	Approximate likelihood-ratio test/ branch test
SIME	Single island marine endemic species
SJZ	São Jorge Island
<i>s.l.</i>	<i>sensu lato</i>
SLS	Sea-Level Sensitive dynamic model
SMA	Santa Maria Island
SMG	São Miguel Island
SNP	Single Nucleotide Polymorphism
<i>s.s.</i>	<i>sensu strictum</i>
SSR	Microsatellite/ Simple Sequence Repeat
SSR-GBAS	Simple Sequencing Repeats – Genotyping by Amplicon
SST	Sea-surface temperature
STR	Short Tandem Repeats
TER	Terceira Island
UFboot	Ultrafast bootstraps
UPGMA	Unweighted Pair Group Method with Arithmetic mean
VTM	Vitória-Trindade Chain Model
WAI	Whole Amplicon Information

Chapter 1

General Introduction

General Introduction

1.1 Island Biogeography

Volcanic oceanic islands have long been considered interesting areas for the study of biological and geological processes and species evolution, as early as in the contributions of Darwin (1859), Hooker (1862), and Wallace (1880). These islands are among the most dynamic geological structures on Earth, arising *de novo* from the bottoms of the sea as a consequence of peculiar geotectonic settings and being morphologically carved by the interaction of volcanic, erosional, depositional, tectonic, isostatic, eustatic, and mass-wasting processes (see review by Ramalho et al., 2013). The occurrence of geomorphological processes at dynamic rates and states constantly established new ecological niches and affect the biodiversity of insular biota (Whittaker et al., 2008; Ávila et al., 2018).

Following the pioneer work of MacArthur & Wilson (1967), island biogeography rapidly became a hot-topic on insular biology research (for extensive reviews see Fernández-Palacios et al., 2015; Patiño et al., 2017; Santos et al., 2016; Warren et al., 2015; Whittaker et al., 2017). Nevertheless, most studies focused on the distribution and evolution of terrestrial organisms, that led to the formulation of important theories in this field such as the Equilibrium Theory of Island Biogeography (ETIB). ETIB suggests a direct species-area relationship: bigger islands have a higher carrying capacity due to larger habitat heterogeneity, promoting lower extinction rates and consequently maintenance of higher species richness; the opposite occurs in smaller islands. Distance also plays an important role in shaping species richness, such that a higher degree of isolation reduces the chances of immigration and species turnover, with an expected decrease in the number of species (MacArthur & Wilson, 1967).

To account for the role of ontogenetic changes in the biogeographical patterns of classic hotspot islands, such as the Hawaiian Archipelago, Whittaker et al. (2008, 2010) proposed the General Dynamic Model (GDM): terrestrial species richness will depend upon the island area and ontogeny, which promotes changes in habitat heterogeneity influencing migration, speciation and extinction rates. During the early stages of an island, carrying capacity and speciation rates increase due to the habitat heterogeneity achieved by volcanic and erosive processes. Immigration also increases with the carrying capacity, contributing for its colonization. Higher speciation rates and immigrant are the key contributors to increase in species richness. The carrying capacity, speciation, and species richness peak when the island reaches its maximum topographic

complexity, promoting a turnover in the immigration-extinction rates. At an advanced stage of erosion the island will lose habitats, thus increasing extinction rates are expected, reducing species richness until the island is fully submerged and disappears, along with all its terrestrial life (Whittaker et al., 2008, 2010).

The role of Quaternary climate change and sea-level variation on the islands' configurations, was later addressed by Rijdsdijk et al. (2014) and by Fernández-Palacios et al. (2016), resulting in the proposal of the Glacial Sensitive Model (GSM). The GSM relates immigration and extinction rates for the same island during glacial and interglacial periods, assuming no volcanic and geomorphic activity and without considering isostatic effects or island subsidence/uplift. The decrease in sea-level during glacial periods leads to an increase of the insular terrestrial area, lower isolation, and new niches, thus promoting higher immigration rates. On the other hand, during interglacial periods, island size is reduced due to sea-level rise, increasing its isolation and extinction rates. Overall, it is expected that terrestrial species richness is higher during glacial periods (Fernández-Palacios et al., 2016). Borregaard et al. (2017) expanded the GDM to archipelagos that do not conform to the simple ontogenetic model – classic hotspot archipelago (e.g. merging island during low stands: Mahan, formed by Lanzarote and Fuerteventura in the Canaries; Maui Nui once formed by the merging of O'ahu, Moloka'i, Lana'i, Maui, and Kaho'olawe in Hawaii). A reduced functional diversity is expected for young islands at an initial stage of colonization, although it will progressively increase due to speciation and habitat heterogeneity. Erosive processes in older islands cause habitat loss and reduce available niches, thus enhancing the extinction rates of specialized species and competition, with consequent extinction of species with similar functional traits (Borregaard et al., 2017). Insular biodiversity patterns in archipelagos were recently modelled by Aguilée et al. (2021), considering the influence of inter-island dispersal ("archipelago effect") and its variation over time ("history effect"), which might affect differently islands within the same archipelago. The GDM and its extensions have mostly been tested or modelled for insular terrestrial biota (for a review see Aguilée et al., 2021; Borregaard et al., 2017), whereas biogeographical studies on the marine realm have only recently gained importance worldwide (e.g. Ávila et al., 2018, 2019; Barroso et al., 2016; Hachich et al., 2015; Hart & Pearson, 2011; Pinheiro et al., 2017).

1.1.1. Marine Island Biogeography

The processes affecting the distribution and diversity of organisms – immigration, speciation, and extinction – are common to both the terrestrial and marine realms, but

how they interact with each other is different in the marine systems, resulting in different biogeographical patterns that the GDM cannot fully explain (Ávila et al., 2018; Hachich et al., 2015, 2020). Thus, holistic approaches are crucial to develop a theory of island biogeography that unifies ecological and evolutionary/historical processes expanding to the marine realm (Ávila et al., 2019).

New models have been recently proposed: the Vitória-Trindade Chain Model (VTCM; Pinheiro et al., 2017) and the Sea-Level Sensitive dynamic model (SLS; Ávila et al., 2019). The SLS was formulated based on the variation of littoral area of 11 Atlantic archipelagos, including the Azores (Ávila et al., 2019), the main focus of this thesis. Both models consider the role of island ontogeny and littoral area in biogeographical processes in the shallow-water marine systems, and compare the predicted rates of immigration, extinction, and speciation, and subsequent carrying capacity and species richness, with GDM's expectations (see Hachich et al., 2020 for a comparison). Overall, the VTCM and the SLS describe similar biogeographical processes and patterns in insular shallow-water habitats, as described next. The definition of littoral area of volcanic oceanic islands according to Ávila et al. (2018, 2019) is the submarine area between mean sea level (0 m) at any given time and its contemporaneous 50-m isobath. At -50 m there is a turnover of the algal composition and associated fauna, which transitions to intermediate and deep-water communities (Ávila et al., 2018, 2019). Contrary to observed in the terrestrial realm, biogeographical processes in marine insular systems start once the new volcanic edifice reaches the -50 m threshold, creating new shallow-water habitats, increasing the carrying capacity (K) of the island and promoting higher immigration (I) rates, with an expected high species richness. During the subaerial constructive phase, despite the changes in the emerged area, the littoral area (0 to 50 m depth) will remain stable, with an expected reduction in the K and I , providing an opportunity for speciation events. Once this phase is over and erosive processes become predominant, habitat heterogeneity, K and species richness increase substantially. During the seamount stage, achieved from erosional decay of old edifices, greater littoral area is available, which might allow a higher species richness in shallow-waters, although not as a rule (Ávila et al., 2019; Hachich et al., 2020; Pinheiro et al., 2017). Extinction rates will increase as the seamount is eroded, reducing the species richness until all processes stop when the edifice subsides below 50 m depths (Ávila et al., 2019).

The SLS dynamic model is the only one that discusses the influence of historical sea-level oscillations associated to Pleistocene climate change, integrating both the GDM and GSM frameworks in the context of biogeography of shallow-water marine organisms

in volcanic oceanic islands located in tropical, subtropical and temperate settings (Ávila et al., 2019). Glacial-interglacial cycles and associated sea-level variation lead to changes in the littoral area and associated biogeographical patterns: during interglacial high-stands littoral area increases, with an expected increase of species richness and speciation rates; during glacial periods, lower sea-levels cause the reduction of littoral area in emerged islands and promote higher extinction rates in shallow-water organisms, although deeper seamounts can reach the 50-m threshold and become newly available for shallow-water biota (Ávila et al., 2019).

1.1.2. Marine Island Biogeography in the NE Atlantic archipelagos

Testing the applicability of island biogeography models and theories to the marine realm is important, namely with different taxa, biogeographic regions, and spatial scales (Hachich et al., 2020). Studies have been developed aiming to better understand the processes and patterns operating in the NE Atlantic, especially in the remote islands in this area. Historically, five Atlantic archipelagos located between latitudes 15 and 39° N – Azores, Madeira, Selvagens, Canaries, and Cabo Verde (Figure 1.1) – have been recognized as forming the “Macaronesia”, which rapidly became a controversial concept (Freitas et al., 2019 and references therein). Even in the terrestrial realm, compositional differences are observed in the typical fauna and flora of these five archipelagos, especially this singular biodiversity and biogeography of several organismal groups in Cabo Verde (Freitas et al., 2019).

In the marine realm, Spalding et al. (2007) distinguished Azores, Madeira, and the Canaries as a single ecoregion within the Lusitanian province, whereas Cabo Verde and the Sahelian Upwelling ecoregions were part of the West African Transition province. Recently, Freitas et al. (2019) proposed the restructuring of ‘Macaronesia’ biogeographic unit, on the grounds of the expectations under the SLS model (Ávila et al., 2019), coupled with biogeographical analysis in six recent marine groups. From the marine perspective, the concept of Macaronesia as a coherent marine biogeographical unit is not valid, requiring its split into the Azores and Webbnesia (Madeira, Selvagens, and Canaries) ecoregions, both comprised within the Lusitanian Province of Spalding et al. (2007), and Cabo Verde subprovince, included in the West African Transition Province. Nowadays, differences in the mean SSTs, varying from 17°C north of the Azores to 24°C south of Cabo Verde (Figure 1.1), and oceanographic features (see Section 1.2 for details) constitute important barriers to the dispersal of marine taxa among archipelagos, although exchanges can occur during “windows of opportunity” or rare chance events

(Ávila et al., 2009, 2015; Baptista et al., 2019, 2021a; Melo et al., 2022a). The biogeographical structuring of the NE Atlantic Archipelagos has major implications in the connectivity and population structure of marine organisms in the NE Atlantic (Freitas et al., 2019; Melo et al., 2022b).

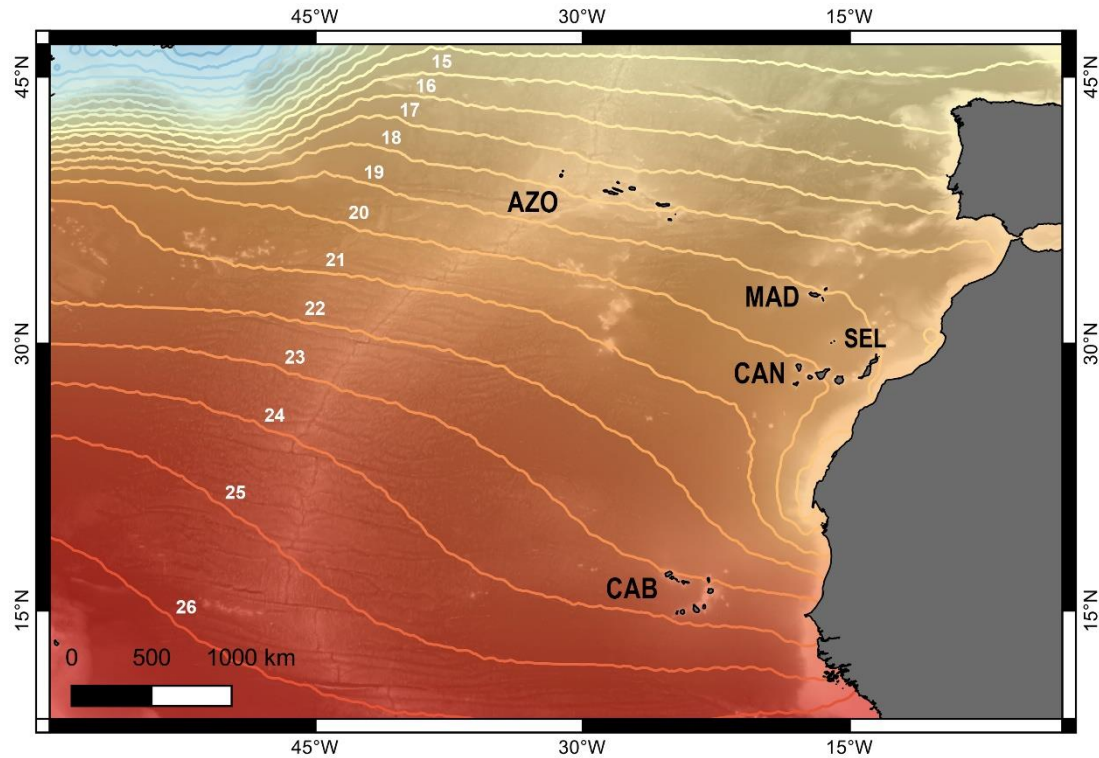


Figure 1.1. Northeast Atlantic archipelagos in study and Sea Surface Temperatures (SST). Acronyms correspond to the archipelagos: AZO (Azores); MAD (Madeira), SEL (Selvagens), CAN (Canaries), CAB (Cabo Verde). Isotherms in Celsius degrees (°C) in the Mediterranean Sea are labelled at a 1°C scale. Coastline delimitation according to available data from the Portuguese Hydrographic Institute (<https://www.hidrografico.pt/op/33>), background digital elevation model generated from GEBCO 2020 (https://www.gebco.net/data_and_products/gridded_bathymetry_data/) and mean SSTs from COBE-SST2 data provided by the NOAA/OAR/ESRL PSL (Hirahara et al., 2014; <https://psl.noaa.gov/data/gridded/data.cobe2.html>).

The colonization of the volcanic NE Atlantic archipelagos, located far from other emerged landmasses (see Ávila et al., 2018 for details) and under complex oceanographic and climatic dynamics, depend upon long distance oceanic dispersal (Ávila et al., 2013; Baptista et al., 2021a; Freitas et al., 2019). This results in peculiar and often impoverished insular biological assemblages (Ávila et al., 2015; Hawkins et al., 2000), with unusual patterns of colonization and diversification that make oceanic islands prime laboratories for the study of biogeography and evolution. In the Azores, the “Azorean Biogeographical Paradox” (Ávila, 2006, 2013) constitutes one of the most puzzling conundrums in marine island biogeography. Despite the predominantly eastbound sea-surface circulation, most of the Azorean marine taxa show strong

biogeographic affinities to the eastern Atlantic and the Mediterranean Sea (Freitas et al., 2019; Meireles et al., 2014; Rebelo et al., 2021; for details see Baptista et al., 2021a). The processes and patterns arising from the mismatch between the biogeographical affinities and the predominant direction of sea-surface circulation become difficult to explain, especially for marine species with short-lived larvae (Ávila et al., 2000).

1.2 Geotectonic and oceanographical setting of the Azores Archipelago

In the Northeast Atlantic Ocean, five oceanic volcanic archipelagos – Azores, Madeira, Selvagens, Canaries, and Cabo Verde – emerge from the bottoms of the sea. The Azores is the most isolated archipelago in the central North Atlantic, ranging across the mid-ocean ridge between latitudes 36°55' and 39°43' N, and longitudes 24°46' and 31°16' W. These islands are distanced 1,370 km to the Iberian Peninsula on the East, 840 km to Madeira southeast, and 1,500 km to the nearest North American shelf (Newfoundland) on the West. Nine islands and several small islets spread over 620 km along a west-northwest to east-southeast axis, divided in three island groups: Eastern (Santa Maria and São Miguel), Central (Terceira, Graciosa, São Jorge, Pico, Faial), Western (Flores and Corvo) (Figure 1.2). These islands emerge from an ocean basin at depths of ~ 4,000 m and co-exist with over 400 seamounts scattered in the Azores basin, of which 63 are large-size structures (Morato et al., 2008; Sala et al., 2016). The archipelago is part of the 400,000 km² area designated as Azores Plateau, which spans approximately on both sides of the Mid-Atlantic Ridge (MAR) close to the triple junction of American, Eurasian and African plates and is characterized by elevated topography (< 2,000 m depth) and thickened oceanic crust (~14 km) (Adam et al., 2013; Hildenbrand et al., 2014; Métrich et al., 2014; Silveira et al., 2006). Formed over 13 myr, beginning 20 Ma, under processes still debated, it is most widely accepted that the formation of the Azores Plateau is a complex case of ridge-hotspot interaction (Adam et al., 2013; Gente et al., 2003; Silveira et al., 2006; Yang et al., 2006). Although sitting in a much older oceanic crust and with several older volcanic edifices fully submerged, the geological ages of subaerial edifices, i.e. emerged islands, vary between 0.186 Ma (Pico; Costa et al., 2015) and 6.01 Ma (Santa Maria; Ramalho et al., 2017).

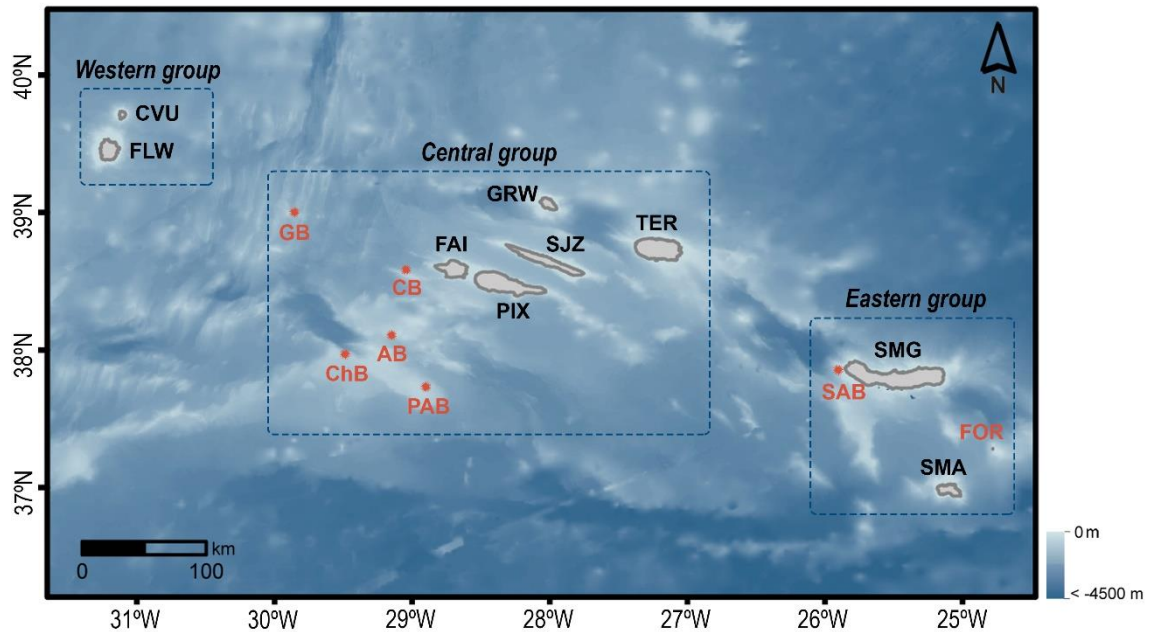


Figure 1.2. Geographical setting of the Azores Archipelago. Azorean islands, in black: SMA (Santa Maria), SMG (São Miguel/Sabrina), TER (Terceira), SJZ (São Jorge), GRW (Graciosa), PIX (Pico), FAI (Faial), FLW (Flores), and CVU (Corvo). Orange circles represent the seamounts sampled for this thesis: FOR (Formigas), SAB (Sabrina), PAB (Princess Alice Bank), AB (Azores Bank), ChB (Chino Bank), CB (Condor Bank), and GB (Gigante Bank). Island/seamount groups in the Azores (Eastern, Central, and Western groups) are delimited by dashed blue boxes. Coastline from the Portuguese Hydrographic Institute (<https://www.hidrografico.pt/op/33>) and bathymetry derived from GEBCO 2020 (https://www.gebco.net/data_and_products/gridded_bathymetry_data/).

Oceanographic circulation in the NE Atlantic basin is peculiar (Figure 1.3), as the North Atlantic's subtropical gyre is bisected by the Azores Current. The geostrophic circum-gyre circulation is composed of the Gulf Stream on the west, the North Atlantic Current system on the north, the Portugal and Canary Currents on the east, and finally, the North Equatorial Current on the south. Close to Cape Hatteras, located over 3,800 km west-southwest from the Azores, the Gulf Stream deflects into the North Atlantic Current system to the north and the Azores Current to the east in direction to the homonymous archipelago, later meandering southeast towards Madeira and the Gulf of Cadiz (Carracedo et al., 2014; Käse & Krauss, 1996; Klein & Siedler, 1989). In the southeastern area, the Azores Current transitions into the Canary Current (Carracedo et al., 2014; Machín et al., 2006), with its northern section being often designated as Madeira Current. Southward branches, three of which are occurring east of the Mid-Atlantic Ridge, are formed by mesoscale eddy activity that promotes recirculation into the central gyre (Klein & Siedler, 1989). East of the Azores, the Azores Current and associated Azores Front circulate eastwards between 30 and 37.5°N on monthly to decadal scales (mean $33.9 \pm 1.3^\circ\text{N}$) (Fründt & Waniek, 2012; Frazão et al., 2022). The

Azores Front-Current system defines the border between contrasting biogeochemical regimes: cold and highly productive temperate waters in the North from warm oligotrophic waters in the south (Frazão et al., 2022; Fründt & Waniek, 2012). The baroclinic instability along the Azores Current induces the formation of large mesoscale structures, with dimensions up to 200 km of diameter and lifetimes of 16 weeks. These structures are mostly anticyclonic north of the jet, whilst cyclonic to the south (i.e., propagating eastward and westward, respectively) (Frazão et al., 2022, Silva-Fernandes & Peliz, 2020). The recent slowdown of the Gulf Stream and Atlantic Meridional Overturning Circulation, detected since mid-20th century, are tightly connected and detectable in the behaviour and temporal variability of the Azores Front-Current system (Frazão et al., 2022). North of the Azores Front, the Azores Counter Current flows westward, mostly at subsurface levels and centred at around 36°N, apparently becoming stronger in Spring (Alves & Colin de Verdière, 1999; Carracedo et al., 2014; Comas-Rodriguez et al., 2011; Onken, 1993). Fine scale circulation patterns and hydrological conditions within the Azores Archipelago are, however, not yet fully comprehended but are probably intrinsically associated with upwelling and mixing processes (Sala et al., 2013, 2016; Silva et al., 2013). Lagrangian transport pathways in the NE Atlantic, through oceanic currents and associated eddies, meanders and filaments, dictate the dispersion and retention of particles at various levels of the water column in the Azores. Considering these pathways might be useful to understand potential rafting routes in the open ocean and role of islands in marine colonization and speciation (Sala et al., 2013, 2016).

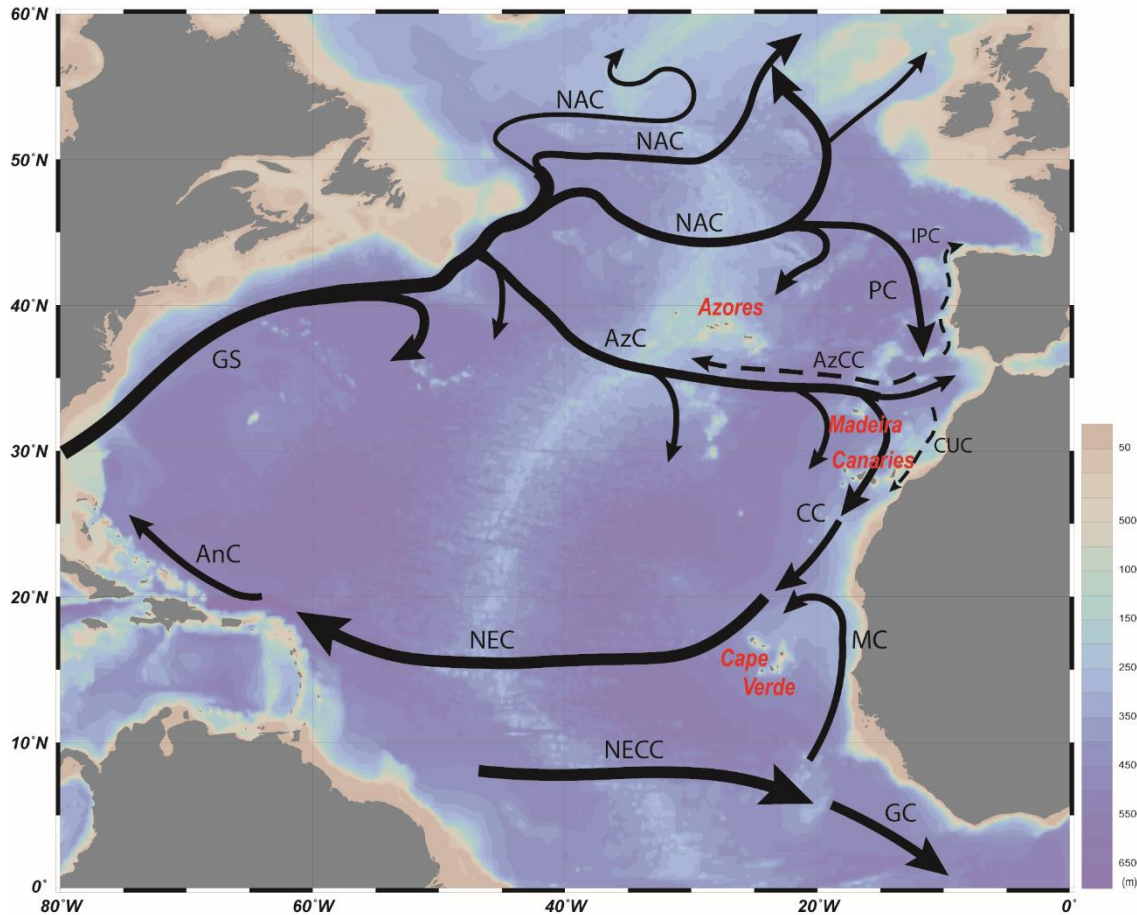


Figure 1.3. Major surface circulation patterns in the North and Central Atlantic Ocean. The NE archipelagos studied in this thesis are identified: Azores, Madeira, Canaries, and Cabo Verde. Gulf Stream recirculation and circulation north of 40°N based on Daniault et al. (2016) Shown as dashed lines are the seasonal Iberian Poleward Current and Canary Upwelling Current, as well as the Azores Counter Current predominantly as a subsurface flow. AnC – Antilles Current, AzC – Azores Current, AzCC – Azores Counter Current, CC – Canary Current, CUC – Canary Upwelling Current, GC – Guinea Current, GS – Gulf Stream, IPC – Iberian Poleward Current, MC – Madeira Current, NAC – North Atlantic Current, NEC – North Equatorial Current, NECC – North Equatorial Counter Current, PC – Portugal Current. Adapted from Baptista et al. (2021a).

1.3 Marine invertebrates as case study of biogeographical processes in remote islands

Invertebrates account for 97 % of all animal species on our planet, and nearly every phyla (31 out of 32) occur in the ocean (May, 1988; Zhang et al., 2021). The detailed study of invertebrates allows insights on the underlying evolutionary and phylogenetic molecular mechanisms of the kingdom Animalia (Zhang et al., 2021). Marine invertebrates can be ideal candidates for ecological and biogeographical studies, considering their diversity, abundance, and crucial roles played in ecosystems. A

panoply of life-histories is displayed across marine invertebrates, namely regarding their reproductive processes, usually short life cycles, and modes of development (Marshall et al., 2012; Strathmann, 1985). However, the variation of life-history traits even among closely related taxa suggests the overall absence of phylogenetic constraints, which is particularly evident in the duration and feeding ability of the larvae (Marshall et al., 2012; Strathmann, 1985). This characteristic turns marine invertebrates into powerful tools for biogeographical studies, as congruent biogeographical patterns are expected for different taxa sharing life-history traits. Despite the growing interest by the scientific community, there is still a negative bias on the knowledge and study of large-scale biogeographic patterns in the marine realm (Ávila et al., 2019).

1.3.1. Larval development and dispersal of marine invertebrates

Larval development of marine invertebrates was first classified from an ecological perspective by Thorson (1945, 1950) into several categories based mostly on the feeding mode and duration of the planktonic phase. Despite the following revisions, Thorson's studies on larval development remain the basis of classification nowadays, distinguishing developmental types in a simplistic view (Levin & Bridges, 1995) as: 1) planktotrophic larvae with a free-swimming feeding stage; 2) non-planktotrophic larvae, lacking the aforementioned stage, in which lecithotrophic larvae depend on yolk reserves for nutrition and energy; 3) direct development, hatching fully formed juveniles. The influence of the type and duration of larval development of marine invertebrates in their dispersal ability, with important biogeographic and evolutionary implications, was firstly proposed by Scheltema (1986, 1989, 1995) (but see also Levin & Bridges, 1995). Marine invertebrates with planktotrophic larvae can easily disperse and expand their distribution ranges by natural means during the free-feeding swimming stage, whereas non-planktotrophy constrains dispersal and restricted distribution ranges are expected (Ávila, 2006, 2013; Scheltema, 1986, 1989, 1995).

Dispersal of shallow-water marine invertebrates can be achieved by pelagic larvae or by phoresy of juveniles and adults (i.e., attachment to bird feathers, common in some intertidal molluscs). Moreover, mechanisms as drifting, rafting, hitchhiking, creeping, and hopping, such that successful dispersal of non-planktotrophic species depends on a panoply of biotic and abiotic factors [for a review see Winston (2012) and references therein]. Rafting occurs when egg-masses, juveniles or adults attach to floating biotic or abiotic materials, which in turn are transported by sea currents and trade winds (Ávila, 2013; Winston, 2012). Following extensive reviews by Thiel & Gutow (2005a, 2005b)

and Thiel & Haye (2006), it is known that rafter species usually share some life traits, namely small size, substratum and food generalist, asexual reproduction or direct development. Rafting constitutes the most significant process in temperate Atlantic waters and, in the open ocean, episodic routes provide unique opportunities to cross biogeographic barriers, acting as “pathways” for the dispersal of non-planktotrophic marine larvae and floating organisms (Ávila et al., 2009a, 2019; Scheltema, 1986; Thiel & Haye, 2006).

The complexity and diversity of life traits in marine invertebrates raises many biogeographic questions, especially in what regards the arrival of species with limited dispersal ability to remote areas (Ávila et al., 2019). Therefore, biogeographic and phylogeographic studies on marine invertebrates are important to clarify the biogeographical processes and patterns, the factors shaping them, and better understand marine life in remote islands. This thesis follows the need to expand biogeographic studies of marine invertebrates inhabiting remote islands in the NE Atlantic Ocean, with an emphasis on gastropods and bryozoans. The reasons behind the choice of these target groups are given below. The patterns retrieved in the studies are highly informative for the target groups, and the complementary interpretations allowed to get a wider perspective of the marine biogeography of remote NE Atlantic Archipelagos and expanded the knowledge on marine diversity and evolution in general.

1.3.2 Gastropods as case study

Gastropods are the most diverse class of the phylum Mollusca, having first appeared ~ 500 Ma and counting 75% of the ~ 110,000 currently known mollusc species occurring in marine, freshwater, and terrestrial environments (Brown & Lydeard, 2010; Frýda, 2021). Most marine gastropods are found in shallow-water benthic communities, occupying different habitats from the intertidal to subtidal (< 50 m) depths (Frýda, 2021). Evolutionary radiations and species complexes are widespread in marine gastropods, as is the case of *Ocenebrina aciculata* (Lamarck, 1822), *Talisman scrobilator* (Linnaeus, 1758), *Stramonita haemastoma* (Linnaeus, 1767), *Dendropoma petraeum* (Monterosato, 1884), genus *Conus* Linnaeus, 1758 in Cabo Verde, to name a few (Barco et al., 2018; Bowen et al., 2020; Calvo et al., 2009, 2015; Claremont et al., 2011; Crocetta et al., 2020; Cunha et al., 2005, 2008; Duda & Rólan, 2005; Smriglio et al., 2019). From a paleontological perspective, their calcified shell is prone to fossilization in high densities and numbers, providing crucial insights into their evolution and paleoecology (Ávila et al., 2019; Frýda, 2021). The duration of the larval stage can easily be inferred with the analyses of the protoconch in recent and fossil material (Jablonski & Lutz, 1980). Marine

gastropods are one of the preferred proxies to represent the evolutionary, ecological, and biogeographical patterns of marine invertebrates for the reasons above mentioned, coupled with their abundance and diversity in the littoral area of volcanic oceanic islands (Ávila et al., 2019; Frýda, 2021). Despite being mobile, long-distance dispersal ability of marine gastropods is intrinsically linked to their larval development and habitat occupied (see section 1.3.1). In that regard, the processes will affect intertidal and subtidal species differently, with influence in the biogeographical history.

In this thesis, several gastropod species were studied, as differences in their life-history traits are ideal to address the biogeographical questions posed. Representatives of the family Rissoidae, namely *Alvania mediolittoralis* Gofas, 1898, *A. sleursi* (Amati, 1987), and *Cingula trifasciata* (J. Adams, 1800), as well as pheasant shells of the genus *Tricolia* Risso, 1826 (family Phasianellidae), the red-mouthed rock shell *Stramonita haemastoma* (Linnaeus, 1767) (family Muricidae), and the top shell *Phorcus sauciatus* (Koch, 19845) (family Trochidae) were studied (Figure 1.4). All these species commonly occur in the Azores Archipelago, the main area of study (see section 1.2), and in neighbouring areas in the NE Atlantic and Mediterranean Sea. Moreover, as most of these species are restricted to intertidal habitats, their arrival to the Azores Archipelago could only have taken place after the first islands became emerged. Biogeographical analyses of these target species allowed to infer connectivity levels and population genetic structure, aiming a better understanding of the “Azorean Biogeographical Paradox” (Ávila, 2000, 2006, 2013). Finally, the target species display different modes of larval development (all non-planktotrophic except *S. haemastoma*), which allowed to compare biogeographical patterns among species with the same larvae type and between planktotrophic and non-planktotrophic species.

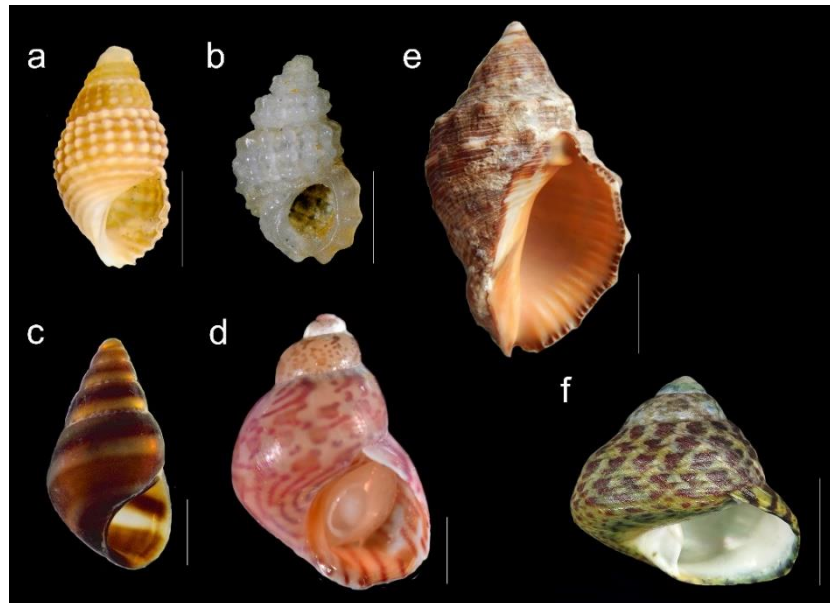


Figure 1.4. Gastropod species used to address biogeographical questions in the present thesis. **a.** *Alvania mediolittoralis* Gofas, 1898 (family Rissoidae); **b.** *A. sleursi* (Amati, 1987) (family Rissoidae); **c.** *Cingula trifasciata* (J. Adams, 1800) (family Rissoidae); **d.** genus *Tricolia* Risso, 1826 (family Phasianellidae); **e.** *Stramonita haemastoma* (Linnaeus, 1767) (family Muricidae); **f.** *Phorcus sauciatus* (Koch, 19845) (family Trochidae). Scale bar represents 1 mm in a-d and 1 cm in e-f.

1.3.3 Bryozoans as case study

Bryozoans are colonial, benthic, suspension-feeding invertebrates with modular construction that are ubiquitous in marine and freshwater environments from polar to tropical regions. Although composed of small zooids, usually under 1 mm in length, the colonies reach considerable sizes up to a few decimetres and are visible with the naked eye (Taylor, 2020). Marine bryozoans mostly occur in subtidal environments, attached to hard or flexible substrata, commonly rocks, shells, and plants or algae (Taylor, 2020). Around 80% of the over 6,500 known extant bryozoan species are assigned to the order Cheilostomatida, which first appeared during the Late Jurassic (Taylor, 1988). Bryozoans play crucial roles in the ecosystems by acting as bioconstructors and providing habitat for other invertebrates (Casoli et al., 2020; Cocito, 2004), by supplying food for micropredators (Lidgard, 2008), and by constituting carbonate sediments factories in shallow-water environments (Bone & James, 1993; Wisshak et al., 2015). Despite their importance in the ecosystems, bryozoans are still among the least studied metazoans, such that integrative taxonomic studies are needed to fully uncover and characterize their diversity.

Within the cheilostomatid family Phidoloporidae, the sessile genus *Reteporella* Busk, 1884 is the most speciose, with representatives living in shallow coastal to upper bathyal

benthic habitats worldwide, and with the erect, lace-like colonies attaching to hard substrata (Hayward & Ryland, 1999; Hayward & Winston, 2011) (Figure 1.5). In the Azores Archipelago, five *Reteporella* species have been historically reported – *R. atlantica* (Busk, 1884), *R. gracilis* (Jullien, 1903), *R. oceanica* (Jullien, 1903), *R. rara* (Jullien, 1903), and *R. tristis* (Jullien, 1903) – but new diversity is expected to be discovered in future studies. This genus was targeted for the present biogeographical studies due to its diversity and easy to spot three dimensional colonies. Moreover, *Reteporella* larvae have non-planktotrophic development, enabling a comparison of biogeographical patterns of Azorean gastropods with the same development and related dispersal restrictions.

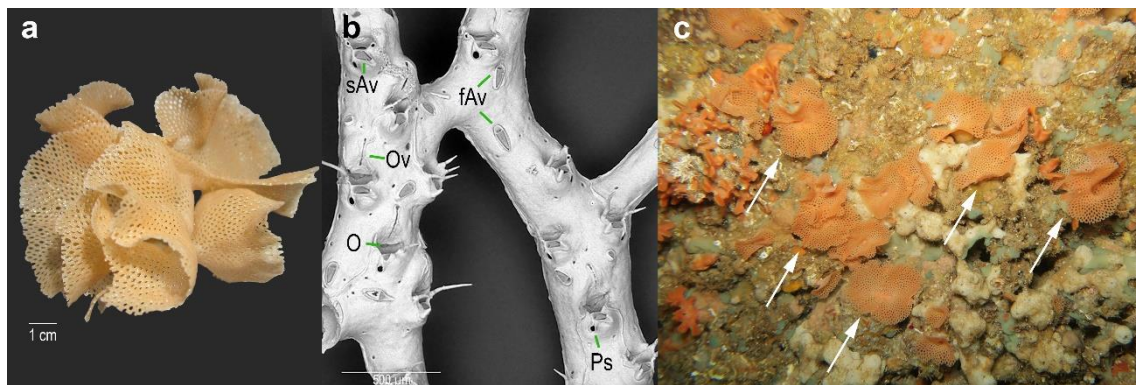


Figure 1.5. Genus *Reteporella* is used to address biogeographical questions in the Azores Archipelago. **a.** Colony of *Reteporella atlantica* sampled in shallow waters around Santa Maria Island. **b.** Scanning-electron microscopy image of *R. atlantica*, depicting several morphological structures often diagnostic at the species level: fAv – frontal avicularia, O – orifice, Ov – ovicell, Ps – pseudosinus, sAv – suboral avicularia. **c.** *Reteporella* colonies *in situ* in Pula, Croatia (Mediterranean Sea), in a similar condition as observed by scuba diving in Azorean shallow waters; photography by Sebastian Decker (Wien University).

Bryozoans were the sessile marine invertebrate group chosen for the biogeographical inferences in this thesis for their many advantages. Other sessile organisms commonly occur in the study area (Azores Archipelago and NE Atlantic) but were not chosen for several reasons. Barnacles (Crustacea: Cirripedia) of several families are characterized by an extended planktotrophic larval phase and are also commonly transported via fouling on the hull of ships or as larvae in ballast water, which promote the success of global dispersal of this group (Southward, 1998; Torres et al., 2012). In the Azores, corals and sponges usually form cold-water assemblages at depths of over 200 m (Rakka et al., 2021; Xavier et al., 2021). At these depths, sampling becomes a difficult and often opportunistic task (Braga-Henriques et al., 2013; Xavier et al., 2021). Therefore, ensuring an adequate sampling effort for population genetic analysis would not be possible.

Furthermore, barnacles and corals typically have a long larval phase, causing homogenization of the gene pool and erasing biogeographical signatures in smaller geographical scales of the remote islands, thus rendering them unsuitable candidates for the population genetic approaches aimed in this thesis.

1.4 Molecular approaches for the study of phylogenetic relationships and population genetics

The study of phylogenetic relationships, molecular variability and population genetic structure is a powerful tool to test biogeographical hypothesis and models with marine invertebrates. However, these depend on the characteristics of the molecular markers chosen for the analyses. Molecular tools reflect the gradual accumulation of modifications on the genome, their transmission mode throughout generations, and how evolutionary forces such as selection and gene flow shape diversity at various taxonomic levels and distinct resolution (Avice, 2004; Patwardhan et al., 2014). Therefore, accounting for the information contained within different molecular markers allows a more complete view of the evolutionary history of populations and species (Avice, 2004; Patwardhan et al., 2014). For the studies in the scope of this thesis, mitochondrial (mtDNA) and nuclear (nDNA) DNA fragments, plus microsatellites (SSR) were used to infer phylogeographic patterns and population genetic dynamics.

Mitochondrial DNA has historically been a powerful marker for evolutionary and population studies in animals due to its unique properties: uniparental (usually maternal) inheritance and absence of genetic recombination allow to trace each lineage as a single evolutionary history, whereas the fast evolutionary rate relative to other regions of the genome, susceptibility to the effects of genetic drift, and absence of introns [but exceptions in bryozoans were recently described by Jenkins et al. (2022)] allow intra- and interpopulation comparisons (Avice, 2004; Ladoukakis & Zouros, 2017; Yang et al., 2014). Its high copy number and existence of conserved regions among distant taxa allow the design of universal primers and inexpensive genetic studies, without the need of a priori knowledge on the target species' mtDNA (Ladoukakis & Zouros, 2017). Hebert et al. (2003) proposed the DNA barcoding approach, relying on intra- and interspecific genetic distances of a small mtDNA fragment for species identification across animal phyla. The protein-coding Cytochrome C Oxidase subunit I (COI) is the preferred barcode for intraspecific analyses and population dynamics inference in animals, as well as for the reconstruction of phylogenetic relationships at the specific and generic levels (Avice, 2004; Ladoukakis & Zouros, 2017; Patwardhan et al., 2014; see Radulovici et al.,

2010 for a review of DNA barcode on marine organisms). Other mitochondrial markers, as the conserved 16S ribosomal RNA (rRNA) gene, are also used in phylogenetic reconstructions, providing a broader perspective of the mitochondrial dynamics and inference of phylogenetic histories up to the family level (Patwardhan et al., 2014, Yang et al., 2014).

The inaccuracy of phylogenetic reconstructions solely inferred with mitochondrial markers can be addressed with the inclusion of nDNA markers in the dataset, characterized by biparental inheritance, a slower mutation rate that allows the existence of conserved and variable domains, and occurrence of recombination events at different rates (Patwardhan et al., 2014; Zhang & Hewitt, 2003). These properties make nDNA markers adequate to retrieve deeper evolutionary relationships among distantly related taxa, since they are less prone to homoplastic events which is not the case of fast evolving mtDNA markers (Zhang and Hewitt, 2003). The ribosomal nDNA markers 28S rRNA, particularly the widely studied D2 expansion region, and internal transcribed spacer 2 (ITS2) are characterized by longer sequences and formation of secondary structures, which can themselves be informative of evolutionary paths (Koetschan et al., 2010; Patwardhan et al., 2014).

Microsatellites, also known as simple sequence repeats (SSRs) or short tandem repeats (STRs) are iterations of tandem repeated nucleotides (1 to 6 bp), ubiquitous in the genome (Ellegren, 2004). With polymorphisms derived from variability in length rather than in the sequence, high heterozygosity levels, codominant nature, biparental inheritance, and existence of multiple alleles, SSRs are powerful markers for the inference of population genetics dynamics and intraspecific studies (Bhargava & Fuentes, 2010; Ellegren, 2004; Putman & Carbone, 2014). Advancing sequencing technologies transformed the discovery and genotyping of SSRs, as well as their application into new systems and scientific research questions in a fast and cost-effective way (Castoe et al., 2012; Curto et al., 2019; Darby et al., 2016; De Barba et al., 2016; Farrell et al., 2016; Guichoux et al., 2011). A higher statistical power and detailed inferences of population genetic structure even with a low sample size (individuals and/or markers) and understudied non-model species are now possible to attain resorting to next-generation sequencing (Darby et al., 2016; De Barba et al., 2016; Farrell et al., 2016; Guichoux et al., 2011; Vartia et al., 2016). The recent SSR-GBAS (Simple Sequencing Repeats—Genotyping by Amplicon Sequencing) workflow calls alleles based on the whole amplicon information (WAI) of the loci analyses, including the flanking region, allele length (AL) and potential SNPs (Single Nucleotide Polymorphism). This approach improves the overall resolution of the SSR dataset, resulting in a higher

number of alleles recovered, increased marker variability, information content, and lower homoplasy (De Barba et al., 2016; Curto et al., 2019; Vartia et al., 2016). A pipeline for analysis of SSR-GBAS data was originally developed by De Barba et al. (2016), and several others were later developed. Curto et al. (2019) described a pipeline that differs by allowing manual correction of the fragment size during a first filtering step that reduces the complexity of the dataset for genotyping, a source of powerful data for biogeographical inferences. This pipeline can also be consulted in detail in Tibihika et al. (2019), Lanner et al. (2021), and Baptista et al. (2021b).

References

- Adam, C., Madureira, P., Miranda, J.M., Lourenço, N., Yoshida, M., & Fitzenz, D. (2013). Mantle dynamics and characteristics of the Azores plateau. *Earth and Planetary Science Letters*, 362, 258–271. <https://doi.org/10.1016/j.epsl.2012.11.014>
- Aguilée, R., Pellerin, F., Soubeyrand, M., Choin, J., & Thébaud, C. (2021). Biogeographic drivers of community assembly on oceanic islands: The importance of archipelago structure and history. *Journal of Biogeography*, 48(10), 2616–2628. <https://doi.org/10.1111/JBI.14228>
- Alves, M. L., & Colin de Verdière, A. (1999). Instability dynamics of a subtropical jet and applications to the Azores Front Current System: eddy-driven mean flow. *Journal of Physical Oceanography*, 29(5), 837–864.
- Ávila, S. P. (2000). Shallow-water marine molluscs of the Azores: biogeographical relationships. *Arquipélago. Life and Marine Sciences, Supplement 2(Part A)*, 99–131.
- Ávila, S. P. (2006). Oceanic islands, rafting, geographical range and bathymetry: a neglected relationship. In *Proceedings of the 5th International Symposium on the Fauna and Flora of Atlantic Islands, Vol. 9*, 22–39. Irish Biogeographical Society.
- Ávila, S. P. (2013). Unravelling the patterns and processes of evolution of marine life in oceanic islands: a global framework. In Fernández-Palacios, JM, Nascimento, L, Hernández, JC, Clemente, S., González, A & Díaz-González, JP (Eds.) *Climate Change perspectives from the Atlantic: past, present and future*. Universidad de La Laguna, Tenerife, 95–125.
- Ávila, S. P., Cordeiro, R., Madeira, P., Silva, L., Medeiros, A., Rebelo, A. C., Melo, C., Neto, A. I., Haroun, R., Monteiro, A., Rijdsdijk, K., & Johnson, M. E. (2018). Global change impacts on large-scale biogeographic patterns of marine organisms on Atlantic oceanic islands. *Marine Pollution Bulletin*, 126, 101–112. <https://doi.org/10.1016/j.marpolbul.2017.10.087>
- Ávila, S. P., Melo, C., Berning, B., Nuno, S., Quartau, R., Rijdsdijk, K. F., Ramalho, R. S., Cordeiro, R., De Sá, N. C., Pimentel, A., Baptista, L., Medeiros, A., Gil, A., & Johnson, M. E. (2019). Towards a ‘Sea-Level Sensitive’ dynamic model: impact of island ontogeny and glacio-eustasy on global patterns of marine island biogeography. *Biological Reviews*, 94(3), 1116–1142. <https://doi.org/10.1111/brv.12492>
- Ávila, S. P., Melo, C., Silva, L., Ramalho, R. S., Quartau, R., Hipólito, A., Cordeiro, R., Rebelo, A. C., Madeira, P., Rovere, A., Hearty, P. J., Henriques, D., Silva, C. M. da, Martins, A. M. de F., & Zazo, C. (2015). A review of the MIS 5e highstand deposits from Santa Maria Island (Azores, NE Atlantic): Palaeobiodiversity, palaeoecology and palaeobiogeography. *Quaternary Science Reviews*, 114, 126–148. <https://doi.org/10.1016/j.quascirev.2015.02.012>
- Ávila, S. P., Silva, C. M. da, Schiebel, R., Cecca, F., Backeljau, T., & Martins, A. M. F. (2009). How did they get here? Palaeobiogeography of the Pleistocene marine molluscs of the Azores. *Bulletin of the Geological Society of France*, 180, 201–213.
- Avice, J.C. (2004). *Molecular markers, natural history and evolution*. 2nd edition. Sinauer Associates Inc., Sunderland (Massachusetts). xv+684 pp.
- Baptista, L., Meimberg, H., Ávila, S. P., Santos, A. M., & Curto, M. (2021b). Dispersal ability,

- habitat characteristics, and sea-surface circulation shape population structure of *Cingula trifasciata* (Gastropoda: Rissoidae) in the remote Azores Archipelago. *BMC Ecology and Evolution*, 21(1), 128. <https://doi.org/10.1186/s12862-021-01862-1>
- Baptista, L., Santos, A. M., Cabezas, M. P., Cordeiro, R., Melo, C., & Ávila, S. P. (2019). Intertidal or subtidal/circalittoral species: which appeared first? A phylogenetic approach to the evolution of non-planktotrophic species in Atlantic Archipelagos. *Marine Biology*, 166, 1–16. <https://doi.org/10.1007/s00227-019-3536-y>
- Baptista, L., Santos, A. M., Melo, C. S., Rebelo, A. C., Madeira, P., Cordeiro, R., Botelho, A. Z., Hipólito, A., Pombo, J., Voelker, A. H. L., & Ávila, S. P. (2021a). Untangling the origin of the newcomer *Phorcus sauciatu*s (Mollusca: Gastropoda) in a remote Atlantic archipelago. *Marine Biology*, 168(1), Article number: 9. <https://doi.org/10.1007/s00227-020-03808-5>
- Barco, A., Aissaoui, C., Houart, R., Bonomolo, G., Crocetta, F., & Oliverio, M. (2018). Revision of the *Ocenebrina aciculata* species complex (Mollusca: Gastropoda: Muricidae) in the northeastern Atlantic Ocean and Mediterranean Sea. *Journal of Molluscan Studies*, 84, 19–29. <https://doi.org/10.1093/MOLLUS/EYX039>
- Barroso, C. X., Lotufo, T. M. C., Bezerra, L. E. A., & Matthews-Cascon, H. (2016). A biogeographic approach to the insular marine ‘prosobranch’ gastropods from the southwestern Atlantic Ocean. *Journal of Molluscan Studies*, 82, 558–563. <https://doi.org/10.1093/mollus/eyw015>
- Bhargava, A., & Fuentes, F. (2010). Mutational Dynamics of Microsatellites. *Molecular Biotechnology*, 44, 250–266. <https://doi.org/10.1007/s12033-009-9230-4>
- Bone, Y., & James, N. P. (1993). Bryozoans as carbonate sediment producers on the coolwater Lacedpede Shelf, southern Australia. *Sedimentary Geology*, 86, 247–271.
- Borregaard, M. K., Amorim, I. R., Borges, P. A. V., Cabral, J. S., Fernández-Palacios, J. M., Field, R., Heaney, L. R., Kreft, H., Matthews, T. J., Olesen, J. M., Price, J., Rigal, F., Steinbauer, M. J., Triantis, K. A., Valente, L., Weigelt, P., & Whittaker, R. J. (2017). Oceanic Island biogeography through the lens of the general dynamic model: assessment and prospect. *Biological Reviews*, 92(2), 830–853. <https://doi.org/10.1111/brev.12256>
- Bowen, B. W., Forsman, Z. H., Whitney, J. L., Faucci, A., Hoban, M., Canfield, S. J., Johnston, E. C., Coleman, R. R., Copus, J. M., Vicente, J., & Toonen, R. J. (2020) Species radiations in the sea: what the flock? *Journal of Heredity*, 111, 70–83. <https://doi.org/10.1093/jhered/esz075>
- Braga-Henriques, A., Porteiro, F. M., Ribeiro, P. A., de Matos, V., Sampaio, I., Ocaña, O., & Santos, R. S. (2013). Diversity, distribution and spatial structure of the cold-water coral fauna of the Azores (NE Atlantic). *Biogeosciences Discussions*, 10, 529–590. <https://doi.org/10.5194/bgd-10-529-2013>
- Brown, K.M., & Lydeard, C. (2010). Chapter 10 - Mollusca: Gastropoda. In Thorp, J.H., Covich, A.P. (Eds.) *Ecology and Classification of North American Freshwater Invertebrates*. 3rd edition, Academic Press, pp. 277–306. <https://doi.org/10.1016/B978-0-12-374855-3.00010-8>
- Calvo, M., Alda, F., Oliverio, M., Templado, J., & Machordom, A. (2015). Surviving the Messinian Salinity Crisis? Divergence patterns in the genus *Dendropoma* (Gastropoda: Vermetidae) in

- the Mediterranean Sea. *Molecular Phylogenetics and Evolution*, 91, 17–26.
<https://doi.org/10.1016/J.YMPEV.2015.05.004>
- Calvo, M., Templado, J., Oliverio, M., & MacHordom, A. (2009). Hidden Mediterranean biodiversity: molecular evidence for a cryptic species complex within the reef building vermetid gastropod *Dendropoma petraeum* (Mollusca: Caenogastropoda). *Biological Journal of the Linnean Society*, 96, 898–912. <https://doi.org/10.1111/J.1095-8312.2008.01167.X>
- Carracedo, L. I., Gilcoto, M., Mercier, H., & Pérez, F. F. (2014). Progress in Oceanography Seasonal dynamics in the Azores – Gibraltar Strait region : A climatologically-based study. *Progress in Oceanography*, 122, 116–130. <https://doi.org/10.1016/j.pocean.2013.12.005>
- Casoli, E., Piazzzi, L., Nicoletti, L., Jona-Lasinio, G., Cecchi, E., Mancini, G. Belluscio, A., & Ardizzone, G. (2020). Ecology, distribution and demography of erect bryozoans in Mediterranean coralligenous reefs. *Estuarine, Coastal and Shelf Science*, 235, 106573. <https://doi.org/10.1016/j.ecss.2019.106573>
- Castoe, T. A., Poole, A. W., de Koning, A. P. J., Jones, K. L., Tomback, D. F., Oyler-McCance, S. J., Fike, J. A., Lance, S. L., Streicher, J. W., Smith, E. N., & Pollock, D. D. (2012). Rapid microsatellite identification from illumina paired-end genomic sequencing in two birds and a snake. *PLoS ONE*, 7(2), e30953. <https://doi.org/10.1371/journal.pone.0030953>
- Claremont, M., Williams, S.T., Barraclough, T.G., & Reid, D.G. (2011). The geographic scale of speciation in a marine snail with high dispersal potential. *Journal of Biogeography*, 38, 1016–1032. <https://doi.org/10.1111/j.1365-2699.2011.02482.x>
- Cocito, S. (2004). Bioconstruction and biodiversity: their mutual influence. *Scientia Marina*, 68(Suppl. 1), 137–144.
- Comas-Rodríguez, I., Hernández-Guerra, A., Fraile-Nuez, E., Martínez-Marrero, A., Benítez-Barrios, V. M., Pérez-Hernández, M. D., & Vélez-Belchí, P. (2011). The Azores Current System from a meridional section at 24.5 W. *Journal of Geophysical Research: Oceans*, 116(C9). <https://doi.org/10.1029/2011jc007129>
- Costa, A. C. G., Hildenbrand, A., Marques, F. O., Sibrant, A. L. R., & de Campos, A. S. (2015). Catastrophic flank collapses and slumping in Pico Island during the last 130 kyr (Pico-Faial ridge, Azores Triple Junction). *Journal of Volcanology and Geothermal Research*, 302, 33–46. <https://doi.org/10.1016/j.jvolgeores.2015.06.008>
- Crocetta, F., Caputi, L., Paz-Sedano, S., Tanduo, V., Vazzana, A., & Oliverio, M. (2020). High genetic connectivity in a gastropod with long-lived planktonic larvae. *Journal of Molluscan Studies*, 86(1), 42–55. <http://dx.doi.org/10.1093/mollus/eyz032>
- Cunha, R.L., Castilho, R., Rüber, L., & Zardoya, R. (2005). Patterns of cladogenesis in the venomous marine gastropod genus *Conus* from the Cape Verde islands. *Systematic Biology*, 54, 634–650
- Cunha, R.L., Tenorio, M.J., Afonso, C., Castilho, R., & Zardoya, R. (2008). Replaying the tape: recurring biogeographical patterns in Cape Verde *Conus* after 12 million years. *Molecular Ecology*, 17, 885–901
- Curto, M., Winter, S., Seiter, A., Schmid, L., Scheicher, K., Barthel, L. M. F., Plass, J., &

- Meimberg, H. (2019). Application of a SSR-GBS marker system on investigation of European Hedgehog species and their hybrid zone dynamics. *Ecology and Evolution*, 9(5), 2814–2832. <https://doi.org/10.1002/ece3.4960>
- Darby, B. J., Erickson, S. F., Hervey, S. D., & Ellis-felege, S. N. (2016). Digital fragment analysis of short tandem repeats by high-throughput amplicon sequencing. *Ecology and Evolution*, 6(13), 4502–4512. <https://doi.org/10.1002/ece3.2221>
- Darwin, C.R., 1859. *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*. 1st edition, John Murray, London.
- de Barba, M., Miquel, C., Lobréaux, S., Quenette, P. Y., Swenson, J. E., & Taberlet, P. (2016). High-throughput microsatellite genotyping in ecology: improved accuracy, efficiency, standardization and success with low-quantity and degraded DNA. *Molecular Ecology Resources*, 17(3), 492–507. <https://doi.org/10.1111/1755-0998.12594>
- Duda, T. F., & Rólan, E. (2005). Explosive radiation of Cape Verde *Conus*, a marine species flock. *Molecular Ecology*, 14(1), 267–273. <http://dx.doi.org/10.1111/j.1365-294X.2004.02397.x>
- Ellegren, H. (2004). Microsatellites: simple sequences with complex evolution. *Nature Reviews Genetics*, 5(6), 435–445. <https://doi.org/10.1038/nrg1348>
- Farrell, E. D., Carlsson, J. E. L., & Carlsson, J. (2016). Next gen pop gen: Implementing a high-throughput approach to population genetics in boarfish (*Capros aper*). *Royal Society Open Science*, 3(12), 160651. <https://doi.org/10.1098/rsos.160651>
- Fernández-Palacios, J. M., Kueffer, C., & Drake, D. R. (2015.) A new golden era in Island biogeography. *Frontiers of Biogeography*, 7(1), 14–20. <https://doi.org/10.21425/F57124493>
- Fernández-Palacios, J. M., Ridjdsdijk, K. F., Norder, S. J., Otto, R., de Nascimento, L., Fernández-Lugo, S., Tjørve, E., & Whittaker, R. J. (2016). Towards a glacial-sensitive model of Island biogeography. *Global Ecology and Biogeography*, 25, 817–830. <https://doi.org/10.1111/geb.12320>
- Frazão, H. C., Prien, R. D., Schulz-Bull, D. E., Seivod, D., & Waniek, J. J. (2022). The forgotten Azores Current: a long-term perspective. *Frontiers in Marine Science*, 628: 842251. <https://doi.org/10.3389/FMARS.2022.842251>
- Freitas, R., Romeiras, M., Silva, L., Cordeiro, R., Madeira, P., González, J. A., Wirtz, P., Falcón, J. M., Brito, A., Floeter, S. R., Afonso, P., Porteiro, F., Viera-Rodríguez, M. A., Neto, A. I., Haroun, R., Farminhão, J. N. M., Rebelo, A. C., Baptista, L., Melo, C. S., Martínez, A., Núñez, J., Berning, B., Johnson, M. E., Ávila, S. P. (2019). Restructuring of the ‘Macaronesia’ biogeographic unit: A marine multi-taxon biogeographical approach. *Scientific Reports*, 9(1), 1–18. <https://doi.org/10.1038/s41598-019-51786-6>
- Fründt, B., & Waniek, J. J. (2012). Impact of the Azores Front propagation on deep ocean particle flux. *Central European Journal of Geosciences*, 4(4), 531–544. <https://doi.org/10.2478/s13533-012-0102-2>
- Frýda, J. 2021. Gastropods. In Alderton, D., Elias, S.A. (Eds.) *Encyclopedia of Geology*. 2nd edition, Academic Press, pp.299–310. <https://doi.org/10.1016/B978-0-08-102908-4.00193-4>

- Gente, P., Dymont, J., Maia, M., & Goslin, J. (2003). Interaction between the Mid-Atlantic Ridge and the Azores hot spot during the last 85 Myr: Emplacement and rifting of the hot spot-derived plateaus. *Geochemistry, Geophysics, Geosystems*, 4(10).
- Guichoux, E., Lagache, L., Wagner, S., Chaumeil, P., Léger, P., Lepais, O., Lepoittevin, C., Malausa, T., Revardel, E., Salin, F., & Petit, R. J. (2011). Current trends in microsatellite genotyping. *Molecular Ecology Resources*, 11(4), 591–611. <https://doi.org/10.1111/j.1755-0998.2011.03014.x>
- Hachich, N. F., Bonsall, M. B., Arraut, E. M., Barneche, D.R., Lewinsohn, T. M., & Floeter, S. R. (2015). Island biogeography: patterns of marine shallow-water organisms in the Atlantic Ocean. *Journal of Biogeography*, 42, 1871–1882. <https://doi.org/10.1111/jbi.12560>
- Hachich, N. F., Ferrari, D. S., Quimbayo, J. P., Pinheiro, H. T., & Floeter, S. R. (2020). Island biogeography of marine shallow-water organisms. In *Encyclopedia of the World's Biomes* (Vols. 1–5, pp. 61–75). Elsevier. <https://doi.org/10.1016/B978-0-12-409548-9.11947-5>
- Hart, P.J.B., & Pearson, E. (2011). An application of the theory of Island biogeography to fish speciation on seamounts. *Marine Ecology Progress Series*, 430, 281–288. <https://doi.org/10.3354/meps08948>
- Hawkins, S. J., Corte-Real, H. B. S. M., Pannacciulli, F. G., Weber, L. C., & Bishop, J. D. D. (2000). Thoughts on the ecology and evolution of the intertidal biota of the Azores and other Atlantic islands. *Hydrobiologia*, 440, 3–17. <https://doi.org/10.1023/A:1004118220083>
- Hayward P. J., & Ryland, J. S. (1996). Some British Phidoloporidae (Bryozoa: Cheilostomatida). *Zoological Journal of the Linnean Society*, 117, 103–112.
- Hayward P. J., & Winston, J. E. (2011). Bryozoa collected by the United States Antarctic Research Program: new taxa and new records. *Journal of Natural History*, 45, 2259–2338. <https://doi.org/10.1080/00222933.2011.574922>
- Hebert, P. D. N., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, 270(1512), 313–332. <https://doi.org/10.1098/rspb.2002.2218>
- Hildenbrand, A., Weis, D., Madureira, P., Marques, F. O. (2014). Recent plate re-organization at the Azores Triple Junction: Evidence from combined geochemical and geochronological data on Faial, S. Jorge and Terceira volcanic islands. *Lithos*, 210-211, 27-39. <https://doi.org/10.1016/j.lithos.2014.09.009>
- Hirahara, S., Ishii, M., & Fukuda, Y. (2014). Centennial-scale sea surface temperature analysis and its uncertainty. *Journal of Climate*, 27(1), 57–75. <https://doi.org/10.1175/JCLI-D-12-00837.1>
- Hooker, J. D. (1862). Outlines of the distribution of Arctic plants. *Transactions of the Linnean Society*, 23, 251–348.
- Jablonski, D., & Lutz, R. A. (1980). Molluscan larval shell morphology: ecological and paleontological applications. In Rhoads, D. C., Lutz, R. A. (Eds.) *Skeletal Growth of Aquatic Organisms*. Plenum, New York, pp. 323–377.

- Jenkins, H. L., Graham, R., Porter, J. S., Vieira, L. M., Almeida, A. C. S., Hall, A., O'Dea, A., Coppard, S. E., & Waeschenbach, A. (2022). Unprecedented frequency of mitochondrial introns in colonial bilaterians. *Scientific Reports*, 12, 10889. <https://doi.org/10.1038/s41598-022-14477-3>
- Käse, R. H., & Krauss, W. (1996). The Gulf Stream, the North Atlantic Current, and the origin of the Azores Current. In W. Krauss (Ed.), *The warmwatersphere of the North Atlantic Ocean* (pp. 291–337). Gebrüder Borntraeger.
- Klein, B., & Siedler, G. (1989). On the origin of the Azores Current. *Journal of Geophysical Research: Oceans*, 94, 6159–6168. <https://doi.org/10.1029/JC094iC05p06159>
- Koetschan, C., Förster, F., Keller, A., Schleicher, T., Ruderisch, B., Schwarz, R., Müller, T., Wolf, M., & Schultz, J. (2010). The ITS2 Database III Sequences and structures for phylogeny. *Nucleic Acids Research*, 38, D275–D279. <https://doi.org/10.1093/nar/gkp966>
- Ladoukakis, E. D., & Zouros, E. (2017). Evolution and inheritance of animal mitochondrial DNA: rules and exceptions. *Journal of Biological Research-Thessaloniki*, 24(1), 1–7. <https://doi.org/10.1186/s40709-017-0060-4>
- Lanner, J., Gstöttenmayer, F., Curto, M., Geslin, B., Huchler, K., Orr, M. C., Pachinger, B., Sedivy, C., & Meimberg, H. (2021). Evidence for multiple introductions of an invasive wild bee species currently under rapid range expansion in Europe. *BMC Ecology and Evolution*, 21, 71. <https://doi.org/10.1186/s12862-020-01729-x>
- Levin, L., & Bridges, T. (1995). Pattern and diversity in reproduction and development. In McEdward, L (Ed.) *Marine invertebrate larvae*. CRC Press, Oxford, 1–47.
- Lidgard, S., Carter, M. C., Dick, M. H., Gordon, D. P., & Ostrovsky, A. N. (2005). Division of labor and recurrent evolution of polymorphisms in a group of colonial animals. *Evolutionary Ecology*, 26, 233–257. <https://doi.org/10.1007/s10682-011-9513-7>
- MacArthur, R.H., & Wilson, E.O. (1967). *The Theory of Island Biogeography*. Princeton University Press, Princeton.
- Machín, F., Hernández-Guerra, A., & Pelegrí, J. L. (2006). Mass fluxes in the Canary Basin. *Progress in Oceanography*, 70(2-4), 416–447. <https://doi.org/10.1016/j.pocean.2006.03.019>
- Marshall, D. J., Krug, P. J., Kupriyanova, E., Byrne, M., & Emlet, R.B. (2012). The Biogeography of Marine Invertebrate Life Histories. *Annual Review of Ecology and Systematics*, 43, 97–114. <http://dx.doi.org/10.1146/annurev-ecolsys-102710-145004>
- May, R. M. (1988). How many species are there on earth? *Science*, 241, 1441–1449.
- Melo, C. S., Martín-González, E., Silva, C.M., Galindo, I., González-Rodríguez, A., Baptista, L., Rebelo, A. C., Madeira, P., Voelker, A. H. L., Johnson, M. E., Arruda, S. A., & Ávila, S.P. (2022a). Range expansion of tropical shallow-water marine molluscs in the NE Atlantic during the Last Interglacial (MIS 5e): causes, consequences and utility of ecostratigraphic indicators for the Macaronesian archipelagos. *Quaternary Science Reviews*, 278, 107377. <https://doi.org/10.1016/j.quascirev.2022.107377>
- Melo, C. S., Martín-González, E., Silva, C. M. da, Galindo, I., González-Rodríguez, A., Baptista, L., Rebelo, A. C., Madeira, P., Voelker, A. H. L., Johnson, M. E., Arruda, S. A., & Ávila, S. P.

- (2022b) Reply to the comment by Meco et al. on “Range expansion of tropical shallow-water marine molluscs in the NE Atlantic during the last interglacial (MIS 5e): Causes, consequences and utility of ecostratigraphic indicators for the Macaronesian archipelagos”. *Quaternary Science Reviews*, 288, 107535. <https://doi.org/10.1016/j.quascirev.2022.107535>
- Métrich, N., Zanon, V., Créon, L., Hildenbrand, A., Moreira, M., & Marques, F.O. (2014). Is the “Azores hotspot” a wetspot? Insights from the geochemistry of fluid and melt inclusions in olivine of Pico basalts. *Journal of Petrology*, 55(2), 377–393.
- Morato, T., Machete, M., Kitchingman, A., Tempera, F., Lai, S., Menezes, G., Pitcher, T. J., & Santos, R. S. (2008). Abundance and distribution of seamounts in the Azores. *Marine Ecology Progress Series*, 357, 17–21. <https://doi.org/10.3354/meps07268>
- Onken, R. (1993). The Azores Countercurrent. *Journal of Physical Oceanography*, 23(8), 1638–1646.
- Patiño, J., Whittaker, R. J., Borges, P. A. V., Fernández-Palacios, J. M., Ah-Peng, Claudine Araújo, M. B., Ávila, S. P., Cardoso, P., Cornuault, J., de Boer, E. J., de Nascimento, L., Gil, A., González-Castro, A., Gruner, D. S., Heleno, R., Hortal, J., Illera, J. C., Kaiser-Bunbury, C. N., Matthews, T. J., Papadopoulou, A., Pettorelli, N., Price, J. P., Santos, A. M. C., Steinbauer, M. J., Triantis, K. A., Valente, L., Vargas, P., Weigelt, P., & Emerson, B.C. (2017). A roadmap for island biology: 50 fundamental questions after 50 years of The Theory of Island Biogeography. *Journal of Biogeography*, 44, 963–983. <https://doi.org/10.1038/10.1111/jbi.12986>
- Patwardhan, A., Ray, S., & Roy, A. (2014). Molecular markers in phylogenetic studies – a review. *Journal of Phylogenetics & Evolutionary Biology*, 2. <https://doi.org/10.4172/2329-9002.1000131>
- Pinheiro, H., Bernardi, G., Simon, T., Joyeux, J.-C., Macieira, R. M., Gasparini, J. L., Rocha, C., Rocha, L. A. (2017). Island biogeography of marine organisms. *Nature*, 549(7670), 82–85. <https://doi.org/10.1038/nature23680>
- Putman, A. I., & Carbone, I. (2014). Challenges in analysis and interpretation of microsatellite data for population genetic studies. *Ecology and Evolution*, 4(22), 4399–4428. <https://doi.org/10.1002/ece3.1305>
- Radulovici A. E., Archambault P., & Dufresne F. (2010). DNA Barcodes for Marine Biodiversity: Moving Fast Forward? *Diversity*, 2, 450–472. <https://doi.org/10.3390/d2040450>
- Rakka, M., Sampaio, I., Colaço, A., & Carreiro-Silva, M. (2021). Reproductive biology of two deep-sea octocorals in the Azores Archipelago. *Deep Sea Research Part I: Oceanographic Research Papers*, 175, 103587. <https://doi.org/10.1016/j.dsr.2021.103587>
- Ramalho, R. S., Helffrich, G., Madeira, J., Cosca, M., Thomas, C., Quartau, R., Hipólito, A., Rovere, A., Hearty, P. J., & Ávila, S. P. (2017). Emergence and evolution of Santa Maria Island (Azores) – The conundrum of uplifted islands revisited. *The Geological Society of America Bulletin*, 129(3-4), 372-390.
- Ramalho, R. S., Quartau, R., Trenhaile, A.S., Mitchell, N. C., Woodroffe, C. D., & Ávila, S. P. (2013). Coastal evolution in oceanic islands: a complex interplay between volcanism, erosion,

- sedimentation and biogenic production. *Earth Science Reviews* 127, 140–170. <https://doi.org/10.1016/j.earscirev.2013.10.007>
- Rijsdijk, K. F., Hengl, T., Norder, S., Otto, R., Emmerson, B. C., Ávila, S. P., López, H., van Loon, E. E., Tjørve, E., & Fernández–Palacios, J. M. (2014). Quantifying surface area changes of volcanic islands driven by Pleistocene Sea level cycles: biogeographic implications for Macaronesian archipelagos, Atlantic Ocean. *Journal of Biogeography*, 41, 1242–1254. <https://doi.org/10.1111/jbi.12336>
- Sala, I., Caldeira, R. M. A., Estrada-Allis, S. N., Froufe, E., & Couvelard, X. (2013). Lagrangian transport pathways in the northeast Atlantic and their environmental impact. *Limnology and Oceanography: Fluids and Environments*, 3, 40–60 <http://dx.doi.org/10.1215/21573689-2152611>
- Sala, I., Harrison, C. S., & Caldeira, R. M. A. (2016). The role of the Azores Archipelago in capturing and retaining incoming particles. *Journal of Marine Systems*, 154, 146–156. <https://doi.org/10.1016/j.jmarsys.2015.10.001>
- Santos, A. M. C., Field, R., & Ricklefs, R.E. (2016). New directions in Island biogeography. *Global Ecology and Biogeography*, 25, 751–768. <https://doi.org/10.1111/geb.12477>
- Scheltema, R. S. (1986). On dispersal and planktonic larvae of benthic invertebrates: An eclectic overview and summary of problems. *Bulletin of Marine Science*, 39(2), 290–322.
- Scheltema, R. S. (1989). Planktonic and non-planktonic development among prosobranch gastropods and its relationship to the geographic range of species. In J. S. Ryland & P. A. Tyles (Eds.), *Reproduction, Genetics and Distribution of Marine Organisms* (pp. 183–188). Fredensborg: Olsen and Olsen.
- Scheltema, R. S. (1995). The relevance of passive dispersal for the biogeography of Caribbean mollusks. *American Malacological Bulletin*, 11, 99–115.
- Silva, A., Brotas, V., Valente, A., Sá, C., Diniz, T., Patarra, R. F., Álvaro, N. V., & Neto, A. I. (2013). Coccolithophore species as indicators of surface oceanographic conditions in the vicinity of Azores islands. *Estuarine, Coastal and Shelf Science*, 118, 50–59. <https://doi.org/10.1016/j.ecss.2012.12.010>
- Silva-Fernandes, S. M., & Peliz, A. J. (2020). The turbulent structure of the Azores Current system: a statistical analysis. *Journal of Geophysical Research: Oceans*, 125, e2020JC016327. <https://doi.org/10.1029/2020JC016327>
- Silveira, G., Stutzmann, E., Davaille, A., Montagner, J.-P., Mendes-Victor, L., & Sebai, A. (2006). Azores hotspot signature in the upper mantle. *Journal of Volcanology and Geothermal Research*, 156(1), 23–34.
- Smriglio, C., Furfaro, G., Trillò, P., Appolloni, M., & Mariottini, P. (2019). A review of the Atlantic-Mediterranean *Bursa scrobilator* (Linnaeus, 1758) species complex. *Molluscan Research*, 39, 341–354. <https://doi.org/10.1080/13235818.2019.1600397>
- Southward, A.J. (1998). New observations on barnacles (Crustacea: Cirripedia) of the Azores region. *Arquipélago. Life and Marine Sciences*, 16A, 11–27.

- Spalding, M. D., Fox, H. E., Allen, G. R., Davidson, N., Ferdaña, Z.A., Finlayson, M., Halpern, B. S., Jorge, M. A., Lombana, A., Lourie, S. A., Martin, K. D., McManus, E., Molnar, J., Recchia, C. A., & Robertson, J. (2007). Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. *BioScience*, 57, 573–583. <https://doi.org/10.1641/B570707>
- Strathmann, R. R. (1985). Feeding and non-feeding larval development and life-history evolution in marine invertebrates. *Annual Review of Ecology and Systematics*, 16, 339–61.
- Taylor, P. D. (1988). Major radiation of cheilostome bryozoans: triggered by the evolution of a new larval type? *Historical Biology*, 1, 45–64.
- Taylor, P.D., 2020. *Bryozoan Paleobiology*. 1st edition. Wiley-Blackwell, Hoboken. pp. 336.
- Thiel, M., & Haye, P. A. (2006). The ecology of rafting in the marine environment. III. Biogeographical and evolutionary consequences. *Oceanography and Marine Biology*, 44, 323–429.
- Thiel, M., & Gutow, L.(2005a). The ecology of rafting in the marine environment. I. The floating substrata. *Oceanography and Marine Biology*, 42, 181–264.
- Thiel, M., & Gutow, L. (2005b). The ecology of rafting in the marine environment. II. The rafting organisms and community. *Oceanography and Marine Biology*, 43, 279–418.
- Thorson, G. (1946). Reproduction and larval development of Danish marine bottom invertebrates. *Meddelelser fra Kommissionen for Danmarks Fiskeri- og Havundersøgelser, Serie Plankton*, 4, 1–523.
- Thorson, G. (1950). Reproduction and larval ecology of marine bottom invertebrates. *Biological Reviews of the Cambridge Philosophical Society*, 25, 1–45.
- Tibihika, P. D., Curto, M., Dornstaeder-Schrammel, E., Winter, S., Alemayehu, E., Waidbacher, H., & Meimberg, H. (2019). Application of microsatellite genotyping by sequencing (SSR-GBS) to measure genetic diversity of the East African *Oreochromis niloticus*. *Conservation Genetics*, 20(2), 357–372. <https://doi.org/10.1007/s10592-018-1136-x>
- Torres, P., Costa, A. C., & Dionísio, M. A. (2012). New alien barnacles in the Azores and some remarks on the invasive potential of Balanidae. *Helgoland Marine Research*, 66, 513–522. <https://doi.org/10.1007/s10152-011-0287-7>
- Vartia, S., Villanueva-Cañas, J. L., Finarelli, J., Farrell, E. D., Collins, P. C., Hughes, G. M., Carlsson, J. E. L., Gauthier, D. T., McGinnity, P., Cross, T. F., Fitzgerald, R. D., Mirimin, L., Crispie, F., Cotter, P. D., & Carlsson, J. (2016). A novel method of microsatellite genotyping-by-sequencing using individual combinatorial barcoding. *Royal Society Open Science*, 3, 150565. <https://doi.org/10.1098/rsos.150565>
- Wallace, A. R. (1880). *Island Life: Or, the Phenomena and Causes of Insular Faunas and Floras, Including a Revision and Attempted Solution of the Problem of Geological Climates*. Macmillan & Co, London.
- Warren, B. H., Simberloff, D., Ricklefs, R. E., Aguilée, R., Condamine, F. L., Gravel, D., Morlon, H., Mouquet, N., Rosindell, J., Casquet, J., Conti, E., Cornuault, J., Fernández-Palacios, J. M., Hengl, T., Norder, S.J., Rijdsdijk, K. F., Sanmartín, I., Strasberg, D., Triantis, K. O., Valente, L. M., Whittaker, R. J., Gillespie, R. G., Emerson, B. C., & Thébaud, C. (2015). Islands as

- model systems in ecology and evolution: prospects fifty years after MacArthur–Wilson. *Ecology Letters*, 18, 200–217. <https://doi.org/10.1111/ele.12398>
- Whittaker, R. J., Triantis, K. A., & Ladle, R. J. (2010). A general dynamic theory of oceanic island biogeography: extending the MacArthur–Wilson theory to accommodate the rise and fall of volcanic islands. In Losos, J. B., Ricklefs, R. E. (Eds.) *The Theory of Island Biogeography Revisited*. Princeton University Press, Princeton, pp. 88–115. <https://doi.org/10.1111/10.1515/9781400831920.88>
- Whittaker, R. J., Triantis, K. A., & Ladle, R. J. (2008). A general dynamic theory of oceanic Island biogeography. *Journal of Biogeography*, 35, 977–994. <https://doi.org/10.1111/j.1365-2699.2008.01892.x>
- Whittaker, R. J., Fernández-Palacios, J. M., Matthews, T. J., Borregaard, M. K., & Triantis, K. A. (2017). Island biogeography: Taking the long view of nature’s laboratories. *Science*, 357, 6354. <https://doi.org/10.1126/science.aam8326>.
- Winston, J. E. (2012). Dispersal in marine organisms without a pelagic larval phase. *Integrative and Comparative Biology*, 52(4), 447–457. <https://doi.org/10.1093/icb/ics040>
- Wisshak, M., Berning, B., Jakobsen, J., & Freiwald, A. (2015.) Temperate carbonate production: biodiversity of sclerobionts from intertidal to bathyal depths (Azores). *Marine Biodiversity*, 45, 87–112. <https://doi.org/10.1007/s1>
- Xavier, J. R., David, J. R., Pereira, R., Colaço, A., Pham, C. K., & Carvalho, F. C. (2021). Diversity, distribution and phylogenetic relationships of deep-Sea lithistids (Porifera, Heteroscleromorpha) of the Azores Archipelago. *Frontiers in Marine Science*, 8, 600087. <https://doi.org/10.3389/fmars.2021.600087>
- Yang, L., Tan, Z., Wang, D., Xue, L., Guan, M., Huang, T., & Li, R. (2014). Species identification through mitochondrial rRNA genetic analysis. *Scientific Reports*, 4, 4089. <https://doi.org/10.1038/srep04089>
- Yang, T., Shen, Y., van der Lee, S., Solomon, S. C., & Hung, S.-H. (2006). Upper mantle structure beneath the Azores hotspot from finite-frequency seismic tomography. *Earth and Planetary Science Letters*, 250(1), 11–26. <https://doi.org/10.1016/j.epsl.2006.07.031>
- Zhang, D.-X., & Hewitt, G. M. (2003). Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. *Molecular Ecology*, 12, 563–584.
- Zhang, Y., Wang, J., Lv, M., Gao, H., Meng, L., Yunga A., Seim, I., Zhang, H., Liu, S., Zhang, L., Liu, X., Xu, X., Yang, H. M10K+ Consortium, & Fan, G. (2021). Diversity, function and evolution of marine invertebrate genomes. *bioRxiv* 2021.10.31.465852. <https://doi.org/10.1101/2021.10.31.465852>

Chapter 2

Objectives and thesis outline

2.1 General objectives

The main goal of this thesis was to increase the current knowledge about the marine diversity, as well as to better understand biogeographical processes and patterns in remote archipelagos and factors shaping them in the marine realm. With a special focus in the Azores Archipelago, this study aims to contribute to unravelling of the “Azorean Biogeographical Paradox” (Ávila, 2006,2013). General goals were defined as follows: 1) test the usefulness of marine invertebrates as proxies to study biogeographical questions; 2) compare the population structure of species with different larval development and between two organismal groups (gastropods versus bryozoans); 3) assess the factors shaping connectivity, evolution and, ultimately, biogeography in NE Atlantic populations (intra-archipelagic and NE Atlantic scales).

2.2 Thesis outline

This dissertation is divided in six chapters. Chapter 1 comprises an introduction to the topics relevant to the following chapters, starting with a review of the history of Island Biogeography and Marine Island Biogeography as scientific disciplines, as well as biogeographical studies and questions in the NE Atlantic Archipelagos, namely the so-called “Azorean Biogeographical Paradox”. Then, a brief description of the geotectonic and oceanographical context of the main study area – the Azores Archipelago – is provided. The importance of studying marine invertebrates to unravel biogeographical processes and patterns in remote islands, as the Azores Archipelago, is explored with details on the use of gastropods and bryozoans as case studies in this thesis. Emphasis is given to the relationship between the mode of larval development and dispersal ability of marine invertebrates. Finally, the principles and methodological approaches used to infer the evolutionary and biogeographical history using molecular information of the target species are presented.

In Chapter 2, the dissertation outline, main objectives, and particular questions addressed in this thesis are described.

Chapter 3 focuses on the importance of marine invertebrates as proxies to study biogeographical questions and is composed of two sections. The mode of larval development of marine invertebrates (non-planktotrophic versus planktotrophic) is expected to affect dispersal and biogeographical patterns. Therefore, two species of

gastropods were studied, aiming to evaluate the biogeographical information provided by species with contrasting life-traits during the larval stage.

Section 3.1 focuses on testing a theoretical biogeographical hypothesis, already supported by ecological and paleontological data, with genetic data of two non-planktotrophic congeneric gastropod species (*Alvania mediolittoralis* and *A. sleursi*) occupying different bathymetrical ranges and occurring in the Azores and Madeira archipelagos. The multidisciplinary analyses support a link between larval development, bathymetrical range, geographical range, and evolutionary time, supporting the usefulness of microgastropods as proxies for evolutionary and biogeographical questions, related to the origin, dispersal, and colonization of new areas by invertebrates with low dispersal ability. The findings of this study were published in:

- Baptista, L., Santos, A. M., Cabezas, M. P., Cordeiro, R., Melo, C. & Ávila, S. P. (2019). Intertidal or subtidal/circalittoral species: who appeared first? A phylogenetic approach to the evolution of non-planktotrophic species in Atlantic Archipelagos. *Marine Biology*, 166(7), 1-16. <https://doi.org/10.1007/s00227-019-3536-y>

Section 3.2 focuses on the population structure of the planktotrophic gastropod *Stramonita haemastoma* in the NE Atlantic. As expected for a species with a long larval stage, no genetic structure was detected across the geographical area studied, showing that planktotrophic species are not as useful to infer biogeographical processes and patterns in remote islands as they tend to provide a less clear signal.

Chapter 4 explores the factors shaping the connectivity of intertidal populations in a remote archipelago and is divided in two sections, both focusing in non-planktotrophic gastropod species.

Section 4.1 investigates the factors behind the population structure of a non-planktotrophic microgastropod – *Cingula trifasciata* – in the Azores Archipelago. How dispersal ability, habitat characteristics, and small-scale oceanographic currents shape intra-archipelagic dynamics of intertidal species was published in the following manuscript:

- Baptista, L., Meimberg, H., Ávila, S. P., Santos, A. M. & Curto, M. (2021). Dispersal ability, habitat characteristics, and sea-surface circulation shape population structure of *Cingula trifasciata* (Gastropoda: Rissoidae) in the remote Azores Archipelago. *BMC Ecology and Evolution*, 21, 128. <https://doi.org/10.1186/s12862-021-01862-1>

Section 4.2 intends to determine the potential origin and mechanisms of dispersal of *Phorcus sauciatus*, a non-planktotrophic gastropod that recently arrived to the Azores (first report in 2013). The role of episodic disruptions of circulation patterns and extreme weather events were presented in the following publication:

- Baptista, L., Santos, A. M., Melo, C., Rebelo, A. C., Madeira, P., Cordeiro, R., Botelho, A. Z., Hipólito, A., Pombo, J., Voelker, A. H. L. & Ávila, S. P. (2021). Untangling the origin of the newcomer *Phorcus sauciatus* (Mollusca: Gastropoda) in a remote northeast Atlantic archipelago. *Marine Biology*, 168, Article number: 9. <https://doi.org/10.1007/s00227-020-03808-5>

Chapter 5, also divided in two sections, addresses the factors shaping the connectivity of deep-water populations in a remote archipelago, studying the bryozoan *Reteporella* genus that occurs from 11 to 820 m depth.

The first section evaluates the diversity of *Reteporella* species in the remote archipelago of the Azores, based on molecular and morphological analyses. The importance of integrative taxonomy in revealing diversity from deep waters in a remote archipelago and the use of molecular phylogenies as backbones for the characterization of the genus can be consulted in the following manuscript:

- Baptista, L., Berning, B., Waeschenbach, A., Curto, M., Meimberg, H., Santos A. M. & Ávila, S. P. (2022). Morphospecies and molecular diversity of 'lace corals': the genus *Reteporella* (Bryozoa: Cheilostomatida) in the central North Atlantic Azores Archipelago. *BMC Ecology and Evolution*, 22, Article 128. <https://doi.org/10.1186/s12862-022-02080-z>

The phylogenetic background on the genus *Reteporella* was crucial to delineate a population genetic workflow, applied in the second section. This study explored how bathymetry and sea-currents in deep waters affect intraspecific connectivity and promote differentiation across the genus *Reteporella*:

In Chapter 6, a discussion of the topics and interpretations drawn in this thesis is presented, with an emphasis on the major findings and future research challenges arising from it.

Chapter 3

Importance of marine invertebrates to study biogeographical questions

3.1 Intertidal or subtidal/circalittoral species: who appeared first? A phylogenetic approach to the evolution of non-planktotrophic species in Atlantic Archipelagos

Introduction

Remote volcanic oceanic archipelagos gather ideal conditions to study evolution in ecosystems formed *de novo*, as life appeared and thrived in unique conditions of isolation (Paulay, 1994). The exceptional contexts in which organisms have reached such islands, often located far from potential colonization sources, have intrigued generations of researchers devoted to understand the biogeographic patterns that emerge from marine and terrestrial taxa inhabiting remote insular areas (see Patiño et al., 2017 for a review; Ávila et al., 2018, 2019). Unlike continental taxa that benefit from physical continuity of landmasses for stepwise migration, for marine organisms, the deep waters and the distance between continental landmasses and oceanic islands usually act as barriers for the dispersal of insular young and adult specimens (Ávila, 2013).

Dispersal ability of marine invertebrates, and consequently its geographical distribution, is directly linked to the duration and mode of larval development, with major consequences at evolutionary and biogeographical levels: species with long planktotrophic stages easily disperse, whilst non-planktotrophic larvae usually have restricted ranges due to the absence of a free-swimming feeding stage (Scheltema, 1986a, 1989, 1995; Scheltema et al., 1996; Scheltema & Williams, 1983). Therefore, the presence of benthic, non-planktotrophic, shallow-water gastropod species, with a geographical distribution restricted to two remote, oceanic archipelagos (e.g. Azores/Madeira, 840 km apart) is not a common feature. One of the authors of this work (R. Cordeiro) compiled and reviewed a database of the 5,284 shallow-water (<50 m depth) gastropod species reported for the entire North Atlantic and adjacent seas (see Supplementary Table A1.1). Rissoidae (Mollusca: Gastropoda) is one of the most diverse families, with a total of 266 shallow-water species, most of them (~77.0%) being np-developers. However, only 10 np-rissoid species (3.8%) occur simultaneously and exclusively in two of the Macaronesian archipelagos (1 species in the Azores and Madeira; 1 in the Azores and Canaries; 4 in Madeira and Selvagens; 1 in Madeira and Canaries; and 3 in Selvagens and Canaries).

Evolutionary history of the family Rissoidae

For decades, the family Rissoidae has been studied to clarify its evolutionary history and relationships among its members. The earlier taxonomic reviews of the family were based mainly on the similarity of conchological characters (Coan, 1964; Thiele, 1929; Wenz, 1938). The classification was subsequently revised by Ponder (1967), with the proposal of four distinct subfamilies. More recently, Ponder (1984) refined the taxonomic position of Rissoidae genera, becoming the most consensual classification scheme for several years by recognizing the subfamilies Rissoinae and Rissoininae. Finally, the phylogenetic status of the superfamily Rissooidea was investigated based on molecular data (16S and 28S; Criscione & Ponder, 2013), and the authors proposed the elevation of Rissoininae (*sensu* Ponder, 1984) to the family level. Subsequent work led to the most comprehensive molecular phylogeny of the family Rissoidae to date, based on 16S and 28S markers (Criscione et al., 2016), with a thorough revision of Rissoidae as the former subfamily Rissoinae, excluding the genera *Merelina* Iredale, 1915 and *Lironoba* Iredale, 1915. This work provided a new broadly accepted classification scheme for this peculiar family, based on genetic data.

Congeneric non-planktotrophs in remote archipelagos: a theoretical hypothesis

In the Northeast Atlantic Ocean, the evolutionary and biogeographical relationships of shallow-water marine molluscs from volcanic oceanic islands and seamounts are well-studied and, particularly, the Azores Archipelago has been considered a model to study the patterns and processes of arrival, colonization, and potential speciation of these species (see Ávila 2005, 2013; Ávila et al., 2009). Although most np-species are geographically limited to small areas (e.g. the rissoids *Alvania angioyi* van Aartsen, 1982 endemic to the Azores, and *Alvania freitasi* Segers, Swinnen & de Prins, 2009 endemic to the Selvagens), some species can occur in more than one archipelago, as mentioned before, or be distributed over large areas as the Northeast Atlantic Ocean (e.g.: the intertidal np-rissoid *Cingula trifasciata* (J. Adams, 1800) has its northern distribution limit in Scandinavia and the southern in Madeira archipelago; Ávila (2005)).

A plausible explanation for these observations would be to consider the speciation of a np-developer in a remote archipelago, where its ancestral first established and later speciated, occupying a suitable, free ecological niche. Through time, the “new” species so far restricted to one remote archipelago, would potentially engage in dispersive processes to remote locations (e.g.: another remote archipelago, located far from the area in which the species first appeared) by mediated (e.g., birds) or non-mediated

dispersal events. If it successfully established in the new area, a np-species would then have extended its geographical range to more than one remote archipelago. Ávila (2005), and later Ávila et al. (2012), studied in detail the marine benthic microgastropod family Rissoidae in the North Atlantic Ocean, clarifying possible mechanisms for the dispersal of its shallow-water species. A total of 124 rissoids are presently reported from the archipelagos of the Azores, Madeira, Selvagens, Canaries, and Cabo Verde. Of these insular rissoids, the majority (78.9%) possess np-larvae, and their small size (≤ 5 mm) and presence amongst algae, from which they can suspend themselves, make them potential candidates for dispersal by rafting. Thus, when algae are pulled out from the substratum by wave action, intertidal and shallow-water rissoids can be rafted along. However, the initiation of a rafting process does not guarantee that dispersal will be well succeeded, especially in remote volcanic oceanic islands where the distance between the source and sink areas difficult the dispersal of young and adult rissoids (Ávila, 2013; Ávila et al., 2012).

Ávila (2006, 2013) suggested the addition of a vertical dimension — the bathymetric/ecological zonation — to the considerations of Scheltema on the relationships between modes of larval development and geographical range distribution (Scheltema 1986b, 1989, 1995). Considering rafting as a key mechanism for the dispersal of epibenthic, shallow-water (< 50 m depth), np-species in Atlantic waters, three main assumptions were pointed out by Ávila (2006): (1) intertidal insular species are more prone to be rafted than subtidal/circalittoral species, due to the increased likelihood of being disturbed by natural events that trigger their passive dispersal (see Figure 3.1a); (2) bathymetry/ecological zonation and geographical distribution of species are directly correlated, with intertidal species generally having wider ranges than sublittoral species (e.g., 3-6 m depth) and these, in turn, having wider ranges than subtidal/circalittoral species (e.g., 20-30 m depth) (see Figure 3.1b); (3) the chances of a successful dispersal are higher for micromolluscs in which the juveniles/adults are the rafting stage, as they are usually associated with algae and remain attached even after the algal substrate is ripped by wave-action. Therefore, the smaller the species, the higher the likelihood of dispersal and range expansion, as rafting is more likely to be well-succeeded if the species remains attached to the substrate (Ávila, 2006).

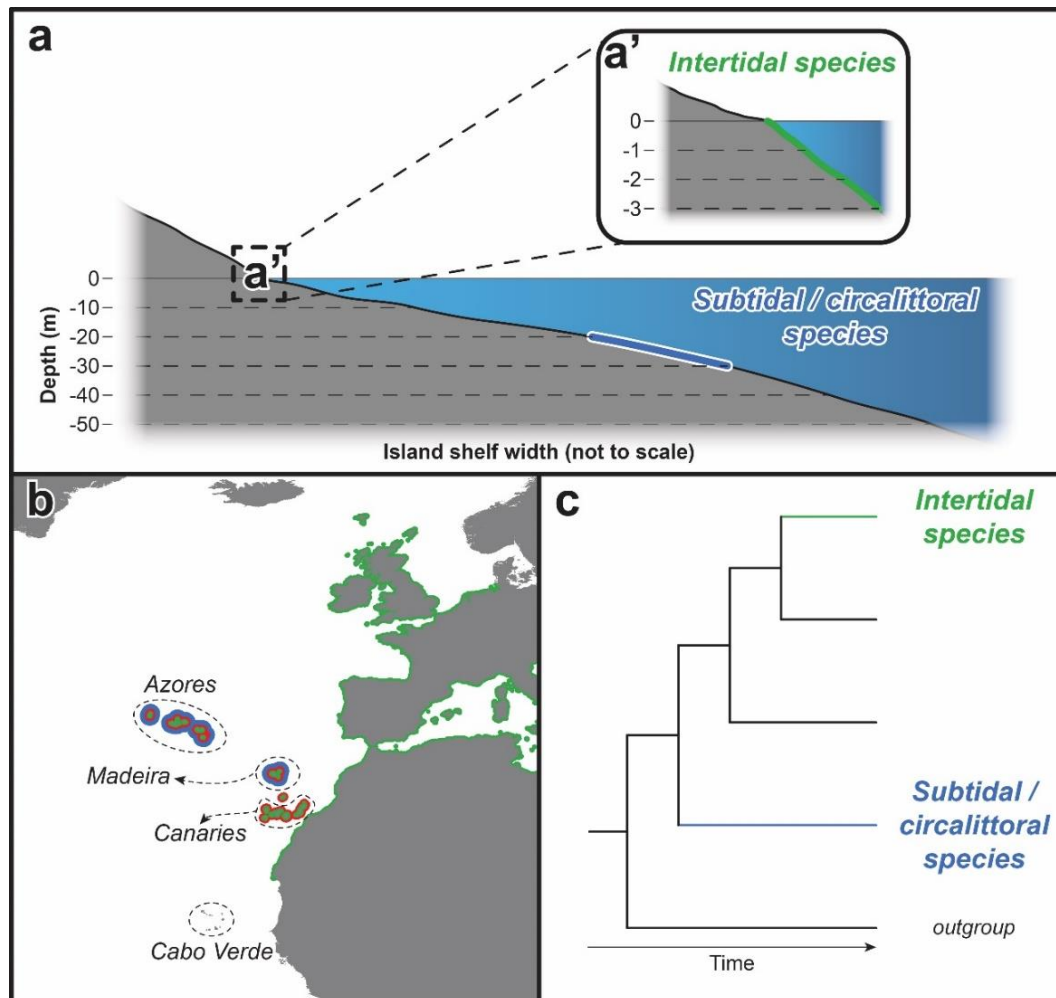


Figure 3.1. Graphical illustration of Ávila's (2013) biogeographical hypothesis. **a.** bathymetric range/ecological zonation of intertidal (in green) and subtidal/circalittoral species (in blue); sublittoral species (not represented) have an intermediate bathymetric zonation (3-6 meters). Intertidal species are more exposed to disturbing agents (e.g. waves); **b.** intertidal species are expected to have a wider geographical distribution (in green) than sublittoral species (in red), which in turn have a wider range than subtidal/circalittoral species (in blue), due to the likelihood of initiating rafting; **c.** when premises of Ávila's (2013) theory are granted, the subtidal/circalittoral species is expected to be evolutionarily older than the intertidal species, conceding enough time for both to disperse successfully.

This relation was later completed by Ávila (2013) and resulted in the formulation of a new biogeographical hypothesis, with the addition of evolutionary time as the fourth and last variable influencing the dispersal and evolution of shallow-water marine molluscs. Based on the compilation of the geographical distribution of rissoids in the North Atlantic Ocean by Ávila et al. (2012), this relationship was formulated to address the simultaneous existence of two congeneric np-species – *Alvania sleursi* (Amati, 1987) and *A. mediolittoralis* Gofas, 1989 – in two nearby archipelagos (Azores and Madeira), which differ only in their bathymetric range (subtidal/circalittoral and intertidal, respectively). Following this premise, as both species presently occur in two nearby

archipelagos, it is expected that the one inhabiting deeper water is evolutionarily older than the shallow-water/intertidal species (Ávila, 2013). Both species may successfully colonize and establish in the same far-away location under this scenario, but the subtidal/circalittoral species inhabits less disturbed environments being less prone to engage dispersal, whereas the intertidal is fustigated by wave-action, inducing rafting more often. Thus, we expect the subtidal/circalittoral lineage to have appeared earlier in the geological scale, granting it more time to successfully expand its distributional range to the same extent as the intertidal congeneric (see Figure 3.1c).

This biogeographical hypothesis was later supported by ecological and palaeontological studies in Madeira archipelago and in the Azores, where Santa Maria is the oldest (~ 6.01 Ma; Ramalho et al., 2017) and the only island having known exposed marine fossiliferous outcrops. In Santa Maria Island, fossils of the subtidal/circalittoral rissoid *A. sleursi* were found in Pliocene (4.78 ± 0.13 to 4.13 ± 0.19 Ma; Ramalho et al., 2017) and Late Pleistocene (last interglacial Marine Isotopic Stage 5e - MIS 5e; 130-116 ka; Ávila et al., 2015) outcrops. For the intertidal species *A. mediolittoralis*, fossilized remains are only observed in Azorean MIS 5e sediments (Ávila et al., 2015), suggesting it is evolutionarily younger. Despite the earlier emergence of Madeira Islands in the northeast Atlantic Ocean — Porto Santo ~18.8 Ma (Mata et al., 2013) and Madeira's re-emergence ~7 Ma (Ramalho et al., 2015) — the fossiliferous outcrops in this archipelago are assigned to only two geological epochs. Reef limestones from the Pliocene are found in Madeira Island, and bioclastic or bio-edified limestones from the Middle Miocene as well as Pleistocene biogenic carbonate sandstone are present in Porto Santo Island (Mata et al., 2013). Nevertheless, specimens of *A. mediolittoralis* and *A. sleursi* are not reported for Madeiran fossiliferous outcrops (Gerber et al., 1989; Ávila et al., 2015).

Notwithstanding its relevance, Ávila's (2013) hypothesis remains untested from a phylogenetic perspective. Therefore, the main aim of our work is to address this evolutionary/biogeographical link between non-planktotrophic larval development, geographic range, bathymetric/ecological zonation and evolutionary time with molecular phylogenetic tools, targeting *A. mediolittoralis*, *A. sleursi* and other rissoids presently occurring in the Azores and/or Madeira archipelagos. The family Rissoidae was chosen as a study-model because of the current broad knowledge at the geological and ecological levels, but also due to the high number of representatives, susceptibility to rafting and distributional range in Atlantic islands (Ávila et al., 2012). We hereby propose a multidisciplinary, combined approach of molecular phylogenetics and palaeontological data to unveil evolutionary relationships of insular rissoids and to assess the prediction of an earlier evolutionary divergence of the subtidal/circalittoral lineage.

Material and methods

Taxon sampling and laboratory analyses

Samples of rissoids naturally occurring in the Azores Archipelago – *Alvania angioyi*, *A. formicarum* Gofas, 1989, *A. mediolittoralis*, *A. sleursi*, *Cingula trifasciata*, *Crisilla postrema* (Gofas, 1990), *Rissoa guernei* Dautzenberg, 1889 and *Setia subvaricosa* Gofas, 1990 – were retrieved from the marine molluscs reference collection of the Department of Biology of the University of the Azores (DBUA), stored in 96% ethanol and collected in several coastal sites of the archipelago. We have also used samples of *Pisinna glabrata* (von Mühlfeld, 1824) from DBUA as the outgroup in the phylogenetic reconstructions. DNA was extracted from whole individuals with the commercial kit PureLink® Genomic DNA Mini Kit (Invitrogen™), following the manufacturer's instructions.

Fragments of the mitochondrial gene cytochrome oxidase subunit I (COI) and the nuclear 28S ribosomal RNA (28S rRNA) were amplified by PCR, performed in 25 µl volumes, entailing 3 µl of DNA, -10x buffer MgCl₂ free, 2.5 mM MgCl₂, 0.2 mM dNTP, 10 µM of each primer, 0.1 µg µl⁻¹ Bovine Serum Albumin (BSA, Promega) and 0.3U Platinum Taq DNA polymerase. PCR conditions and primers are provided as Supplementary Table A1.2. Electrophoretic runs in 2% (w/v) agarose gel with GelRed (DNA fluorescent dye, BioTarget™) were performed to assess the efficiency of the amplification reactions and the quality of the DNA products. PCR products purification and bi-directional Sanger sequencing with amplification primers were realized by a commercial facility — Genewiz, London, UK.

Sequence data

A manual check of misreads in COI and 28S chromatograms was performed with BioEdit v.7.0.5.3 for Windows (Hall, 1999). Sequences of the mitochondrial coding COI gene were translated into aminoacids with ExPASy Translate Tool (<https://web.expasy.org/translate/>), allowing to inspect the existence of stop codons and thus the presence of pseudogenes. For the phylogenetic analyses of COI and 28S genes, we have also included sequences of species reported for the Northeast Atlantic Ocean, deposited in GenBank. All the sequences analysed for the reconstruction of the gene trees are provided in Supplementary Table A1.3. Sequences of the mitochondrial COI marker were aligned using Clustal Omega algorithm via Web Services by EMBL-EBI (McWilliam et al., 2013), whereas the alignment of 28S sequences was performed using the MAFFT program (Katoh et al., 2005). Poorly aligned positions and divergent regions that often obscure the phylogenetic signal (Xia & Lemey, 2009) were removed

from the alignment of the nuclear 28S marker resorting to GBlocks Server v0.91b (Castresana, 2000).

Phylogenetic analyses of COI and 28S

The software PartitionFinder v1.1.1 (Lanfear et al., 2012) was used to determine the best-fit partitioning schemes and models of molecular evolution to each dataset, according to the Akaike's Information Criterion (Akaike, 1973). Data partitioning by codon was applied to COI gene to minimize saturation effects of codon positions on phylogenetic reconstructions (Salemi, 2009) and to account for different rates of evolution of each codon position (Pond et al., 2009). The following models of molecular evolution were applied to COI partitions: HKY+G (1st partition), GTR+G (2nd partition), HKY+I (3rd partition). Bayesian inference (BI) and Maximum Likelihood (ML) methods were used to reconstruct the Rissoidae phylogeny. For BI analyses, performed in MrBayes v3.2.6 software (Ronquist et al., 2012), two independent runs (each with four chains for 2×10^7 generations) were performed. Trees and parameters were sampled every 1,000 generations, with the heating parameter set to 0.25. Stationarity was considered to be reached when the average standard deviation of split frequencies was lower than 0.01. Majority-rule consensus trees were estimated from both analyses, after discarding 25% of the total samples as burn-in. ML analyses in Garli v2.0.1 (Zwickl, 2006) were performed with a 10 independent search replicates and 1,000 bootstrap replicates. The evaluation of log-likelihood values across searches allowed to check the convergence between the topologies of the trees generated. SumTrees program of the DendroPy package (Sukumaran & Holder, 2010) was used to summarize non-parametric bootstrap support (BS) values for the best tree, after generating a majority-rule consensus tree. Consensus trees inferred for each molecular marker were visualized and rooted – setting *P. glabrata* as the outgroup for the COI dataset and *Anabathron contabulatum* (Frauenfeld, 1867) as the outgroup for the 28S dataset – resorting to FigTree v1.4.3 software (Rambaut, 2006).

Species tree reconstruction and estimation of divergence times

Species tree reconstruction can be achieved by distinct phylogenetic methodologies. Maximum likelihood methods with concatenated sequences estimate a single species tree that best fits the combined alignment, according to the phylogenetic likelihood function (Felsenstein, 1981; Ogilvie et al., 2017). However, inferences drawn concatenated datasets have been associated with inconsistent results and low reliability, including high bootstrap support even in incorrectly estimated branches (Gadagkar et

al., 2005; Kubatho & Degan, 2007), due to the influence of model misspecification or long-branch attraction (Liu et al., 2015; Ogilvie et al., 2017) and the incomplete lineage sorting phenomenon that causes systematic errors on the estimation of branch lengths and divergence times (Mendes & Hahn, 2016; Ogilvie et al., 2016, 2017). Therefore, a fully Bayesian multispecies coalescent method — StarBEAST2 framework (Ogilvie et al., 2017) — was applied to the dataset comprising both COI and 28S genes, as implemented in BEAST v2.4.5 software (Bouckaert et al., 2014), for the inference of the evolution of eight rissoid species and their divergence time. This approach, based on the co-estimation of multi-individual multi-locus sequence data, provides a reliable estimation of the species tree by searching and embedding each gene tree within a single shared species tree that likely best reflects the evolutionary history of the taxa (Ogilvie et al., 2017), thus avoiding the issues associated with concatenated datasets (Mendes & Hahn, 2016; Ogilvie et al., 2016). Due to the requirements of the chosen phylogeny reconstruction framework, we have only considered rissoid species represented by two or more individuals (Heled & Drummond, 2010), for which sequences of both COI and 28S were available (Table 3.1): *Alvania angioyi*, *A. formicarum*, *A. mediolittoralis*, *A. sleursi*, *Cingula trifasciata*, *Crisilla postrema*, *Rissoa guernei*, and *Setia subvaricosa*. According to the currently accepted phylogeny of the superfamily Rissooidea, based on molecular data (*sensu* Criscione & Ponder, 2013), *Pisinna glabrata* was selected as an outgroup in the species reconstruction for the same reasons.

Table 3.1. Sequences of mitochondrial COI and nuclear 28S genes of Rissoidae species and of the outgroup *Pisinna glabrata*, included in the species tree reconstruction following StarBEAST2 framework. Accession numbers (AN) are provided; all the sequences used were obtained in this study.

	COI AN	28S AN
<i>A. angioyi</i> van Aartsen, 1982	MG652373-5 MG652378	MG663173-4 MG663176 MG663178
<i>A. formicarum</i> Gofas, 1989	MG652379-80	MG663179
<i>A. mediolittoralis</i> Gofas, 1989	MG652382-3	MG663180-1
<i>A. sleursi</i> (Amati, 1987)	MG652386-7	MG663182-3
<i>Cingula trifasciata</i> (J. Adams, 1800)	MG652396-7 MG652401 MG652403-4 MG652406	MG663191-6
<i>Crisilla postrema</i> (Gofas, 1990)	MG652392 MG652394	MG663185-6
<i>Rissoa guernei</i> Dautzenberg, 1889	MG652408-9 MG652410-13 MG652419-21	MG663197-203 MG663207 MG663209
<i>Setia subvaricosa</i> Gofas, 1990	MG652429-30	MG663215-6
<i>Pisinna glabrata</i> (von Mühlfeld, 1824)	MG652432-33 MG652435	MH047302-4

The software jModelTest v2.1.10 (Darriba et al., 2012) yielded the following substitution models and parameters as the best-fitted to the data analyzed, according to the Akaike's Information Criterion (Akaike, 1973): HKY+I+G model, with gamma shape=0.6100, proportion of invariants=0.5500 and kappa=18.7407 for the COI marker; GTR+I+G model, with gamma shape=0.4710, proportion of invariants=0.508, rate AC=0.3118, rate AG=2.1462, rate AT=0.7970, rate CG=0.7967, rate CT=6.8066 and rate GT=1.0000 for the 28S marker.

Four fossil calibration points were assigned to estimate the timing of the split events, according to the presence/absence of the species analysed in fossiliferous outcrops in Santa Maria Island (Azores), dated from the Pliocene and Pleistocene (MIS 5e). Their attribution followed the recommendations of Forest (2009) and Parham et al. (2012) for species tree reconstructions analyses, being defined as the minimum age of the clades to which they were assigned and according to the most recent and valid literature available for the fossil taxa in question (Meireles et al., 2013; Cordeiro et al., 2015; Ávila et al., 2015, 2016; Ramalho et al., 2017), to ensure the accurate temporal placement of the lineage in the phylogenetic analyses. *Alvania sleursi* is an extant species, also found in fossiliferous sediments dated back to the Pliocene, meaning that it was already inhabiting Santa Maria shores during those periods. One must assume its speciation prior to the formation of the Pliocene palaeoenvironment, otherwise, fossil specimens of *A. sleursi* would not be present there. In Santa Maria, the ancient habitat where *A. sleursi* existed was covered by lava during a period of transgressive volcanism, promoting the preservation of the Pliocene fossiliferous outcrop. The lava formation on top of the Pliocene sediments was dated as 4.13 ± 0.19 Ma (Ramalho et al., 2017), which was designated as the minimum age for the divergence among *A. sleursi*, *A. mediolittoralis* and *A. formicarum* as, based on the fossil record, *A. sleursi* already existed but none of its conspecific related-taxa. On the other hand, considering their presence only in more recent fossil sediments from Pleistocene (MIS 5e) (Cordeiro & Ávila, 2015; Ávila et al., 2015, 2016), a minimum age of 0.12 Ma (the estimated age of 116 ka was rounded up to 0.12 Ma) was set up for: (1) the split between *A. formicarum* and *A. mediolittoralis*, (2) the divergence among *C. trifasciata* and the endemic species *A. angioyi* and *C. postrema*; and (3) the split between *R. guernei* and *S. subvaricosa*. Although the low number of fossil calibration points used ($n=4$) and their inference from fragmented fossil record from a single island are not considered ideal practices on a species tree reconstruction (Parham et al., 2012), the information to calibrate this inference is only available from the fossiliferous outcrops of Santa Maria Island in the Azores Archipelago.

All this information was manually included in the input file for StarBEAST2, created using BEAUti package manager. We have tested the use of Uncorrelated Lognormal molecular clocks, as well as using the Calibrated Yule model of speciation, but the analyses did not convergence in any of the attempts, reaching ESS (Effective sample size) values under 50 in most parameters. For that reason, and after evaluating convergence, we have defined a strict clock for both genes, unlinking the molecular clock and tree priors between the markers, and defined Yule model of speciation for 10^8 generations, with parameters sampled every 10,000 generations. Burn-in was set for 10%, after graphically checking the achievement of stationarity in Tracer v1.6 software (Rambaut et al., 2014). The convergence and ESS were also assessed with Tracer v1.6. After discarding the burn-in samples, the final species tree with node ages was plotted with TreeAnnotator v2.4.5 (Rambaut & Drummond, 2002) and visualized with the FigTree v1.4.3 software (Rambaut, 2006). Posterior modifications of the phylogeny were performed resorting to Inkscape v0.92 software (<http://www.inkscape.org>).

Results

Sequenced data and phylogenetic analyses

All the rissoid sequences obtained in this study were deposited in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>), under the accession numbers: MG652373-87, MG652392-422, and MG652429-31 for the COI sequences; MG663173-83, MG663185-209 for the 28S rRNA marker. Sequences were also obtained from *Pisinna glabrata*: MG652432-36 (COI) and MH047302-4 (28S rRNA). The alignment of COI marker had a total length of 658 bp, whereas the alignment of 28S marker had a length of 1015 bp after the removal of uninformative gaps (total length of 1074 bp prior to the GBlocks analysis).

The phylogenetic trees obtained for COI (Figure 3.2) and 28S (Figure 3.3) displayed similar topologies and acceptable resolution in clarifying systematic positions among Rissoidae taxa. Within each marker, BI and ML analyses produced phylogenetic trees with similar topologies for most relationships, thus only the topology inferred with ML methods is shown (Bayesian Inference gene trees of COI and 28S markers are provided as Supplementary Figures A1.1 and A1.2, respectively). Posterior probabilities (PP) tended to be higher than BS values at most nodes, but it is known that PP are typically higher than BS values (Suzuki et al., 2002). The major relationships were concordant between the mtDNA COI (Figure 3.2) and nDNA 28S (Figure 3.3) phylogenies, as three clades were supported by PP values. Clade A comprised several *Alvania* spp. in both gene trees (PP 99-100%), whereas in Clade B (PP 96% in the COI phylogeny; PP 88%

in the 28S phylogeny) *Cingula trifasciata* occupied a basal position, closely related with *Crisilla postrema* and some *Alvania* spp. Clade C (PP 99% in COI and 28S gene trees) comprised taxa assigned to *Pusillina* and *Rissoa* genera, as well as *Setia ambigua* in the 28S phylogeny. Moreover, the basal phylogenetic position of *Setia subvaricosa* in relation to the remaining rissoids was supported in the COI gene tree (BS and PP 100%), but for the 28S its position within Clade C was not supported. In the COI phylogeny, another group comprising *Pseudosetia* and *Onoba* genera was also retrieved, but no further considerations were made regarding its phylogenetic position as it was not supported by the analyses. The polyphyly of the genus *Alvania* was depicted and well-supported in the two markers analysed.

In both phylogenies, *A. formicarum*, *A. mediolittoralis*, and *A. sleursi* were depicted as closely related, with an earlier divergence of the subtidal/circalittoral *A. sleursi*. In the COI gene tree, the split between *Alvania mediolittoralis* and *A. formicarum* was supported by ML and BI analyses (BS 79%, PP 100%), whereas the older splits were only supported by PP (>90%). In the 28S phylogeny, splits within Clade A were only supported by PP values (>85%).

Species tree reconstruction

In the species tree reconstruction using StarBEAST2 framework, the effective sample sizes of every parameter reached values higher than 200, and assessment of parameter statistics in Tracer v1.6 suggested the convergence of the analysis. Posterior probabilities higher than 99% were recovered for all but one node (PP 56.8%, broad credibility interval ranging from 12.82 to 23.17 Ma) (Figure 3.4). Confidence intervals were calculated for the age of all the splits inferred and, the older the split event, the lower the associated confidence level.

The species tree reconstruction (Figure 3.4) showed early diversification of the studied species during the Early Miocene, ca. 17.52 Ma, resulting in the separation of the lineage that originated *Rissoa* and *Setia* genera and the one that leads to the speciation of *Alvania*, *Cingula* and *Crisilla* genera. Curiously, *A. angioyi* was depicted as more closely related to *Crisilla postrema* and *Cingula trifasciata* than to other *Alvania* species. Three major clades can be distinguished in the species tree: (1) Clade A estimated the first diversification of three species during the Middle Pliocene (ca. 4.34 Ma), by splitting *Alvania sleursi* and the lineage that later (ca. 360 ka, during the Pleistocene) originated *A. formicarum* and *A. mediolittoralis*. As both splits were calibrated, narrow Highest Posterior Density (HPD) intervals were estimated for both split events providing high confidence in this inference: credibility interval of 0.73 Ma for the

oldest event and ranging from 0.12 to 0.61 Ma in the youngest; (2) Clade B comprised a first split event (calibrated) during the Late Miocene (ca. 7.67 Ma) between *C. trifasciata* and the ancestor of *A. angioyi* and *C. postrema*, and a divergence between these two taxa around 2.5 Ma later. Both estimations were associated with a low degree of uncertainty, with credibility intervals ranging from 5.04 to 10.12 Ma for the older split, and between 3.5 and 7.33 Ma for the most recent event; and (3) Clade C showed the split between *Rissoa guernei* and *Setia subvaricosa* sometime between 20.48 and 5.14 Ma, which decreased the credibility of this inference.

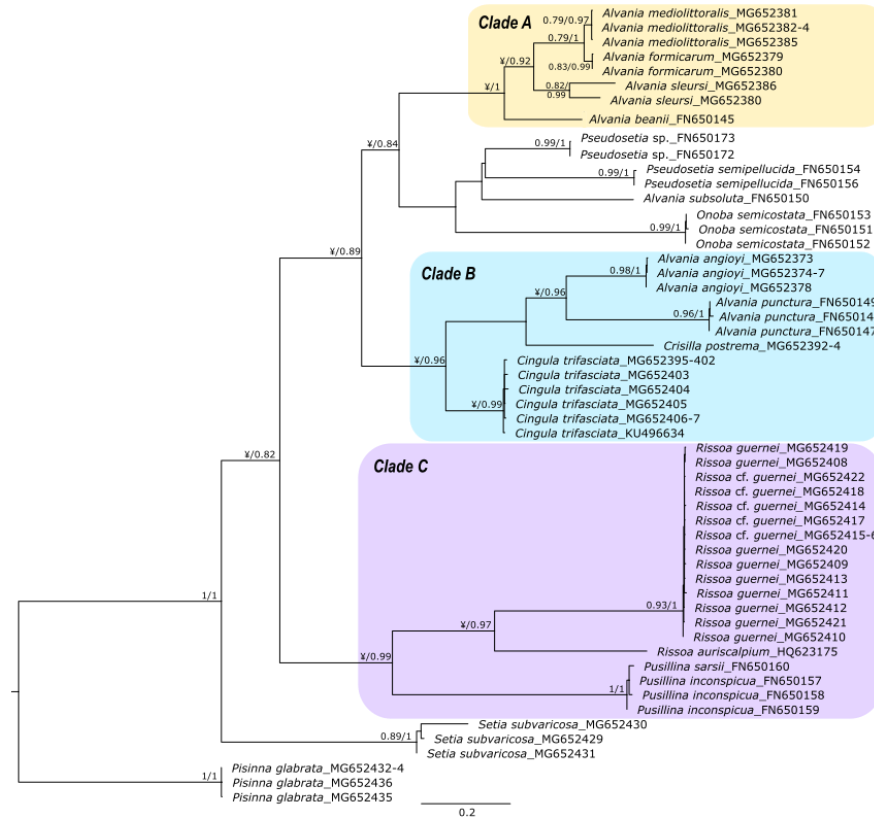


Figure 3.2. Maximum Likelihood tree obtained for mtDNA COI gene. Values at the nodes correspond to Bootstrap Support (BS) values and Posterior Probability (PP). Clades A-C are identified. Accession Numbers of sequences are provided, after the species designation. Yen (¥) symbol is used to represent support values inferior to 0.7. Colours and names assigned to the clades match between gene trees and species tree.

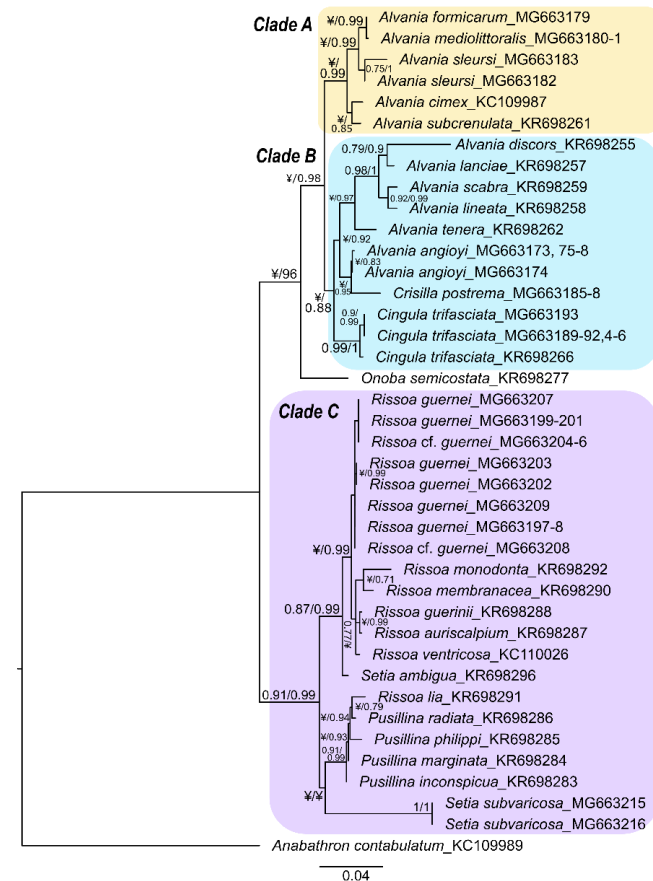


Figure 3.3. Maximum Likelihood tree obtained for nDNA 28S rRNA gene. Values at the nodes correspond to Bootstrap Support (BS) values and Posterior Probability (PP). Clades A-C are identified. Accession Numbers of sequences are provided, after the species designation. Yen (¥) symbol is used to represent support values inferior to 0.7. Colours and names assigned to the clades match between gene trees and species tree.

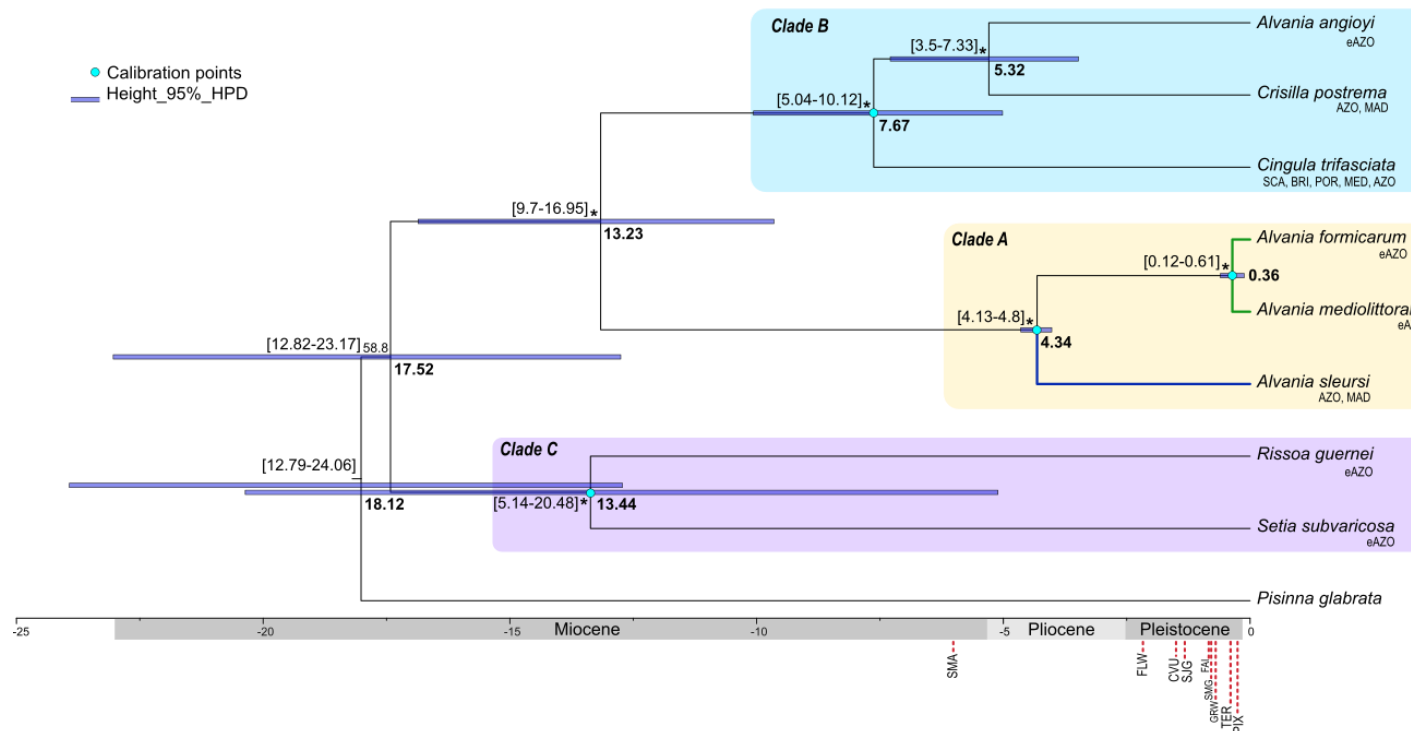


Figure 3.4. Rissoidae species tree, estimated according to a fully Bayesian multispecies coalescent method implemented in StarBEAST2 software with datasets of mtDNA COI gene and nDNA 28S rRNA gene. Four calibrations were added to some nodes and represented by a light blue circle. Clades A-C are depicted by different colours; colours and names assigned match between gene trees and species tree. Within Clade A, the coloured branches follow the same colour scheme as Figure 3.1 to represent the ecological zonation of the species: intertidal (green) or subtidal/circalittoral (blue). Posterior probability values are displayed at the nodes and hereby represented by an asterisk (*) when higher than 99%. Values at the nodes indicate the estimated age of the split event and blue node bars represent 95% of the Highest Posterior Density interval. Numbers within brackets represent the 95% credibility interval associated with the node bars and, thus, split events. The reported geographical distribution of the species is provided under each species name, at the tip of the species tree: eAZO – endemic to the Azores; AZO – Azores; BRI – British Isles; MAD – Madeira; POR – Portuguese shores; SCA – Scandinavia. The appearance of the Azorean Islands is depicted chronologically: SMA – Santa Maria, 6.01 Ma (Ramalho et al., 2017); FLW – Flores, 2.16 Ma (Azevedo, 1998); CVU – Corvo, 1.50 Ma (França et al., 2002); SJG – São Jorge, 1.32 Ma (Hildenbrand et al., 2014); FAI – Faial, 0.85 Ma (Hildenbrand et al., 2014); SMG - São Miguel, 0.79 Ma (Sibrant et al., 2015); GRW – Graciosa, 0.70 Ma (Sibrant et al., 2014); TER – Terceira, 0.40 Ma (Hildenbrand et al., 2014); PIX – Pico, 0.27 Ma (Demand et al., 1982).

Discussion

Phylogenetic analyses of COI and 28S markers

The phylogenetic analyses performed for Rissoidae taxa supported the monophyly of the family Rissoidae. The genus *Alvania* was shown to be polyphyletic in both phylogenies, with species assigned clades A and B and in accordance with previous work by Criscione et al. (2017). Within Clade A, we highlight the relationship established between the intertidal *A. mediolittoralis* and the subtidal/circalittoral *A. sleursi*, highly supported in both ML and BI analyses (BS and PP >75% in COI gene tree; PP 99% in 28S gene tree). The systematic position of *A. sleursi*, currently reported for the Azores and Madeira archipelagos, in a molecular phylogeny of the family Rissoidae had already been hypothesized by Ávila (2013) as expected to occupy a basal position in relation to *A. mediolittoralis*. This hypothetical systematic position of *A. sleursi* was corroborated by both COI and 28S phylogenies.

Within Clade B, *A. punctura* and the Azorean endemic *A. angioyi* were positioned as sister species in the COI dataset, whereas the endemic taxon was depicted as closely related to the subclade containing *A. discors*, *A. lanciae*, *A. lineata* and *A. scabra* in the 28S phylogeny. Some controversy is attributed to the classification of the latter four species as *Alvania*, as they form a lineage distantly related to other *Alvania* spp.. Although no formal attempt has been made to reclassify it, we support Criscione's et al. (2017) suggestion of revision as *Alvaniella* Sacco, 1895 or *Alvanolira* Nordsieck, 1972. On the other hand, *Crisilla postrema*, currently found in relatively shallow water of the Azores and Madeira (Cordeiro et al., 2015), was positioned as sister species to the Azorean endemic *A. angioyi*. The type species of *Crisilla* – *C. semistriata* (Montagu, 1808) – was considered a subgenus of *Alvania* for decades due to morphological similarities (Gofas, 2016; Ponder, 1984). Although with low support, the phylogenetic affinities between these two species might call for the need to review their classification. Clade C comprises *Pusillina* spp., *Rissoa* spp. and *Setia* spp., which is concordant with the relationships within Clade E retrieved by Criscione et al. (2017). The phylogenetic position of *Setia ambigua* suggests it to be more closely related with *Rissoa* spp. than to the congeneric Azorean endemic *S. subvaricosa*. Morphological and molecular distinction of *S. ambigua* relatively to other *Setia* spp., displaying accentuated differences in the shell features, suggests the need of a taxonomic revision of this taxon, as already stated by Criscione et al. (2017).

Evolutionary history of the Azorean Rissoidae species

The species tree obtained in this study revealed that the lineage that later originated *Alvania angioyi*, *A. formicarum*, *A. mediolittoralis*, *A. sleursi*, *Cingula trifasciata*, *Crisilla postrema*, *Rissoa guernei*, and *Setia subvaricosa*, first started to diversify during the Early Miocene, ca. 17.52 Ma. However, this estimate is not reliable as the low support for this node (PP 56.8%) broadens the credibility interval to ages ranging from 12.82 to 23.17 Ma. Rissoidae-like organisms are known since the Lower Jurassic (Conti et al., 1993; Criscione et al., 2016; Criscione & Ponder, 2013) and some genera were already diversified in the Central Paratethys during the Miocene (Kowalke & Harzhauser, 2004), thus, despite not being able to assertively define an age for this divergence event, we believe it is possible that the divergence of these lineages occurred sometime within the estimated range, as it agrees with the fossil record of rissoids worldwide. The concordance in the typology of the species tree inferred (Figure 3.4) and the gene trees (Figures 3.2 and 3.3), all revealing a clade comprising *A. sleursi*, *A. mediolittoralis* and *A. formicarum* with an earlier divergence of the subtidal/circalittoral *A. sleursi*, increases the confidence in its phylogenetic position among the rissoids.

Different confidence intervals were retrieved for the several splits inferred. Large credibility intervals (e.g. Clade C) obscure the estimation of a trustworthy age for the split event analyzed, turning inferences regarding the taxonomic position and temporal range of existence of the species involved quite dubious. Even when calibrated, older splits were associated with lower confidence levels. This scenario might reflect a discrepancy between the placement of the calibration point and its true position in the phylogeny, as the further the calibration point is assigned relative to the node of interest, the greater the uncertainty associated with the estimations (Forest, 2009). As this work constitutes a phylogenetic study, split events reflect the timing of divergence of the lineages that later originated modern assemblages and not the time of arrival of rissoids or their ancestors to the Azores Archipelago's vicinity.

Nevertheless, the divergence dates and relationships proposed by the species' tree obtained seem reasonable based on the worldwide fossil record of the family Rissoidae, the geological history of the Azores Archipelago, and the evolutionary history revealed by the main patterns retrieved from mtDNA COI and nDNA 28S phylogenies (see Figures 3.2 and 3.3). As expected, the relationships inferred in the gene trees are, sometimes, not concordant among phylogenies (e.g. in the COI phylogeny *Setia subvaricosa* is not included in the clade comprising *Rissoa guernei*, but it is in the 28S gene tree). Nuclear and mitochondrial molecules have different evolutionary rates, which influences the evolutionary history inferred by a gene tree (Degnan & Rosenberg, 2006). Particularly,

due to its faster evolutionary rate, mitochondrial markers are expected to show higher saturation levels than the nuclear genes and will, therefore, obscure the estimations of older relationships by overestimating them. In salamanders, the overestimation of the divergence events by mitochondrial markers has reached levels of 3-10 times for recent events and 2-3 times for ancient ones, when compared with the nuclear markers (Zheng et al., 2011), whereas in a study of ray-fishes it ranged from 1-1.5 times (Hurley et al., 2007). Overestimation is not exclusive to mitochondrial markers, as it also occurs in nuclear genes, which require longer times to coalesce. Nevertheless, these biases are particularly misleading in cases of few calibration points and studies of deep phylogenies in higher taxonomic levels, where the time span is wide and the lineages are considerably old (Zheng et al., 2011).

Due to stochastic events during speciation, discordance between the topologies of the gene trees and the species tree is expected under the coalescent model, especially when assuming the most frequently observed gene tree typology as an estimate of the species tree (Degnan & Rosenberg, 2006; Kubatho & Degnan, 2007). Reasons pointed out as responsible for the discordance between gene trees and species trees include horizontal transfer, incomplete lineage sorting, and gene duplication and extinction (Maddison, 1997). The larger the time between speciation events, associated with longer internal branches in the species tree, and the older the species origins, reduces the chances for gene-species tree discordance. However, in the case of recent diversifications, the time elapsed is insufficient to grant the fixation of gene lineages by genetic drift, and discordance between gene and species tree will arise due to deep coalescence of gene lineages from non-closely related species (Knowles, 2009). Nevertheless, when comparing the major patterns of the species tree with the ones inferred in the gene trees, the Clades A-C are found in the three phylogenies, despite some differences in the relationships within. To avoid the incorrect use of gene trees as a proxy for species phylogeny and to obtain a more reliable estimation of the “true” topology of the rissoid’s phylogeny, we opted to apply StarBEAST2 framework, which allows an integrated analysis of each marker’s evolution and embeds each tree within a most-likely single shared species tree. With this approach, we avoided the interference of biasing phenomena such as overestimation by mitochondrial markers and incomplete lineage sorting (Ogilvie et al., 2017). This constitutes the first time that a species tree is obtained for rissoids in the Azores, as well as the first approach to its evolution in an integrated perspective that includes clues about past environments, given by the palaeontological record.

Testing Ávila's (2013) biogeographical hypothesis

Throughout this study, Ávila's (2013) theory was tested by the reconstruction of the evolutionary history of eight Rissoidae species (Figure 3.4), including the two species which observations encouraged the early formulation of the idea. Within the species tree inferred, Clade A must be emphasized to test this hypothesis, as it comprises *A. mediolittoralis* and *A. sleursi*, two np-species currently found in the Azores and Madeira archipelagos. Nowadays, *A. mediolittoralis* is commonly found in the intertidal algal turf, with much rarer reports from depths of up to 24 m (Ávila, 2005, 2013; Cordeiro et al., 2015), whereas *A. sleursi* frequently occurs in deeper waters from 15-25 m down to 40 m depth (Ávila, 2013; Cordeiro et al., 2015).

The inferred species tree indicates that the divergence between the lineages that originated *A. sleursi* and the shallow-water species occurred 4.8 to 4.13 Ma ago. By this time, the volcanic edifice of Santa Maria Island (Azores), which first emerged at ~6.01 Ma (Ramalho et al., 2017), is believed to have been under a period of waning volcanism, erosion, and subsidence leading to the formation of a truncated, top-flat seamount (Ramalho et al., 2017). Thus, during this period, Santa Maria's edifice became a shallow-water sandy shoal where volcanic activity was rare, mostly submarine (Ramalho et al., 2017). A wide variety of marine environments existed in this guyot, yielding diverse organisms typical from open-water marine environments (see Uchman et al., 2018 and references therein) and benthic species from shallower waters, including rissoids and other molluscs (Ávila et al., 2015).

According to Ávila's (2013) hypothesis, subtidal/circalittoral species would be less prone to be rafted than intertidal ones, which would be expected to have also wider geographical distributions. Despite these predictions, both species are currently found in the same geographical areas — they are common species in all the islands of the Azores and are reported for the coastal areas of the Madeira Islands (Cordeiro et al., 2015). The palaeontological record of both archipelagos holds valuable clues to interpret the results of the phylogenetic approach used in this study and better understand the current distribution of the non-planktotrophic *A. mediolittoralis* and *A. sleursi*.

The finding of fossilized specimens of *A. sleursi* (Meireles et al., 2013) on Pliocene sediments (4.78 ± 0.135 to 4.13 ± 0.19 Ma; Ramalho et al., 2017) of Santa Maria Island suggests that the divergence of the lineages encompassed within Clade A occurred earlier than the fossilization event, and that, by that time, viable populations of *A. sleursi* were already established in the Azores. As all the species assigned to Clade A are endemic to one (the Azores) or two (the Azores and Madeira) Atlantic archipelagos and are not found in fossiliferous outcrops outside of the Azores, it is plausible to assume

that they do not constitute relicts of a former wider distribution. Therefore, and considering the climatic and oceanographic conditions of the last 5 Ma, we hypothesize that this divergence occurred in the Azores vicinities, where the ancient lineage diversified in, at least, three species – *A. sleursi*, *A. mediolittoralis* and *A. formicarum* – that established in the Azores and have only recently dispersed to Madeira. The finding of *A. sleursi* representatives also in MIS 5e sediments, with an age of 130-116 ka (Ávila, 2005; Cordeiro & Ávila, 2015; Ávila et al., 2015, 2016) suggests that this subtidal/circalittoral rissoid has been inhabiting this region for a considerable time, where it supposedly established long before *A. mediolittoralis* speciated. The posterior divergence of lineages that originated *A. formicarum* and *A. mediolittoralis* is estimated to have taken place ca. 360 ka ago, during the Pleistocene. Considering that representatives of both species are found in MIS 5e highstand deposits of Santa Maria Island (Ávila et al., 2009, 2015, 2016), the age estimated for the split event leading to their speciation can be accepted. The divergence is also believed to have happened in the Azores Archipelago, as *A. formicarum* is endemic to this region, with no knowledge of previous wider distribution (Cordeiro et al., 2015). Despite the older age of Porto Santo Island, its Miocene fossiliferous outcrops, mainly composed by massive coral reefs, rhodoliths, echinoderms and some molluscs (Mata et al., 2013), are similar in taxonomic composition to Santa Maria's Pliocene outcrops (Ávila et al., 2016 and references therein), with the exception of the massive coral reefs, which are only represented at Santa Maria by small, solitary corals. Although efforts to evaluate the palaeontological record of Madeira Archipelago have been made, there are still no reports of fossilized *A. mediolittoralis* and *A. sleursi* in Madeiran region (Gerber et al., 1989; Ávila et al., 2015). This absence suggests that these molluscs were still not present in the latter archipelago during the Pliocene or Pleistocene, when they had already established in the Azores (Ávila et al., 2015, 2016), having reached the Madeira Archipelago later. Therefore, based on the evolutionary history inferred and data provided by ecological and palaeontological observations, we propose early speciation within the Azores Archipelago's vicinity, followed by their dispersal to Madeira.

From an evolutionary perspective, this phylogenetic approach allowed us to test if the subtidal/circalittoral species was indeed longer-lived than the intertidal species. Clade A revealed that the oldest split event was the one leading to the divergence of *A. sleursi*, recently followed by the speciation of the intertidal *A. mediolittoralis* during the Pleistocene. The early speciation of the subtidal/circalittoral species provided enough time for individuals to engage dispersal, even though less prone to do so, settle and successfully colonize another archipelago, and thus, to widen its geographical range.

For the first time, Ávila's theoretical biogeographical hypothesis, proposed to explain the simultaneous existence of two congeneric sp-species with different bathymetric ranges in two nearby archipelagos, is hereby corroborated with genetic data from rissoids, the marine mollusc gastropods that inspired its formulation. Observations in distinct scientific fields — phylogenetics, palaeontology and ecology — contribute to increase the robustness of its theory. Further studies involving other marine invertebrates with similar biogeographical patterns, should be addressed to further sustain Ávila's (2013) hypothesis.

Conclusions

Out of 266 shallow-water (<50 m depth) rissoid species reported for the entire North Atlantic and adjacent seas, a total of 12 rissoid species are reported simultaneously and exclusively for three archipelagos (Madeira, Selvagens, and the Canaries). This shows that the dispersal of formerly endemic species to one of the archipelagos, albeit infrequent, is probably a more common event than previously thought. A consequence of this work is the prediction that, for marine species having similar ecological traits (ecological zonation depths, mode of larval development, habitat), those having wider geographical ranges are expected to be older (in an evolutionary sense) in comparison with species that occur in only two, or are restricted to just one, archipelago.

Extending and testing of this biogeographical hypothesis to other marine phyla will impact our current knowledge of inter-archipelagic patterns and processes of dispersal, as well as the evolutionary times of speciation of shallow-water marine organisms living in remote volcanic oceanic islands, thus providing a more complete and robust understanding for recent marine island biogeography theory (see Ávila et al., 2018, 2019).

3.2 Population structure of *Stramonita haemastoma* (Linnaeus, 1767) in the NE Atlantic Ocean

Introduction

The influence of larval development in the dispersal ability of marine invertebrates has long been of interest for marine biogeographers. Species with a long free-swimming feeding larval stage are expected to easily disperse during this stage and expand to broader distribution ranges, whereas the dispersal of non-planktotrophic larvae is often linked to extrinsic chance events (e.g. storms and rafting events), being geographically more restricted (Ávila, 2006, 2013; Scheltema, 1971a, 1986a, 1986b; Winston, 2012). The widely known oyster drill *Stramonita haemastoma* (Linnaeus, 1767) complex is widespread in warm-temperate and tropical latitudes of the Atlantic and Pacific Oceans, and Mediterranean Sea (Claremont et al., 2011), possessing planktotrophic larvae able to survive in the water column for at least 2-3 months (Scheltema, 1977; Kool, 1993). The wide geographic distribution of *S. haemastoma*, although not surprising for a mollusc with planktotrophic larvae (Ávila, 2013; Scheltema, 1971a, 1986b), has raised taxonomic and biogeographical questions related to its geographical scale of speciation. Phylogenetic studies (Claremont et al., 2011; Vermeij, 2001; Vermeij & Carlson, 2000) have shown this group to be a good example of a species complex, with seven subspecies currently recognized, almost entirely allopatric (Claremont et al., 2011). The European species, *S. haemastoma*, occurs in the continental eastern Atlantic, from West Africa to the English Channel, and Mediterranean coasts and in the NE Atlantic archipelagos (Azores, Madeira, Selvagens, Canaries, Cabo Verde), and is likely the only amphi-Atlantic representative of the complex (Claremont et al., 2011; Clench, 1947; Pezy et al., 2019).

The recent split of Macaronesia into the Azores and Webbsnesia (Madeira, Selvagens, and Canaries archipelagos) ecoregions, and Cabo Verde biogeographic subprovince (Freitas et al., 2019) poses questions regarding the connectivity of planktotrophic marine invertebrates among these regions. Therefore, population genetic analyses based on mitochondrial and microsatellite markers were conducted in a dataset of *S. haemastoma* from the Azores, Madeira, the Canaries, Cabo Verde, and Mediterranean Sea, with the goal of assessing the genetic structure and population dynamics of a planktotrophic marine invertebrate across a wide geographical area.

Material and methods

Samples of *Stramonita haemastoma* were collected in the intertidal/infralittoral (0-5 m) of the Azores, and Cabo Verde archipelagos. Individuals from Madeira, the Canaries, and Ligurian Sea (Mediterranean) were provided by collaborators. All newly collected samples were deposited in the marine molluscs' reference collection at the Department of Biology of the University of the Azores (DBUA), which was also visited to retrieve specimens from Azorean islands where fieldwork was not possible (Graciosa). Details on the populations sampled, as well as coordinates of the sampling sites, are provided in Table 3.2. For a map of the NE Atlantic and archipelagos in study, please consult Figures 1.1 and 1.2.

Mitochondrial dataset

DNA extraction from *S. haemastoma* was performed with foot tissue, following the manufacturer's protocol for the commercial EasySpin® Genomic DNA Tissue Kit (Citomed, Lisbon, Portugal). The fragment of the mitochondrial gene Cytochrome C Oxidase subunit I (COI) was amplified in a 25 µl reaction [12.5 µl of QIAGEN Multiplex PCR Master Mix (Qiagen, CA, USA), 5 µL of each 2 µM primer, and 2.5 µL of gDNA], with the primer pair jgLCO1490/jgHCO2198 (Geller et al 2013) under the following cycling profile: 95 °C for 15 min; 35 cycles of 95 °C for 30 s, 50 °C for 1 min, 72 °C for 30 s; 72 °C for 10 min. The efficiency of the PCR and quality of its products were confirmed in electrophoretic runs of 2% (w/v) agarose gels stained with GelRed (DNA fluorescent dye, BioTarget™). Purification of the PCR product and bi-directional Sanger sequencing with the amplification primers were realised by Genewiz, Leipzig, Germany.

All chromatograms were manually checked for potential misreads with Geneious 8.1.9 (Kearse et al., 2012) and the COI sequences inspected for premature stop codons and/or frameshift mutations with AliView v1.26 (Larsson, 2014). The dataset, comprising 96 sequences, was aligned with Clustal Omega algorithm from Web Services by EMBL-EBI (McWilliam et al., 2013). Estimates of the raw p-distances among regions were performed in MEGA11 (Tamura et al., 2021). The genetic structure between *S. haemostoma* haplotypes from the NE Atlantic was examined with the reconstruction of a statistical parsimony haplotype network at the 95% connection limit with TCS v1.21 (Clement et al., 2000). The resulting haplotype networks were rendered with tcsBU (Santos et al., 2016), considering archipelagos/regions or the individual localities as groups. Pairwise F_{ST} between NE Atlantic regions were estimated with Arlequin v3.5 (Excoffier & Lischer, 2010), with statistical significance tested by 1023 permutations and considered below the critical p-value of 0.003, adjusted for multiple pairwise comparisons.

following Bonferroni correction (Rice, 1989). A hierarchical analysis of molecular variance (AMOVA; Excoffier et al., 1992) was also conducted in Arlequin v3.5 to test several hypotheses of the genetic structure of *S. haemastoma* in the NE Atlantic and Mediterranean: A) Azores vs. Madeira+Canaries (Webbnesia sensu Freitas et al., 2019) vs. Cabo Verde vs. Mediterranean; B) Azores+Madeira+Canaries vs. Cabo Verde vs. Mediterranean; C) NE Atlantic archipelagos vs. Mediterranean.

Microsatellite dataset

The microsatellite dataset was generated following established workflows of genotyping by sequencing (SSR-GBAS) (Baptista et al., 2021a; Curto et al., 2019; Lanner et al., 2021; Tibihika et al., 2019). Two low-coverage Illumina MiSeq runs from two *S. haemastoma* individuals (ES-PDH5144 from the Canaries and PT-MOS4510 from the Azores) were conducted for marker discovery from paired-end reads of 300 bp, after preparation of the library at the Genomics Service Unit, Ludwig-Maximilian's-Universität (LMU, München, Germany). Details on the processing of the raw reads and identification of sequences containing potential repeats, plus design and individual test reactions of the newly designed primers can be found elsewhere in this thesis (cf. Chapter 4.1) or in published literature (Baptista et al., 2021a; Curto et al., 2019; Lanner et al., 2021; Tibihika et al., 2019). A total of 47 primers were designed for *S. haemastoma*, of which 20 passed the quality control steps (cf. Chapter 4.1) and were combined in two mixes of 10 primer pairs, each with a final concentration of 1 µM (Supplementary Table A2.1). Multiplex amplification for all the *S. haemastoma* samples, pooling, PCR clean-up with the Agencourt AMPure XP PCR Purification kit (Beckman Coulter Inc., Brea, CA, USA), and incorporation of unique dual indexes combinations to each sample followed already established protocols (Baptista et al., 2021a; Curto et al., 2019; cf. Chapter 4.1). Sequences to be analysed by the genotyping by amplicon sequencing (GBAS) protocol were generated using the pooled index-PCR products as input for an PE 300 bp Illumina MiSeq run, conducted at the Genomics Service Unit at LMU. These sequences, downloaded from Illumina BaseSpace, were processed by several software, as described in Baptista et al. (2021a) and references therein. For every SSR loci and sample, the genotype was determined using the four scripts comprised in the SSR_GBAS_pipeline.py (Curto et al., 2019; Tibihika et al., 2019), available at <https://github.com/mcurto/SSR-GBS-pipeline>.

Population genetic analyses and assessment of genetic structure were conducted on the codominant matrix resulting from the SSR-GBAS genotyping, after removal of individuals and loci with more than 60% missing data from the dataset. Genetic diversity

indices, heterozygosity levels, and deviations to Hardy-Weinberg Equilibrium (HWE) for each geographical region (Azores, Madeira, Canaries, Cabo Verde, Mediterranean) were estimated in the whole amplicon information (WAI) dataset resorting to GenAIEx v6.51 (Peakall & Smouse, 2006). The frequency of null alleles per loci and population was assessed with FreeNA (Chapuis & Estoup, 2007). The genetic structure in the dataset of *S. haemastoma* from the NE Atlantic Archipelagos and Mediterranean was assessed with a principal component analysis (PCoA), as implemented in GenAIEx v6.51, and STRUCTURE v2.3.4 (Pritchard et al., 2000) analyses. For it, the following settings were defined: burn-in of 50000, 200000 replications of Markov Chain Monte Carlo (MCMC), 10 iterations, maximum of 10 clusters (K), keeping the default for admixture model and allele frequencies. The validation of the optimal K-value and graphical summarization of the outputs were conducted with Structure Harvester and CLUMPAK (Earl & VonHoldt, 2012; Kopelman et al., 2015). Differentiation between the regions in study was assessed with AMOVA (Excoffier et al., 1992) and estimates of pairwise F_{ST} values, as implemented in GenAIEx v6.5, with statistical significance tested by 1023 permutations and considered below the critical p-value of 0.003, adjusted for multiple pairwise comparisons following Bonferroni correction (Rice, 1989).

Table 3.2. Details of the populations of *S. haemastoma* and number of specimens considered in this study. Includes the region, localities, sampling date, coordinates in decimal degrees (WGS84), and voucher assigned in the marine molluscs' reference collection at the Department of Biology of the University of the Azores (DBUA). Question mark (?) indicates details unknown.

Region	Island/ Area	Localities	#individuals (COI/SSR-GBAS)	Sampling date	Coordinates (DD)	Collection voucher
Azores	Santa Maria	Praia Formosa	3/ 2	08 Sep 2018	36.951, -25.101	DBUA 1487
		Poça do Carro, Anjos	4/ 1	07 Aug 2018	37.005, -25.157	DBUA 1488
	São Miguel	Ponta do Castelo	4/ 2	08 Aug 2018	36.929, -25.018	DBUA 1489
		Ponta dos Mosteiros	4/ 3	25 Jul 2018	37.891, -25.825	DBUA 1195
		Cerco da Caloura	5/ 4	26 Jan 2019	37.707, -25.510	DBUA 1486
		Porto de Santa Cruz	4/ 0	10-11 Jun 2004	?	DBUA 1490-1
	Graciosa	Baixa do Ferreiro	2/ 1	08 Jun 2004	?	DBUA 1492
		Lajes do Pico	7/ 4	18 May 2018	38.390, -28.251	DBUA 1493
	Pico	Lajes do Pico	7/ 4	18 May 2018	38.390, -28.251	DBUA 1493
	Faial	Feteira	6/ 3	11 Jun 2018	38.523, -28.671	DBUA 1494
	São Jorge	Piscina de Velas	9/ 7	26 Jun 2019	38.678, -28.207	DBUA 1412
	Flores	Fajã Grande	4/ 4	12 Aug 2018	39.449, -31.266	DBUA 1495
		Piscinas Naturais do Boqueirão	5/ 3	11 Aug 2018	39.463, -31.128	DBUA 1496
	Formigas Islet	-	3/ 4	28 Aug 2018	37.271, -24.781	DBUA 1497
Madeira	Madeira	Quinta do Lorde	10/ 4	10-11 Dec 2018	32.741, -16.711	DBUA 1483/5
Canaries	Tenerife	Punta del Hidalgo	10/ 5	24 Sep 2019	?	DBUA 1604
Cabo Verde	Boa Vista	?	7/ 7	Jun 2018	?	DBUA 1155
Mediterranean	Liguria, Genoa	Santa Margherita Spiaggia di Prelo	8/ 8	16 Apr 2019	?	DBUA 1498

Results and Discussion

Mitochondrial dataset

A total of 95 COI sequences of *S. haemastoma* were generated, representing 12 islands of the NE Atlantic Archipelagos (8 from the Azores, 1 from Madeira, 1 from the Canaries, 1 from Cabo Verde) and one locality from the Mediterranean Sea. Raw p-distances among NE Atlantic archipelagos were of the same magnitude (0.6-0.8%) for all pairwise comparisons, similar to the differentiation between them and the Mediterranean population (0.8-0.9%). The levels of differentiation within the Mediterranean population were slightly higher (1.1%). The reconstruction of haplotype networks, considering the archipelagos/regions (Figure 3.5a) or each island as a distinct population (Figure 3.5b), show no visible population structure and a rich haplotype diversity. Star-shaped haplogroups and the complex reticulate connection among haplotypes suggests the occurrence of demographic expansions, coupled with extensive gene flow in the broad area of study in the NE Atlantic and Mediterranean.

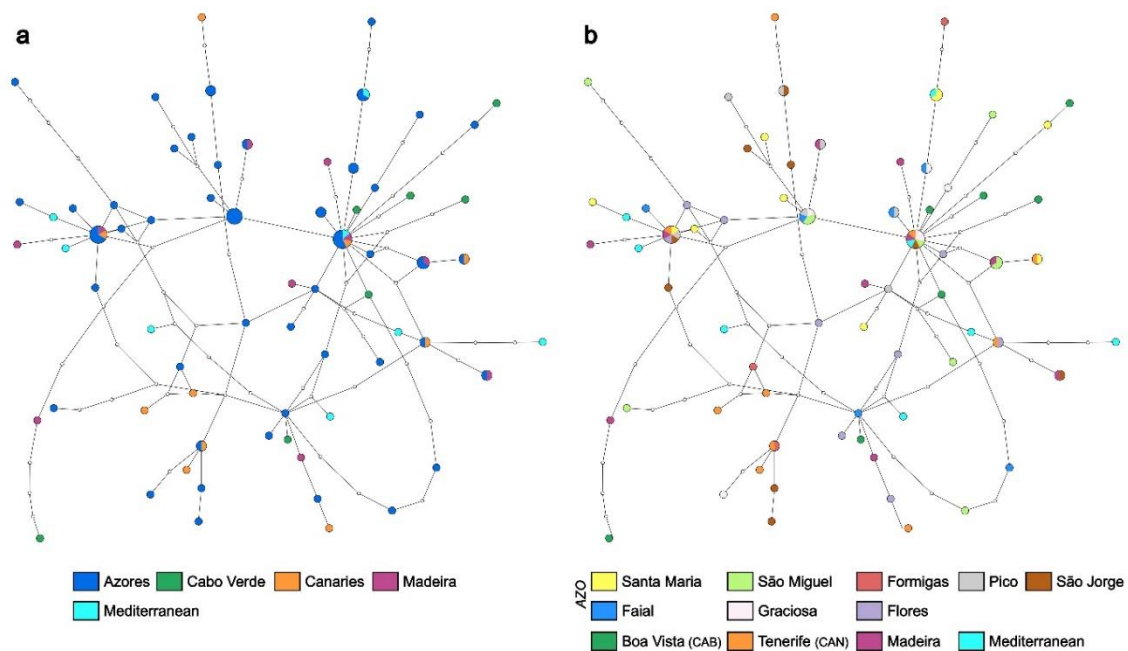


Figure 3.5. Haplotypic network of mitochondrial data of *S. haemastoma*, based on 95 COI sequences at 95% parsimony connection limit with haplotype circles coloured according to two criteria: **a.** Five archipelagos/regions studied: Azores, Cabo Verde, Canaries, Madeira, and Mediterranean. **b.** 12 populations in study by island/locality: eight at the Azores (Santa Maria, São Miguel, Formigas, Pico, São Jorge, Faial, Graciosa, Flores), Boa Vista at Cabo Verde, Tenerife at the Canaries, Madeira Island, and the Mediterranean. Size of the circles is proportional to the frequency of each haplotype; small uncoloured circles represent non-observed haplotypes; each line connecting haplotypes represents a single mutational change. Network obtained with TCS v1.21 (Clement et al., 2000) and tcsBU (Santos et al., 2016).

Pairwise F_{ST} levels between regions (four NE Atlantic archipelagos and Mediterranean) did not surpass 0.06 and were non-significant for all comparisons. The three scenarios of population structure tested with AMOVA were discarded, as over 98% of the variation is found within the populations and all fixation indices, close to zero, were non-significant (Table 3.3).

Table 3.3. Results of the hierarchical AMOVA tests comparing variation in the mitochondrial COI dataset under three scenarios of population structure: a. Azores vs. Webbnesia (Madeira+Canaries) vs. Cabo Verde vs. Mediterranean; **b.** Azores+Madeira+Canaries vs. Cabo Verde vs. Mediterranean; **c.** NE Atlantic archipelagos vs. Mediterranean. Significance tests derived from 1023 permutations.

a. Azores vs. Webbnesia vs. Cabo Verde vs. Mediterranean						
Source	d.f.	SS	Var.	% var.	Fixation Indices	p-value
Among groups	3	7.027	-0.05182	-2.36	$F_{CT} = -0.02363$	0.70968
Among pops within groups	1	2.900	0.07283	3.32	$F_{SC} = 0.03245$	0.18964
Within populations	90	195.452	2.17169	99.04	$F_{ST} = 0.00958$	0.20235
Total	94	205.379	2.19271			

b. Azores+Webbnesia vs. Cabo Verde vs. Mediterranean						
Source	d.f.	SS	Var.	% var.	Fixation Indices	p-value
Among groups	2	5.056	0.01386	0.63	$F_{CT} = 0.00630$	0.40665
Among pops within groups	2	4.871	0.01623	0.74	$F_{SC} = 0.00742$	0.26295
Within populations	90	195.452	2.17169	98.63	$F_{ST} = 0.01367$	0.20430
Total	94	205.379	2.20178			

c. NE Atlantic archipelagos vs. Mediterranean						
Source	d.f.	SS	Var.	% var.	Fixation Indices	p-value
Among groups	1	1.436	-0.08502	-3.99	$F_{CT} = -0.03986$	1.00000
Among pops within groups	3	8.490	0.04619	2.17	$F_{SC} = 0.02083$	0.07429
Within populations	90	195.452	2.17169	101.82	$F_{ST} = -0.01821$	0.20821
Total	94	205.379	2.13286			

Microsatellite dataset

The Illumina MiSeq runs produced libraries containing 51,448 and 790,134 raw reads, respectively for PT-MOS4510 and ES-PDH5144. After quality control, 151,656 and 240,799 paired reads, respectively, were merge into a total of 121,175 and 140,230 reads. These data were screened for SSR motifs, comprising 7,589 and 11,403 reads, respectively, whose motifs complied with the criteria defined for the SSR search pipeline: 2,220 di-, 1,870 tri-, 3,031 tetra-, and 438 pentanucleotide repeats for PT-MOS4510; 3,422 di-, 2,809 tri-, 4,492 tetra-, and 680 pentanucleotide repeats for ES-PDH5144. A total of 47 primer pairs for *S. haemastoma* were designed from sequences containing penta- and tetranucleotide repeats, of which 20 were included in multiple primer mixes

(Supplementary Table A2.1). For the SSR-GBAS genotyping, a total of 5,277,296 paired reads were produced and reduced to 590,557 after quality control, merging and primer demultiplex steps. The number of reads per marker ranged between 0 (SH1, SH3, and SH38) 246,439 (SH40), whilst the number of sequences per sample varied from 48 (PC4485) to 17,116 (PBA4969). The markers SH1_TTTA, SH3_ATTT, SH5_GATT, SH16_ACAG, SH17_ACCT, and SH38_GTCAA were excluded for yielding more than 60% missing data. The dataset was also filtered to exclude individuals with more than 60% missing data across all loci, reducing it to 38 individuals from the Azores, four from Madeira, five from the Canaries, seven from Cabo Verde and eight from the Mediterranean. The imbalance in the number of samples from each locality is a consequence of an intensive sampling across the Azores Archipelago, aiming to assess intra-archipelagic population structure patterns. As these were not detected in our preliminary analyses, we decided to group them as a single population “Azores” and focus on inter-archipelago comparisons. Further population genetic analyses were conducted in a dataset of 62 specimens of *S. haemastoma* and 14 markers.

Tests for Hardy–Weinberg Equilibrium (HWE; Table 3.4) per markers and population revealed deviations at most loci (nine) in the Azores, four in Cabo Verde, one in the Canaries and Mediterranean, none in Madeira. Across the five regions studied, we estimated a lower-than-expected heterozygosity for each markers (data not shown) or overall (Figure 3.6), indicating an excess of homozygotes in the dataset. The estimated frequency of null alleles (Table 3.4) is relatively low across loci and populations, not exceeding 45% (SH35_AACA in Madeira). The higher putative incidence of null alleles in most loci in the Azores can explain the deviations to HWE (Faria et al., 2016), although it can also be a consequence of allele dropout, small effective population size, demographic changes, or non-panmixia in the archipelago. The Azores is one of the most remote archipelagos in the Atlantic Ocean (840 km to Madeira), only surpassed by Ascension and Saint Helena (1290 km between each other) (Ávila et al., 2018). Consequently, the first colonizers of a species had to arrive from external sources going through founder effect and genetic bottleneck. *Stramonita haemastoma* successfully invaded the Azores at least 120 kya, before the Marine Isotope Stage 5 (MIS5), according to the fossil record available in Santa Maria Island (Ávila et al., 2002, 2009). Nevertheless, the geographical distance to other archipelagos/mainland and the complex oceanographical circulation in the NE Atlantic nowadays can constitute effective barriers to, or at least reduce, gene flow, even within the archipelago (Baptista et al., 2021a, 2021b; Desiderato et al., 2019; Quinteiro et al., 2015, 2020; Vieira et al., 2019, 2022). The AMOVA analysis and estimates of pairwise F_{ST} assigned the variation in the

dataset to differences among (48%) or within (52%) individuals, with no significant population structure detected between the archipelagos based on the microsatellite dataset. Pairwise F_{ST} levels did not exceed 0.013 (between Madeira and Cabo Verde), but all comparisons were non-significant. Inbreeding coefficients were significantly moderate ($F_{IS}=0.478$ and $F_{IT}=0.479$; $p=0.001$), suggesting that non-random mating might occur. The PCoA (Figure 3.7) and STRUCTURE (Supplementary Figure A2.1) analyses were consistent, revealing no population structure of *S. haemastoma* across the NE Atlantic Archipelagos and Mediterranean Sea. The optimal K-value of 9 was estimated for the STRUCTURE analysis, although no visible structure is detected with clear admixture among all populations. The lack of genetic structure within and among archipelagos excludes the possibility of HWE deviations being a consequence of Wahlund effect. Instead, allele dropout, non-panmixia, or demographic changes might explain the HWE deviations, particularly evident in the Azorean populations.

Table 3.4. Estimates of Hardy–Weinberg Equilibrium tests and frequency of null alleles per marker and population of *S. haemastoma*. Significant ($p < 0.05$) deviations to HWE are highlighted in bold, monomorphic loci are identified as *mono*.

	Azores		Madeira		Canaries		Cabo Verde		Mediterranean	
	HWE	f(null)	HWE	f(null)	HWE	f(null)	HWE	f(null)	HWE	f(null)
SH4_ CTGT	0.001	0.0498	0.423	0.0000	0.293	0.0005	0.450	0.0000	0.573	0.0000
SH6_ TATT	0.001	0.0669	0.609	0.0000	0.572	0.0000	0.542	0.0537	0.879	0.0000
SH8_ AATAC	0.023	0.1600	0.261	0.1111	0.343	0.1756	0.168	0.1054	0.412	0.0007
SH9_ CTAT	0.336	0.0000	0.451	0.0000	0.532	0.0000	0.464	0.0000	0.472	0.0000
SH12_ TCTG	0.000	0.1693	0.609	0.0008	0.100	0.3000	0.062	0.1813	0.148	0.0000
SH18_ TACA	0.000	0.3378	0.174	0.1575	0.172	0.1667	0.043	0.2063	0.054	0.2222
SH19_ TTTA	0.240	0.1247	<i>mono</i>	0.1437	0.958	0.0000	0.248	0.0939	0.382	0.0010
SH20_ ATAA	0.000	0.3854	0.174	0.3333	0.157	0.3333	0.157	0.4286	0.062	0.2222
SH24_ TTTC	0.805	0.0000	0.679	0.0384	0.994	0.0000	0.970	0.0155	0.414	0.0000
SH28_ GCAG	0.874	0.0487	<i>mono</i>	0.0000	0.729	0.0000	0.804	0.1465	0.222	0.0010
SH35_ AACA	0.000	0.2956	0.172	0.4497	0.375	0.0667	0.002	0.3357	0.017	0.1667
SH39_ TCCAA	0.000	0.2916	0.174	0.2449	0.172	0.1667	0.039	0.1590	0.112	0.2222
SH40_ AATAC	0.057	0.1271	0.349	0.1497	0.170	0.1333	0.170	0.0763	0.253	0.0000
SH47_ AGGTC	0.000	0.3075	0.261	0.4032	0.046	0.3333	0.006	0.3364	0.003	0.0007

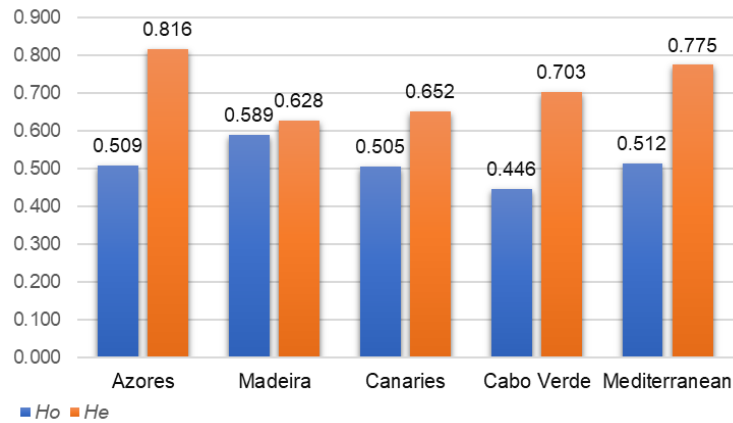


Figure 3.6. Observed (H_o) and expected (H_e) heterozygosity in the five populations of *S. haemastoma* studied, estimated as implemented in GenAlEx v6.5 (Peakall & Smouse, 2012).

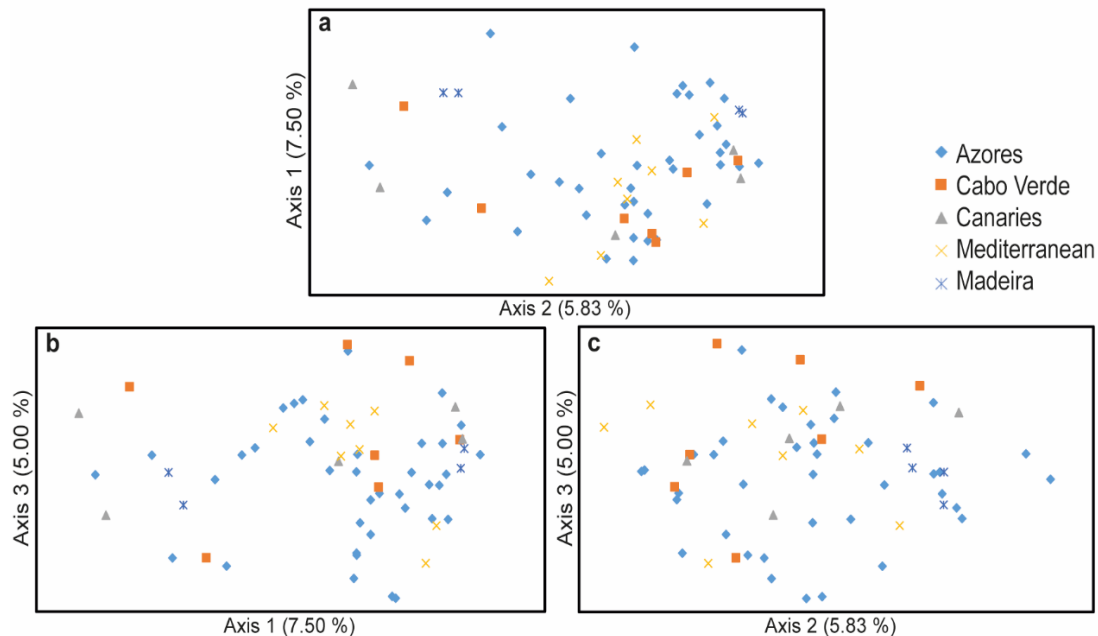


Figure 3.7. Principal coordinates analysis from the microsatellite dataset of *S. haemastoma*. a. Variance explained by axis 1 (7.50%) and axis 2 (5.83%); b. Variance explained by axis 1 (7.59%) and axis 3 (5.00%); c. Variance explained by axis 2 (5.83%) and axis 3 (5.00%). PCoA conducted as implemented in GenAlEx v6.5 (Peakall & Smouse, 2012); populations in study are coded by colour and icon shape.

Species with planktotrophic larvae are expected to easily disperse during the larval stage, occupying large geographical areas and being able to maintain gene flow between localities far apart (Scheltema, 1971b, 1986a, 1986b). The larvae of *S. haemastoma* survive in the water column for at least 2-3 months (Scheltema, 1971a; Vermeij & Carlson, 2000), providing enough time to be transported to new localities and potentially resulting in the complete absence of population structure in the distribution range. The isolation of the Azores from the remaining NE Atlantic archipelagos is a recurrent genetic pattern, even in planktotrophic marine invertebrates (Faria et al., 2017; Quinteiro et al.,

2007, 2015, 2020; Riesgo et al., 2019; Sá-Pinto et al., 2008; Sasson et al., 2012), as it is the differentiation of Cabo Verdean populations (Quinteiro et al., 2007, 2015, 2020; Van Den Broeck et al., 2008). However, none of the latter patterns are observed in *S. haemastoma*. The absence of genetic structure can be a consequence of temporal variation in the sea-surface circulation patterns in the NE Atlantic Ocean, favouring different dispersal pathways and low self-recruitment. In this case, genetic structure will reflect the time of arrival and source population, rather than the current geographical partitioning. This idea can be tested in the future by estimating the age of the individuals comprised in this dataset, plotting the results by age instead of the geographical origin of the sample.

We inferred close genetic patterns between NE Atlantic archipelagos and Mediterranean localities, as reported in other organisms and also related with the survival capacity of teleplanic larvae to cross Atlanto-Mediterranean biogeographical barriers (Crocetta et al., 2020; Fourdrilis & Backeljau, 2019; Patarnello et al., 2007; Pérez-Portela et al., 2010; Quinteiro et al., 2020). Similarly, several marine invertebrates with distinct modes of larval development are able to maintain connectivity between Atlantic archipelagos, such as *Lasaea* spp. T. Brown, 1827, *Phorcus sauciatus* (Koch, 1845), *Megabalanus azoricus* (Pilsbry, 1916), and *Phorbas fictitius* (Bowerbank, 1866) (Baptista et al., 2021b; Ó Foighil et al., 2001; Quinteiro et al., 2015; Xavier et al., 2010). *Stramonita haemastoma* seems to be panmictic across the NE Atlantic archipelagos and Mediterranean Sea, without signals of phylogeographic or haplotype structuring across the sampled range in both the mitochondrial and microsatellite datasets.

The dataset comprises a high number of specimens collected around the Azores, as we aimed to assess population structure within the archipelago (data not shown) and compare with the results from the non-planktotrophic *C. trifasciata* (Baptista et al., 2021a, cf. Chapter 4.1). Clear population structure of the microgastropod *C. trifasciata* was detected within the Azores Archipelago, influenced by the larval development, type of habitat occupied, and sea-surface circulation patterns established in the area (Baptista et al., 2021b). The overall absence of structure in *S. haemastoma* suggests that planktotrophic marine invertebrates might not be as useful to study biogeographic patterns and processes operating in remote archipelagos, as their larvae are easily dispersed and gene flow is maintained over broad geographical areas. For this reason, the following chapters of this thesis are focused on non-planktotrophic gastropods and bryozoans, used in molecular analyses and methodological approaches that contribute to a better understanding of marine life in the remote Azores Archipelago and surrounding areas.

References

- Aboim, M. A., Rogers, A. D., Menezes, G. M., Maggioni, R., & Pearson, C. V. M. (2003). Isolation of polymorphic microsatellite markers for the demersal fish *Helicolenus dactylopterus* (Dela Roche 1809). *Molecular Ecology Notes*, 3(1), 18–20. <https://doi.org/10.1046/J.1471-8286.2003.00337.X>
- Akaike, H. (1973). Information Theory and an Extension of the Maximum Likelihood Principle. *Proceedings of the 2nd International Symposium on Information Theory.*, 267–281.
- Ávila, S. P. (2005). Processos e padrões de dispersão e colonização nos Rissoidae (Mollusca: Gastropoda) dos Açores. [unpublished Ph.D. dissertation]. University of the Azores.
- Ávila, S. P. (2006). Oceanic islands, rafting, geographical range and bathymetry: a neglected relationship? *Occasional Publication of the Irish Biogeographical Society*, 9, 22–39.
- Ávila, S. P. (2013). Unravelling the patterns and processes of evolution of marine life in oceanic islands: a global framework. In J. M. Fernández-Palacios, L. Nascimento, J. C. Hernández, S. Clemente, A. González, & J. P. Díaz-González (Eds.), *Climate Change Perspectives From the Atlantic: Past, Present and Future* (pp. 99–125). Universidad de La Laguna.
- Ávila, S. P., Amen, R., Azevedo, J. M. N., Cachão, M., & García-Talavera, F. (2002). Checklist of the Pleistocene marine molluscs of Praínha and Lagoínhas (Santa Maria, Azores). *Açoreana*, 9(4), 343–370.
- Ávila, S. P., Cachão, M., Ramalho, R. S., Botelho, A. Z., Madeira, P., Rebelo, A. C., Cordeiro, R., Melo, C., Hipólito, A., Ventura, M. A., & Lipps, J. H. (2016). The Palaeontological Heritage of Santa Maria Island (Azores: NE Atlantic): a Re-evaluation of Geosites in GeoPark Azores and Their Use in Geotourism. *Geoheritage*, 8(2), 155–171. <https://doi.org/10.1007/s12371-015-0148-x>
- Ávila, S. P., da Silva, C. M., Schiebel, R., Cecca, F., Backeljau, T., & Martins, A. M. F. (2009). How Did They Get Here? Palaeobiogeography of the Pleistocene Marine Molluscs of the Azores. *Bulletin de La Société Géologique de La France*, 180(4), 295–307. <https://doi.org/10.2113/gssgfbull.180.4.295>
- Ávila, S. P., Cordeiro, R., Madeira, P., Silva, L., Medeiros, A., Rebelo, A. C., Melo, C., Neto, A. I., Haroun, R., Monteiro, A., Rijdsdijk, K., & Johnson, M. E. (2018). Global change impacts on large-scale biogeographic patterns of marine organisms on Atlantic oceanic islands. *Marine Pollution Bulletin*, 126, 101–112. <https://doi.org/10.1016/j.marpolbul.2017.10.087>
- Ávila, S. P., Goud, J., & De Frias Martins, A. M. (2012). Patterns of diversity of the Rissoidae (Mollusca: Gastropoda) in the Atlantic and the Mediterranean region. In *The Scientific World Journal* (Vol. 2012). <https://doi.org/10.1100/2012/164890>
- Ávila, S. P., Madeira, P., Mendes, N., Rebelo, A., Medeiros, A., Gomes, C., García-Talavera, F., da Silva, C. M., Cachão, M., Hillaire-Marcel, C., & Martins, A. M. F. (2008). Mass extinctions in the Azores during the last glaciation: Fact or myth? *Journal of Biogeography*, 35(6), 1123–1129. <https://doi.org/10.1111/j.1365-2699.2008.01881.x>
- Ávila, S. P., Melo, C., Sá, N., Quartau, R., Rijdsdijk, K., Ramalho, R. S., Berning, B., Cordeiro, R.,

- de Sá, N. C., Pimentel, A., Baptista, L., Medeiros, A., Gil, A., & Johnson, M. E. (2019). Towards a “Sea-Level Sensitive Marine Island Biogeography” model: the impact of glacio-eustatic oscillations in global marine island biogeographic patterns. *Biological Reviews*, 94(2), 1116–1142. <https://doi.org/10.1111/brv.12492>
- Ávila, S. P., Melo, C., Silva, L., Ramalho, R. S., Quartau, R., Hipólito, A., Cordeiro, R., Rebelo, A. C., Madeira, P., Rovere, A., Hearty, P. J., Henriques, D., Silva, C. M. da, Martins, A. M. F., & Zazo, C. (2015). A review of the MIS 5e highstand deposits from Santa Maria Island (Azores, NE Atlantic): Palaeobiodiversity, palaeoecology and palaeobiogeography. *Quaternary Science Reviews*, 114, 126–148. <https://doi.org/10.1016/j.quascirev.2015.02.012>
- Azevedo, J. (1998). *Geologia e Hidrogeologia da Ilha das Flores (Açores – Portugal)*. University of Coimbra, Coimbra.
- Baptista, L., Meimberg, H., Ávila, S. P., Santos, A. M., & Curto, M. (2021a). Dispersal ability, habitat characteristics, and sea-surface circulation shape population structure of *Cingula trifasciata* (Gastropoda: Rissoidae) in the remote Azores Archipelago. *BMC Ecology and Evolution*, 21(1), 128. <https://doi.org/10.1186/s12862-021-01862-1>
- Baptista, L., Santos, A. M., Melo, C. S., Rebelo, A. C., Madeira, P., Cordeiro, R., Botelho, A. Z., Hipólito, A., Pombo, J., Voelker, A. H. L., & Ávila, S. P. (2021b). Untangling the origin of the newcomer *Phorcus sauciatatus* (Mollusca: Gastropoda) in a remote Atlantic archipelago. *Marine Biology*, 168(1), Article number: 9. <https://doi.org/10.1007/s00227-020-03808-5>
- Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C. H., Xie, D., Suchard, M. A., Rambaut, A., & Drummond, A. J. (2014). BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. *PLoS Computational Biology*, 10(4), e1003537. <https://doi.org/10.1371/journal.pcbi.1003537>
- Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, 17(4), 540–552.
- Chapuis, M. P. M., & Estoup, A. (2007). Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution*, 24(3), 621–631. <https://doi.org/10.1093/molbev/msl191>
- Claremont, M., Williams, S. T., Barraclough, T. G., & Reid, D. G. (2011). The geographic scale of speciation in a marine snail with high dispersal potential. *Journal of Biogeography*, 38(6), 1016–1032. <https://doi.org/10.1111/j.1365-2699.2011.02482.x>
- Clement, M., Posada, D., & Crandall, K. A. (2000). TCS: A computer program to estimate gene genealogies. *Molecular Ecology*, 9(10), 1657–1659. <https://doi.org/10.1046/j.1365-294X.2000.01020.x>
- Clench, W. (1947). The genera *Purpura* and *Thais* in the Western Atlantic. *Johnsonia*, 2, 61–90.
- Coan, E. (1964). A proposed revision of the Rissoacean families Rissoidae, Rissoinidae, and Cingulopsidae (Mollusca: Gastropoda). *The Veliger*, 6(3), 164–171.
- Conti, M. A., Monari, S., & Oliverio, M. (1993). Early rissoid gastropods from the Jurassic of Italy: the meaning of first appearances. *Scripta Geologica, Special Is*, 67–74.
- Cordeiro, R., & Ávila, S. P. (2015). New species of Rissoidae (Mollusca, Gastropoda) from the

- Archipelago of the Azores (northeast Atlantic) with an updated regional checklist for the family. *ZooKeys*, 480, 1–19. <https://doi.org/10.3897/zookeys.480.8599>
- Cordeiro, R., Borges, J. P., Martins, A. M. F., & Ávila, S. P. (2015). Checklist of the littoral gastropods (Mollusca Gastropoda) from the Archipelago of the Azores (NE Atlantic). In *Biodiversity Journal* (Vol. 6, Issue 4).
- Criscione, F., & Ponder, W. F. (2013). A phylogenetic analysis of rissooidean and cingulopsoidean families (Gastropoda: Caenogastropoda). *Molecular Phylogenetics and Evolution*, 66(3), 1075–1082. <https://doi.org/10.1016/j.ympev.2012.11.026>
- Criscione, F., Ponder, W. F., Köhler, F., Takano, T., & Kano, Y. (2016). A molecular phylogeny of Rissoidae (Caenogastropoda: Rissooidea) allows testing the diagnostic utility of morphological traits. *Zoological Journal of the Linnean Society*, 179(1), 23–40. <https://doi.org/10.1111/zoj.12447>
- Crocetta, F., Caputi, L., Paz-Sedano, S., Tanduo, V., Vazzana, A., & Oliverio, M. (2020). High genetic connectivity in a gastropod with long-lived planktonic larvae. *Journal of Molluscan Studies*, 86(1), 42–55. <https://doi.org/10.1093/MOLLUS/EYZ032>
- Curto, M., Winter, S., Seiter, A., Schmid, L., Scheicher, K., Barthel, L. M. F., Plass, J., & Meimberg, H. (2019). Application of a SSR-GBS marker system on investigation of European Hedgehog species and their hybrid zone dynamics. *Ecology and Evolution*, 9(5), 2814–2832. <https://doi.org/10.1002/ece3.4960>
- Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9(8), 772. <https://doi.org/10.1038/nmeth.2109>
- Degnan, J. H., & Rosenberg, N. A. (2006). Discordance of species trees with their most likely gene trees. *PLoS Genetics*, 2(5), 762–768. <https://doi.org/10.1371/journal.pgen.0020068>
- Demand, J., Fabriol, R., Gerard, F., Lundt, F., & Chovelon, P. (1982). *Prospection géothermique, îles de Faial et de Pico (Açores). Rapport géologique, géochimique et gravimétrique. Technical Report BRGM 82 SGN 003 GTH.*
- Desiderato, A., Costa, F. O., Serejo, C. S., Abbiati, M., Queiroga, H., & Vieira, P. E. (2019). Macaronesian islands as promoters of diversification in amphipods: The remarkable case of the family Hyalidae (Crustacea, Amphipoda). *Zoologica Scripta*, 48(3), 359–375. <https://doi.org/10.1111/zsc.12339>
- Earl, D. A., & VonHoldt, B. M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4(2), 359–361. <https://doi.org/10.1007/s12686-011-9548-7>
- Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10, 564–567.
- Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of Molecular Variance Inferred from Metric Distances Among DNA Haplotypes: Application. *Genetics*, 491, 479–491.
- Faria, J., Martins, G. M., Pita, A., Ribeiro, P. A., Hawkins, S. J., Presa, P., & Neto, A. I. (2017).

- Disentangling the genetic and morphological structure of *Patella candei* complex in Macaronesia (NE Atlantic). *Ecology and Evolution*, 7(16), 6125–6140. <https://doi.org/10.1002/ECE3.3121>
- Faria, J., Pita, A., Rivas, M., Martins, G. M., Hawkins, S. J., Ribeiro, P., Neto, A. I., & Presa, P. (2016). A multiplex microsatellite tool for conservation genetics of the endemic limpet *Patella candei* in the Macaronesian archipelagos. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 26(4), 775–781. <https://doi.org/10.1002/AQC.2651>
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution*, 17(6), 368–376. <https://doi.org/10.1007/BF01734359>
- Forest, F. (2009). Calibrating the tree of life: Fossils, molecules and evolutionary timescales. *Annals of Botany*, 104(5), 789–794. <https://doi.org/10.1093/aob/mcp192>
- Fourdrilis, S., & Backeljau, T. (2019). Highly polymorphic mitochondrial DNA and deceiving haplotypic differentiation: Implications for assessing population genetic differentiation and connectivity. *BMC Evolutionary Biology*, 19(1). <https://doi.org/10.1186/s12862-019-1414-3>
- França, Z., Nunes, J., Cruz, J., Duarte, J., & Forjaz, V. (2002). Preliminary study of the Corvo Island volcanism, Azores. *3o Assembleia Luso-Espanhola de Geodesia e Geofísica*, S09: 727–730.
- Freitas, R., Romeiras, M., Silva, L., Cordeiro, R., Madeira, P., González, J. A., Wirtz, P., Falcón, J. M., Brito, A., Floeter, S. R., Afonso, P., Porteiro, F., Viera-Rodríguez, M. A., Neto, A. I., Haroun, R., Farminhão, J. N. M., Rebelo, A. C., Baptista, L., Melo, C. S., Martínez, A., Núñez, J., Berning, B., Johnson, M. E. & Ávila, S. P. (2019). Restructuring of the ‘Macaronesia’ biogeographic unit: A marine multi-taxon biogeographical approach. *Scientific Reports*, 9(1), 1–18. <https://doi.org/10.1038/s41598-019-51786-6>
- Gadagkar, S., Rosenberg, M.S., & Kumar, S. (2005). Inferring species phylogenies from multiple genes: concatenated sequence tree versus consensus gene tree. *Journal of Experimental Biology*, 304B, 64–74.
- Gerber, J., Hemmen, J., & Groh, K. (1989). Eine pleistozäne marine Molluskenfauna on Porto Santo (Madeira-Archipel). *Mitteilungen Der Deutschen Malakozoologischen Gesellschaft*, 44(45), 19–30.
- Gofas, S. (2016). *The littoral Rissoidae and Anabathridae of São Miguel, Azores*. <https://www.researchgate.net/publication/235424077>
- 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.
- Heled, J., & Drummond, A.J. (2010). Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution*, 27(3), 570–580.
- Hildenbrand, A., Weis, D., Madureira, P., & Marques, F. (2014). Recent plate re-organization at the Azores Triple Junction: Evidence from combined geochemical and geochronological data on Faial, S. Jorge and Terceira volcanic islands. *Lithos*, 210–211, 27–39. <https://doi.org/10.1016/j.lithos.2014.09.009>
- Hurley, I., Mueller, R., Dunn, K., Schmidt, E., Friedman, M., Ho, R., Prince, V., Yang, Z., Thomas,

- M., & Coates, M. (2007). A new time-scale for ray-finned fish evolution. *Proceedings of the Royal Society B*, 274, 489–498. <https://doi.org/10.1098/rspb.2006.3749>
- Katoh, K., Kuma, K., Toh, H., & Miyata, T. (2005). MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research*, 33(2), 511–518. <https://doi.org/10.1093/nar/gki198>
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., & Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28(12), 1647–1649.
- Knowles, L.L. (2009). Estimating species trees: methods of phylogenetic analysis when there is incongruence across genes. *Systematic Biology*, 58, 463–467.
- Kool, S. P. (1993). Phylogenetic analysis of the Rapaninae (Neogastropoda: Muricidae). *Malacologia*, 35, 155–259.
- Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, 15(5), 1179–1191. <https://doi.org/10.1111/1755-0998.12387>
- Kowalke, T., & Harzhauser, M. (2004). Early ontogeny and palaeoecology of the Mid-Miocene rissoid gastropods of the Central Paratethys. *Acta Palaeontologica Polonica*, 49(1), 111–134.
- Kubatko, L.S., Degnan, J.H. (2007). Inconsistency of phylogenetic estimates from concatenated data under coalescence. *Systematic Biology*, 56, 17–24.
- Lanfear, R., Calcott, B., Ho, S. Y. W., & Guindon, S. (2012). PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, 29(6), 1675–1701.
- Lanner, J., Gstöttenmayer, F., Curto, M., Geslin, B., Huchler, K., Orr, M. C., Pachinger, B., Sedivy, C., & Meimberg, H. (2021). Evidence for multiple introductions of an invasive wild bee species currently under rapid range expansion in Europe. *BMC Ecology and Evolution*, 21, 71. <https://doi.org/10.1186/s12862-020-01729-x>
- Larsson, A. (2014). AliView: A fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics*, 30(22), 3276–3278. <https://doi.org/10.1093/bioinformatics/btu531>
- Liu, L., Xi, Z., Wu, S., Davis, C. C., & Edwards, S. V. (2015). Estimating phylogenetic trees from genome-scale data. *Annals of the New York Academy of Sciences*, 1360(1), 36–53.
- Maddison, W.P. (1997). Gene trees in species trees. *Systematic Biology*, 46, 523–536.
- Mata, J., Fonseca, P. E., Prada, S., Rodrigues, D., Martins, S., Ramalho, R. S., Madeira, J., Cachão, M., da Silva, C. M., & Matias, M. J. (2013). O Arquipélago da Madeira. In R. Dias, A. Araújo, P. Terrinha, & J. C. Kullberg (Eds.), *Geologia de Portugal* (1st ed., pp. 691–746). Escolar Editora.
- McWilliam, H., Li, W., Uludag, M., Squizzato, S., Park, Y. M., Buso, N., Cowley, A. P., & Lopez, R. (2013). Analysis tool web services from the EMBL-EBI. *Nucleic Acids Research*, 41(W1), W597–W600.

- Meireles, R. P., Quartau, R., Ramalho, R. S., Rebelo, A. C., Madeira, J., Zanon, V., & Ávila, S. P. (2013). Depositional processes on oceanic island shelves - Evidence from storm-generated Neogene deposits from the mid-North Atlantic. *Sedimentology*, 60(7), 1769–1785. <https://doi.org/10.1111/sed.12055>
- Mendes, F. K., & Hahn, M. W. (2016). Gene tree discordance causes apparent substitution rate variation. *Systematic Biology*, 65(4), 711–721. <https://doi.org/10.1093/sysbio/syw018>
- Ó Foighil, D., Jennings, R., Park, J. K., & Merriwether, D. A. (2001). Phylogenetic relationships of mid-oceanic ridge and continental lineages of *Lasaea* spp. (Mollusca: Bivalvia) in the northeastern Atlantic. *Marine Ecology Progress Series*, 213, 165–175. <https://doi.org/10.3354/MEPS213165>
- Ogilvie, H. A., Bouckaert, R. R., & Drummond, A. J. (2017). StarBEAST2 brings faster species tree inference and accurate estimates of substitution rates. *Molecular Biology and Evolution*, 34(8), 2101–2114. <https://doi.org/10.1093/molbev/msx126>
- Ogilvie, H. A., Heled, J., Xie, D., & Drummond, A. J. (2016). Computational performance and statistical accuracy of *BEAST and comparisons with other methods. *Systematic Biology*, 65(3), 381–396. <https://doi.org/10.1093/sysbio/syv118>
- Parham, J. F., Donoghue, P. C. J., Bell, C. J., Calway, T. D., Head, J. J., Holroyd, P. A., Inoue, J. G., Irmis, R. B., Joyce, W. G., Ksepka, D. T., Patané, J. S. L., Smith, N. D., Tarver, J. E., Van Tuinen, M., Yang, Z., Angielczyk, K. D., Greenwood, J. M., Hipsley, C. A., Jacobs, L., Makovicky, P. J., Müller, J., Smith, K. T., Theodor, J. M., Warnock, R. C. M., & Benton, M. J. (2012). Best practices for justifying fossil calibrations. *Systematic Biology*, 61(2), 346–359. <https://doi.org/10.1093/sysbio/syr107>
- Patarnello, T., Volckaert, F. A. M. J., & Castilho, R. (2007). Pillars of Hercules: Is the Atlantic-Mediterranean transition a phylogeographical break? *Molecular Ecology*, 16, 4426–4444. <https://doi.org/10.1111/j.1365-294X.2007.03477.x>
- Patiño, J., Whittaker, R. J., Borges, P. A. V., Fernández-Palacios, J. M., Ah-Peng, C., Araújo, M. B., Ávila, S. P., Cardoso, P., Cornuault, J., de Boer, E. J., de Nascimento, L., Gil, A., González-Castro, A., Gruner, D. S., Heleno, R., Hortal, J., Illera, J. C., Kaiser-Bunbury, C. N., Matthews, T. J., Papadopoulou, A., Pettorelli, N., Price, J. P., Santos, A. M. C., Steinbauer, M. J., Triantis, K. A., Valente, L., Vargas, P., Weigelt, P., & Emerson, B. C. (2017). A roadmap for island biology: 50 fundamental questions after 50 years of The Theory of Island Biogeography. *Journal of Biogeography*, 44(5), 963–983. <https://doi.org/10.1111/jbi.12986>
- Paulay, G. (1994). Biodiversity on oceanic islands: Its origin and extinction. *Integrative and Comparative Biology*, 34(1), 134–144. <https://doi.org/10.1093/icb/34.1.134>
- Peakall, R., & Smouse, P. E. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6, 288–295. <https://doi.org/10.1111/j.1471-8286.2005.01155.x>
- Peakall, R., & Smouse, P. E. (2012). GenALEX 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics*, 28, 2537–2539. <https://doi.org/10.1111/j.1471-8286.2005.01155.x>

- Pérez-Portela, R., Villamor, A., & Almada, V. (2010). Phylogeography of the sea star *Marthasterias glacialis* (Asteroidea, Echinodermata): Deep genetic divergence between mitochondrial lineages in the north-western mediterranean. *Marine Biology*, 157(9), 2015–2028. <https://doi.org/10.1007/S00227-010-1470-0/TABLES/6>
- Pezy, J. P., Raoux, A., Kerckhof, F., & Dauvin, J. C. (2019). First record of the gastropod *Stramonita haemastoma* (Linnaeus, 1767) in the English Channel. *BioInvasions Records*, 8(2), 266–272. <https://doi.org/10.3391/bir.2019.8.2.08>
- Pond, S. L. K., Poon, A. F. Y., & Frost, S. D. W. (2009). Estimating selection pressures on alignments of coding sequences. In P. Lemey, M. Salemi, & A. Vandamme (Eds.), *The Phylogenetic Handbook: A Practical Approach to Phylogenetic Analysis and Hypothesis* (2nd ed., pp. 419–451). Cambridge University Press.
- Ponder, W. F. (1967). Classification of Rissoidae and Orbitestellidae with descriptions of some new taxa. *Transactions of the Royal Society of New Zealand - Zoology*, 9(17), 193–224.
- Ponder, W. F. (1984). A review of the Genera of the Rissoidae (Mollusca: Mesogastropoda: Rissoacea). *Records of the Australian Museum, Suppl.* 4, 1–221. <https://doi.org/10.3853/j.0812-7387.4.1985.100>
- Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155, 945–959.
- Quinteiro, J., Manent, P., Pérez-Diéguez, L., González, J. A., Almeida, C., Lopes, E., Araújo, R., Carreira, G. P., Rey-Méndez, M., & González-Henríquez, N. (2015). Phylogeography of a Marine Insular Endemic in the Atlantic Macaronesia: The Azorean Barnacle, *Megabalanus azoricus* (Pilsbry, 1916). *PLOS ONE*, 10(4), e0124707. <https://doi.org/10.1371/JOURNAL.PONE.0124707>
- Quinteiro, J., Rodríguez-Castro, J., & Rey-Méndez, M. (2007). Population genetic structure of the stalked barnacle *Pollicipes pollicipes* (Gmelin, 1789) in the northeastern Atlantic: Influence of coastal currents and mesoscale hydrographic structures. *Marine Biology*, 153(1), 47–60. <https://doi.org/10.1007/S00227-007-0783-0/FIGURES/6>
- Quinteiro, J., Rodríguez-Castro, J., Rey-Méndez, M., & Henríquez, N. G. (2020). Phylogeography of the insular populations of common octopus, *Octopus vulgaris* Cuvier, 1797, in the Atlantic Macaronesia. *PLoS ONE*, 15(3). <https://doi.org/10.1371/journal.pone.0230294>
- Ramalho, R. S., da Silveira, A. B., Fonseca, P. E., Madeira, J., Cosca, M., Cachão, M., Fonseca, M. M., & Prada, S. N. (2015). The emergence of volcanic oceanic islands on a slow-moving plate: the example of Madeira Island, NE Atlantic. *Geochemistry, Geophysics, Geosystems*, 16(2), 522–537. <https://doi.org/10.1002/2014GC005657>
- Ramalho, R. S., Helffrich, G., Madeira, J., Cosca, M., Thomas, C., Quartau, R., Hipólito, A., Rovere, A., Hearty, P. J., & Ávila, S. P. (2017). Emergence and evolution of Santa Maria Island (Azores) – The conundrum of uplifted islands revisited. *Bulletin of the Geological Society of America*, 129(3–4), 372–391. <https://doi.org/10.1130/B31538.1>
- Rambaut, A. (2006). *FigTree v1.4.2 Tree Figure Drawing Tool*. Available from tree.bio.ed.ac.uk/software/figtree/.

- Rambaut, A., & Drummond, A. J. (2002). *TreeAnnotator v2.4.5 MCMC Output analysis*. Available from <http://tree.bio.ed.ac.uk/software/beast>
- Rambaut, A., Suchard, M. A., Xie, D., & Drummond, A. J. (2014). *Tracer v1.6*. Available from <http://beast.bio.ed.ac.uk/Tracer>
- Rice, W. R. (1989). Analysing tables of statistical tests. *Evolution*, 43, 223–225.
- Riesgo, A., Taboada, S., Pérez-Portela, R., Melis, P., Xavier, J. R., Blasco, G., & López-Legentil, S. (2019). Genetic diversity, connectivity and gene flow along the distribution of the emblematic Atlanto-Mediterranean sponge *Petrosia ficiformis* (Haplosclerida, Demospongiae). *BMC Evolutionary Biology*, 19. <https://doi.org/10.1186/S12862-018-1343-6>
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., B., L., Liu, L., & Suchard, M.A. Huelsenbeck, J. P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61(3), 539–542.
- Sá-Pinto, A., Branco, M., Sayanda, D., & Alexandrino, P. (2008). Patterns of colonization, evolution and gene flow in species of the genus *Patella* in the Macaronesian Islands. *Molecular Ecology*, 17(2), 519–532. <https://doi.org/10.1111/J.1365-294X.2007.03563.X>
- Salemi, M. (2009). Genetic distances and nucleotide substitution models. In P. Lemey, M. Salemi, & A. Vandamme (Eds.), *The Phylogenetic Handbook: A Practical Approach to Phylogenetic Analysis and Hypothesis* (2nd ed., pp. 126–140). Cambridge University Press.
- Santos, A. M., Cabezas, M. P., Tavares, A. I., Xavier, R., & Branco, M. (2016). tcsBU: a tool to extend TCS network layout and visualization. *Bioinformatics*, btv636. <https://doi.org/10.1093/bioinformatics/btv636>
- Sasson, N., Simon-Blecher, N., & Achituv, Y. (2012). New molecular markers for revealing the population structure of *Chthamalus stellatus* in the Mediterranean and the eastern Atlantic. *Marine Ecology Progress Series*, 459, 99–107. <https://doi.org/10.3354/MEPS09735>
- Scheltema, R. S. (1971a). Larval dispersal as a means of genetic exchange between geographically separated populations of shallow-water benthic marine gastropods. *Biological Bulletin*, 140(2), 284–322.
- Scheltema, R. S. (1971b). The Dispersal of the Larvae of Shoal-Water Benthic Invertebrate Species Over Long Distances by Ocean Currents. In D. J. Crisp (Ed.), *Fourth European Marine Biology Symposium* (pp. 7–28). Cambridge University Press.
- Scheltema, R. S. (1977). Dispersal of marine invertebrate organisms: paleogeographic and biostratigraphic implications. *Concepts and Methods in Biostratigraphy*, 73–108.
- Scheltema, R. S. (1986a). Long-distance dispersal by planktonic larvae of shoal-water benthic invertebrates among central Pacific islands. *Bulletin of Marine Science*, 39(2), 241–256.
- Scheltema, R. S. (1986b). On dispersal and planktonic larvae of benthic invertebrates: An eclectic overview and summary of problems. *Bulletin of Marine Science*, 39(2), 290–322.
- Scheltema, R. S. (1989). Planktonic and non-planktonic development among prosobranch gastropods and its relationship to the geographic range of species. In J. S. Ryland & P. A. Tyles (Eds.), *Reproduction, Genetics and Distribution of Marine Organisms* (pp. 183–188). Fredensborg: Olsen and Olsen.

- Scheltema, R. S. (1995). The relevance of passive dispersal for the biogeography of Caribbean mollusks. *American Malacological Bulletin*, 11, 99–115.
- Scheltema, R. S., & Williams, I. P. (1983). Long-distance dispersal of planktonic larvae and the biogeography and evolution of some Polynesian and western Pacific mollusks. *Bulletin of Marine Science*, 33(3), 545–565.
- Scheltema, R. S., Williams, I. P., & Lobel, P. S. (1996). Retention around and long-distance dispersal between oceanic islands by planktonic larvae of benthic gastropod Mollusca. *American Malacological Bulletin*, 12(1–2), 67–75.
- Sibrant, A., Hildenbrand, A., Marques, F., Weiss, B., Boulesteix, T., Hübscher, C., Lüdmann, T., Costa, A., & Catalão, J. (2015). Morpho-structural evolution of a volcanic island developed inside an active oceanic rift: S. Miguel Island (Terceira Rift, Azores). *Journal of Volcanology and Geothermal Research*, 301, 90–106. <https://doi.org/10.1016/j.jvolgeores.2015.04.011>
- Sibrant, A., Marques, F., & Hildenbrand, A. (2014). Construction and destruction of a volcanic island developed inside an oceanic rift: Graciosa Island, Terceira Rift, Azores. *Journal of Volcanology and Geothermal Research*, 284(1), 32–45. <https://doi.org/10.1016/j.jvolgeores.2014.07.014>
- Sukumaran, J., & Holder, M. . (2010). DendroPy: a Python library for phylogenetic computing. *Bioinformatics*, 26, 1569–1571.
- Suzuki, Y., Glazko, G. V, & Nei, M. (2002). Overcredibility of molecular phylogenies obtained by Bayesian phylogenetics. *Proceedings of the National Academy of Sciences USA*, 99, 16138–16143. <https://doi.org/10.1073/pnas.212646199>
- Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, 38(7), 3022–3027. <https://doi.org/10.1093/molbev/msab120>
- Tibihika, P. D., Curto, M., Dornstauder-Schrammel, E., Winter, S., Alemayehu, E., Waidbacher, H., & Meimberg, H. (2019). Application of microsatellite genotyping by sequencing (SSR-GBS) to measure genetic diversity of the East African *Oreochromis niloticus*. *Conservation Genetics*, 20(2), 357–372. <https://doi.org/10.1007/s10592-018-1136-x>
- Thiele, J. (1929). *Handbuch der Systematischen Weichtierkunde*. Gustav Fischer, Jena.
- Uchman, A., Torres, P., Johnson, M.E., Berning, B., Ramalho, R.S., Rebelo, A.C., Melo, C.S., Baptista, L., Madeira, P., Cordeiro, R., & Ávila, S.P. (2018). Feeding traces of recent ray fish and occurrences of the trace fossil *Piscichnus waitemata* from the Pliocene of Santa Maria Island, Azores (Northeast Atlantic). *Palaos*, 33, 361–375. <https://doi.org/10.2110/palo.2018.027>
- Van Den Broeck, H., Breugelmans, K., De Wolf, H., & Backeljau, T. (2008). Completely disjunct mitochondrial DNA haplotype distribution without a phylogeographic break in a planktonic developing gastropod. *Marine Biology*, 153(3), 421–429. <https://doi.org/10.1007/S00227-007-0820-Z/TABLES/4>
- Vermeij, G. J. (2001). Distribution, history, and taxonomy of the *Thais* clade (Gastropoda: Muricidae) in the Neogene of tropical America. *Journal of Paleontology*, 75(3), 697–705.

- Vermeij, G. J., & Carlson, S. J. (2000). The muricid gastropod subfamily Rapaninae: phylogeny and ecological history. *Paleobiology*, 26(1), 19–46. [https://doi.org/10.1666/0094-8373\(2000\)0262.0.CO;2](https://doi.org/10.1666/0094-8373(2000)0262.0.CO;2)
- Vieira, P. E., Desiderato, A., Azevedo, S. L., Esquete, P., Costa, F. O., & Queiroga, H. (2022). Molecular evidence for extensive discontinuity between peracarid (Crustacea) fauna of Macaronesian islands and nearby continental coasts: over fifty candidate endemic species. *Marine Biology*, 169(5), 1–13. <https://doi.org/10.1007/S00227-022-04051-W/FIGURES/3>
- Vieira, P. E., Desiderato, A., Holdich, D. M., Soares, P., Creer, S., Carvalho, G. R., Costa, F. O., & Queiroga, H. (2019). Deep segregation in the open ocean: Macaronesia as an evolutionary hotspot for low dispersal marine invertebrates. *Molecular Ecology*, 28(7), 1784–1800. <https://doi.org/10.1111/mec.15052>
- Wenz, W. (1938). Gastropoda. Teil 1, Allgemeiner Teil und Prosobranchia. In O. H. Schindewolf (Ed.), *Handbuch der Paläozoologie* (Vol. 6, pp. 1–231). Gebrüder Bornträger.
- Winston, J. E. (2012). Dispersal in marine organisms without a pelagic larval phase. *Integrative and Comparative Biology*, 52(4), 447–457. <https://doi.org/10.1093/icb/ics040>
- Xavier, J. R., Van Soest, R. W. M., Breeuwer, J. A. J., Martins, A. M. F., & Menken, S. B. J. (2010). Phylogeography, genetic diversity and structure of the poecilosclerid sponge *Phorbas fictitius* at oceanic islands. *Contributions to Zoology*, 79(3), 119–129. <https://doi.org/10.1163/18759866-07903004>
- Xia, X., & Lemey, P. (2009). Assessing substitution saturation with DAMBE. In P. Lemey, M. Salemi, & A. Vandamme (Eds.), *The Phylogenetic Handbook: A Practical Approach to Phylogenetic Analysis and Hypothesis* (pp. 615–630). Cambridge University Press. <https://doi.org/10.1017/CBO9780511819049.022>
- Zheng, Y., Peng, R., Kuro-o, M., & Zeng, Z. (2011). Exploring Patterns and Extent of Bias in Estimating Divergence Time from Mitochondrial DNA Sequence Data in a Particular Lineage: A Case Study of Salamanders (Order Caudata). *Molecular Biology and Evolution*, 28, 2521–2535. <https://doi.org/10.1093/molbev/msr072>
- Zwickl, D. J. (2006). *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. University of Texas, Austin.

Chapter 4

Factors shaping the connectivity of intertidal populations in a remote archipelago

4.1 Dispersal ability, habitat characteristics, and sea-surface circulation shape population structure of *Cingula trifasciata* (Gastropoda: Rissoidae) in the remote Azores Archipelago

Introduction

Species' ranges are shaped by multiple biotic and abiotic factors; however, in the marine realm, dispersal ability is one of the main factors influencing the distribution of taxa, with significant evolutionary and biogeographic implications (Ávila, 2013; Robinson et al., 2011). Dispersal is frequently related to the duration and behaviour of marine invertebrates' larval stages, classified either as planktotrophic or non-planktotrophic (np), the latter comprising lecithotrophic and direct developers (Thorson, 1946, 1950). Among the dispersal strategies reviewed by Winston (2012), rafting is the most relevant mechanism followed by epibenthic, shallow-water (< 50 m depth) np-invertebrates in temperate Atlantic waters (Ávila, 2013; Scheltema, 1986). Details concerning the larval development are only known for a few gastropods (e.g. Ávila et al., 2008; Crothers, 2001; Johannesson, 1988; Modica et al., 2017; Oliverio, 1995; Pechenik, 1984), but are unclear for most species, in particular small ones. Dispersal pathways and processes are therefore only poorly understood in many marine invertebrates. In particular, the characteristics of dispersal could play a role to explain differentiation and genetic structure in response to ecological and geographical constraints.

Rissoidae Gray, 1847 is one of the best-known family of microgastropods. Research has been conducted over the past decades regarding distinct aspects of their biology, ecology, and evolution (Ávila, 2000; Ávila et al., 2008, 2012, 2015a; Baptista et al., 2019; Cordeiro & Ávila, 2015; Costa & Ávila, 2001; Criscione et al., 2016; Criscione & Ponder, 2013; Davis et al., 1998; Gofas, 1990, 2007; Ponder, 1984). The family comprises the largest number of small-sized, marine gastropod species, conspicuous around the world and with 546 species in the Atlantic Ocean and Mediterranean Sea (Ávila et al., 2012; Criscione et al., 2016; Ponder, 1984). Among these, *Cingula trifasciata* (J. Adams, 1800) is found throughout the Northeast Atlantic Ocean, being reported for the Azores Archipelago, Iberian Peninsula, up to the Bay of Biscay and British Isles (Baptista et al., 2019; Borges et al., 2016; Cordeiro et al., 2015; Davidson, 2005; Duff & Hily, 2001; Miralles et al., 2016). This microgastropod species is commonly found at intertidal areas with low hydrodynamics and mesotidal regimes, which get exposed during low tide and

submerged during high tide. A typical habitat for *C. trifasciata* is protected and enclosed gravel intertidal areas (Figure 4.1a), especially beneath gravel/boulders that provides shelter and environmental stability (Ávila, 1998; Gofas, 1990; Ponder, 1984). The species is also present in the algal turf (Figure 4.1b), which in turn provide protection to wave action, predation, and desiccation during low tide (Buršić et al., 2019). Several features of the rissoid *C. trifasciata* increase the likelihood of initiate dispersal by rafting, namely: 1) its minute size (<5 mm); 2) its high abundance; 3) its association with intertidal habitats; and, 4) the secretion of mucus from a posterior pedal gland that allows juveniles and adult rissoids to suspend themselves from the surface film (Ávila, 2013).



Figure 4.1. Typical intertidal habitats of *Cingula trifasciata*. **a.** Enclosed gravel/boulder areas, protected from wave action; photo at Fajã da Caldeira de Santo Cristo (São Jorge Island, Azores) by Sérgio P. Ávila; **b.** In the algal turf; photo at Horta (Faial Island, Azores) by Lara Baptista. Permission to publish these images was granted by the photographers and people displayed in them.

Nevertheless, *C. trifasciata* is a np-species (direct developer), laying one to four eggs in algae (Ponder, 1984). It is expected to be geographically restricted, as the lack of a free-swimming stage negatively affects the dispersal ability at early-life stages (Scheltema, 1986, 1989, 1995). Its wide distribution across the NE Atlantic Ocean and reports on the Mediterranean Sea - Ceuta (Ponder, 1990; Tringali, 2001); Adriatic Sea (Buršić et al., 2019) - challenge the expectations for a np-species (Baptista et al., 2019), although examples of other np-species with wide biogeographic ranges exist (e.g. *Lasea* spp. (Ó Foighil, 1989), Calyptraeid gastropods (Collin, 2003), trochids (Donald et al., 2005)). The np nature of *C. trifasciata* suggests that long-distance dispersal strategies other than rafting are unlikely. If that is the case, surface water currents should play a major role in shaping *C. trifasciata* genetic variation. These patterns may be especially intricate in a remote volcanic oceanic island system such as the Azores Archipelago.

Using molecular tools, important biogeographical questions, can be addressed, such as: (1) is there gene flow between continental and insular populations of the same species?; (2) how is the species dispersing within the archipelago, among distant populations?; (3) what are the drivers and factors influencing dispersal in the Azores? Similar questions have been posed in previous studies that intended to clarify patterns and processes affecting marine gastropods in the Azores (Baptista et al., 2019, 2021).

During the past decades, microsatellite sets have been developed and characterized for marine gastropods, especially for abundant, commercially interesting or threatened species (e.g. Beldade et al., 2012; Cárdenas et al., 2007; Dupont & Viard, 2003; Kawai et al., 2001; López-Márquez et al., 2018; McInerney et al., 2009; Vega-Retter et al., 2016; Weetman et al., 2005). These sets of SSR markers appear to be skewed towards large-sized genera, such as *Nucella* (Röding, 1798), *Littorina* (Férussac, 1822), *Buccinum* (Linnaeus, 1758), *Haliotis* (Linnaeus, 1758), and *Concholepas* Lamarck, 1801. SSR markers, sometimes coupled with other molecular markers, have mainly been used in marine gastropods for paternity studies (Brante et al., 2011; le Cam et al., 2014; Xue et al., 2014), phylogeography and species delimitation (Kemppainen et al., 2009; Teske et al., 2017), population structure (Cahill & Viard, 2014; Dupont et al., 2007; Pálsson et al., 2013; Ribeiro et al., 2010; Wort et al., 2019), as well as dispersal and connectivity associated to larval development (Bell, 2008; Donald et al., 2011, 2020; Quintero-Galvis et al., 2020). Microgastropods tend to be understudied when compared to larger relatives, requiring special attention in field surveys and still associated with taxonomic classification issues (Albano et al., 2011). This negative bias in the knowledge of microgastropods is also found in the application of molecular techniques to improve the study of population dynamics and phylogenetic questions.

In this work we investigate the processes shaping the genetic diversity and structure patterns of *C. trifasciata*, with a special focus on the influence of sea surface circulation. This was done by applying a traditional analysis of the COI molecular marker from several populations and developing a set of primers for microsatellite markers for this non-model species, using the SSR-GBAS approach, a method that relies on amplicon sequencing with second generation sequencing techniques to determine genotypes at microsatellite containing loci. By doing so, we aim to increase the current knowledge of the widespread rissoid *C. trifasciata*, as well as to clarify the intraspecific genetic structure and population dynamics of this species in the NE Atlantic Ocean, with an emphasis on its behaviour in the remote Azorean islands. With this study, we intend to understand the role of habitat and small-scale surface currents among islands in shaping the genetic structure in a remote archipelago.

Material and Methods

With this work we aim to investigate the processes shaping the intraspecific genetic diversity and population dynamics of *C. trifasciata* in the NE Atlantic Ocean, with a special focus on its behaviour and factors shaping the genetic structure in the remote Azores Archipelago.

Sampling and DNA isolation

Cingula trifasciata specimens from the Azores Archipelago (Portugal) and Vigo (Spain) were used in this study, either recently collected by the authors in intertidal habitats or retrieved from collections (Table 4.1). Fresh samples from Flores Island were obtained collected by integral algal scrapping with 20x20 cm squares. In other Azorean localities, numerous populations of *C. trifasciata* are sheltered under coastal boulder deposits in intertidal pools: Santa Maria, São Miguel, São Jorge, and Pico. All the recently collected specimens were stored in 96% ethanol and deposited in the Marine Molluscs Collection of the Department of Biology of the University of the Azores (DBUA). Permits for sampling were issued by the respective authorities in the Azores (Direção Reginal da Ciência e Tecnologia, Governo Regional dos Açores; AMP 2018/014, CCIP 24/2019/DRCT, CCIP 35/2019/DRCT). Samples from two localities in Graciosa island and from a NE Atlantic population in Vigo were loaned, respectively, from the DBUA and CIBIO-InBIO molluscs' collections. A total of 75 samples were considered in this study.

Due to its minute size, total genomic DNA (gDNA) was extracted from the entire animal, removed from the shell when possible, following the manufacturer's instructions for the column-based commercial kit PureLink® Genomic DNA (Invitrogen™). gDNA was eluted in a volume of 40 µl and its quality assessed based on the absorbance ratios measured with Nanodrop®2000. An electrophoretic run in agarose gel 0.8% at 100 V for 50 minutes was performed to evaluate DNA integrity.

Table 4.1. Samples of *Cingula trifasciata* used in this study.

Island/Region	Locality	Habitat type	Coordinates (DD)	Collection voucher	#ind. (COI)	#ind. (SSR)
Santa Maria, Azores	Praia de Lobos	G/B	37.004, -25.166	DBUA 1400	7	7
São Miguel, Azores	Caloura	G/B	37.71, -25.51	DBUA 1474	11	9
	Mosteiros	G/B	37.891, -25.825	DBUA 1194	11	2
São Jorge, Azores	Fajã da Caldeira de Santo Cristo	G/B	36.63, -27.927	DBUA 1415	7	6
Pico, Azores	Poça da Barra	G/B	38.391, -28.254	n.a./DBUA 1510	12	11
Graciosa, Azores	1-Poças de Santa Cruz	A	39.088 ^a , -27.997 ^a	DBUA 1184	4	2
	2-Praia de São Mateus	A	39.087 ^a , -28.006 ^a	DBUA 1186/1190	10	3
Flores, Azores	Poça da Salema	A	39.46, -31.127	DBUA 1339	7	1
Galícia, Spain	Vigo	U	n.a.	VIG4395-9	6	3

^a approximate coordinates; n.a. – non-applicable; G/B – Gravel/Boulder; A – Algal mat; U – Unknown; DD – Decimal Degrees. Number of individuals studied for each population resorting to COI and SSR datasets are indicated.

Sequence data and genetic analyses

The fragment of the mitochondrial cytochrome oxidase subunit I (COI) were amplified in 25 µl volumes, containing 12.5 µl of QIAGEN Multiplex PCR Master Mix (Qiagen, CA, USA), 5 µL of each primer at a concentration of 2 µM, and 2.5 µL of gDNA. The amplification of COI was achieved with the primers LCO1490/ HCO1490 (Folmer et al., 1994) or jgLCO1490/jgHCO2198 (Geller et al., 2013). The following cycling profile was applied to both markers: 95°C for 15 minutes; 35 cycles of 95°C for 30 seconds, 50/55°C for 1 minute, 72°C for 30 seconds; 72°C for 10 minutes. PCR products were checked by electrophoresis. Purification of the PCR products and bi-directional Sanger sequencing were realized by a commercial facility (Genewiz, Leipzig, Germany) using the same primers as for PCR.

Geneious 8.1.9 (Kearse et al., 2012) was used for manual check of potential misreads in the generated chromatograms. The reviewed mitochondrial coding COI sequences were inspected for the existence of stop codons and putative pseudogenes by translating into amino acids with ExPASy Translate Tool (<https://web.expasy.org/translate/>). All the sequences generated during this study were deposited at GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>), under the accession numbers MW518062-117 and MW518858-63. Additional sequences of *C. trifasciata* from the Azores, publicly available at GenBank, were included in the COI dataset: MG652395-MG562406. The COI dataset was aligned with Clustal Omega algorithm via Web Services by EMBL-EBI (McWilliam et al., 2013) and the 75 sequences were reduced to 44 haplotypes. Raw (p) distances among haplotypes of *C. trifasciata* were calculated in MEGA v7 (Kumar et al., 2016) to estimate evolutionary divergence and sequence diversity. Frequency, distribution, and genetic structure between the haplotypes in the populations was further examined at a statistical parsimony haplotype network at the 95% connection limit, generated with the software TCS v1.21 (Clement et al., 2000). The output was rendered using tcsBU web-based program (Santos et al., 2016), allowing to overlap the genetic structure retrieved by TCS with the geographical structure of the populations studied. A UPGMA tree of *C. trifasciata* haplotypes (Sneath & Sokal, 1973) was obtained with Geneious 8.1.9 (Kearse et al., 2012), and bootstraps calculated with 1,000 replicates. The evolutionary divergence levels within Rissoidae family were further evaluated with a maximum-likelihood analysis conducted in RAxML v8.2.7 (Stamatakis, 2014), under the GTR model of substitutions and based on 18 COI sequences of different rissoid species retrieved from GenBank (cf. Supplementary Table A3.1 for details) and *C. trifasciata* from the Azores and Vigo. Pairwise F_{ST} estimates with COI haplotypes of *C. trifasciata* and Rissoidae species were performed in Arlequin v3.5 (Excoffier & Lischer, 2010), with

statistical significance tested by 1023 permutations and considered for p-values below 0.01.

SSR marker discovery

Two low-coverage Illumina MiSeq runs, from two *C. trifasciata* individuals (PSM4350 from Graciosa and FSC5131 from São Jorge), were conducted for marker development and raw reads can be accessed through the BioProject PRJNA702169, at GenBank. Library preparation and sequencing using shot-gun genomic libraries without enrichment on the Illumina MiSeqs paired-end (PE) 300 bp were performed at the Genomics Service Unit, Ludwig-Maximilian University Munich, Germany. The resulting reads, after quality check with FastQC software (Andrews, 2010), were processed by Trimmomatic v0.39 (Bolger et al., 2014) to trim adapters and low-quality regions (Phred score > 20) in forward and reverse reads, which were then merged using Usearch v11 (Edgar, 2010). Merged reads were used as input for the identification of sequences containing microsatellite (SSR) motifs with the SSR_pipeline's script SSR_search.py (Miller et al., 2013). For the SSR search, the following parameters of quality control were defined to ensure that the sequence contains: 1) a minimum of 30 bp flanking regions on both sides of the motif; 2) a minimum of five repeats for penta- and tetranucleotides; 3) a minimum of seven repeats for trinucleotides; 4) a minimum of nine repeats for dinucleotides. These less stringent parameters allowed the extraction of a considerable number of SSR motif containing sequences, which were manually checked to remove sequences containing either interrupted motifs, more than one repetitive motif, and long mononuclear stretches (> 6 bp). If this filtering step resulted in a low final number of usable reads, sequences containing mononucleotide repeats were maintained.

Primer3 (Untergasser et al., 2012) as implemented in Geneious 8.1.9 (Kearse et al., 2012) was chosen to design primers, as a batch under manual control. The following parameters were set for the primer design: length of 19 to 22 bp, optimal melting temperature of 55°C, GC content between 20% and 80% and optimal as 50%, and amplification product size between 300 and 450 bp. Only primers producing amplicons comprising the complete SSR repetitive motif in the first or last 300 bp were selected, ensuring its coverage by one of MiSeq's paired reads. This is crucial to avoid problems in the overlap of the paired reads during the merging step of the bioinformatics pipeline (Curto et al., 2019; Tibihika et al., 2019). We added recognition sequences corresponding to the Illumina adapter to the selected primer pairs: part of the P5 motif (TCTTTCCCTACACGACGCTCTTCCGATCT) elongated the forward primer, whereas part of the P7 motif (CTGGAGTTCAGACGTGTGCTCTTCCGATCT) was added to the

reverse primer. These recognition sequences serve as linkers for a second index PCR using primers containing the eight bp indexes and TrueSeq adapters (P5: AATGATACGGCGACCACCGAGATCTACAC [Index] ACACTCTTTCCCTACACGACG; and P7: CAAGCAGAAGACGGCATACGAGAT [Index] TGAAGTGGAGTTCAGACGTGT) (Curto et al., 2019; Tibihika et al., 2019). The primers designed from the initial two Illumina MiSeq runs were individually tested using gDNA for two specimens of *C. trifasciata*. PCR reactions were conducted for a final volume of 10 μ L: 5 μ L of QIAGEN Multiplex PCR Master Mix (Qiagen, CA, USA), 1 μ L of each primer at 1 μ M, 1 μ L of diluted gDNA in a 1:3 proportion, and water. The cycling profile was applied as follows: 95°C for 15 min; 30 cycles of 95°C for 30 s, 55°C for 1 min, and 72°C for 1 min; and a final extension at 72°C for 10 min. The PCR results were visualized after an electrophoretic run in 1.5% agarose gel and 80 V. Primers that successfully generated amplicons of the expected size were combined in three mixes of eight primer pairs and one mix of seven primer pairs, each with a final concentration of 1 μ M (Supplementary Table A3.1).

Multiplex PCR and Illumina sequencing

Multiplex amplification was achieved with PCR reactions of 5 μ L, containing 2.5 μ L of QIAGEN Multiplex PCR Master Mix (Qiagen, CA, USA), 0.5 μ L of each primer mix, and 2 μ L of gDNA diluted in 1:3 proportion. Cycling conditions were the same as used in the single PCR to test the primers individually. For each sample, and following Curto et al.'s (2019) protocol, equal volumes of PCR products from different primers mixes were pooled into a final volume of 6 μ L. PCR clean-up, aiming the removal of unused primers and primer-dimer products, was conducted using the magnetic bead technology offered by the Agencourt AMPure XP PCR Purification kit (Beckman Coulter Inc., Brea, CA, USA), applying some modifications to the standard protocol. The total volume of pooled PCR product (6 μ L) was mixed with 4.3 μ L of AMPure XP beads, followed by a 5 min incubation period at room temperature. The beads, to which DNA bound after the first step, were captured by an inverted magnetic bead extraction device, VP407-AM-N (V&P Scientific, INC.), and afterwards washed twice for 45 s in 200 μ L of 80% ethanol. The beads were then dried at room temperature for 5 min and the DNA finally eluted in 17 μ L of elution buffer (10 mM Tris-HCl, pH 8.3) at 65°C.

Once purified, the multiplex PCR product underwent a second PCR, whereby designated as index-PCR, for the assignment of indexes to each sample. A unique combination of forward and reverse indexes was carefully chosen, so that each sample can be unambiguously identified after the MiSeq Run. A 10 μ L PCR reaction was performed, comprising 5 μ L of QIAGEN Multiplex PCR Master Mix (Qiagen, CA, USA),

1 μ L of each index primer at 1 μ M, and 1 μ L of pooled purified PCR product. The cycling conditions were applied as follows: 95°C for 15 min; 10 cycles of 95°C for 30 s, 58°C for 60 s, and 72°C for 60 s; 72°C for 5 min. The index-PCR product, from 5' to 3', entails: P5 motif for flow cell hybridization, index 1 with a length of 8 bp, P5 sequencing primer, specific forward primer, target DNA for sequence, specific reverse primer, P7 sequencing primer, 8 bp long index 2, P7 motif for flow cell hybridization. After visualizing the index-PCR products in 1.5% agarose gel stained with GelRed (Biotium), all the samples were pooled in equal volumes of 2 μ L. The Illumina MiSeq run for PE 300 bp sequencing at the Genomics Service Unit at Ludwig Maximilian Universität, München, Germany, using the pooled index-PCR product as input, produced sequences to be analysed with a genotyping by amplicon sequencing (GBAS) protocol.

SSR data analysis and SSR-GBAS genotyping

Raw FASTQ sequence data (reads R1 and R2), automatically extracted by the MiSeq equipment based on the index combinations and each corresponding to a different sample, were downloaded from Illumina BaseSpace. These Illumina reads, containing all sequences per index, underwent the quality control and merging procedures resorting to FastQC, Trimmomatic v0.39, Usearch v11 (cf. “SSR discovery” section). Genotyping and analysis of each sample for all the SSR loci was performed with the SSR_GBS_pipeline scripts available at GitHub (<https://github.com/mcurto/SSRGBS-pipeline>). Script 1 (primer_demultiplex.py) allowed to demultiplex merged fastq files by identifying the primers on both sides of the merged reads and sort them by locus. Script 2 (CountLengths.sh) calculated the number of occurrences of each sequence length, excluding sequences below the 250 bp threshold. With Script 3, potential alleles and histograms were plotted according to sequence length, setting a minimum of 10 reads to define a valid genotype. The stutter effect in the defined alleles is also assessed by Script 3, as described previously (Curto et al., 2019; Tibihika et al., 2019). A codominant matrix in .csv format, based on the allele length information of each sample is generated and used as input for the downstream recovery of sequence alleles, which considers SNP variation within alleles of the same size along with the sequence length variation determined at this point. Sequence_Allele_Call.py (Script 4) constitutes the second part of the SSR_GBS_pipeline.py, for the extraction of the corresponding reads and definition of consensus sequence for each length-based allele. It also allows the detection of possible SNP variation and calls alleles based on sequence information. This information set is called in the following WAI.

Population structure analyses

Descriptive population genetics and marker variability analyses, as well as evaluation of genetic structure patterns, were performed on the codominant WAI dataset of *Cingula trifasciata*. The two populations of Graciosa island were analysed together, as no major differences were found between them. Genetic diversity indices, null alleles and deviations to Hardy-Weinberg Equilibrium (HWE) in the WAI dataset were estimated only for populations with five or more individuals analysed – Santa Maria, Caloura, Graciosa, Pico, and São Jorge. FreeNA (Chapuis & Estoup, 2007) was used to estimate the frequency of null alleles per marker and population, using the AL dataset as required by the software. GenAlEx v6.5 (Peakall & Smouse, 2006, 2012) allowed to check the number of alleles per marker and allelic patterns per population – number of alleles (N_a), number of private (N_{pa}) and effective (N_e) alleles, observed (H_o) and expected (H_e) heterozygosity. Although excluded from the inference of genetic diversity, monomorphic markers were kept in the dataset for downstream analyses, as they are informative (Holla et al., 2014). Tests for deviations to HWE and Wright's Fixation index (F_{IS}) were performed by marker and population resorting to GenAlEx. The remaining analyses based on the WAI dataset included information of all individuals studied, belonging to the seven populations sampled. The software Cervus v3.0.7 (Kalinowski et al., 2007) was used to estimate the overall polymorphism information content (PIC) of each marker. Genetic structure patterns in the dataset were inferred in a Principal Coordinate Analysis (PCoA), as implemented in GenAlex v6.5 (Peakall & Smouse, 2006, 2012), and STRUCTURE v2.3.4 (Hubisz et al., 2009; Pritchard et al., 2000) with the complete dataset, allowing to assess the informativeness of the developed SSR markers. PCoA, performed with the complete dataset and excluding Vigo to clarify the position of the Azorean populations, evaluates genetic structure among populations without assumptions of HWE and based on absolute genetic distances between individuals. With the number of clusters (K) varying between 2 and 10, STRUCTURE ran for 10 independent replicates for 500,000 generations, following a burn-in period of 100,000 and maintaining the default settings for the admixture model and correlated allele frequencies (Falush et al., 2003). The online program Structure Harvester (Earl & VonHoldt, 2012) was used to validate multiple K -values for optimal detection of genetic structure, according to the Delta- K method and inferring the K that best suits the data from hundreds of iterations. The results from STRUCTURE across the K -values were summarized and graphically displayed resorting to the online pipeline CLUMPAK (Kopelman et al., 2015). A hierarchical analysis of molecular variance (AMOVA; Excoffier et al. (1992), as implemented in GenAlEx v6.5 (Peakall & Smouse, 2006, 2012), was

performed to evaluate the differentiation between populations and regions, with a simultaneous estimate of pairwise population F_{ST} values.

Results

Sequence data and genetic analyses

The COI dataset comprised a total of 75 sequences, with seven to 14 individuals per population. A total of 44 haplotypes were distinguished in the 658 bp alignment, none shared between populations from the Azores and Vigo. Among Azorean haplotypes, the estimates of evolutionary divergence ranged from 0.2% to 2.6% (see Supplementary Table A3.2). Unexpected high levels of divergence (~2%) within the populations at Graciosa and Santa Maria are detected, but a manual check of the chromatograms ensured that the reported variation is real and not a consequence of misreads. Differences between the two populations at Graciosa Island seem to be negligible, thus being considered a single population for interpretation purposes. The lowest inter-locality differentiation levels are found in the comparisons of haplotypes from the central group, namely São Jorge, Pico, Graciosa islands, and some haplotypes from the western locality Mosteiros in São Miguel island. Haplotypes from Vigo show low within population divergence (average 0.23%), but considerably high differentiation in relation divergence to the Azorean haplotypes, ranging from 3.6 to 5%.

The distribution of mitochondrial haplotypes (Figure 4.2) reflects the estimates of evolutionary divergence, with congruent relationships inferred by the TCS network and UPGMA tree. Only two haplotypes are shared by two or more populations (H14 among Mosteiros (São Miguel Island) and both populations of Graciosa Island; H16 among Mosteiros, Graciosa, Pico, and São Jorge), the remaining exclusive to the population in which they are found. This level of genetic differentiation and uniqueness of the mitochondrial set is observed even within the same island, as Mosteiros and Caloura (São Miguel Island) share no haplotypes, although the first shares haplotypes with more distant central islands. The closely related haplotypes from Pico, São Jorge, and Graciosa form one major star-shaped group in the TCS network (Figure 4.2a) that also includes one haplotype exclusive to Santa Maria Island (H4), another exclusive to Caloura (H11) and several from Mosteiros. The remaining haplotypes form a disperse second group, connected by several inferred mutation steps, which comprises haplotypes from the eastern (Santa Maria and most Caloura and Mosteiros' haplotypes from São Miguel) and western (Flores) group of Azorean islands. Haplotypes 25 (Pico) and 28 (São Jorge) were found in this second group of the network, while haplotypes from Graciosa, Mosteiros and Caloura can be found in both groups. Haplotypes from

Vigo (H42-44) form an isolated cluster, not connected to the Azorean haplotypes. The reconstruction of a Rissoidae phylogenetic tree (Figure 4.3) allowed to clarify the divergence levels within the family and to ascertain the position of *C. trifasciata* from the Azores and Vigo. The separation of the samples from the mainland and the islands is well-supported (99%) in the phylogeny. A BLASTN search (Zhang et al., 2000) of VIG sequences revealed 100% cover and 95-96% identity with several *C. trifasciata* haplotypes from the Azores, but 99-100% identity with a shorter Galician sequence deposited at the database (KU695304; 55% query cover).

Pairwise F_{ST} statistics between populations were estimated for the COI dataset (cf. Supplementary Table A3.3 for details). Comparatively high and significant levels of differentiation, over 0.8, were inferred between Vigo and Azorean populations. Among Graciosa, Pico, and São Jorge Islands, the F_{ST} levels were low and non-significant, whereas Mosteiros (São Miguel Island, eastern group) shows only significant differences with Graciosa and Pico from the central group. The analysis revealed significant and high F_{ST} values in the pairwise comparisons with Flores Island. In comparison with the remaining estimates, the pairwise comparisons involving Flores, Santa Maria, and Caloura retrieved higher F_{ST} levels, suggesting higher isolation of these populations.

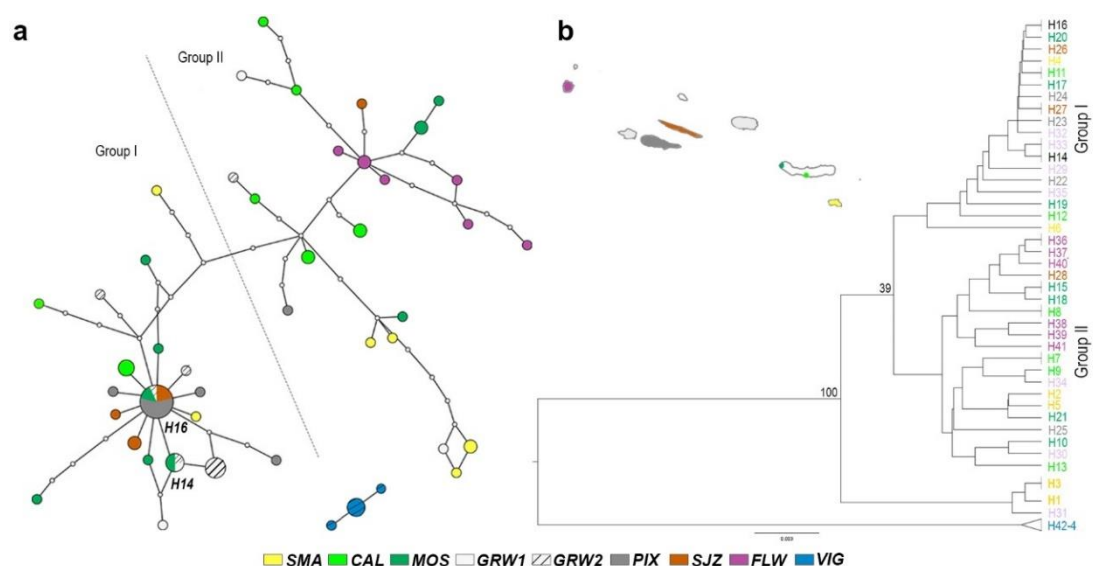


Figure 4.2. Distribution of mitochondrial haplotypes of *Cingula trifasciata*, based on 75 COI sequences/44 haplotypes. **a.** Haplotypic network of nine colour-coded populations, at 95% parsimony connection limit. Size of the circles proportional to the frequency of each haplotype; small uncoloured circles represent non-observed haplotypes; each line connecting haplotypes represents a single mutational change. Network obtained with TCS v1.21 (Clement et al., 2000) and tcsBU (Santos et al., 2016); **b.** UPGMA tree (Sneath & Sokal, 1973), as implemented in Geneious 8.1.9 (Kearse et al., 2012) and considering 1000 replicates for the bootstrap inference. Haplotypes coloured according to the population in which they are detected, black indicates haplotypes present in more than one population. Geographical representation of Azorean islands/localities colour-coded; distances among island groups not to scale.



SSR marker discovery

For the SSR marker discovery, the MiSeq runs for the two samples produced 766,960 and 1,382,293 paired reads, respectively for PSM4350 and FSC5131. A total of 445,578 and 248,419 reads, which passed the quality control and merging steps were screened for SSR motifs. Of these, respectively, 450 and 8,673 reads containing SSR motifs complied with the criteria defined for the SSR search pipeline. These comprised 203 di-, 123 tri-, 107 tetra-, and 17 pentanucleotide repeats for PSM4350; and 2168 di-, 1477 tri-, 1303 tetra-, and 254 pentanucleotide repeats for FSC5131. A total of 42 primer pairs for *C. trifasciata* were designed only from sequences containing penta- and tetranucleotide repeats. Eleven out of the 42 designed primers failed to amplify in the

single PCR test phase. The remaining primers were included in 4 multiplex primer mixes and are provided in Supplementary Table A3.1.

SSR data analysis and SSR-GBAS genotyping

For the SSR-GBAS genotyping, a total of 2,543,202 paired reads were produced, which were reduced to 1,008,047 after quality control, merging and primer demultiplex steps. Raw reads can be accessed through the BioProject PRJNA702169. The number of reads per marker ranged from 1,794 to 121,460, with CT42_TAAA being the one with highest number of sequences. Regarding the number of sequences per sample, it varied between 38 (PS4380) and 56,441 (CAL5184). After filtering the matrix and exclude the samples with more than 60% missing data across all markers, the dataset was reduced to seven individuals from Santa Maria, nine from Caloura and two from Mosteiros (São Miguel), six from São Jorge, 11 from Pico, five from Graciosa, one from Flores, and three from Vigo (Galicia, Spain). As only one sample was successful in SSR-GBAS genotyping, the population from Flores island was excluded from further analyses. Although they had been successfully amplified in the single PCR test, the following markers were excluded due to failure in the multiplex step or Illumina MiSeq, resulting in data unfit for further analysis or missing data: CT6_TTGT and CT17_TTTG were excluded due to non-specific amplification; CT1_TGTT, CT3_GAATA, and CT10_GTTT were excluded for yielding over 60% missing data. Thus, a total of 26 markers and 43 samples were suitable for population genetic analyses of *C. trifasciata*.

Population structure analyses

Genetic diversity measures

In the WAI (Whole Amplicon Information) dataset, the markers analysed had between 6 and 41 alleles (Supplementary Figure A3.1a), which can be found in GenBank Database (<https://www.ncbi.nlm.nih.gov/genbank/>) under the accession numbers MW623085-363. The polymorphic information content (PIC) per locus ranged between 0.43 and 0.954, with only 4 out of 26 markers displaying PIC values below 0.50. Although monomorphic markers for the complete WAI dataset were not detected, some of the markers were monomorphic in some populations (Table 4.2): CT23_TTTA in Caloura; CT24_CAAA in Graciosa; CT31_AATA in Graciosa; CT38_TCAT in Caloura; plus eight monomorphic markers in Vigo population (data not shown). Not accounting monomorphic markers across the studied populations, the complete WAI dataset showed *Ho* varying from 0.235 to 0.921 and *He* from 0.448 to 0.956. Within each of the five populations in study, with five or more individuals (Supplementary Figure A3.1b), the number of alleles (*Na*) ranged from 3.346 (São Jorge) to 4.538 (Santa Maria) all with a

frequency over 5%, except in Pico. The number of effective alleles (N_e) was lower to N_a in all populations, ranging from 2.357 (Pico) to 3.317 (Santa Maria). H_o and H_e followed the same trend across all populations when analysing the dataset per population, with H_o levels varying between 0.394 (São Jorge) and 0.620 (Santa Maria), and H_e between 0.498 (São Jorge) and 0.623 (Santa Maria). Private alleles were detected in all populations.

Tests for Hardy-Weinberg Equilibrium (HWE; Table 4.2) per markers and population revealed deviations at one locus in Caloura (CT5_TGAA) and three loci in Pico (CT23_TTTA, CT33_CATT, CT38_CATT). The potential phenomena causing it were further evaluated with estimates of null alleles' frequency and Wright's Fixation index (F_{IS}). Errors in the assignment of genotypes causing deviations to HWE were discarded after checking the markers plots generated after the Allele Length calling script. The deviations were either caused by the dominance of null alleles (CT5_TGAA in Caloura) inferred from FreeNA and checked in the original matrix, excess of homozygotes indicated by F_{IS} (CT33_CATT in Pico), or both (CT23_TTTA in Pico). A total of six out of 26 markers deviated from HWE in the population from São Jorge (CT14_CAGA, CT18_AAAG, CT23_TTTA, CT24_CAAA, CT31_AATA, CT37_AAAT) due to excess of homozygotes suggested by the F_{IS} levels, which led to higher frequencies of null alleles despite the absence of real null alleles. In Vigo population (data not shown), three loci failed to amplify (CT23_TTTA, CT30_TGTC, and CT5_TGAA) resulting in null alleles across all the samples of this population; the negative values of F_{IS} in the several loci suggest an excess of heterozygotes in this population.

Table 4.2. Estimates of Hardy-Weinberg Equilibrium tests, frequency of null alleles and Wright's Fixation index.

	Santa Maria			Caloura			Graciosa			Pico			São Jorge		
	HWE Test	f(Null)	F _{IS}	HWE Test	f(Null)	F _{IS}	HWE Test	f(Null)	F _{IS}	HWE Test	f(Null)	F _{IS}	HWE Test	f(Null)	F _{IS}
CT11_GTTT	0.296	0.189	0.378	0.284	0.001	0.357	0.164	0.000	0.565	0.218	0.119	0.371	0.828	0.001	0.077
CT13_GTTG	0.787	0.000	0.091	0.967	0.000	-0.185	0.821	0.001	-0.304	0.567	0.001	0.263	0.446	0.000	-0.212
CT14_CAGA	0.784	0.000	0.103	0.223	0.001	-0.091	0.577	0.072	0.000	0.562	0.000	0.083	0.008**	0.420	1.000
CT15_TTTG	0.208	0.157	0.138	0.120	0.042	0.385	0.256	0.153	0.333	0.740	0.000	-0.100	0.804	0.001	-0.111
CT16_TTTG	0.904	0.000	-0.321	0.987	0.000	-0.091	0.804	0.000	-0.111	0.218	0.001	0.371	0.341	0.001	0.429
CT18_AAAG	0.659	0.000	-0.167	0.093	0.066	0.032	0.958	0.000	-0.176	0.987	0.000	-0.183	0.007**	0.251	0.429
CT19_GACA	0.907	0.000	-0.200	0.388	0.081	0.091	0.704	0.000	-0.250	0.113	0.063	0.173	0.824	0.001	-0.091
CT21_CAAA	0.594	0.000	-0.014	0.143	0.153	0.123	0.475	0.000	0.143	0.725	0.000	-0.111	0.775	0.000	-0.143
CT22_ACAG	0.435	0.000	0.082	0.777	0.000	-0.400	0.353	0.001	0.040	0.786	0.000	-0.158	0.775	0.000	-0.143
CT23_TTTA	0.682	0.001	-0.371	<i>mono</i>	0.001	-	0.351	0.153	0.467	0.01**	0.164	0.807	0.034*	0.082	0.429
CT24_CAAA	0.540	0.000	-0.373	0.480	0.001	-0.011	<i>mono</i>	0.001	-	0.763	0.030	0.091	0.025*	0.001	1.000
CT26_TGTT	0.270	0.138	0.417	0.326	0.053	0.024	0.120	0.269	0.167	0.724	0.001	-0.077	0.931	0.000	-0.231
CT28_CTGT	0.995	0.000	-0.296	0.524	0.000	-0.043	0.704	0.000	-0.250	0.791	0.000	-0.245	0.244	0.000	0.143
CT29_AAAT	0.166	0.000	0.111	0.666	0.000	0.217	0.062	0.290	1.000	0.958	0.000	-0.132	0.125	0.269	0.412
CT30_TGTC	0.138	0.000	0.125	0.207	0.197	0.652	0.393	0.000	0.000	1.000	0.000	-0.200	0.132	0.001	0.231
CT31_AATA	0.270	0.001	0.417	0.850	0.001	-0.067	<i>mono</i>	0.001	-	0.774	0.001	-0.266	0.014*	0.251	1.000
CT32_TTTG	0.188	0.000	-0.463	0.470	0.000	0.000	0.764	0.000	-0.250	0.541	0.000	-0.176	0.666	0.000	0.217
CT33_CATT	0.119	0.001	0.054	0.051	0.001	0.576	0.861	0.000	-0.333	0.002**	0.307	1.000	0.112	0.001	0.333
CT34_ACCA	0.112	0.290	1.000	0.692	0.000	0.143	0.079	0.001	0.032	0.461	0.000	0.067	0.414	0.001	-0.333
CT35_TTTG	0.511	0.000	-0.089	0.166	0.047	0.265	0.152	0.223	0.500	0.650	0.000	0.210	0.999	0.000	-0.263
CT37_AAAT	0.126	0.042	0.408	0.865	0.000	-0.220	0.958	0.000	-0.176	0.376	0.089	0.267	0.025*	0.001	1.000
CT38_TCAT	0.814	0.000	-0.120	<i>mono</i>	0.001	-	0.847	0.000	0.063	0.001**	0.000	0.057	0.704	0.000	-0.250
CT39_CATT	0.451	0.143	-0.250	0.752	0.001	-0.167	0.891	0.001	-0.471	0.054	0.000	-0.618	0.216	0.251	-0.111
CT40_ATCC	0.272	0.000	-0.235	0.549	0.000	-0.200	0.135	0.000	-0.212	0.131	0.119	0.436	0.062	0.082	0.538
CT42_TAAA	0.471	0.000	-0.273	0.804	0.000	-0.159	0.172	0.000	-0.724	0.461	0.000	-0.222	0.824	0.001	-0.091
CT5_TGAA	0.637	0.000	-0.333	0.046*	0.333	1.000	0.963	0.000	-0.333	0.850	0.000	-0.067	0.572	0.001	-0.600

Analysis per marker and population with at least five individuals. Significant (* $p < 0.05$, ** $p < 0.01$) deviations to HWE highlighted in bold. Monomorphic loci (*mono*) and loci which completely failed genotyping (*all null*) also depicted.

Genetic and spatial structure analyses

The PCoA analysis of the complete WAI dataset (Figure 4.4a-c) revealed a clear distinction of populations from the Azores Archipelago (Santa Maria, Caloura, Mosteiros, Graciosa, Pico, São Jorge) and the one from Vigo (Galicia, Spain). The repetition of the PCoA without Vigo population allowed to clarify the similarities among Azorean populations (Figure 4.4d-f), each forming smaller, distinct subclusters. In both dimensions, Mosteiros (São Miguel) is positioned between populations from Graciosa or Pico, whereas Pico and Caloura show considerable dispersion along all the axis. Except for Mosteiros' population, the eastern populations seem to be more divergent when compared to the remaining.

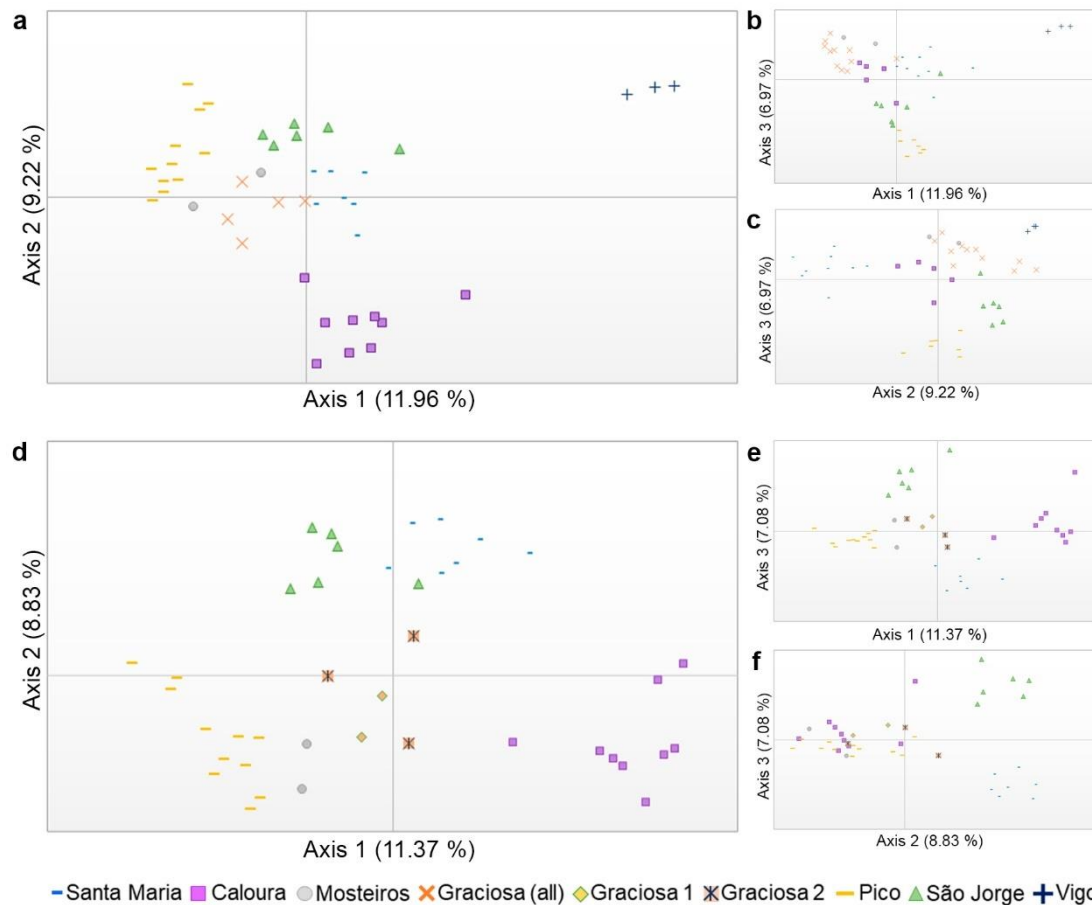


Figure 4.4. Principal coordinates analysis from complete WAI dataset of *Cingula trifasciata* (a-c) and Azorean localities (d-f). a. Variance explained by axis 1 (11.96%) and axis 2 (9.22%); b. Variance explained by axis 1 (11.96%) and axis 3 (6.97%); c. Variance explained by axis 2 (9.22%) and axis 3 (6.97%); d. Variance explained by axis 1 (11.37%) and axis 2 (8.83%) for the Azorean localities; e. Variance explained by axis 1 (11.37%) and axis 3 (7.08%), for the Azorean localities. f. Variance explained by axis 2 (9.22%) and axis 3 (6.97%), excluding Vigo population. PCoAs conducted as implemented in GenAlEx v6.5 (Peakall & Smouse, 2006, 2012); populations in study are coded by colour and icon shape.

The STRUCTURE analysis was congruent with the PCoA results, with K-value of 6 estimated as optimal (Figure 4.5), separating each population individually except for Pico and Mosteiros, which cluster together. At this K-value, Pico, Santa Maria, and Vigo populations each form a well-defined cluster, agreeing with the pattern retrieved from the PCoA. We have also considered lower K-values to evaluate potential correspondence between the hierarchy cluster and geographical distribution of the populations studied (Figure 4.5). At K=2, Vigo shares similar patterns of variation with the Azorean populations of Caloura and Santa Maria, whereas the second cluster is dominated by Pico and São Jorge individuals. The remaining populations, located in between these groups, show a considerable degree of admixture. At K=3, Santa Maria and Vigo are assigned to one new cluster, while the other two clusters show evidences of admixture in some populations. Vigo forms a new cluster when K=4 and, finally, at K=5 it is assigned to an isolated cluster with no signal in other populations. From K=3 to K=5, the pattern roughly corresponds to a gradual transition of clusters from West to East. The assignment of Graciosa to a new cluster only when K=6 falls out of this pattern, as does the unexpected placement of Mosteiros samples in the cluster together with Pico. For K-values over 7 (cf. Supplementary Figure A3.2), cluster assignment becomes increasingly unclear for Azorean populations, and no further population sub-structure is inferred; Vigo population remains isolated.

The AMOVA analysis (Table 4.3), performed among regions (Azores and Vigo) and populations, revealed that 25% of the variation in the complete WAI dataset is found among regions, whereas the differentiation among populations accounts for 13% of the overall diversity. Regarding the F-statistics, a significant ($p < 0.01$) F_{ST} value of 0.385 was estimated for the overall WAI dataset, whereas the pairwise F_{ST} estimates for populations are detailed in Supplementary Table A3.4 (cf. Figure 4.7 for a summary). As expected, the highest differentiation is detected between Vigo and all the Azorean populations, with F_{ST} values over 35% in all pairwise comparisons. The lowest F_{ST} values are observed in Mosteiros vs. Pico (0.110), Mosteiros vs. Graciosa (0.119), and Graciosa vs. Pico (0.122), in congruence with the previous results of PCoA and STRUCTURE, suggesting gene flow between these populations.

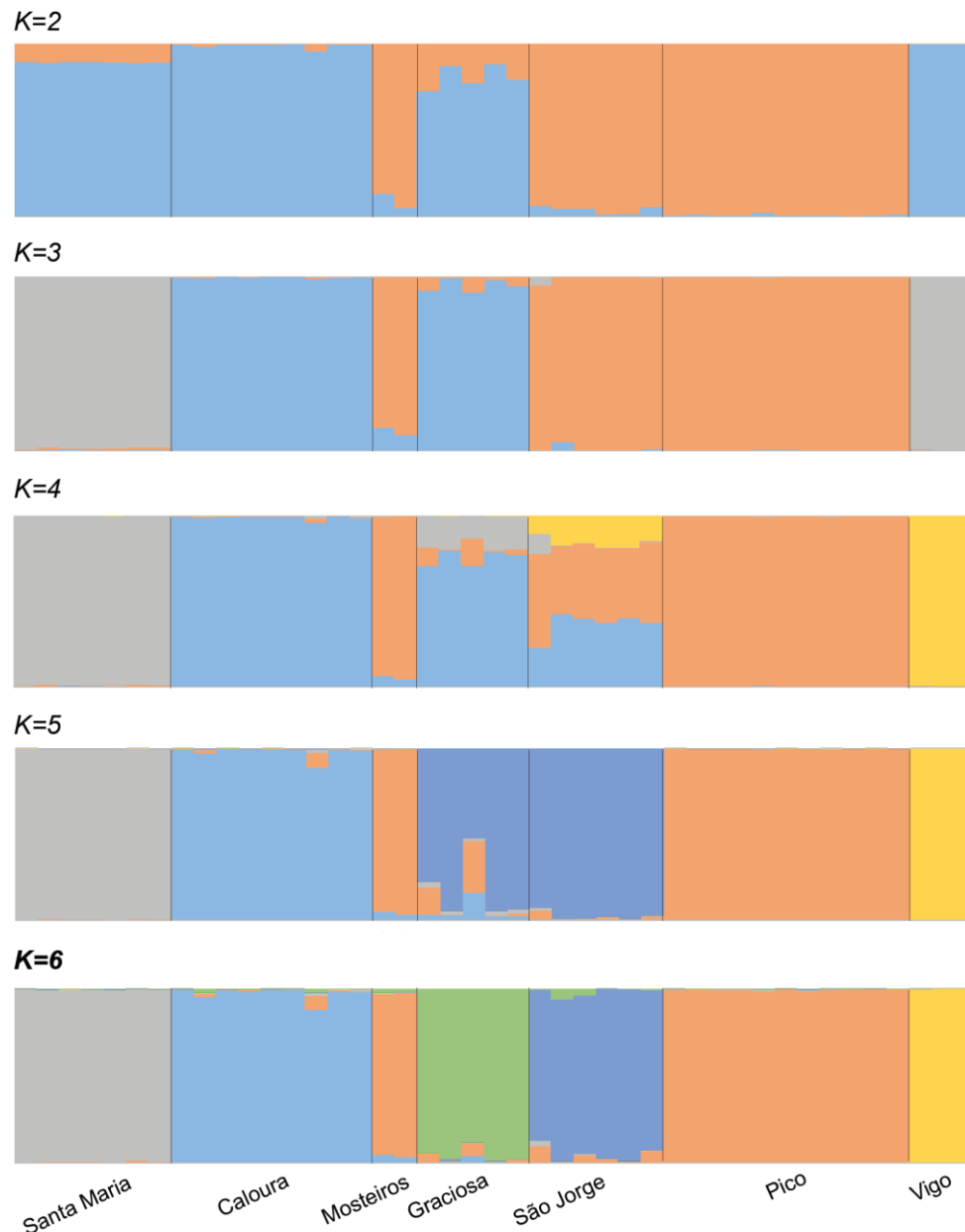


Figure 4.5. Genetic structure analysis of the complete WAI dataset of *Cingula trifasciata*. Inferred with STRUCTURE v2.3.4 (Hubisz et al., 2009; Pritchard et al., 2000) and reporting the results from K=2 up to the optimal K=6.

Table 4.3. Hierarchical analysis of Molecular Variance among *Cingula trifasciata*.

Source	df	SS	MS	Est. Var.	%
Among Regions	1	58.016	58.016	3.269	25%
Among Populations	5	160.353	32.071	1.714	13%
Among Individuals	36	362.468	10.069	2.104	16%
Within Individuals	43	252.000	5.860	5.860	45%
Total	85	832.837		12.947	100%

Analysis performed among populations and among geographical regions (Azores, Portugal vs. Galicia, Spain).

Discussion

With this work we address biogeography and the processes shaping the genetic structure and evolutionary dynamics of *Cingula trifasciata* in the NE Atlantic Ocean, with a special focus in the Azores Archipelago. The results indicate a divergence between insular and continental populations of this microgastropod and the existence of genetic structure within the Azores Archipelago on a small spatial scale. This indicates dispersal barriers for the species with non-planktotrophic larvae that could form the starting point of population differentiation. To our knowledge, this is the first time that the SSR-GBAS protocol generates a numerous set of *de novo* SSR primers for a marine microgastropod. Comparing to previous studies in other marine invertebrate taxa, a higher number of loci is herewith amplified contributing to an informative dataset even with a low number of individuals. Thus, this methodology has the potential to be applied in similar study systems, increasing the number of markers successfully developed for marine taxa. These topics will be further discussed in the next sections.

A *de novo* SSR primer dataset for *Cingula trifasciata*

In this study, a total of 26 SSR loci of *C. trifasciata* were developed and characterized and genotyped using sequence information of Illumina MiSeq according to the SSR-GBAS methodology described earlier (Curto et al., 2019; Lanner et al., 2021; Tibihika et al., 2019). Second-generation sequencing technologies revolutionized the discovery and genotyping of microsatellites, solving technical drawbacks posed by the traditional genotyping techniques (Castoe et al., 2012; Curto et al., 2019; Darby et al., 2016; de Barba et al., 2016; Farrell et al., 2016; Guichoux et al., 2011). The application of next-generation sequencing (NGS) to non-model species, to which a reference genome is usually not available, is attained by downsizing approaches for the analysis of a small subset of loci, ultimately contributing to the development of genotyping by sequencing (Darby et al., 2016; Farrell et al., 2016). The approach is frequently used in population genetics studies, detecting more microsatellite markers per sample and unique alleles in a fast and cost-effective way (Curto et al., 2019; Darby et al., 2016; de Barba et al., 2016; Farrell et al., 2016; Tibihika et al., 2019; Vartia et al., 2016). Due to its characteristics, amplicon sequencing increases the statistical power even with a low number of sequenced markers and the resolution of detailed population genetic structure, suitable even in large-scale *de novo* population genetic studies in non-model species (Darby et al., 2016; Farrell et al., 2016; Guichoux et al., 2011). Moreover, this approach allows to obtain the complete sequence of the loci analysed, including the flanking region, the repetition motif, and potential SNPs (Single Nucleotide Polymorphism), overcoming

homoplasmy typical of SSR studies (Curto et al., 2019; de Barba et al., 2016; Vartia et al., 2016). The recent SSR-GBAS (Simple Sequencing Repeats - Genotyping by Amplicon Sequencing) workflow calls alleles based on the whole amplicon information (WAI), including allele length (AL) and SNPs. It results in a higher number of alleles recovered, marker variability, information content, and lower influence of homoplasmy, which together provide a better resolution of the SSR dataset. A script for analysis of data was provided by de Barba et al. (2016) and another later described by Curto et al. (2019). Relying on approaches suggested by Illumina, the SSR-GBAS methodology follows a cost-effective optimization of a high level of multiplexing, up to 10 markers. The results are easily reproduced and analysed resorting to bioinformatic tools, allowing the automation of allele calling and reduction of traditional SSR artefacts. The SSR-GBAS method is useful for the study of small-scale systems, non-model organisms, and specific research questions, yielding potential to become an important tool to be incorporated in long-term screening projects and meta-analysis, along with data from other sources/teams, comparable to phylogenetic data collections. The SSR-GBAS methodology has been described in detail by Curto et al. (2019), Lanner et al. (2021), Tibihika et al. (2019), and summarized herewith.

In congruence with the previous studies using SSR-GBAS, a set of highly informative markers was obtained for the non-model, marine microgastropod, with moderate to high PIC values according to the classification scheme (Botstein et al., 1980). Only loci with penta- and tetranucleotide repeats were included to reduce the complexity of PCR and artefacts that interfere with the call of alleles (de Barba et al., 2016). Following this approach, allele call could be done unambiguously with no need to include manual edits after visual control of genotypes (Curto et al., 2019). Dinucleotides more frequently produce stutter bands that make allele determination difficult and, in the case of SSR-GBAS, additionally result in a higher error rate in the determination of single nucleotide polymorphisms, when alleles that differ by one repeat unit contain a SNP (Curto et al., 2019; Ginot et al., 1996; Litt et al., 1993). The additive signal of allele and stutter influences the base frequency at the SNP position. In our case, the penta- and tetranucleotide repeat loci comprised a high amount of information so the inclusion of the shorter repeat unit motifs was not necessary.

In addition to being highly informative, this newly generated set of SSR markers retrieved overall diversity parameters consistent with values reported for other marine gastropods (Donald et al., 2011, 2020; López-Márquez et al., 2018). Deviations from HWE in some populations – Caloura, Pico, São Jorge, and Vigo –, are frequently reported in marine invertebrates (Addison & Hart, 2005; Cahill & Viard, 2014; Dupont et

al., 2007; Pálsson et al., 2013). Reproductive patterns have been suggested to contribute to the excess of homozygotes in marine invertebrates (Cahill & Viard, 2014; Zouros & Foltz, 1984). *Cingula trifasciata* possesses np-larvae with low dispersal ability, which contributes in one hand to the high genetic structure detected but also to homozygote excess in some populations. Nevertheless, this should not affect the biogeographical interpretations regarding the Azorean populations, as their prevalence in other gastropod species has been shown to not influence the population structure inferred (e.g. Cahill & Viard, 2014; Kemppainen et al., 2009). High variability in the flanking regions might contribute to the inference of null alleles in the Vigo population (Papetti et al., 2018). Our results suggest that Azorean and Vigo populations constitute distinct evolutionary lineages, thus the specificity of the SSR primers, designed from Azorean samples, might be lower for individuals from Vigo. Therefore, the application of this set of SSR primer in Vigo's individuals is comparable to a situation of cross-species amplification, with the potential to negatively affect the success of the protocol in the related species (Curto et al., 2019; Turini et al., 2014).

Although laboratory and analytical methodologies have developed fast in the past decades, molecular markers for molluscs are scarce and mostly based on traditional methods, as microsatellites and allozymes (Cahill & Viard, 2014; Donald et al., 2011; Weersing & Toonen, 2009), also as a consequence of the problems arising during DNA extraction of molluscan tissues (Sokolov, 2000). The combination of markers with different information content has been shown to improve the sensitivity of population genetic inferences (Sunnucks, 2000). However, the few datasets so far available do not truly represent the genetic and geographic differentiation in marine gastropods, and the lack of widely used molecular markers hampers comparative analyses of markers' suitability in a study system (Teske et al., 2018). The development of de novo molecular markers, ideally transversal to related taxa, is urgently needed to increase our understanding on connectivity in the marine realm and evolution of invertebrates with different ecological and biological characteristics. SSR-GBAS methodology can be an important tool to achieve these goals, being easy to implement and fast to develop, as shown here with *C. trifasciata* (Curto et al., 2019; Tibihika et al., 2019). Datasets generated with this methodology, comprising variability from the repetition motif and SNP in the flanking region, are informative in distinguishing evolutionary entities. In understudied groups, as marine invertebrates, this type of markers might even be useful to uncover cryptic patterns of diversity, as detected in this work and further discussed in the next section.

Differentiation between insular and continental populations

The analysis of the mitochondrial marker COI of *Cingula trifasciata* revealed differentiation between samples from the Azores Archipelago and Vigo (Galicia, Spain). A complete separation of the Azorean and Vigo networks at 95% connection limit (Figure 4.2) has been previously pointed as indicative of deep differences, even considered by some as different species (Hart & Sunday, 2007). Divergence levels between haplotypes from the Azores and Vigo ranged from 3.6 to 5% (cf. Supplementary Table A3.2) and this distinction becomes evident at a phylogenetic tree (Figure 4.3). Within the superfamily Rissoidae, to which *C. trifasciata* belongs, the COI marker is an adequate diagnostic tool for species status by its strong and reliable phylogeographic signal (Wilke & Falniowski, 2001). We have further assessed evolutionary divergence levels of COI within the family Rissoidae, ranging from 2.9 to 19.9% within the several genera analysed (Supplementary Table A3.5). The divergence between *C. trifasciata* Azores and Vigo falls within this interval, surprisingly high for conspecific individuals. A similar level of differentiation (2.9%) occurs between *Alvania mediolittoralis* (Gofas 1989) and *A. formicarum* (Gofas 1989), two recognized species occurring in the Azores Archipelago with distinctive morphological characters. The inclusion of these *Alvania* species in a calibrated molecular phylogeny of Rissoidae has shown them to have diverged quite recently, at about 0.36 million years (Ma), within a range uncertainty of 0.12-0.61 Ma (Baptista et al., 2019), explaining the relatively low divergence levels. Following this rationale, we propose that a divergence event is currently acting on *C. trifasciata*, causing a split between the evolutionary lineages from the Azores and Vigo. The differentiation between insular and mainland (Vigo) populations is supported by the COI and WAI datasets, and evident both in clustering analyses (Figures 4.4 and 4.5). In the STRUCTURE analysis, samples from Vigo cluster together with no signs of admixture even when $K=2$, and at $K=4$ it forms an individual cluster, not admixed in other populations. The maintenance of this pattern and absence of further sub-structuring for $K > 6$ supports the uniqueness of Vigo's samples relative to Azorean populations.

Although true allopatric isolation in the marine realm is difficult to accomplish, the high distances across deep-water and unsuitable habitats between locations could hamper dispersal and reduce gene flow between populations though to have similar ecological requirements and means of dispersal (Ávila, 2013; Ávila et al., 2012; Palumbi, 1994). Lower dispersal leads to the accumulation of genetic variants exclusive to each population and perhaps partial isolation of the insular populations in relation to the mainland (Palumbi, 1994), here reflected in the genetic differences in the COI and SSR datasets. This scenario of insular isolation is not exclusive to *C. trifasciata*. Differentiation

of Azorean populations, often together with high intra-archipelagic diversity levels, have been described for other marine organisms as the gastropod genus *Patella* (Sá-Pinto et al., 2007) and several fishes (Aurelle et al., 2003; Domingues et al., 2006, 2007; Francisco et al., 2014; González-Wangüemert et al., 2010; Stefanni et al., 2015), but also for terrestrial organisms [e.g. *Nyctalus azoreum* (Salgueiro et al., 2004, 2008); starlings (Neves et al., 2010)].

Morphological differences between Azorean and Iberian individuals of *C. trifasciata* have previously been reported (Gofas, 1990), in easily observable characters (e.g. broader brown bands on the whitish shell; cf. Figure 4.6) and in detailed observations of the tentacles and snout. Doubts regarding the assignment of Azorean *C. trifasciata* to the European taxon raised two decades ago are now supported by molecular data of mitochondrial and SSR markers. These results reinforce the importance to couple morphological, ecological, and molecular data to have a broader perspective of biodiversity, especially in such an understudied environment as the marine realm. Following the results of this study and life-traits of *C. trifasciata*, we propose that the insular and mainland populations are currently experiencing a split, constituting distinct evolutionary lineages. As *C. trifasciata* is the only known representative of the genus in the NE Atlantic Ocean, further studies including more populations across the established distribution range of the species in the NE Atlantic Ocean, as well as from the putative populations in the Mediterranean Sea, might clarify questions regarding its current taxonomic classification.

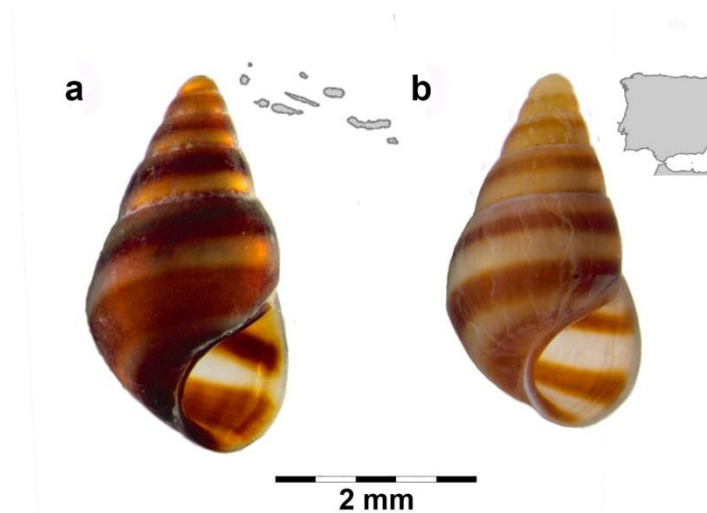


Figure 4.6. *Cingula trifasciata* specimens from insular and mainland populations. **a.** Cerco da Caloura, São Miguel Island, Azores (PT-CAL3; DBUA 1474); **b.** Vigo, Galicia, Spain (ES-VIG4400).

Population structure of *Cingula trifasciata* in the Azores Archipelago

The analyses of mitochondrial and SSR datasets provide insights at two different time scales, and both point to a strong genetic structure of *C. trifasciata* within the Azores Archipelago, suggesting low dispersal among islands. For Graciosa, Flores, and Mosteiros the discordant number of individuals analysed for both datasets can be explained by older samples or poorer preservation, causing their degradation and easier amplification of the COI. The main findings regarding the relationships among Azorean populations of *C. trifasciata* are summarized in a geographical map of the archipelago (Figure 4.7).

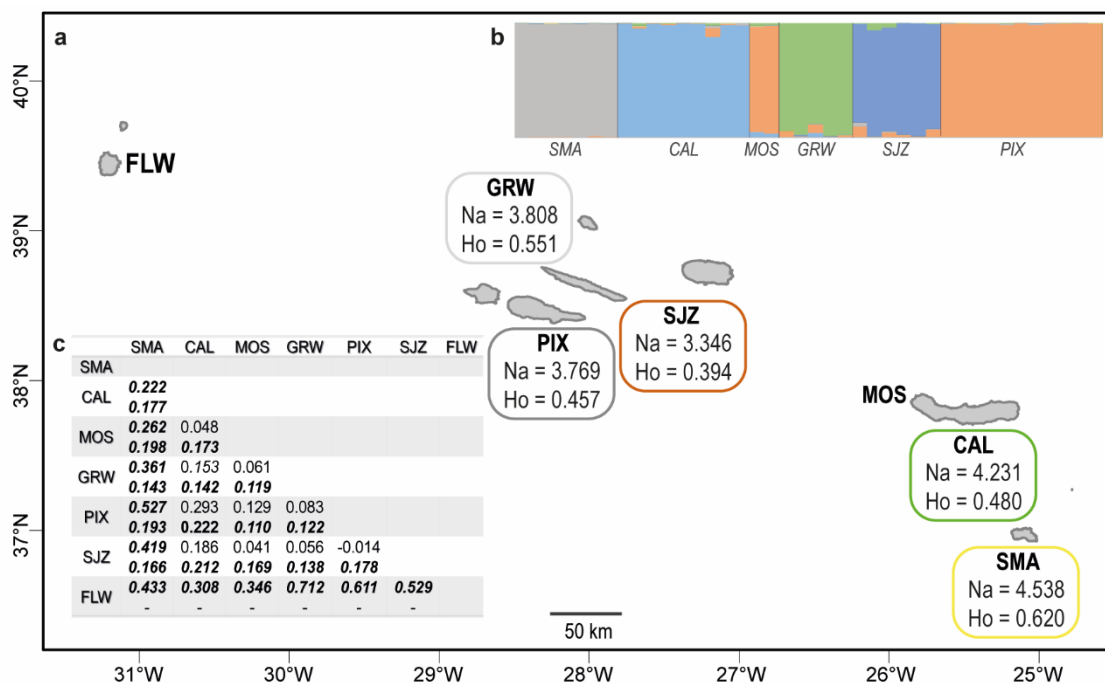


Figure 4.7. Summary of the relationships among Azorean populations of *Cingula trifasciata*. **a.** Geographical map of the Azores Archipelago, with identification of the populations studied: SMA (Santa Maria), CAL (Caloura), MOS (Mosteiros), PIX (Pico), SJZ (São Jorge), GRW (Graciosa), and FLW (Flores). Coastline from the Portuguese Hydrographic Institute 2014 (<https://www.hidrografico.pt/op/33>) and bathymetry derived from GEBCO 2019. (<https://www.gebco.net>). The number of alleles (N_a) and observed heterozygosity (H_o), based on the WAI dataset are presented for the populations with more than five individuals; **b.** STRUCTURE v2.3.4 (Hubisz et al., 2009; Pritchard et al., 2000) plot for the optimal $K=6$; and **c.** Pairwise F_{ST} values between populations of *C. trifasciata*; for each comparison, top values refer to the COI dataset, bottom values are based on the WAI dataset; bold italic indicates significant pairwise F_{ST} values ($p < 0.01$).

Mitochondrial loci as COI are indicated for the study of older divergence events and molecular differentiation of species. In their turn, SSR are a multi-locus method in which differences are defined via allele frequency, making them especially useful to study spatial patterns of diversity at smaller time and geographic scales (Teske et al., 2018).

In that regard, the mitochondrial results suggest the occurrence of two haplogroups: 1) a star-like distribution mainly from the central group of the Azores (Pico, São Jorge, and Graciosa) suggest a recent differentiation in the region; 2) a linear branched haplogroup mainly from the eastern (Santa Maria and São Miguel) and western (Flores) groups, as a sign of stable, isolated populations with less frequent gene flow. Several explanations can be hypothesised for this observation such as the retention of ancestral polymorphisms, as Flores is separated from Santa Maria and São Miguel by more than 585 km of deep, unsuitable waters. Restrictions to gene flow of Flores' populations had already been reported in the marine realm for *Patella candei* d'Orbigny, 1839 and *P. ulyssiponensis* Gmelin, 1791 (Sá-Pinto et al., 2007). Future fieldwork in more islands and localities in the Azores might allow more accurate inferences and reduce the number of unknown/unsampled mitochondrial haplotypes. Based on the WAI dataset, a stronger genetic structure is detected among populations. The clustering pattern in STRUCTURE roughly corresponds to a gradual East-West transition, except for the assignment of Graciosa and Mosteiros samples (cf. Figure 4.5). These deviations from the geographical pattern indicate a recent colonization from the Central group or the recent exchange of individuals due to unusual patterns of circulation between these island groups. Therefore, even though the Azorean islands are relatively isolated for long periods of times, occasional dispersal events of rafting and extreme weather might allow the exchange of individuals among distant populations (Ávila, 2013; Baptista et al., 2021; Waters & Craw, 2018).

Dispersal ability, habitat type, and oceanic circulation patterns within the archipelago, appear to be the main drivers of population structure in the Azores. The finding of strong genetic structure of *C. trifasciata* in the Azores is consistent with its np-larval development and habitat preferences of this microgastropod (Bell, 2008; Cahill & Viard, 2014; Donald et al., 2020; Kojima et al., 2004; Lee & Boulding, 2009; Wilke & Davis, 2000; Wort et al., 2019). The lack of a free-swimming dispersal stage during the larval development of *C. trifasciata* likely hampers dispersal ability at early-life stages (Scheltema, 1986, 1989, 1995). Considering the direct development strategy, most of the dispersal must be ensured by early juveniles and adults in occasional rafting in algal patches (Ávila, 2013; Thiel & Haye, 2006; Winston, 2012), as reported from other direct developers [e.g. *Batillaria cumingi* (Crosse, 1862) (Kojima et al., 2004)]. *Cingula trifasciata* gathers the common characteristics of rafters (Thiel & Gutow, 2005a, 2005b), namely its minute size and presence among/close to algal patches in the intertidal. Rafting can be the process behind some inter-island dispersal events in the Azores Archipelago.

In the Azores, *C. trifasciata* and other microgastropod fauna are easily found in protected sites, under boulders in enclosed tide pools with low hydrodynamics and mesotidal regimes [e.g. Cerco da Caloura, Poça da Barra, and Fajã da Caldeira do Santo Cristo (Ávila et al., 2011a), Mosteiros]. *Cingula trifasciata* has been reported in similar habitats outside of the Azores [Lough Hyne, Ireland (Davidson, 2005)]. This rissoid has also been reported in Ceuta (Ponder, 1990) and Praia de Lobos (Santa Maria Island) associated with gravel/boulders, which get uncovered during low tide but prone to wave action in bad weather conditions or high tide. In the absence of such conditions, they are found in lower numbers in algal substrates or among big boulders in protected intertidal areas of the Azorean islands, as reported for Flores and Graciosa islands (Gofas, 1990). The influence of habitat in the likelihood of dispersal events of juveniles and adults by rafting, thus affecting population structure, has been proposed to *Steromphala* spp. (Wort et al., 2019). In our study system, one expects juveniles to drive genetic exchange in the gravel/boulder habitats, depending on the oceanographic features in the area. If the fauna is mainly associated with algal substrate, the chances of rafting in algae to distant localities are higher (Ávila, 2013; Scheltema, 1986). The habitat type seems to play a major role at São Miguel Island, particularly at the enclosed locality of Caloura (Ávila, 1998; Gofas, 1990; Ponder, 1990). Migration events from and to Caloura seem to be rare, as the environment might hamper dispersal of juveniles from leaving the protected area and the low algal coverage reduces the chances of adult rafting. The surprisingly low connectivity between Caloura and Mosteiros, just 45 km apart, can be attributed to the geomorphological characteristics of the southern coast of São Miguel Island, which features long stretches of unsuitable sandy habitats for *C. trifasciata*, making gene flow virtually impossible between these populations. Mosteiros closely resembles populations from Graciosa and Pico (central group), located 215 to 225 km far, respectively. The sea-surface circulation regimes nearby the westernmost tip of São Miguel Island, where Mosteiros is located, might favour the frequent dispersal of individuals to or from the Central Group. Within the central islands, gene flow seems to be frequent and results point to recent haplotype differentiation. The distance separating the islands sampled in the central group (20 km Pico-São Jorge; 35 km São Jorge-Graciosa) is probably small enough to not constitute a barrier for the occasional migration and demographic expansion, supported by the low mitochondrial differentiation in levels comparable to other gastropods (Brante et al., 2011; Weersing & Toonen, 2009).

The success of rafting episodes and maintenance of connectivity are influenced by the complex sea-surface circulation patterns and hydrological conditions around the Azores Archipelago (Sala et al., 2013, 2016; Silva et al., 2013). Although surface spatial differences between islands are attributed to large-scale circulation in the NE Atlantic, patterns at fine scale close to island shores are far from well-understood but likely related to colder waters from upwelling or mixing processes (Silva et al., 2013). Lagrangian transport pathways in the NE Atlantic Ocean are useful to understand potential rafting routes and major transport directions in the open ocean (Sala et al., 2013, 2016). These studies show the complexity of superficial (0-5 m) connectivity in the Azores area and among island groups, supporting our inferences regarding gene flow and exchange of individuals among populations: the western islands are the most isolated with lower changes of exchange with the remaining; high connectivity levels are expected within the Central Group; the Eastern Group is isolated to some extent but with some degree of connectivity with the Central Group (Sala et al., 2013, 2016). While the direction and circulation of the transport pathways within and among island groups await further studies, one cannot ignore the potential role of temporary disruptions of these circulation patterns in the episodic long-distance transports of individuals (Baptista et al., 2021), often due to extreme weather events lashing the Azores. Future long-term studies regarding oceanographic circulation and extreme events in the NE Atlantic might provide the necessary data to better explain the genetic structuring of *C. trifasciata* and other np marine invertebrates in the Azores Archipelago.

Conclusions

Combining mitochondrial and SSR datasets provide the opportunity to detect hidden patterns of cryptic diversity and to get a clearer perspective of gene flow among populations. Differentiation between *C. trifasciata* from the Azores and Vigo suggest the existence of potential cryptic diversity within the genus. Based on genetic data and life-history traits, these constitute distinct evolutionary lineages. Deep insights into spatial genetic structure of a microgastropod in the isolated Azores Archipelago were also reached with this study, providing crucial knowledge to better understand gene flow and connectivity among islands and considerably isolated populations. Several aspects seem to work in concert to shape the genetic structure of *C. trifasciata* in the Azores. Its np nature hinders dispersal during larval stage, but occasional exchange of individuals must occur through processes of rafting or by extreme weather events. Our results, especially the SSR dataset, reveal a preliminary analysis of these patterns in the remote islands of the Azores. A most comprehensive interpretation can only be achieved with

further studies in related research areas, namely oceanography, but also on expanding molecular approaches to other marine invertebrates. Without a thorough understanding of fine-scale circulation patterns within the Azores, unexpected connections, as the one detected between Mosteiros and Graciosa, become difficult to justify. The application of the SSR-GBAS approach to other marine invertebrates might be the key to generate comparative datasets and to determine how widespread are the patterns inferred for *C. trifasciata*. How transversal is the complex interaction between the larval development type, ecological traits related to the habitat occupied, and the sea-surface circulation patterns in the population dynamics and genetic structure of marine invertebrates can be further addressed by expanding the taxa studied. Understanding differentiation levels and patterns of diversity at the regional scale might be a useful proxy for other intertidal invertebrate species, and to implement conservation and management strategies of the shore habitats in a way that connectivity in the remote Azores Archipelago will be preserved.

4.2 Untangling the origin of the newcomer *Phorcus sauciatu*s (Mollusca: Gastropoda) in a remote Atlantic archipelago

Introduction

The marine topshells of the genus *Phorcus* (Trochidae, Gastropoda) are common grazers at the intertidal rocky shores of the Mediterranean Sea and Northeast Atlantic Ocean (Rubal et al., 2014). The interest of the scientific community in the various *Phorcus* species is not uniform, as most studies on the genus focus on the widely distributed *P. lineatus* (da Costa 1778). Nevertheless, in recent years, attention to a different species – *P. sauciatu*s (Koch, 1841) – has been growing as its cultural importance for human consumption increases, especially in North Atlantic archipelagos (Sousa et al., 2019a). Moreover, the ecological importance of *Phorcus* as algal grazers shaping communities and the range of environmental pressures faced by the individuals (Sousa et al., 2018) prompted for many studies aiming at a better understanding of the ecology and evolution of the genus (e.g., Crothers, 2001; Donald et al., 2012a; Mieszkowska et al., 2007; Ramírez et al., 2005, 2009; Rubal et al., 2014; Sousa, Delgado, González, Freitas, & Henriques, 2018; Sousa et al., 2019a, 2019b).

As many other successful intertidal algal grazers, *P. sauciatu*s occurs from supratidal to subtidal zones in rocky shores, apparently with high tolerance to wave action (Crothers, 2001; Rubal et al., 2014; Sousa et al., 2019b). It is currently found along the temperate-subtropical shores of the Lusitanian Province sensu Freitas et al. (2019), including the coasts of the oceanic volcanic archipelagos of the Azores, Madeira, and the Canaries (Donald et al., 2012; Ávila et al., 2015b) (Figure 4.8). The northern boundary of its distribution range is at the Iberian Peninsula (Fischer-Piétte, 1963) being most probably ruled by sea-surface temperatures (Rubal et al., 2014). Studies on *P. sauciatu*s have been conducted about the population structure and geographic distribution of the species in the Iberian Peninsula (Fischer-Piétte, 1963; Rubal et al., 2014), Canaries (Ramírez et al., 2005, 2009), and Madeira (Sousa et al., 2019b), based in historical records and measures of life history traits' related parameters.

The most recent and complete work dealing with the life history traits of *P. sauciatu*s is Sousa et al. (2019b). *Phorcus sauciatu*s, like other *Phorcus* species, is a broadcast spawner (Sousa et al., 2018). Relying on external fertilization could be considered risky due to the mating limitation or Allee effect, as the reproductive success depends on the local density of spawning animals (Allee, 1931). About 29 hours after fertilization, a free-

swimming, non-feeding veliger hatches (Crothers, 2001; Desai, 1966); at the fourth day its motility is reduced; on the sixth day, a juvenile snail completely formed acquires the creeping habit (data for *P. lineatus*; Crothers, 2001). This information coupled with a 1.0-1.25 whorl protoconch (Supplementary Material in Ávila et al., 2015b) leads us to assume that *P. saucius* has a non-planktotrophic (lecithotrophic) mode of larval development, with short-lived veliger larvae for 6-7 days (Crothers, 2001; Desai, 1966; Hickman, 1992). *Phorcus saucius* relies on the yolk to complete metamorphosis without external feeding during the stay in the water column (Levin & Bridges, 1995). The median lifespan of *P. saucius* (in Madeira) was estimated to be ~10 years (Sousa et al., 2019b).

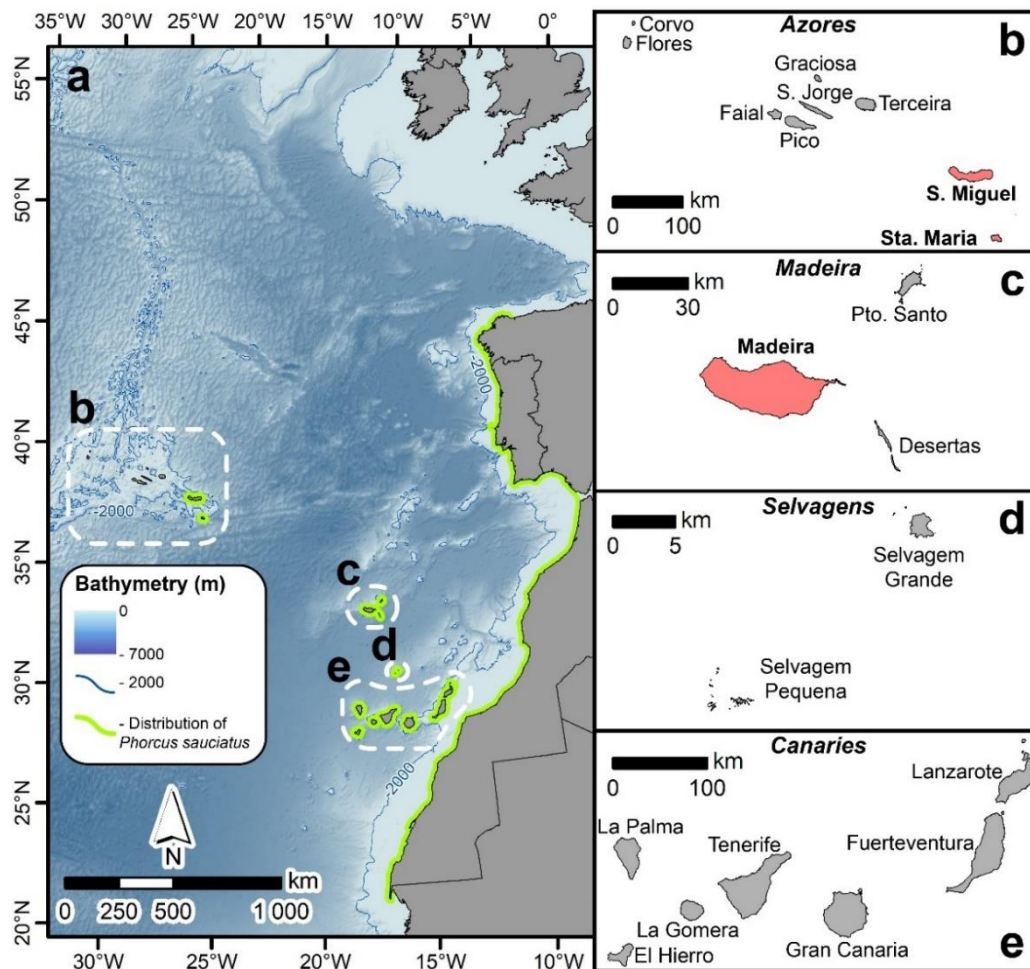


Figure 4.8. a. Geographical location of Azores, Madeira, Selvagens, and Canaries archipelagos within the Northeast Atlantic Ocean. The Azores distances 1,370 km to the Iberian Peninsula and 840 km to Madeira; Madeira is located about 285 km north of Selvagens and 630 km northwest of the northwest African coast; the Canaries are located 430 km south of Madeira. Detailed geographical distribution of the islands composing the **b. Azores Archipelago**; **c. Madeira Archipelago**; **d. Selvagens Archipelago**; and **e. Canaries Archipelago**. The blue line in Figure 4.8a marks the 2000 m isobath. Green banding indicates the known geographical distribution of *Phorcus saucius*. Islands where sampling was performed to obtain new individuals (Santa Maria, São Miguel, and Madeira) are highlighted in light red in b and c. Coastline from the Portuguese Hydrographic Institute (2014; <https://www.hidrografico.pt/op/33>) and bathymetry derived from GEBCO (2019; <https://www.gebco.net>)

For decades, extensive surveys of recent communities in Azorean island's shores and seamounts, as well as the publication of detailed checklists of gastropods in the Azores have never reported the occurrence of any *Phorcus* species in the archipelago (Ávila et al., 1998, 2000, 2007, 2011b; Cordeiro et al., 2015; Dautzenberg, 1889; Delongueville et al., 2019; Hawkins et al., 2000; Nobre, 1924, 1930). Similarly, detailed studies of the fossil record of the late Pleistocene of Santa Maria Island (Last Interglacial) did not yield any species of this genus (Ávila et al., 2002, 2009, 2015a). In July 2013, for the first time, the presence of seven individuals of *P. sauciatus* was documented in basalt crevices and intertidal pools at the upper intertidal zone of Prainha, Santa Maria Island (Ávila et al., 2015b). The year after, in June 2014, 668 specimens with ages estimated between 1 and 9 years were counted at the same area, having spread their range to a 1.45 km stretch in less than one year after the first report (Ávila et al., 2015b). Although no other individuals were found in other sectors of the island coast, the range expansion over a year reflects the success of the colonization of Santa Maria Island, where the ecological requirements of *P. sauciatus* were met. The resemblance between the population structure of Santa Maria and those of the well-established continental (A Mariña's, North Spain; Rubal et al., 2014) and insular (Madeira; Sousa et al., 2019b) populations denotes self-recruitment and a very successful colonization of a new island and archipelago by *P. sauciatus* (Ávila et al., 2015b). Starting in 2013, sporadic observations of *P. sauciatus* in the neighbouring island of São Miguel were reported, first at Borrallho locality, nearby Livramento (I. Moniz, pers. comm. March 2020). In 2015, two individuals were first observed in yet another locality, Caloura, where nowadays an abundant population is established. Recent observations during fieldtrips report *P. sauciatus* throughout most of São Miguel island shoreline since 2015, in Pico by 2021, and in São Jorge and Terceira by 2022.

How the top shell *P. sauciatus* reached the most remote archipelago of the Macaronesian geographic area (sensu Freitas et al., 2019) is yet to be explained. In this study, a molecular dataset of the mitochondrial Cytochrome Oxidase subunit I (COI) marker was produced to evaluate the genetic structure of *P. sauciatus* from the Azores and other locations in the Northeast Atlantic Ocean, for which genetic data was available. With this approach, this paper aims to: 1) enlighten the potential origin of the first colonizers of *P. sauciatus* that reached the Azores; and 2) propose a mechanism to explain how a non-planktotrophic gastropod was able to cross important, extensive biogeographic barriers, based on phenomena observed in the archipelago and Northeast Atlantic area over the past decades. Furthermore, we discuss the role of climate change as a trigger for the successful arrival and settlement of *P. sauciatus* in the Azores.

Material and Methods

Sample collection

Samples of *Phorcus sauciatu*s were collected during low-tide in five intertidal locations at Santa Maria Island (Azores Archipelago) – Cabrestantes, Porto de São Lourenço, Costa Sul, Ribeira Seca and Laracha – on July of 2016 and 2017, as well as August 2020. Sampling at São Miguel (Azores Archipelago) was done in three locations – Cerco da Caloura in July 2019 and 2020, Porto dos Batéis and Praia do Pópulo in July 2020 – whereas at Madalena do Mar (Madeira Archipelago) on July 2019. Details on the populations sampled, as well as coordinates of the sampling sites, are provided in Table 4.4. The samples were frozen overnight, stored in ethanol 96% and deposited in the marine molluscs' reference collection at the Department of Biology of the University of the Azores (DBUA): Cerco da Caloura – DBUA 1478/1607; Madalena do Mar – DBUA 1481; Cabrestantes – DBUA 1499; Porto de São Lourenço – DBUA 1500; Ribeira Seca – DBUA 1501; Costa Sul – DBUA 1502; Praia do Pópulo – DBUA 1608; Porto dos Batéis – DBUA 1609; Laracha – DBUA 1621.

Laboratory procedures and sequence data

DNA extraction was performed with foot tissue, following the manufacturer's protocol for the commercial EasySpin® Genomic DNA Tissue Kit (Citomed, Lisbon, Portugal). Fragments of the mitochondrial gene Cytochrome Oxidase subunit I (COI) were amplified by PCR, performed in 25 µl volumes, entailing 3 µl of DNA, – 10 × buffer MgCl₂ free, 2.5 mM MgCl₂, 0.2 mM dNTP, 10 µM of each primer, 0.1 µg µl⁻¹ bovine serum albumin (BSA, Promega) and 0.3 U platinum Taq DNA polymerase. Primers LCO1490/HCO2198 (Folmer et al., 1994) were used with the following cycling profile: 94°C for 4 minutes; 35 cycles of 94°C for 40 seconds, 48°C for 1 minute, 72°C for 1 minute; 72°C for 4 minutes. Electrophoretic runs in 2% (w/v) agarose gel with GelRed (DNA fluorescent dye, BioTarget™) were performed to assess the efficiency of the amplification reactions and the quality of the DNA products. PCR product purification and bi-directional Sanger sequencing with amplification primers were realised by a commercial facility—Genewiz, Leipzig, Germany.

A manual check of misreads in COI chromatograms was performed with BioEdit v.7.0.5.3 for Windows (Hall, 1999). Sequences of the mitochondrial coding COI gene were translated into amino acids with ExPASy Translate Tool (<https://web.expasy.org/translate/>), allowing to inspect the existence of stop codons and thus the presence of pseudogenes. All the COI *P. sauciatu*s sequences obtained in this study were deposited in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>), under the

accession numbers MT474920-38. Mitochondrial COI sequences of *P. sauciatus* from additional locations, as well as of *P. lineatus* for further comparison of population and genetic parameters between conspecifics, publicly available in GenBank, were also included in the dataset (Table 4.4). All the COI sequences were aligned using Clustal Omega algorithm via Web Services by EMBL-EBI (McWilliam et al., 2013).

Table 4.4. Populations of *Phorcus sauciatus* and *P. lineatus* defined in the scope of this study, localities and region of study of each population, as well as the coordinates in decimal degrees (WGS84) and COI accession numbers. * This study

	Population	Region	Localities included	Coordinates (DD)	COI Accession numbers
<i>P. sauciatus</i>	SMA	Santa Maria, Azores	Cabrestantes	36.998, -25.175	* MT474920-3
			Porto de S. Lourenço	36.995, -25.054	* MT474924-7
			Costa Sul	36.947, -25.089	* MT474928-31
			Ribeira Seca	36.944, -25.165	* MW094272-5
			Laracha	36.9477, -25.0893	* MW094276-9
	SMG	São Miguel, Azores	Cerco da Caloura	37.707, -25.509	* MT474932-3; * MW094284-90
			Praia do Pópulo	37.749, -25.619	* MW094280-3
			Porto dos Batéis	37.804, -25.802	* MW094291-3
	MAD	Madeira	Madalena do Mar	32.703, -17.139	* MT474934-8; * MW094294-304
			Funchal	-	JN686306
			São Vicente	-	JN686300
	CAN	Canaries	El Socorro, Tenerife	-	KC990609-16; KC990625-31
			San Cristobal, Gran Canaria	-	JN686310-1
			Jameous de Agua, Lanzarote	-	JN686301-5
	GAL	Galicia	Ria de Aldan	-	JN686309
			Cabo Estai	-	JN686299
			Playa de La Atalaya	-	JN241992-4
			Luarca	-	JN241995
			Asturias	-	KJ818223
	MOR	Morocco	Anza	-	JN686307-8
			Tifnit	-	JN686312-3
<i>P. lineatus</i>	POR	Portugal mainland	Praia de São Torpes	-	KF369166
	ESP	Spain	North of Spain	-	JN241978-91; KU714765-73
	LPOR	Portugal	North+Central Portugal	-	KU496663; KU566798-9
			Lisbon	-	JN686319-20; KU496658-9; KU496664
			Algarve	-	KU496657; KU496661-2
	LMOR	Morocco	Anza	-	JN686325-7

Genetic diversity and population structure

After reducing the dataset to haplotypes, raw (p) distances among haplotypes of *P. sauciatus* were conducted in MEGA version 7 (Kumar et al., 2016) to evaluate sequence diversity and estimate evolutionary divergence between haplotypes. The software TCS v1.21 (Clement et al., 2000) was used to further examine the relationships and genetic structure between all COI haplotypes of *P. sauciatus* and *P. lineatus*, generating a statistical parsimony haplotype network at the 95% connection limit. Information regarding the frequency and distribution of haplotypes in the populations is also depicted in the network, as the circle sizes are proportional to the haplotype frequency in the total sample. The tcsBU web-based program (Santos et al., 2016) was used to overlap the genetic structure retrieved by TCS with the geographical structure, expected to influence the haplotype occurrence. Considering the type of larval development and geographical setting of the study area, we have defined populations of *P. sauciatus* by archipelago/region, as in Table 4.4. Santa Maria and São Miguel Islands are expected to be closely related, the latter expected to have been founded by individuals that migrated from Santa Maria. Nevertheless, we opted to consider them as distinct populations and did not combine them into an “Azores” population, so that they were kept as distinct populations in an archipelago currently under invasion by *P. sauciatus*.

Population genetic analyses

Measures of genetic diversity, haplotype diversity (H_d , Nei 1987) and nucleotide diversity (π , Nei, 1987) of the mtDNA COI marker were estimated at Arlequin version 3.5 (Excoffier & Lischer, 2010) for all the *P. sauciatus* and *P. lineatus* populations, except POR and LMOR due to the reduced number of individuals (three or less). The occurrence of population expansion in *P. sauciatus* was evaluated by conducting neutrality tests on the haplogroups, with the statistical significance obtained by coalescent simulations. Fu's F_s (Fu, 1997) and R_2 (Ramos-Onsins & Rozas, 2002) neutrality tests were conducted in DnaSP v6 (Rozas et al., 2017) to evaluate whether a demographic expansion of the species took place in the past. The significance of these parameters was determined based on 1,000 coalescent simulations under a Standard Neutral Model of population stability conditioned on theta (θ). Fu's F_s and R_2 were chosen as they are powerful neutrality tests; moreover, R_2 is suitable for small sample sizes. Pairwise F_{ST} values were calculated between pairs of *P. sauciatus* populations – excluding POR due to its reduced number of samples that would bias the results – with statistical significance being tested by 1,000 permutations. Bonferroni correction was applied to adjust the level of significance to the number of pairwise multiple comparisons (Rice, 1989).

A hierarchical analysis of molecular variance (AMOVA; Excoffier et al., 1992), based on mitochondrial COI data for *P. sauciatus*, was realised with Arlequin version 3.5 (Excoffier & Lischer, 2010) to evaluate the distribution of genetic variability between populations. Based on the preliminary results of the network, we further tested the existence of population structure between two major groups: Group 1 containing SMA, SMG, MAD and POR; Group 2 containing CAN. Four scenarios were tested in the AMOVA, regarding the inclusion of GAL and MOR in the groups: 1) assigning each sequence to the respective group, indicated by the haplotype distribution; 2) excluding GAL and MOR; 3) including all in Group 1; 4) including all in Group 2.

Results

Genetic diversity and population structure

A total of 80 haplotypes were identified in the 620 bp COI dataset of *Phorcus sauciatus*, originally with 86 sequences. The *P. lineatus* COI dataset containing 38 sequences was reduced to 23 haplotypes. Estimates of evolutionary divergence in the COI dataset revealed a mean differentiation of 11% between *P. sauciatus* and *P. lineatus*. Within the *P. sauciatus* dataset (Supplementary Table A4.1), the genetic divergence among the haplotypes found across the northeast Atlantic ranged from 0.2 to 2.3%, with a mean differentiation of 0.91%. The levels of genetic differentiation were lower within CAN and among samples from SMA, SMG, and MAD.

The TCS network analysis (Figure 4.9), based on the COI haplotypes of *P. sauciatus* from the Azores, Madeira, Canaries, Morocco and Iberian Peninsula, retrieved two major clusters, separated by two mutational steps, with some derivative haplotypes: 1) Haplogroup 1 - "Azores/Madeira", mainly includes sequences from SMA, SMG, MAD and POR populations; 2) Haplogroup 2 - "Canaries", containing all the sequences from CAN and two from MAD. Sequences from Morocco (MOR) and Galicia (GAL) are found in both haplogroups, whereas no haplotypes are shared between the Azores/Madeira and Canaries. The analysis also separates the network of *P. sauciatus* and *P. lineatus*, the latter displaying evenly distributed haplotypes in a reticulate pattern.

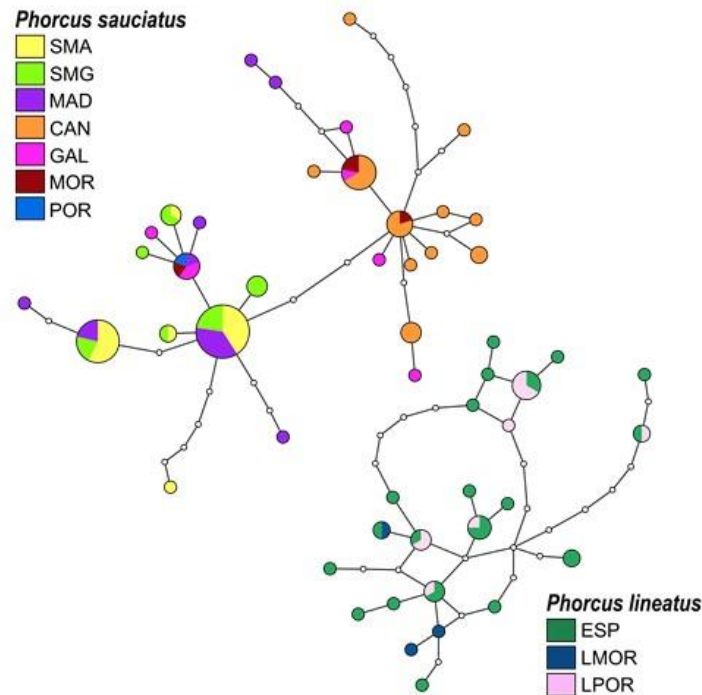


Figure 4.9. Haplotypic network, at 95% parsimony connection limit, for 86 sequences/28 haplotypes of *Phorcus sauciatus* from seven populations and for 38 sequences/23 haplotypes of *Phorcus lineatus* from three populations. Each population is represented by a unique colour. SMA – Santa Maria; SMG – São Miguel; MAD – Madeira; CAN – Canaries; GAL – Galicia; MOR – Morocco; POR – Portugal mainland; LPOR – Portugal for *P. lineatus*; ESP – Spain for *P. lineatus*; LMOR – Morocco for *P. lineatus*. The size of the circles is proportional to the frequency of each haplotype; small uncoloured circles represent non-observed haplotype; each line connecting haplotypes represents a single mutational change. Network obtained with TCS v1.21 (Clement et al., 2000) and tcsBU (Santos et al., 2016)

Population genetic analyses

Estimates of genetic diversity by species and populations are summarized in Table 4.5. The global haplotype diversity (H_d) is over 0.85 in both species analysed, although it is lower in *P. sauciatus* ($H_d=0.894$). In a similar way, the nucleotide diversity (π) is lower in *P. sauciatus* ($\pi=0.006441$). Regarding genetic diversity, SMG, CAN, GAL, and MOR populations show moderately high levels of haplotypic diversity around 80-95%, whereas SMA and MAD show medium haplotypic diversity of 65-70%; the same trend is observed for the nucleotide diversity. Populations of *P. lineatus* are genetically more diverse than the congeneric *P. sauciatus*. Due to the reduced number of sequences, it was not possible to assess these parameters for POR and LMOR populations.

Within *P. sauciatus*, the haplotypes HS1-7 and HS9-13 are restricted to Azores and Madeira Archipelagos; HS14-15, HS17-18, HS20 -24 are only found in samples from Canaries; HS25-28 are exclusive to Galician samples. Haplotype HS8 is shared by samples from MOR, GAL and POR, whereas HS16 includes sequences from MOR, GAL,

and CAN; HS19 is present in MOR and CAN populations. Several haplotypes of *P. lineatus* are shared between the three populations analysed, except for HL22-23 that are private of LMOR.

Table 4.5. Estimates of genetic diversity – number of samples, haplotypes, number of haplotypes (*H*), haplotype diversity (*Hd*), and nucleotide diversity (π) - for COI sequences of *Phorcus sauciatus* and *P. lineatus* for each sampling site

Population	N	Haplotype codes	<i>H</i>	<i>Hd</i>	π
<i>P. sauciatus</i>					
SMA	20	HS1-5	5	0.6632 $\pm$ 0.0689	0.00292 $\pm$ 0.00196
SMG	15	HS1-2, HS4-7	4	0.8381 $\pm$ 0.0612	0.002767 $\pm$ 0.001931
MAD	17	HS1-2, HS8-13	8	0.7721 $\pm$ 0.0972	0.005218 $\pm$ 0.003167
CAN	22	HS14-24	11	0.8918 $\pm$ 0.0442	0.005341 $\pm$ 0.003183
GAL	7	HS8, HS16, HS25-28	6	0.9524 $\pm$ 0.0955	0.007576 $\pm$ 0.004835
MOR	4	HS8, HS16, HS19	3	0.8333 $\pm$ 0.2224	0.006720 $\pm$ 0.005026
POR	1	HS8	1	-	-
Global	86	-	28	0.8936 $\pm$ 0.0212	0.006441 $\pm$ 0.003603
<i>P. lineatus</i>					
ESP	26	HL1-20	20	0.966 $\pm$ 0.022	0.01004 $\pm$ 0.005584
LPOR	9	HL2, HL3, HL4, HL12, HL19, HL21	6	0.889 $\pm$ 0.0091	0.00785 $\pm$ 0.004801
LMOR	3	HL18, HL22-23	3	-	-
Global	38	-	23	0.945 $\pm$ 0.021	0.00956 $\pm$ 0.005278

Neutrality tests were applied to the populations grouped accordingly to the *P. sauciatus* clusters retrieved in the TCS network – “Azores/Madeira” and “Canaries” – to evaluate the occurrence of population expansion. Values for Fu’s F_S (Fu, 1997) and R_2 (Ramos-Onsins & Rozas, 2002) were non-significant in “Azores/Madeira” ($F_S = -2.975$, p-value 0.088; $R_2 = 0.061$, p-value=0.102), showing no departure from neutrality expectations. The neutrality tests in the haplogroup “Canaries” retrieved significant values for Fu’s F_S (-9.45 , p-value=0.0) and R_2 (0.055, p-value=0.0). The analysis of the global dataset showed departures from neutrality expectations, with significance of both parameters ($F_S = -13.832$, p-value=0.0; $R_2 = 0.042$, p-value=0.024).

Pairwise comparisons of F_{ST} values between the populations of *P. sauciatus* revealed levels ranging from -0.12 to 0.55457 (Table 4.6). The significance of the F_{ST} values was maintained for all comparisons except MAD vs. MOR, after applying the sequential Bonferroni correction (adjusted critical p-value=0.003; Table 4.6). The analysis showed, after correction, significant differences between SMA/SMG/MAD and CAN, as well as with GAL; and between SMA/SMG and MOR. Overall, geographically distant regions showed low-intermediate levels of divergence (~35-55%). However, despite the

geographical distance between SMA/SMG and MAD populations (840 km), the F_{ST} values retrieved for these pairwise comparisons are close to zero.

Table 4.6. Pairwise F_{ST} values between populations of *Phorcus sauciatus*, based on mtDNA COI sequences. POR populations were excluded from the analysis due to the low number of samples (three or less individuals). Significance tests derived from 1,023 permutations. Bold numbers indicate $p < 0.05$; Asterisk (*) indicates significance for the adjusted critical $p = 0.003$, after Bonferroni correction

	SMA	SMG	MAD	CAN	GAL	MOR
SMA						
SMG	0.02505					
MAD	0.00995	0.00248				
CAN	0.55457 *	0.53378 *	0.43039 *			
GAL	0.41890 *	0.38127 *	0.22508 *	0.08428		
MOR	0.50590 *	0.48127 *	0.28506	-0.06001	-0.12000	

Hierarchical AMOVA (Excoffier et al., 1992) based on the COI dataset for *P. sauciatus* maximized the population differentiation among groups (49.35%) and the fixation indexes (Table 4.7) when including GAL and MOR sequences in the respective groups, according to the TCS haplotype distribution, although similar levels were obtained when excluding them from the analysis. Differentiation at all the components evaluated is significant.

Table 4.7. Results of the hierarchical AMOVA tests comparing variation in the mitochondrial COI dataset of *Phorcus sauciatus*, grouped according to the haplotype distribution: Group 1 – SMA+SMG+MAD+POR; Group 2 – CAN+MAD; MAD, GAL and MOR sequences assigned to groups according to haplotype distribution. Significance tests derived from 1,023 permutations; Asterisk (*) indicates $p < 0.05$

Source of variation	<i>f</i>	SS	Variance components	% variation	F_{CT}	F_{SC}	F_{ST}
Among groups		56.588	1.35051	49.35	0.49354*		
Among populations		16.06	0.10859	3.97		0.07835*	
Among populations within groups	76	97.073	1.27727	46.68			0.53322*
Total	85	169.721	2.73637				

Discussion

Comparing genetic diversity of *Phorcus sauciatus* and *Phorcus lineatus*

The distinction of *P. sauciatus* and *P. lineatus* as species is clear (Donald et al., 2012) and corroborated by our results, with mean divergence of 11% based on the mitochondrial marker and a complete separation of the networks at 95% connection limit (Figure 4.9; Hart & Sunday, 2007). Both species yield high levels of global genetic diversity, suggested by the parameters Hd and π . The haplotype networks of *P. sauciatus* and *P. lineatus* show distinct patterns: two haplogroups almost in star-like

shape and a complex reticulate pattern, respectively (Figure 4.9). The evenly distributed, reticulate pattern of *P. lineatus* haplotypes supports the frequent occurrence of gene flow across its distribution range and contributes to the high genetic diversity levels calculated within each population. The frequent gene flow in *P. lineatus* can be associated with its geographical distribution range, from North Wales and Ireland to the northwest African coast (Crothers, 2001; Freitas et al., 2019), benefiting from physical continuity across wide continental shore extensions, and with a lecithotrophic larval stage of 6-7 days that allows dispersal to some extent (Crothers, 2001; Hickman, 1992).

Population structure of *Phorcus sauciatus*

Donald et al. (2012) provided the most detailed phylogenetic analysis of *Phorcus* to date, clarifying its confusing and inconsistent taxonomy. Our findings revealed low distinctiveness among the populations of *P. sauciatus* studied, agreeing with Donald et al.'s (2012). In 2012, *P. sauciatus* had not yet been reported in the Azores and the low levels of genetic differentiation between Madeira, the Canaries and northwest African coasts were interpreted as “suggesting either recent or continuing dispersal among these areas” (in Donald et al., 2012). The same explanation is herewith expanded to the low genetic divergence detected also in the Azorean samples, where the species was first spotted in 2013 (Ávila et al., 2015b). Samples from MOR and GAL are found distributed among the haplotypes from populations of the Atlantic Archipelagos, although, as expected due to the much shorter distances involved, MOR is more frequently found among the Canaries. The reduced number of sequences available for these locations could bias any interpretations and confound any significant findings, therefore no further inferences will be made regarding its population dynamics. We have tested the exclusion of MOR, GAL and POR samples from the dataset and the population parameters of diversity suffered only slight changes but the magnitudes were kept, also observed in the fixation indexes and variation retrieved from AMOVA results. For these reasons, it is believed that the inclusion/exclusion of these sequences does not affect significantly the patterns obtained, thus not being often mentioned in further interpretations.

Southern populations (CAN, MOR), as well as GAL, are genetically more diverse than the populations from Azores and Madeira archipelagos (SMA, SMG, MAD). The two haplogroups inferred – designated as “Azores/Madeira” and “Canaries” – also reflect this genetic diversity trend, revealing some degree of differentiation between these groups of populations. The AMOVA results and the, although not high, significant F_{CT} value support the existence of some population structuring. According to the present analysis, half of the variation in the dataset is explained by the separation in haplogroups. Due to

the resemblance of the network's shape to a star, neutrality tests were performed to evaluate population expansion, but F_u 's F_s does not support this scenario for the "Azores/Madeira" haplogroup. For "Canaries" and the global dataset, both neutrality tests performed denote significant deviations from neutrality expectations ($p < 0.05$), suggesting that population expansion might have occurred sometime in the past (Fu, 1997; Ramos-Onsins & Rozas, 2002).

In Canaries Archipelago, *P. sauciatus* is restricted to the easternmost islands – Lanzarote, Fuerteventura, Gran Canaria, and Tenerife (Ramírez et al., 2005). Ramírez et al. (2005) attributed this distribution pattern to the productivity levels and geological age of these islands. As the results from genetic data suggest past demographic expansion, it is offered another explanation that might have operated in conjunction with the hypotheses of Ramírez et al. (2005). We propose that the colonization of Canaries probably happened recently, in a geological scale, from a source in the northwest African coast and expanded to other islands likely in an east to west direction.

The genus *Phorcus* is found in Last Interglacial marine sediments (MIS 5e, 120-130 ka) on the Canaries [misidentified as *Osilinus trappei* Nordsieck, 1974, from Tachero outcrop, Tenerife Island (García-Talavera et al., 1978); Airport, Barranco del Río Cabras outcrop, Fuerteventura Island (Cabero, 2009)] and Selvagens [Selvagem Pequena (García-Talavera & Sánchez-Pinto, 2001)] but not in the fossiliferous deposits of this time period in Madeira (Gerber et al., 1989), although such absence can be attributed to the few and outdated studies on this archipelago. During glacial times, the isolation of Madeira and Canaries decreased by more than 50% and 60%, respectively, in relation to the present distances (Ávila et al., 2018), thus one can hypothesize that gene flow and exchange of individuals between archipelagos was facilitated during this period. The presence of *Phorcus* spp. in the fossiliferous marine sediments of Canaries and Selvagens does not ensure that it was *P. sauciatus* that inhabited these islands in the past, as other species are found there today (Supplementary Material in Freitas et al. 2019), but the historical record undeniably shows that *P. sauciatus* established stable populations and has been exploited in Madeira and Canaries for centuries (Ramírez et al., 2005; Sousa et al., 2019a). Considering the distance from Madeira to La Palma (Canaries) (460 km), climatic similarities and the formal classification of Madeira, Canaries and Selvagens as the Webbsnesia ecoregion (Freitas et al., 2019), we expected gene flow and shared haplotypes between MAD and CAN populations. However, the results here presented go against these expectations. The persistent southward transport of the Canary Current (Klein & Siedler, 1989; Hernández-Guerra et al., 2001; Carracedo et al., 2014) seems to be a potential barrier for migration of species from

Canaries towards Madeira; however, it should provide a means of transport for Madeiran species towards Canary Islands. As the present results indicate absence of the exchange of *P. sauciatus* individuals between the two archipelagos, it is suggested that it is the larval duration of *P. sauciatus* that is key for the patterns here described. Its short duration, less than the 6-7 days veliger stage of the congeneric *P. lineatus* (Crothers, 2001; Desai, 1966; Hickman, 1992) which is used as its proxy, makes southward dispersal of MAD individuals under the influence of the Madeira Current rather unsuitable. We hypothesize that, probably, the ancestral of *Phorcus* that colonized Canaries come from the Moroccan shores, having been transported by the upwelling filaments (such as off Cape Ghir) and related mesoscale/eddy activity, and maybe also by the Canary Upwelling Current. However, as the population of *P. sauciatus* in the Canaries is not the focus of this study, we will not further address the demographic history of this population/haplogroup.

Relationship between Azores and Madeira

The results show the existence of two groups of *P. sauciatus* in the northeast Atlantic, with no haplotypes shared between them. Surprisingly, most samples from Madeira are clustered with Azorean samples, except two (out of eight) haplotypes falling among Canaries' samples. These two groups – “Azores/Madeira” and “Canaries” – go against the preliminary expectations for the population structure of *P. sauciatus* in the northeast Atlantic Ocean. One expected these results to point to a source population of the newly arrived *P. sauciatus* to the Azores somewhere in the Iberian Peninsula, as in general there is a biogeographical pattern for the Azorean marine biota (particularly gastropods) to show strong similarities with the Eastern Atlantic/Mediterranean shores, despite the discordance with the eastward sea-surface circulation patterns of the North Atlantic Gyre (Ávila et al., 2009; Ávila, 2013; Freitas et al., 2019). It could be argued the role of the Azores Counter Current in transporting water masses westward at about 36°N, especially stronger during summer, however it flows at the sub-surface level at average depths of 1,000 m (Carracedo et al., 2014), which is unsuitable for the transport of larvae among plankton. Yet, the strong relation between SMA, SMG and MAD haplotypes shown by our results rather suggests Madeira as the source of the colonizers of Santa Maria Island. The role of the Iberian Peninsula as source of the first *P. sauciatus* reaching the Azores should not be discarded, as in the cases of species moving towards the poles it is common for gaps to exist in the knowledge regarding sources, sinks and routes (Canning-Clode & Carlton, 2017). In fact, we suggest that the relationship between *P. sauciatus* in the Azores and the Iberian Peninsula should be further evaluated, as it was

not possible to accurately test it with the dataset here used due to the scarcity of sequences from that continental region (7 samples from GAL, 1 from POR).

The finding of a closer relationship of Madeira and Azores populations, instead of Madeira with Canaries populations, is also unexpected, as these archipelagos are separated by 840 km of deep-waters and assigned to different ecoregions – Webbsnesia and the Azores, respectively (Freitas et al., 2019). Moreover, during interglacial periods as we are currently living at, the major sea-surface currents in the Macaronesia region predominantly flow from south of the Azores towards the southeast, in direction to Madeira and Canaries (Klein & Siedler, 1989; Machín et al., 2006; Santos et al., 1995) (cf. Figure 1.3). Overall, the Azores are characterised by a lower taxonomic richness when compared with other Macaronesian Archipelagos (Ávila et al., 2005; Freitas et al., 2019). Hawkins et al. (2000) stated “the lack of large intertidal trochids (...) in the intertidal of the Azores” as an evident example of the impoverished biota in the archipelago. By this time, the genus *Phorcus* had not yet been reported for the Azores, which only happened more than one decade later in July 2013 in Santa Maria Island (Ávila et al., 2015b). The absence of the genus in the fossil record of Santa Maria Island (reviewed in Ávila et al. 2015a), despite the high sampling effort and studies performed over the years, sustain the hypothesis that *Phorcus* spp. had never reached the northern latitudes of the Azores before.

The journey of *Phorcus sauciatus* to the Azores

Since its first observation at Prainha, Santa Maria Island, *P. sauciatus* expanded its distribution to all the shoreline of the island in 2016 (Costa et al., 2016). By 2013 it was first reported for São Miguel Island, at Borralho locality (I. Moniz, pers. comm. March 2020); by 2015 at Caloura (J. Faria, pers. comm. March 2020); by 2020, populations are thriving through most of the south coast (L. Baptista, pers. obs. July 2020); by 2021 it was first spotted in Pico and by 2022 in Terceira and São Jorge (pers. obs.). The distribution of *P. sauciatus* in two Azorean islands matches Ávila et al.'s (2015b) prediction of a successful colonization towards the rest of the archipelago. The short duration of the lecithotrophic larval stage has probably hampered the dispersal of *P. sauciatus* to the Azores in the past, against the major circulation patterns, as other gastropods with a slightly longer larval duration have been able to establish in all the Macaronesian Archipelagos (e.g., *Patella* spp. limpets; Donald et al., 2012).

Rafting is of major importance for range expansion of non-planktotrophic shallow-water epibenthic molluscs inhabiting subtropical and temperate Atlantic waters (Ávila et al., 2012; Ávila, 2013; Thiel & Hays 2006). *Phorcus sauciatus* is believed to be a good

candidate for episodic rafting to remote islands for three reasons: 1) its ability to attach to floating substrata, 2) its considerably small sizes (<15 mm) until sexual maturity is reached (Rubal et al., 2014; Sousa et al., 2019b); and 3) inhabiting the intertidal zone, where it is easier to initiate the process (Ávila, 2006). We argue that if at least two different-sexed individuals of *P. sauciatus* attached to the raft (Hawkins et al., 2000), the likelihood of a successful colonization of the new location is increased, as for other external fertilizers that are successful rafters [cf. Thiel & Gutow, 2005b].

One could argue an anthropic mediated introduction via hitchhiking as a more plausible explanation; however, we discard such hypothesis because: 1) *P. sauciatus* is found on rocky shores and, to our knowledge, it has not been observed fouling on boats/ships or motile animals; 2) Prainha is a protected bay with sandy stretches during Summer; 3) Prainha is located more than 3.5 km from the only commercial harbour/marina of Santa Maria Island – Porto de Vila do Porto –surrounded by rocky shores; 4) even today, *P. sauciatus* is not found inside this harbour/marina. The ships' movement in Santa Maria is mostly restricted to Summer months, except for the transport of cargo and essential goods all year-round. From 2005 to 2018, roughly 200 ships of cargo and passengers have entered this harbour annually (official data available at INE - <http://ine.pt> and Portos dos Açores, S. A. - <https://portosdosacores.pt/estatisticas/>). São Miguel and Terceira islands received about 3-4 times more ships in the same periods and, despite the arrival of lonely individuals of other species (e.g. *Mytilus* spp.; Hawkins et al., 2000), they were not the first islands to be invaded by *P. sauciatus*.

Although the knowledge in broad interchangeable topics regarding marine invertebrates' biology and ocean circulation, these were never puzzled together to explain the arrival of *P. sauciatus* to the most remote archipelago in the NE Atlantic. During 2013-2014, a maximum age of 8-9 years was estimated at the founder population at Prainha (Ávila et al., 2015b), placing their arrival to Santa Maria between 2005-2009. In June and July 2006 (J. Brum, pers. comm. March 2020), rafts with dimensions up to 10x8 meters were observed in the Azores, especially around Santa Maria Island (Figure 4.10a-c), mainly composed of the floating pelagic crustacean barnacle *Dosima fascicularis* (Ellis and Solander 1786). This species is formally reported for temperate and subtropical waters but unusual in the European coast (Cavallaro et al., 2020). Nevertheless, irruptions and episodic stranding of buoy barnacles (cf. Figure 4.10d) occur frequently (e.g.: Betti et al., 2017; Cavallaro et al., 2020; Junoy & Junoy, 2014; Mifsud et al., 2005; Ryan & Branch, 2012). Similar rafts, of remarkable dimensions and composition, have not been reported for the Azores before or after this year. Thus, we hereby propose, for the first time, that the first *P. sauciatus* colonizers were mainly

juveniles that arrived at Santa Maria in such rafts, which got stranded on shore. Once founded, the new population could establish, thrive and persist (Hawkins et al., 2000) where the environmental conditions fitted the organisms' needs.

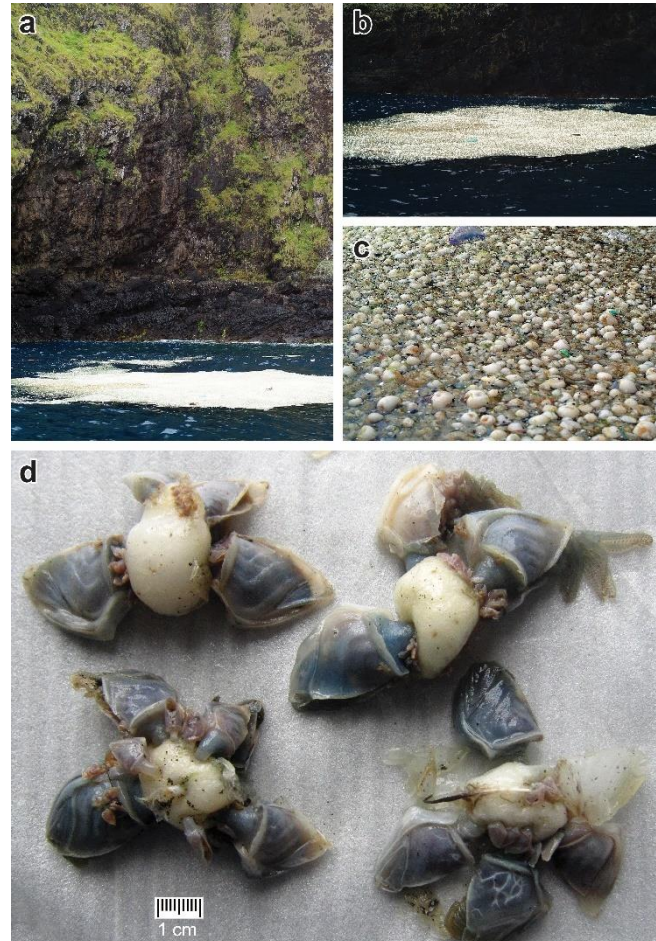


Figure 4.10. a-c. Rafts of the floating pelagic crustacean barnacle *Dosima fascicularis* (Ellis and Solander 1786) around Santa Maria Island, pictures taken in June 2006, by Sérgio P. Ávila. Several rafts were spotted in different locations around the island, reaching dimensions up to 10x8 meters. d. Specimens of *Dosima fascicularis* washed ashore at Faial Island (Praia da Conceição, 22nd of May 2017); photo by João Gonçalves (OKEANOS/Universidade dos Açores)

Climate change and geographical range expansion

It is not the first time that shifts in the distribution of *Phorcus* species are reported. Rubal et al. (2014) studied the expansion of *P. sauciatus* in the Iberian Peninsula, having started from southern Galicia in the early 1990's and arriving to North Portugal in the beginning of the twenty-first century. An increase in the SST during this time period was pointed out as the reason for *P. sauciatus* (Rubal et al., 2014), and other intertidal invertebrates (Lima et al., 2006; Mieszkowska et al., 2006, 2007), to expand their distribution ranges. In recent years, the first occurrence of several fish species typical of

warm (sub)tropical latitudes has been reported for the Azores, also in periods when an increase in the coastal water temperature on the archipelago was detected (Afonso et al., 2013). The thorough study of the Last Interglacial (MIS 5e; 120-130 ka) fossiliferous deposits in Santa Maria revealed the past occurrence of at least eight *Conus* spp. as far north as the Azores (Ávila et al., 2015a). Nowadays, this is a very specious genus in the warm, tropical waters of Cabo Verde, but not a single species is reported in the modern assemblages of the Azores (Ávila et al., 2018). Ávila et al. (2018, 2019) thoroughly reviewed and analysed the impact of climate change and warmer waters on the latitudinal shifts in marine shallow-water organisms. These authors show that, during interglacial times, latitudinal migrations occur, with geographical range expansions of tropical/thermophilic Cabo Verdean species well-documented in the MIS 5e fossil record from the Canaries, Madeira and Azores archipelagos. In the next glacial period that succeeded the MIS 5e, these species locally disappeared from the northern archipelagos as a direct consequence of the drop of the SST; their geographical distribution again confined to the archipelagos in the tropical belt that acted as refugia (Ávila et al., 2018, 2019). *Phorcus sauciatatus* is not present in the fossiliferous outcrops of Santa Maria (Ávila et al., 2015a) and has never been reported in the Azores before, although being reported for the MIS 5e fossil record of the Canaries. Its recent arrival accomplished by episodic rafting and successful establishment at Santa Maria, might have been triggered by chance, and favoured by the warming trend in the subtropical gyre's surface waters south of the Azores Front and occurrence of more northern positions of the Azores Current, the Azores Front and, subsequently, the Azores Counter Current, during the last decades (Fründt & Waniek, 2012; Fründt et al., 2013).

Conclusions

Currently, we are living in an interglacial period in which climate change and global warming are undeniable. New, and more frequent, marine invasions have been recorded all over the world, facilitated by the warmer seawaters and the broader thermal range of invasive species (Canning-Clode & Carlton, 2017; and references therein). In the Azores, the average increase of 1°C in the SST since the beginning of the twenty-first century, promotes the “tropicalization” of the archipelago, where the arrival of southern species and extirpation of northern taxa is expected (Afonso et al. 2013; Ávila et al. 2018, 2019). The non-planktotrophic *P. sauciatatus* is one of the new occurrences reported in the remote islands of the Azores in the past two decades (Ávila et al., 2015b; Delongueville et al., 2019).

In this work it is suggested that the first colonizers arrived at Santa Maria Island in rafts of the floating pelagic crustacean barnacle *Dosima fascicularis*. Due to the genetic similarities inferred in the present analyses, we suggest that these rafts assembled somewhere at Madeira waters, allowing *P. sauciatus* to get trapped and initiate the rafting process along with the debris. We believe that the rafts contained mainly larvae and/or small-sized juveniles, as rafting of large gastropods is unlikely (Ávila et al., 2018). Normally, rafts assembled in Madeira should move southwards with the Madeira Current/Canary Current (Sala et al., 2013; Freitas et al., 2019). However, in early 2004 the Azores Front was located at 30°N and after 2005 the front and current system propagated from a mean position at 35.2°N southward again (Fründt & Waniek, 2012). So, we argue that, given the Azores Current's high eddy activity, rafts were able to be advected north of the Azores Front and then transported north-westward with the Azores Counter Current. Furthermore, periodic disruptions of the surface circulation and subsequent reversal of the system to a north-westward direction, frequently happening during Autumn/Winter (Hawkins et al., 2000; Santos et al., 1995) and major storms/hurricanes (Waters & Crow, 2018), have been pointed as responsible for the arrival of new colonizers to the Azores in the past (Santos et al., 1995). The rafts then got stranded in the southern coast of Santa Maria Island around 2006, close to Prainha, where the first population was detected. For the first time, a mechanism to explain the journey of the topshell *P. sauciatus* to the Azores is proposed, based on genetic signatures, ecological features and occurrence of uncommon oceanographical phenomena in the region, which are puzzled together in a way that potentially explains its arrival. Nevertheless, this hypothesis awaits testing and further support by detailed, long-term studies of the oceanographical features of the Northeast Atlantic Ocean and behaviour of rafts in this region.

Phorcus sauciatus was successful in the settlement of a viable population at the new location, having expanded its range around the entire island's shoreline in just 5 years. Today, it is already found in the nearest island, São Miguel, and other islands of the central group, and one believe, as stated by Ávila et al. (2015b), that it will successfully expand to other Azorean islands in the next years. As a commercially valuable species, prone to competition with other intertidal grazers common in the Azores (e.g. limpet patellids; Sousa et al., 2019b), the establishment of monitoring programmes for the expansion of *P. sauciatus* through the nine islands might be of major importance to better understand the consequences of global warming and effects of new arrivals in intertidal habitats of remote archipelagos.

References

- Addison, J. A., & Hart, M. (2005). Spawning, copulation, and inbreeding coefficients in marine invertebrates. *Biology Letters*, 1(4), 450–453. <https://doi.org/10.1098/rsbl.2005.0353>
- Afonso, P., Porteiro, F. M., Fontes, J., Tempera, F., Morato, T., Cardigos, F., & Santos, R. S. (2013). New and rare coastal fishes in the Azores islands: occasional events or tropicalization process?. *Journal of Fish Biology*, 83(2), 272–294. <https://doi.org/10.1111/jfb.12162>
- Albano, P. G., Sabelli, B., & Bouchet, P. (2011). The challenge of small and rare species in marine biodiversity surveys: Microgastropod diversity in a complex tropical coastal environment. *Biodiversity and Conservation*, 20(13), 3223–3237. <https://doi.org/10.1007/s10531-011-0117-X>
- Allee, W.C. (1931). *Animal Aggregations: A Study in General Sociology*. University of Chicago Press, Chicago.
- Andrews, S. (2010). *FastQC - A quality control tool for high throughput sequence data*. <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>
- Aurelle, D., Guillemaud, T., Afonso, P., Morato, T., Wirtz, P., Santos, R. S., & Cancela, M. L. (2003). Genetic study of *Coris julis* (Osteichthyes, Perciformes, Labridae) evolutionary history and dispersal abilities. *Comptes Rendus - Biologies*, 326(8), 771–785. <https://doi.org/10.1016/j.crv.2003.08.001>
- Ávila, S. P. (1998). Zonação intertidal de uma comunidade malacológica na “Poça da Barra”, uma lagoa localizada na plataforma costeira da Vila das Lajes do Pico, Açores. *Açoreana*, 8(4), 457–485.
- Ávila, S. P. (2000). The shallow-water Rissoiidae (Mollusca, Gastropoda) of the Azores and some aspects of their ecology. *Iberus*, 18(2), 51–76.
- Ávila, S. P. (2006). Oceanic islands, rafting, geographical range and bathymetry: a neglected relationship. In *Proceedings of the 5th International Symposium on the Fauna and Flora of Atlantic Islands, Vol. 9*, 22–39. Irish Biogeographical Society.
- Ávila, S. P. (2013). Unravelling the patterns and processes of evolution of marine life in oceanic islands: a global framework. In J. M. Fernández-Palacios, L. de Nascimento, J. C. Hernández, S. Clemetne, A. González, & J. P. Díaz-González (Eds.), *Climate Change Perspectives From the Atlantic: Past, Present and Future* (pp. 95–125). Universidad de La Laguna.
- Avila, S. P., Azevedo, J., Gonçalves, J. M., Fontes, J., & Cardigos, F. (1998). Checklist of the shallow-water marine molluscs of the Azores: 1-Pico, Faial, Flores and Corvo. *Açoreana*, 8, 487–523
- Ávila, S. P., Azevedo, J., Gonçalves, J. M., Fontes, J., & Cardigos, F. (2000). Checklist of the shallow-water marine molluscs of the Azores: 2-São Miguel Island. *Açoreana*, 9, 139–173.
- Ávila, S. P., Amen, R. G., Azevedo, J., Cachão, M., & García-Talavera, F. (2002). Checklist of the Pleistocene marine molluscs of Praínha and Lagoínhas (Santa Maria Island, Azores). *Açoreana*, 9, 343–370.

- Ávila S. P., Borges, J. P., & Martins, A. M. F. (2011a). The littoral Trochoidea (Mollusca: Gastropoda) of the Azores. *Journal of Conchology*, 40, 408.
- Ávila, S. P., Goud, J., & Martins, A. M. F. (2012). Patterns of Diversity of the Rissoidae (Mollusca: Gastropoda) in the Atlantic and the Mediterranean Region. *The Scientific World Journal*, Article ID, 164890. <https://doi.org/10.1100/2012/164890>
- Ávila, S. P., Medeiros, A., Martins, A. M. F., Silva, A., Melo, C., Gomes, C., Michielsen, G., Madeira, J., Bagaço, L., Silva, L., Fernandes, M., Silva, M., Madeira, P., Cordeiro, R., Meireles, R., & Prieto, R. (2011b). *Lajes do Pico “À Ban-baxe-muro.”* Publiçor.
- Ávila, S. P., Cardigos, F., & Santos, R. S. (2007). Comparison of the community structure of the marine molluscs of the “Banco D. João de Castro” seamount (Azores, Portugal) with that of typical inshore habitats on the Azores archipelago. *Helgoland Marine Research*, 61(1), 43-53.
- Ávila, S. P., Cordeiro, R., Madeira, P., Silva, L., Medeiros, A., Rebelo, A. C., Melo, C., Neto, A. I., Haroun, R., Monteiro, A., Rijdsdijk, K., & Johnson, M. E. (2018). Global change impacts on large-scale biogeographic patterns of marine organisms on Atlantic oceanic islands. *Marine Pollution Bulletin*, 126, 101–112. <https://doi.org/10.1016/j.marpolbul.2017.10.087>
- Ávila, S. P., Melo, C., Silva, L., Ramalho, R. S., Quartau, R., Hipólito, A., Cordeiro, R., Rebelo, A. C., Madeira, P., Rovere, A., Hearty, P. J., Henriques, D., Silva, C. M. da, Martins, A. M. de F., & Zazo, C. (2015a). A review of the MIS 5e highstand deposits from Santa Maria Island (Azores, NE Atlantic): Palaeobiodiversity, palaeoecology and palaeobiogeography. *Quaternary Science Reviews*, 114, 126–148. <https://doi.org/10.1016/j.quascirev.2015.02.012>
- Ávila, S. P., Madeira, P., Zazo, C., Kroh, A., Kirby, M., da Silva, C. M., Cachão, M., & Martins, A. M. F. (2009). Palaeoecology of the Pleistocene (MIS 5.5) outcrops of Santa Maria Island (Azores) in a complex oceanic tectonic setting. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 274(1–2), 18–31. <https://doi.org/10.1016/j.palaeo.2008.12.014>
- Ávila, S. P., Madeira, P., Rebelo, A. C., Melo, C., Hipólito, A., Pombo, J., Botelho, A. Z., & Cordeiro, R. (2015b). *Phorcus sauciatu*s (Koch, 1845) (Gastropoda: Trochidae) in Santa Maria, Azores archipelago: the onset of a biological invasion. *Journal of Molluscan Studies*, 81(4), 516-521. <https://doi.org/10.1093/mollus/eyv012>
- Ávila, S. P., Melo, C., Berning, B., Sá, N., Quartau, R., Rijdsdijk, K. F., Ramalho, R. S., Cordeiro, R., de Sá, N. C., Pimentel, A., Baptista, L., Medeiros, A., Gil, A., & Johnson, M. E. (2019). Towards a ‘Sea-Level Sensitive’ dynamic model: Impact of island ontogeny and glacio-eustasy on global patterns of marine island biogeography. *Biological Reviews*, 94(3), 1116-1142. <https://doi.org/10.1111/brv.12492>
- Ávila, S. P., Melo, P. J., Lima, A., Amaral, A., de Frias Martins, A. M., & Rodrigues, A. (2008). Reproductive cycle of the rissoid *Alvania mediolittoralis* Gofas, 1989 (Mollusca, Gastropoda) at São Miguel Island (Azores, Portugal). *Invertebrate Reproduction and Development*, 52(1–2), 31–40. <https://doi.org/10.1080/07924259.2008.9652269>
- Ávila, S. P., Santos, A. C., Penteado, A. M., Rodrigues, A. M., Quintino, I., & Machado, M. I. (2005). The molluscs of the intertidal algal turf in the Azores. *Iberus*, 23, 67–76.

- Avise, J. C. (2004). *Molecular markers, natural history and evolution* (2nd ed.). Sinauer Associates Inc.
- Baptista, L., Santos, A. M., Cabezas, M. P., Cordeiro, R., Melo, C., & Ávila, S. P. (2019). Intertidal or subtidal/circalittoral species: which appeared first? A phylogenetic approach to the evolution of non-planktotrophic species in Atlantic Archipelagos. *Marine Biology*, 166(7). <https://doi.org/10.1007/s00227-019-3536-y>
- Baptista, L., Santos, A. M., Melo, C. S., Rebelo, A. C., Madeira, P., Cordeiro, R., Botelho, A. Z., Hipólito, A., Pombo, J., Voelker, A. H. L., & Ávila, S. P. (2021). Untangling the origin of the newcomer *Phorcus sauciatu* (Mollusca: Gastropoda) in a remote Atlantic archipelago. *Marine Biology*, 168(1), Article number: 9. <https://doi.org/10.1007/s00227-020-03808-5>
- Beldade, R., Bell, C. A., Raimondi, P. T., George, M. K., Miner, C. M., & Bernardi, G. (2012). Isolation and characterization of 8 novel microsatellites for the black abalone, *Haliotis cracherodii*, a marine gastropod decimated by the withering disease. *Conservation Genetics Resources*, 4(4), 1071–1073. <https://doi.org/10.1007/s12686-012-9709-3>
- Bell, J. J. (2008). Similarity in connectivity patterns for two gastropod species lacking pelagic larvae. *Marine Ecology Progress Series*, 357, 185–194. <https://doi.org/10.3354/meps07301>
- Betti, F., Bavestrello, G., Bo, M., Coppari, M., Enrichetti, F., Manuele, M., & Cattaneo-Vietti, R. (2017). Exceptional strandings of the purple snail *Janthina pallida* Thompson, 1840 (Gastropoda: Epitoniidae) and first record of an alien goose barnacle along the Ligurian coast (western Mediterranean Sea). *The European Zoological Journal*, 84(1), 488-495. <https://doi.org/10.1080/24750263.2017.1379562>
- Bhargava, A., & Fuentes, F. (2010). Mutational Dynamics of Microsatellites. *Molecular Biotechnology*, 44, 250–266. <https://doi.org/10.1007/s12033-009-9230-4>
- Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, 30(15), 2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>
- Borges, L. M. S. S., Hollatz, C., Lobo, J., Cunha, A. M., Vilela, A. P., Calado, G. G., Coelho, R., Costa, A. C., Ferreira, M. S. G. G., Costa, M. H., & Costa, F. O. (2016). With a little help from DNA barcoding: Investigating the diversity of Gastropoda from the Portuguese coast. *Scientific Reports*, 6(1), 20226. <https://doi.org/10.1038/srep20226>
- Botstein, D., White, R., Skolnick, M., & RW, D. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*, 32(3), 314–331.
- Boulajfene, W., Strogyloudi, E., Lasram, M., El Mlayah, A., Vassiliki-Angelique, C., & Zouari-Tlig, S. (2019). Biological and biochemical assessment in *Phorcus articulatus* (Lamarck 1822): contamination and seasonal effect. *Environmental Monitoring and Assessment*, 191(9), 1-16. <https://doi.org/10.1007/s10661-019-7726-3>
- Brante, A., Fernández, M., & Viard, F. (2011). Microsatellite evidence for sperm storage and multiple paternity in the marine gastropod *Crepidula coquimbensis*. *Journal of Experimental Marine Biology and Ecology*, 396, 83–88. <https://doi.org/10.1016/j.jembe.2010.10.001>

- Buršić, M., Iveša, L., Jaklin, A., & Arko Pijevac, M. (2019). A preliminary study on the diversity of invertebrates associated with *Corallina officinalis* Linnaeus in southern Istrian peninsula. *Acta Adriatica*, 60(2), 127–135. <https://doi.org/10.32582/aa.60.2.2>
- Cabero, A. (2009). *Registro costero de los cambios eustáticos y climáticos durante los interglaciares recientes cuaternarios: Sur y Sureste peninsular, islas Baleares, Canarias y Cabo Verde* (Doctoral dissertation, Universidad de Salamanca).
- Cahill, A. E., & Viard, F. (2014). Genetic structure in native and non-native populations of the direct-developing gastropod *Crepidula convexa*. *Marine Biology*, 161(10), 2433–2443. <https://doi.org/10.1007/s00227-014-2519-2>
- Canning-Clode, J., & Carlton, J. T. (2017). Refining and expanding global climate change scenarios in the sea: poleward creep complexities, range termini, and setbacks and surges. *Diversity and Distributions*, 23(5), 463–473. <https://doi.org/10.1111/ddi.12551>
- Cárdenas, L., Daguin, C., Castilla, J. C., & Viard, F. (2007). Isolation and characterization of 11 polymorphic microsatellite markers for the marine gastropod *Concholepas concholepas* (Brugière, 1789). *Molecular Ecology Notes*, 7, 464–466. <https://doi.org/10.1111/j.1471-8286.2006.01619.x>
- Carracedo, L. I., Gilcoto, M., Mercier, H., & Pérez, F. F. (2014). Progress in Oceanography Seasonal dynamics in the Azores – Gibraltar Strait region: A climatologically-based study. *Progress in Oceanography*, 122, 116–130. <https://doi.org/10.1016/j.pocean.2013.12.005>
- Castoe, T. A., Poole, A. W., de Koning, A. P. J., Jones, K. L., Tomback, D. F., Oyler-McCance, S. J., Fike, J. A., Lance, S. L., Streicher, J. W., Smith, E. N., & Pollock, D. D. (2012). Rapid microsatellite identification from illumina paired-end genomic sequencing in two birds and a snake. *PLoS ONE*, 7(2), e30953. <https://doi.org/10.1371/journal.pone.0030953>
- Cavallaro, M., Ammendolia, G., & Battaglia, P. (2020). First record of *Dosima fascicularis* (Ellis & Solander, 1786) (Crustacea, Cirripedia) in the Strait of Messina (Central Mediterranean Sea). *Zootaxa*, 4743(3), zootaxa-4743. <https://doi.org/10.11646/zootaxa.4743.3.14>
- Chapuis, M. P. M., & Estoup, A. (2007). Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution*, 24(3), 621–631. <https://doi.org/10.1093/molbev/msl191>
- Clement, M., Posada, D., & Crandall, K. A. (2000). TCS: A computer program to estimate gene genealogies. *Molecular Ecology*, 9(10), 1657–1659. <https://doi.org/10.1046/j.1365-294X.2000.01020.x>
- Collin, R. (2003). Phylogenetic relationships among calyptraeid gastropods and their implications for the biogeography of marine speciation. *Systematic Biology*, 52, 618–640. <https://doi.org/10.1080/10635150390235430>
- Cordeiro, R., & Ávila, S. P. (2015). New species of Rissoidae (Mollusca, Gastropoda) from the Archipelago of the Azores (northeast Atlantic) with an updated regional checklist for the family. *ZooKeys*, 480, 1–19. <https://doi.org/10.3897/zookeys.480.8599>

- Cordeiro, R., Borges, J. P., Martins, A. M. F., & Ávila, S. P. (2015). Checklist of the littoral gastropods (Mollusca Gastropoda) from the Archipelago of the Azores (NE Atlantic). *Biodiversity Journal*, 6(4), 855–900.
- Costa, A. C., Parente, M., Botelho, A. Z., Monteiro, J., Micael, J., Gabriel, D., Ávila, S. P., Jardim, N., Gillon, A., Figueras, D., Torres, R., Cordeiro, R., Madeira, P., Melo, C. S., Baptista, L., & Raposo, V. (2016). Origem, vetores de introdução e condições ambientais que facilitam a introdução de espécies não indígenas nos Açores. (Technical report, Portugal)
- Costa, A. C., & Ávila, S. P. (2001). Macrobenthic mollusc fauna inhabiting *Halopteris* spp. subtidal fronds in São Miguel island, Azores. *Scientia Marina*, 65(2), 117–126.
- Criscione, F., & Ponder, W. F. (2013). A phylogenetic analysis of rissooidean and cingulopsoidean families (Gastropoda: Caenogastropoda). *Molecular Phylogenetics and Evolution*, 66(3), 1075–1082. <https://doi.org/10.1016/j.ympev.2012.11.026>
- Criscione, F., Ponder, W. F., Köhler, F., Takano, T., & Kano, Y. (2016). A molecular phylogeny of Rissoidae (Caenogastropoda: Rissooidea) allows testing the diagnostic utility of morphological traits. *Zoological Journal of the Linnean Society*, 179(1), 23–40. <https://doi.org/10.1111/zoj.12447>
- Crothers, J. H. (2001). Common topshells: An introduction to the biology of *Osilinus lineatus* with notes on other species in the genus. *Field Studies*, 10, 115–160.
- Curto, M., Winter, S., Seiter, A., Schmid, L., Scheicher, K., Barthel, L. M. F., Plass, J., & Meimberg, H. (2019). Application of a SSR-GBS marker system on investigation of European Hedgehog species and their hybrid zone dynamics. *Ecology and Evolution*, 9(5), 2814–2832. <https://doi.org/10.1002/ece3.4960>
- Darby, B. J., Erickson, S. F., Hervey, S. D., & Ellis-felege, S. N. (2016). Digital fragment analysis of short tandem repeats by high-throughput amplicon sequencing. *Ecology and Evolution*, 6(13), 4502–4512. <https://doi.org/10.1002/ece3.2221>
- Daniault, N., Mercier, H., Lherminier, P., Sarafanov, A., Falina, A., Zunino, P., Pérez, F. F., Ríos, A. F., Ferron, B., Huck, T., Thierry, V., & Gladyshev, S. (2016). The northern North Atlantic Ocean mean circulation in the early 21st century. *Progress in Oceanography*, 146, 142–158. <https://doi.org/10.1016/j.pocean.2016.06.007>
- Dautzenberg, P. (1889). *Contribution a la faune malacologique des Iles Açores: résultats des dragages effectués par le yacht l'Hirondelle pendant sa campagne scientifique de 1887: révision des mollusques marins des Açores*. Imprimerie de Monaco, pp. 112.
- Davidson, I. C. (2005). Structural gradients in an intertidal hard-bottom community: Examining vertical, horizontal, and taxonomic clines in zoobenthic biodiversity. *Marine Biology*, 146(4), 827–839. <https://doi.org/10.1007/s00227-004-1478-4>
- Davis, G. M., Wilke, T., Spolsky, C., Qiu, C. P., Qiu, D. C., Xia, M. Y., Zhang, Y., & Rosenberg, G. (1998). Cytochrome Oxidase I-based phylogenetic relationships among the Pomatiopsidae, Hydrobiidae, Rissoidae and Truncatellidae (Gastropoda: Caenogastropoda: Rissoacea). *Malacologia*, 40(1–2), 251–266

- de Barba, M., Miquel, C., Lobréaux, S., Quenette, P. Y., Swenson, J. E., & Taberlet, P. (2016). High-throughput microsatellite genotyping in ecology: improved accuracy, efficiency, standardization and success with low-quantity and degraded DNA. *Molecular Ecology Resources*, 17(3), 492–507. <https://doi.org/10.1111/1755-0998.12594>
- Delongueville, C., Scaillet, R., & Swinnen, F. (2019). New records of marine littoral Gastropoda and Bivalvia in the Azores Archipelago (Northeast Atlantic Ocean). *Novapex*, 20(1-2), 35-43.
- Desai, B. N. (1966). The biology of *Monodonta lineata* (da Costa). *Journal of Molluscan Studies*, 37(1), 1-17. <https://doi.org/10.1093/oxfordjournals.mollus.a064970>
- Domingues, V. S., Santos, R. S., Brito, A., Alexandrou, M., & Almada, V. C. (2007). Mitochondrial and nuclear markers reveal isolation by distance and effects of Pleistocene glaciations in the northeastern Atlantic and Mediterranean populations of the white seabream (*Diplodus sargus*, L.). *Journal of Experimental Marine Biology and Ecology*, 346, 102–113. <https://doi.org/10.1016/j.jembe.2007.03.002>
- Domingues, V. S., Santos, R. S., Brito, A., & Almada, V. C. (2006). Historical population dynamics and demography of the Eastern Atlantic pomacentrid *Chromis limbata* (Valenciennes, 1833). *Molecular Phylogenetics and Evolution*, 40(1), 139–147. <https://doi.org/10.1016/j.ympev.2006.02.009>
- Donald, K. M., Keeney, D. B., & Spencer, H. G. (2011). Contrasting population makeup of two intertidal gastropod species that differ in dispersal opportunities. *Journal of Experimental Marine Biology and Ecology*, 396(2), 224–232. <https://doi.org/10.1016/j.jembe.2010.10.028>
- Donald, K. M., Kennedy, M., & Spencer, H. G. (2005). Cladogenesis as the result of long-distance rafting events in South Pacific topshells (Gastropoda, Trochidae). *Evolution*, 59, 1701–1711. <https://doi.org/10.1111/j.0014-3820.2005.tb01819.x>
- Donald, K. M., McCulloch, G. A., Dutoit, L., & Spencer, H. G. (2020). Population structure of the New Zealand whelk, *Cominella glandiformis* (Gastropoda: Buccinidae), suggests sporadic dispersal of a direct developer. *Biological Journal of the Linnean Society*, 130(1), 49–60. <https://doi.org/10.1093/biolinnean/blaa033>
- Donald, K. M., Preston, J., Williams, S. T., Reid, D. G., Winter, D., Alvarez, R., Buge, B., Hawkins, S. J., Templado, J., & Spencer, H. G. (2012). Phylogenetic relationships elucidate colonization patterns in the intertidal grazers *Osilinus* Philippi, 1847 and *Phorcus* Risso, 1826 (Gastropoda: Trochidae) in the northeastern Atlantic Ocean and Mediterranean Sea. *Molecular Phylogenetics and Evolution*, 62(1), 35-45. <https://doi.org/10.1016/j.ympev.2011.09.002>
- Duff, M. le, & Hily, C. (2001). La zone intertidale du site Natura 2000 de Guisseny - Inventaire des habitats marins -. <https://doi.org/10.13140/RG.2.1.1314.5364>
- Dupont, L., Bernas, D., & Viard, F. (2007). Sex and genetic structure across age groups in populations of the European marine invasive mollusc, *Crepidula fornicata* L. (Gastropoda). *Biological Journal of the Linnean Society*, 90(20), 365–374. <https://doi.org/10.1111/j.1095-8312.2007.00731.x>

- Dupont, L., & Viard, F. (2003). Isolation and characterization of highly polymorphic microsatellite markers from the marine invasive species *Crepidula fornicata* (Gastropoda: Calyptraeidae). *Molecular Ecology Notes*, 3, 498–500. <https://doi.org/10.1046/j.1471-8286.2003.00491.x>
- Earl, D. A., & VonHoldt, B. M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4(2), 359–361. <https://doi.org/10.1007/s12686-011-9548-7>
- Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, 26(19), 2460–2461. <https://doi.org/10.1093/bioinformatics/btq461>
- Ellegren, H. (2004). Microsatellites: simple sequences with complex evolution. *Nature Reviews. Genetics*, 5(6), 435–445. <https://doi.org/10.1038/nrg1348>
- Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10, 564–567
- Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of Molecular Variance Inferred from Metric Distances Among DNA Haplotypes: Application. *Genetics*, 491, 479–491
- Falush, D., Stephens, M., & Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, 164(4), 1567–1587
- Farrell, E. D., Carlsson, J. E. L., & Carlsson, J. (2016). Next gen pop gen: Implementing a high-throughput approach to population genetics in boarfish (*Capros aper*). *Royal Society Open Science*, 3(12), 160651. <https://doi.org/10.1098/rsos.160651>
- Fischer-Piette, É. (1963). La distribution des principaux organismes intercotidaux Nord-Ibériques en 1954-1955. *Ann l'Institut Océanographique*, 40, 165–312.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3(5), 294–299. <https://doi.org/10.1371/journal.pone.0013102>
- Francisco, S. M., Almada, V. C., Faria, C., Velasco, E. M., & Robalo, J. I. (2014). Phylogeographic pattern and glacial refugia of a rocky shore species with limited dispersal capability: the case of Montagu's blenny (*Coryphoblennius galerita*, Blenniidae). *Marine Biology*, 161, 2509–2520. <https://doi.org/10.1007/s00227-014-2523-6>
- Freitas, R., Romeiras, M., Silva, L., Cordeiro, R., Madeira, P., González, J. A., Wirtz, P., Falcón, J. M., Brito, A., Floeter, S. R., Afonso, P., Porteiro, F., Viera-Rodríguez, M. A., Neto, A. I., Haroun, R., Farminhão, J. N. M., Rebelo, A. C., Baptista, L., Melo, C. S., Martínez, A., Núñez, J., Berning, B., Johnson, M. E. & Ávila, S. P. (2019). Restructuring of the 'Macaronesia' biogeographic unit: A marine multi-taxon biogeographical approach. *Scientific Reports*, 9(1), 1–18. <https://doi.org/10.1038/s41598-019-51786-6>
- Fründt, B., Müller, T. J., Schulz-Bull, D. E., & Waniek, J. J. (2013). Long-term changes in the thermocline of the subtropical Northeast Atlantic (33° N, 22° W). *Progress in Oceanography*, 116, 246–260. <https://doi.org/10.1016/j.pocean.2013.07.004>

- Fründt, B., & Waniek, J. (2012). Impact of the Azores Front propagation on deep Ocean particle flux. *Central European Journal of Geosciences*, 4(4), 531–544. <https://doi.org/10.2478/s13533-012-0102-2>
- Fu, Y. X. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, 147(2), 915–925.
- García-Escárcaga, A., Gutiérrez-Zugasti, I., Schöne, B. R., Cobo, A., Martín-Chivelet, J., & González-Morales, M. R. (2019). Growth patterns of the topshell *Phorcus lineatus* (da Costa, 1778) in northern Iberia deduced from shell sclerochronology. *Chemical Geology*, 526, 49–61. <https://doi.org/10.1016/j.chemgeo.2018.03.017>
- Talavera, F. G., Kardas, S. J., Richards, J., & Richards, H. G. (1978). Quaternary marine mollusks from Tenerife, Canary Islands. *The Nautilus*, 92(3), 97–102.
- García-Talavera, F., & Sánchez-Pinto, L. (2001). Moluscos marinos fósiles de Selvagem Pequenha e Ilhéu de Fora (Islas Selvajes). Descripción de una nueva especie de Neogasterópodo. *Revista de la Academia Canaria de Ciencias*, 13(4), 9–21.
- Geller, J., Meyer, C., Parker, M., & Hawk, H. (2013). Redesign of PCR primers for mitochondrial Cytochrome C Oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Molecular Ecology Resources*, 13(5), 851–861. <https://doi.org/10.1111/1755-0998.12138>
- Gerber, J., Hemmen, J., & Groh, K. (1989). Eine pleistozäne marine Molluskenfauna von Porto Santo (Madeira-Archipel). *Mitteilungen der deutschen malakozoologischen Gesellschaft*, 44(45), 19–30.
- Ginot, F., Bordelais, I., Nguyen, S., & Gyapay, G. (1996). Correction of some genotyping errors in automated fluorescent microsatellite analysis by enzymatic removal of one base overhangs. *Nucleic Acids Research*, 4, 540–541. <https://doi.org/10.1093/nar/24.3.540>
- Gofas, S. (1990). The littoral Rissoidae and Anabathridae of São Miguel, Azores. In A. M. F. Martins (Ed.), *The Marine Fauna and Flora of the Azores. (Proceedings of the First International Workshop of Malacology, Vila Franca Do Campo, São Miguel, Azores)* (pp. 97–134). Açoreana, Supplement 2
- Gofas, S. (2007). Rissoidae (Mollusca: Gastropoda) from northeast Atlantic seamounts. *Journal of Natural History*, 41(13–16), 779–885. <https://doi.org/10.1080/00222930701298085>
- González-Wangüemert, M., Cánovas, F., Pérez-Ruzafa, A., Marcos, C., & Alexandrino, P. (2010). Connectivity patterns inferred from the genetic structure of white seabream (*Diplodus sargus* L.). *Journal of Experimental Marine Biology and Ecology*, 383(1), 23–31. <https://doi.org/10.1016/j.jembe.2009.10.010>
- Guichoux, E., Lagache, L., Wagner, S., Chaumeil, P., Léger, P., Lepais, O., Lepoittevin, C., Malausa, T., Revardel, E., Salin, F., & Petit, R. J. (2011). Current trends in microsatellite genotyping. *Molecular Ecology Resources*, 11(4), 591–611. <https://doi.org/10.1111/j.1755-0998.2011.03014.x>
- Hall, T. A. (1999, January). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98.

- Hart, M. W., & Sunday, J. (2007). Things fall apart: Biological species form unconnected parsimony networks. *Biology Letters*, 3(5), 509–512. <https://doi.org/10.1098/rsbl.2007.0307>
- Hawkins, S. J., Corte-Real, H. B. S. M., Pannacciulli, F. G., Weber, L. C., & Bishop, J. D. D. (2000). Thoughts on the ecology and evolution of the intertidal biota of the Azores and other Atlantic islands. *Hydrobiologia*, 440(1), 3–17. <https://doi.org/10.1023/A:1004118220083>
- Hernández-Guerra, A., López-Laatzén, F., Machín, F., De Armas, D., & Pelegrí, J. L. (2001). Water masses, circulation and transport in the eastern boundary current of the North Atlantic subtropical gyre. *Scientia Marina*, 65(S1), 177–186.
- Hickman, C. S. (1992). Reproduction and development of trochacean gastropods. *Veliger*, 35(4), 245–272.
- Holla, S., Khan, J., Sowjanya, M. S., & Shashidhar, H. (2014). Monomorphic molecular markers are as informative as polymorphic molecular markers. *Indian Journal of Genetics and Plant Breeding*, 74, 596. <https://doi.org/10.5958/0975-6906.2014.00896.7>
- Hubisz, M., Falush, D., Stephens, M., & Pritchard, J. K. (2009). Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources*, 9, 1322–1332. <https://doi.org/10.1111/j.1755-0998.2009.02591.x>
- Johannesson, K. (1988). The paradox of Rockall: why is a brooding gastropod (*Littorina saxatilis*) more widespread than one having a planktonic larval dispersal stage (*L. littorea*)? *Marine Biology*, 99(4), 507–513. <https://doi.org/10.1007/BF00392558>
- Junoy, J., & Junoy, J. (2014). First record of the buoy barnacle *Dosima fascicularis* (Ellis & Solander, 1786) (Crustacea, Cirripedia) from the Galician beaches (NW Spain) after the Prestige oil spill. *Check List*, 10(3), 669–671. <https://doi.org/10.15560/10.3.669>
- Kalinowski, S. T., Taper, M. L., & Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, 16, 1099–1106. <https://doi.org/10.1111/j.1365-294x.2007.03089.x>
- Kawai, K., Hughes, R. N., & Takenaka, O. (2001). Isolation and characterization of microsatellite loci in the marine gastropod *Nucella lapillus*. *Molecular Ecology Notes*, 1, 270–272.
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., & Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28(12), 1647–1649.
- Kemppainen, P., Panova, M., Hollander, J., & Johannesson, K. (2009). Complete lack of mitochondrial divergence between two species of NE Atlantic marine intertidal gastropods. *Journal of Evolutionary Biology*, 22(10), 2000–2011. <https://doi.org/10.1111/j.1420-9101.2009.01810.x>
- Klein, B., & Siedler, G. (1989). On the origin of the Azores Current. *Journal of Geophysical Research: Oceans*, 94, 6159–6168. <https://doi.org/10.1029/JC094iC05p06159>
- Kojima, S., Hayashi, I., Kim, D., Iijima, A., & Furota, T. (2004). Phylogeography of an intertidal direct-developing gastropod *Batillaria cumingi* around the Japanese Islands. *Marine Ecology Progress Series*, 276, 161–172. <https://doi.org/10.3354/meps276161>

- Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, 15(5), 1179–1191. <https://doi.org/10.1111/1755-0998.12387>
- Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33(7), 1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Ladoukakis, E. D., & Zouros, E. (2017). Evolution and inheritance of animal mitochondrial DNA: rules and exceptions. *Journal of Biological Research-Thessaloniki*, 24(1), 1–7. <https://doi.org/10.1186/s40709-017-0060-4>
- Lanner, J., Gstöttenmayer, F., Curto, M., Geslin, B., Huchler, K., Orr, M. C., Pachinger, B., Sedivy, C., & Meimberg, H. (2021). Evidence for multiple introductions of an invasive wild bee species currently under rapid range expansion in Europe. *BMC Ecology and Evolution*, 21, 71. <https://doi.org/10.1186/s12862-020-01729-x>
- le Cam, S., Riquet, F., Pechenik, J. A., & F, V. (2014). Paternity and Gregariousness in the Sex-Changing Sessile Marine Gastropod *Crepidula convexa*: Comparison with Other Protandrous *Crepidula* Species. *Journal of Heredity*, 105(3), 397–406. <https://doi.org/10.1093/jhered/esu002>
- Lee, H. J., & Boulding, E. G. (2009). Spatial and temporal population genetic structure of four northeastern Pacific littorinid gastropods: the effect of mode of larval development on variation at one mitochondrial and two nuclear DNA markers. *Molecular Ecology*, 18, 2165–2184. <https://doi.org/10.1111/j.1365-294X.2009.04169.x>
- Levin, L., & Bridges, T. (1995). Pattern and diversity in reproduction and development. In McEdward, L (Ed.) *Marine invertebrate larvae*. CRC Press, Oxford, 1–47.
- Lima, F. P., Queiroz, N., Ribeiro, P. A., Hawkins, S. J., & Santos, A. M. (2006). Recent changes in the distribution of a marine gastropod, *Patella rustica* Linnaeus, 1758, and their relationship to unusual climatic events. *Journal of Biogeography*, 33(5), 812–822. <https://doi.org/10.1111/j.1365-2699.2006.01457.x>
- Litt, M., Hauge, X., & Sharma, V. (1993). Shadow bands seen when typing polymorphic dinucleotide repeats — Some causes and cures. *BioTechniques*, 15, 280–284.
- López-Márquez, V., García-Jiménez, R., Calvo, M., Templado, J., & Machordom, A. (2018). Isolation of microsatellite loci for the endangered vermetid gastropod *Dendropoma lebeche* using Illumina MiSeq next generation sequencing technology. *Molecular Biology Reports*, 45(6), 2775–2781. <https://doi.org/10.1007/s11033-018-4346-x>
- Machín, F., Hernández-Guerra, A., & Pelegrí, J. L. (2006). Mass fluxes in the Canary Basin. *Progress in Oceanography*, 50(2-4), 416–447. <https://doi.org/10.1016/j.pocean.2006.03.019>
- McInerney, C. E., Allcock, A. L., Johnson, M. P., & Prodöhl, P. A. (2009). Characterization of polymorphic microsatellites for the periwinkle gastropod, *Littorina littorea* (Linnaeus, 1758) and their cross-amplification in four congeners. *Conservation Genetics*, 10, 1417–1420. <https://doi.org/10.1007/s10592-008-9750-7>

- McWilliam, H., Li, W., Uludag, M., Squizzato, S., Park, Y. M., Buso, N., Cowley, A. P., & Lopez, R. (2013). Analysis tool web services from the EMBL-EBI. *Nucleic Acids Research*, 41(W1), W597–W600.
- Mieszkowska, N., Hawkins, S. J., Burrows, M. T., & Kendall, M. A. (2007). Long-term changes in the geographic distribution and population structures of *Osilinus lineatus* (Gastropoda: Trochidae) in Britain and Ireland. *Journal of the Marine Biological Association of the United Kingdom*, 87(2), 537–545. <https://doi.org/10.1017/S0025315407053799>
- Mieszkowska, N., Kendall, M. A., Hawkins, S. J., Leaper, R., Williamson, P., Hardman-Mountford, N. J., & Southward, A. J. (2006). Changes in the range of some common rocky shore species in Britain—a response to climate change?. *Hydrobiologia*, 555, 241–251. <https://doi.org/10.1007/s10750-005-1120-6>
- Mifsud, C. (2005). On the occurrence of the buoy barnacle *Dosima fascicularis* Ellis and Solander, 1786 (Cirripedia: Lepadidae) in Maltese waters with new records of other species of Thoracica. *The Central Mediterranean Naturalist*, 4, 131–133.
- Miller, M. P., Knaus, B. J., Mullins, T. D., & Haig, S. M. (2013). SSR_pipeline: A bioinformatic infrastructure for identifying microsatellites from paired-end Illumina high-throughput DNA sequencing data. *Journal of Heredity*, 104(6), 881–885. <https://doi.org/10.1093/jhered/est056>
- Miralles, L., Ardura, A., Arias, A., Borrell, Y. J., Clusa, L., Dopico, E., de Rojas, A. H., Lopez, B., Muñoz-Colmenero, M., Roca, A., Valiente, A. G., Zaiko, A., & Garcia-Vazquez, E. (2016). Barcodes of marine invertebrates from north Iberian ports: Native diversity and resistance to biological invasions. *Marine Pollution Bulletin*, 112(1–2), 183–188. <https://doi.org/10.1016/j.marpolbul.2016.08.022>
- Modica, M. V., Russini, V., Fassio, G., & Oliverio, M. (2017). Do larval types affect genetic connectivity at sea? Testing hypothesis in two sibling marine gastropods with contrasting larval development. *Marine Environmental Research*, 127, 92–101. <https://doi.org/10.1016/j.marenvres.2017.04.001>
- Nei, M. (1987). *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- Neves, V. C., Griffiths, K., Savory, F. R., Furness, R. W., & Mable, B. K. (2010). Are European starlings breeding in the Azores archipelago genetically distinct from birds breeding in mainland Europe? *European Journal of Wildlife Research*, 56, 95–100. <https://doi.org/10.1007/s10344-009-0316-x>
- Nobre, A. (1924). *Contribuições para a fauna dos Açores*. Anais do Instituto de Zoologia da Universidade do Porto 1, 41–90.
- Nobre, A. (1930). *Materiais para o estudo da fauna dos Açores*. Anais do Instituto de Zoologia da Universidade do Porto 1, 41–91.
- Ó Foighil, D. (1989). Planktotrophic larval development is associated with a restricted geographic range in *Lasaea*, a genus of brooding, hermaphrodite bivalves. *Marine Biology*, 103, 349–358.
- Oliverio, M. (1995). Larval development and allozyme variation in the East Atlantic *Columbella* (Gastropoda: Prosobranchia: Columbellidae). *Scientia Marina*, 59(11), 77–86.

- Pálsson, S., Magnúsdóttir, H., Reynisdóttir, S., Jónsson, Z. O., & Örnólfssdóttir, E. B. (2013). Divergence and molecular variation in common whelk *Buccinum undatum* (Gastropoda: Buccinidae) in Iceland: a trans-Atlantic comparison. *Biological Journal of the Linnean Society*, 111(1), 145–159. <https://doi.org/10.1111/bij.12191>
- Palumbi, S. R. (1994). Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics*, 25, 547–572. <https://doi.org/10.1146/annurev.es.25.110194.002555>
- Papetti, C., Schiavona, L., Milan, M., Lucassen, M., Cavaco, J. A., Paterno, M., Boscari, E., Marino, I. A. M., Congiu, L., & Zane, L. (2018). Genetic variability of the striped venus *Chamelea gallina* in the northern Adriatic Sea. *Fisheries Research*, 201, 68–78. <https://doi.org/10.1016/j.fishres.2018.01.006>
- Peakall, R., & Smouse, P. E. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6, 288–295. <https://doi.org/10.1111/j.1471-8286.2005.01155.x>
- Peakall, R., & Smouse, P. E. (2012). GenALEX 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics*, 28, 2537–2539. <https://doi.org/10.1111/j.1471-8286.2005.01155.x>
- Pechenik, J. A. (1984). The relationship between temperature, growth rate, and duration of planktonic life for larvae of the gastropod *Crepidula fornicata* (L.). *Journal of Experimental Marine Biology and Ecology*, 74(3), 241–257.
- Ponder, W. F. (1984). A review of the Genera of the Rissoidae (Mollusca: Mesogastropoda: Rissoacea). *Records of the Australian Museum, Suppl.* 4, 1–221. <https://doi.org/10.3853/j.0812-7387.4.1985.100>
- Ponder, W. F. (1990). A gravel beach shelled micro-gastropod assemblage from Ceuta, Strait of Gibraltar, with the description of a new truncatelloidean genus. *Bulletin Du Muséum National d'histoire Naturelle. Section A, Zoologie, Biologie et Écologie Animales*, 12(2), 291–311.
- Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155, 945–959.
- Putman, A. I., & Carbone, I. (2014). Challenges in analysis and interpretation of microsatellite data for population genetic studies. *Ecology and Evolution*, 4(22), 4399–4428. <https://doi.org/10.1002/ece3.1305>
- Quintero-Galvis, J. F., Bruning, P., Paleo-López, R., Gomez, D., Sánchez, R., & Cárdenas, L. (2020). Temporal variation in the genetic diversity of a marine invertebrate with long larval phase, the muricid gastropod *Concholepas concholepas*. *Journal of Experimental Marine Biology and Ecology*, 530–531, 151432. <https://doi.org/10.1016/j.jembe.2020.151432>
- Ramírez, R., Tuya, F., & Haroun, R. J. (2009). Efectos potenciales del marisqueo sobre moluscos gasterópodos de interés comercial (*Osilinus* spp. y *Patella* spp.) en el Archipiélago Canario. *Revista de Biología Marina y Oceanografía*, 44(3), 703–714. <https://doi.org/10.4067/S0718-19572009000300016>

- Ramírez, R., Tuya, F., Sánchez-Jerez, P., Fernández-Gil, C., Bersaga, P., Haroun, R. J., & Henández-Brito, J. J. (2005). Population structure and spatial distribution of the gastropod molluscs *Osilinus atrata* and *Osilinus sauciatus* in the rocky intertidal zone of the Canary Islands (Central East Atlantic). *Ciencias Marinas*, 31(4), 697-706. <https://doi.org/10.7773/cm.v31i4.35>
- Ramos-Onsins, S. E., & Rozas, J. (2002). Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution*, 19(12), 2092-2100. <https://doi.org/10.1093/oxfordjournals.molbev.a004034>
- Ribeiro, P. A., Branco, M., Hawkins, S. J., & Santos, A. M. (2010). Recent changes in the distribution of a marine gastropod, *Patella rustica*, across the Iberian Atlantic coast did not result in diminished genetic diversity or increased connectivity. *Journal of Biogeography*, 37(9), 1782–1796. <https://doi.org/10.1111/j.1365-2699.2010.02330.x>
- Rice, W. R. (1989). Analysing tables of statistical tests. *Evolution*, 43, 223–225.
- Robinson, L. M., Elith, J., Hobday, A. J., Pearson, R. G., Kendall, B. E., Possingham, H. P., Richardson, A. J. (2011). Pushing the limits in marine species distribution modelling: lessons from the land present challenges and opportunities. *Global Ecology and Biogeography* 20, 789–802. <https://doi.org/10.1111/j.1466-8238.2010.00636.x>
- Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J. C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S. E., & Sánchez-Gracia, A. (2017). DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution*, 34(12), 3299-3302. <https://doi.org/10.1093/molbev/msx248>
- Rubal, M., Veiga, P., Moreira, J., & Sousa-Pinto, I. (2014). The gastropod *Phorcus sauciatus* (Koch, 1845) along the north-west Iberian Peninsula: filling historical gaps. *Helgoland Marine Research*, 68(1), 169-177. <https://doi.org/10.1007/s10152-014-0379-2>
- Ryan, P. G., & Branch, G. M. (2012). The November 2011 irruption of buoy barnacles *Dosima fascicularis* in the Western Cape, South Africa. *African Journal of Marine Science*, 34(1), 157-162. <https://doi.org/10.2989/1814232X.2012.675130>
- Sala, I., Caldeira, R. M. A., Estrada-Allis, S. N., Froufe, E., & Couvelard, X. (2013). Lagrangian transport pathways in the northeast Atlantic and their environmental impact. *Limnology and Oceanography: Fluids and Environments*, 3(1), 40–60. <https://doi.org/10.1215/21573689-2152611>
- Sala, I., Harrison, C. S., & Caldeira, R. M. A. (2016). The role of the Azores Archipelago in capturing and retaining incoming particles. *Journal of Marine Systems*, 154, 146–156. <https://doi.org/10.1016/j.jmarsys.2015.10.001>
- Salgueiro, P., Coelho, M. M., Palmeirim, J. M., & Ruedi, M. (2004). Mitochondrial DNA variation and population structure of the island endemic Azorean bat (*Nyctalus azoreum*). *Molecular Ecology*, 13(11), 3357–3366. <https://doi.org/10.1111/j.1365-294x.2004.02354.x>
- Salgueiro, P., Palmeirim, J. M., Ruedi, M., & Coelho, M. M. (2008). Gene flow and population structure of the endemic Azorean bat (*Nyctalus azoreum*) based on microsatellites:

- implications for conservation. *Conservation Genetics*, 9, 1163–1171. <https://doi.org/10.1007/s10592-007-9430-z>
- Santos, A. M., Cabezas, M. P., Tavares, A. I., Xavier, R., & Branco, M. (2016). tcsBU: a tool to extend TCS network layout and visualization. *Bioinformatics*, *btv636*. <https://doi.org/10.1093/bioinformatics/btv636>
- Santos, R. S., Hawkins, S., Monteiro, L. R., Alves, M., & Isidro, E. J. (1995). Marine research, resources and conservation in the Azores. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 5(4), 311–354. <https://doi.org/10.1002/aqc.3270050406>
- Sá-Pinto, A., Branco, M., Sayanda, D., & Alexandrino, P. (2007). Patterns of colonization, evolution and gene flow in species of the genus *Patella* in the Macaronesian Islands. *Molecular Ecology*, 17(2), 519–532. <https://doi.org/10.1111/j.1365-294x.2007.03563.x>
- Scheltema, R. S. (1986). On dispersal and planktonic larvae of benthic invertebrates: An eclectic overview and summary of problems. *Bulletin of Marine Science*, 39(2), 290–322.
- Scheltema, R. S. (1989). Planktonic and non-planktonic development among prosobranch gastropods and its relationship to the geographic range of species. In J. S. Ryland & P. A. Tyles (Eds.), *Reproduction, Genetics and Distribution of Marine Organisms* (pp. 183–188). Fredensborg: Olsen and Olsen.
- Scheltema, R. S. (1995). The relevance of passive dispersal for the biogeography of Caribbean mollusks. *American Malacological Bulletin*, 11, 99–115.
- Silva, A., Brotas, V., Valente, A., Sá, C., Diniz, T., Patarra, R. F., Álvaro, N. V., & Neto, A. I. (2013). Coccolithophore species as indicators of surface oceanographic conditions in the vicinity of Azores islands. *Estuarine, Coastal and Shelf Science*, 118, 50–59. <https://doi.org/10.1016/j.ecss.2012.12.010>
- Sneath, P., & Sokal, R. R. (1973). Unweighted Pair Group Method with Arithmetic Mean. In *Numerical Taxonomy* (pp. 230–234). Freeman.
- Sokolov, E. P. (2000). An improved method for DNA isolation from mucopolysaccharide-rich molluscan tissues. *Journal of Molluscan Studies*, 66(4), 573–575. <https://doi.org/10.1093/mollus/66.4.573>
- Sousa, R., Delgado, J., González, J. A., Freitas, M., & Henriques, P. (2018). Marine snails of the genus *Phorcus*: biology and ecology of sentinel species for human impacts on the rocky shores. In Ray, S (Ed.) *Biological Resources of Water*. IntechOpen, pp. 141–167.
- Sousa, R., Vasconcelos, J., Delgado, J., Riera, R., González, J. A., Freitas, M., & Henriques, P. (2019b). Filling biological information gaps of the marine topshell *Phorcus sauciatus* (Gastropoda: Trochidae) to ensure its sustainable exploitation. *Journal of the Marine Biological Association of the United Kingdom*, 99(4), 841–849. <https://doi.org/10.1017/S0025315418001054>
- Sousa, R., Vasconcelos, J., Riera, R., Delgado, J., González, J. A., Freitas, M., & Henriques, P. (2019a). Disentangling exploitation of the intertidal grazer *Phorcus sauciatus* (Gastropoda: Trochidae) in an oceanic archipelago: Implications for conservation. *Marine Ecology*, 40(2), e12540. <https://doi.org/10.1111/maec.12540>

- Stamatakis, A. (2014). RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. *Bioinformatics*, 30(9), 1312–131. <https://doi.org/10.1093/bioinformatics/btu033>
- Stefanni, S., Castilho, R., Sala-Bozano, M., Robalo, J. O., Francisco, S. M., Santos, R. S., Marques, N., Brito, A., Almada, V. C., & Mariani, S. (2015). Establishment of a coastal fish in the Azores: recent colonisation or sudden expansion of an ancient relict population? *Heredity*, 115, 527–537. <https://doi.org/10.1038/hdy.2015.55>
- Sunnucks, P. (2000). Efficient genetic markers for population biology. *Trends in Ecology and Evolution*, 15(5), 199–203. [https://doi.org/10.1016/S0169-5347\(00\)01825-5](https://doi.org/10.1016/S0169-5347(00)01825-5)
- Teske, P. R., Golla, T. R., Sandoval-Castillo, J., Emami-Khoyi, A., van der Lingen, C. D., von der Heyden, S., Chiazari, B., Jansen Van Vuuren, B., & Beheregaray, L. B. (2018). Mitochondrial DNA is unsuitable to test for isolation by distance. *Scientific Reports*, 8(1), 1–9. <https://doi.org/10.1038/s41598-018-25138-9>
- Teske, P. R., Sandoval-Castillo, J., Waters, J., & Beheregaray, L. B. (2017). An overview of Australia's temperate marine phylogeography, with new evidence from high-dispersal gastropods. *Journal of Biogeography*, 44(1), 217–229. <https://doi.org/10.1111/jbi.12783>
- Thiel, M., & Gutow, L. (2005a). The ecology of rafting in the marine environment. I. the floating substrata. In *Oceanography and Marine Biology: An Annual Review* (Vol. 42). <https://doi.org/10.1201/9780203507810>
- Thiel, M., & Gutow, L. (2005b). The ecology of rafting in the marine environment. II. The rafting organisms and community. *Oceanography and Marine Biology*, 43(1), 279–418. <https://doi.org/10.1201/9781420037449.ch7>
- Thiel, M., & Haye, P. A. (2006). The ecology of rafting in the marine environment. III. Biogeographical and evolutionary consequences. *Oceanography and Marine Biology*, 44, 323–429.
- Thorson, G. (1946). Reproduction and larval development of Danish marine bottom invertebrates. *Meddelelser fra Kommissionen for Danmarks Fiskeri- og Havundersøgelser, Serie Plankton*, 4, 1–523.
- Thorson, G. (1950). Reproduction and larval ecology of marine bottom invertebrates. *Biological Reviews of the Cambridge Philosophical Society*, 25, 1–45.
- Tibihika, P. D., Curto, M., Dornstauder-Schrammel, E., Winter, S., Alemayehu, E., Waidbacher, H., & Meimberg, H. (2019). Application of microsatellite genotyping by sequencing (SSR-GBS) to measure genetic diversity of the East African *Oreochromis niloticus*. *Conservation Genetics*, 20(2), 357–372. <https://doi.org/10.1007/s10592-018-1136-x>
- Tringali, L. P. (2001). Marine malacological records (Gastropoda: Prosobranchia, Heterobranchia, Opisthobranchia and Pulmonata) from Torres de Alcalé, Mediterranean Morocco, with the description of a new philinid species. *Bollettino Malacologico*, 37(9–12), 207–222.
- Turini, F. G., Steinert, C., Heubl, G., Bringmann, G., Lombe, B. K., Mudogo, V., & Meimberg, H. (2014). Microsatellites facilitate species delimitation in Congolese *Ancistrocladus*

- (Ancistrocladaceae), a genus with pharmacologically potent naphthylisoquinoline alkaloids. *Taxon*, 63, 329–341.
- Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012). Primer3—New capabilities and interfaces. *Nucleic Acids Research*, 40(15), e115–e115. <https://doi.org/10.1093/nar/gks596>
- Vartia, S., Villanueva-Cañas, J. L., Finarelli, J., Farrell, E. D., Collins, P. C., Hughes, G. M., Carlsson, J. E. L., Gauthier, D. T., McGinnity, P., Cross, T. F., Fitzgerald, R. D., Mirimin, L., Crispie, F., Cotter, P. D., & Carlsson, J. (2016). A novel method of microsatellite genotyping-by-sequencing using individual combinatorial barcoding. *Royal Society Open Science*, 3, 150565. <https://doi.org/10.1098/rsos.150565>
- Vega-Retter, C., Briones, M., & Véliz, D. (2016). Characterization of sixteen microsatellite loci from the marine gastropod *Monetaria caputdraconis* (Gastropoda: Cypraeidae) by next generation sequencing. *Revista de Biología Marina y Oceanografía*, 51(3), 695–698. <https://doi.org/10.4067/S0718-19572016000300021>
- Waters, J. M., & Craw, D. (2018). Cyclone-driven marine rafting: storms drive rapid dispersal of buoyant kelp rafts. *Marine Ecology Progress Series*, 602, 77–85. <https://doi.org/10.3354/meps12695>
- Weersing, K., & Toonen, R. J. (2009). Population genetics, larval dispersal, and connectivity in marine systems. *Marine Ecology Progress Series*, 393, 1–12. <https://doi.org/10.3354/meps08287>
- Weetman, D., Hauser, L., Shaw, P. W., & Bayes, M. K. (2005). Microsatellite markers for the whelk *Buccinum undatum*. *Molecular Ecology Notes*, 5, 361–362. <https://doi.org/10.1111/j.1471-8286.2005.00926.x>
- Wilke, T., & Davis, G. M. (2000). Intraspecific mitochondrial sequence diversity in *Hydrobia ulvae* and *Hydrobia ventrosa* (Hydrobiidae: Rissoidae: Gastropoda): Do their different life histories affect biogeographic patterns and gene flow? *Biological Journal of the Linnean Society*, 70(1), 89–105. <https://doi.org/10.1006/bjil.1999.0388>
- Wilke, T., & Falniowski, A. (2001). The genus *Adriohydrobia* (Hydrobiidae: Gastropoda): polytypic species or polymorphic populations? *Journal of Zoological Systematics and Evolutionary Research*, 39, 227–234. <https://doi.org/10.1046/j.1439-0469.2001.00171.x>
- Winston, J. E. (2012). Dispersal in marine organisms without a pelagic larval phase. *Integrative and Comparative Biology*, 52(4), 447–457. <https://doi.org/10.1093/icb/ics040>
- Wort, E. J. G., Chapman, M. A., Hawkins, S. J., Henshall, L., Pita, A., Rius, M., Williams, S. T., & Fenberg, P. B. (2019). Contrasting genetic structure of sympatric congeneric gastropods: Do differences in habitat preference, abundance and distribution matter? *Journal of Biogeography*, 46(2), 369–380. <https://doi.org/10.1111/jbi.13502>
- Xue, D., Zhang, T., & Liu, J.-X. (2014). Microsatellite Evidence for High Frequency of Multiple Paternity in the Marine Gastropod *Rapana venosa*. *PLoS ONE*, 9(1), e86508. <https://doi.org/10.1371/journal.pone.0086508>

- Zhang, Z., Schwartz, S., Wagner, L., & Miller, W. (2000). A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology*, 7(1–2), 203–214.
<https://doi.org/10.1089/10665270050081478>
- Zouros, E., & Foltz, D. W. (1984). Possible explanations of heterozygote deficiency in bivalve molluscs. *Malacologia*, 25, 583–591.

Chapter 5

Factors shaping the connectivity of deep-water populations in a remote archipelago

5.1 Morphospecies and molecular diversity of ‘lace corals’: the genus *Reteporella* (Bryozoa: Cheilostomatida) in the central North Atlantic Azores Archipelago

Introduction

Bryozoans, although common components of marine benthic communities, remain some of the most understudied metazoans. As a result, detailed taxonomic studies combining morphological and molecular data are still missing for many higher taxa, even those that produce large colonies visible to the naked eye and occur at diving depths. The genus *Reteporella* Busk, 1884 is the most speciose and widespread of the 23 currently accepted genera within the cheilostomatid bryozoan family Phidoloporidae Gabb & Horn, 1862 (Bock, 2022; Hayward & Winston, 2011). Like other phidoloporids, *Reteporella* occur in shallow coastal to upper bathyal benthic habitats throughout the world (Hayward & Ryland, 1999). They often form lace-like, colourful, three-dimensional colonies, which earns them their common, yet misleading, name ‘lace corals’. Colony form can be influenced by environmental factors (Hayward, 2000), enabling them to colonize a diversity of microhabitats (Casoli et al., 2020a). Colony morphology in *Reteporella* is complex, typically displaying erect bilaminar, fenestrated sheets of zooids that form infolded, lobed or widely open calices. Taxonomic classification of *Reteporella* species has hitherto exclusively been based on morphological characters such as the shape of the peristome, morphology of the ovicell, and polymorphisms of the avicularia. However, taxonomic decisions are often hampered by the species’ complex morphology, which includes a pronounced ontogenetic gradient from the colony margin to its base, as well as the difficulty to distinguish interspecific differences from intraspecific variability in response to environmental conditions. This is particularly problematic if only fragmentary material is available for study (Hayward, 2000, 2004). Molecular DNA analyses are therefore important to test the morphospecies taxon concept.

A recent large-scale phylogenetic analysis, which included three *Reteporella* representatives besides a number of other phidoloporid taxa (Orr et al., 2022), suggested that the genus is non-monophyletic, although the family is monophyletic. Precise knowledge of the ecology and (palaeo)biogeography of *Reteporella* species remains scarce, and even in comparatively well-studied regions, such as the North Atlantic and Mediterranean Sea, new taxa are regularly described (e.g. Denisenko, 2022; Harmelin,

1976; Hayward & Ryland, 1996; López de la Cuadra & García Gómez, 2001; Ramalho et al., 2018). Studies on bryozoans from the central North Atlantic archipelago of the Azores peaked in the early 20th century (e.g. Calvet, 1907, 1931; Jullien & Calvet, 1903), while only few studies have made significant contributions to the knowledge of Azorean bryozoan diversity thereafter (e.g. d'Hondt, 1975; Harmelin, 1978). Recent times have seen a renewed interest regarding revisions of historical taxa in the Azores (e.g. Berning et al., 2019, 2021; Harmelin et al., 2019; Reverter-Gil et al., 2015) and the identification of non-indigenous species (e.g. Micael et al., 2014, 2019).

To date, five *Reteporella* species have been described from the Azores: *R. atlantica* (Busk, 1884), *R. gracilis* (Jullien, 1903), *R. oceanica* (Jullien, 1903), *R. rara* (Jullien, 1903), and *R. tristis* (Jullien, 1903). Another four species, which have their type localities outside the Azores [e.g. *Reteporella mediterranea* (Smitt, 1867) from the Mediterranean Sea, or *Reteporella sparteli* (Calvet, 1906) from the Gulf of Cádiz], were also reported to occur in the archipelago (Calvet, 1907, 1931; d'Hondt, 1975; Jullien & Calvet, 1903). However, none of these species have as yet been revised using scanning electron microscopy (SEM) and their identity is unclear at present. With this study we aim to characterize the diversity and biogeographic distribution of *Reteporella* species in the Azores Archipelago, based on molecular and morphological datasets, as well as better understand the relationships with non-Azorean phidoloporids.

Material and Methods

Here we present phylogenetic reconstructions of *Reteporella* species sampled across different benthic habitats in the Azores and adjunct seamounts, complemented with samples from the Mediterranean Sea and North Atlantic. Phylogenies were based on partial sequences of genes cytochrome C oxidase subunit I (COI), small subunit mitochondrial ribosomal RNA (16S) and large subunit nuclear ribosomal RNA (28S). Molecular clades are interpreted in combination with morphological data to characterize the diversity and biogeographic distribution of *Reteporella* species in the Azores Archipelago.

Sampling and morphological characterization

Reteporella specimens from several locations around the Azores Archipelago were either recently sampled by the authors or sub-sampled from collection material (cf. Supplementary Table A5.1 for further details). During the scientific cruise M150 BIODIAZ “Controls in benthic and pelagic BIODiversity of the AZores” with the German RV *Meteor* in 2018, *Reteporella* specimens were collected around the islands of Flores, Terceira,

and Santa Maria, from depths of 140–280 m with a box corer as well as Henning- and Shipek-Grabs (George et al., 2018). Shallow-water sampling around the island of Santa Maria was carried out by SCUBA diving by L. Baptista and B. Berning at depths between 10 and 30 m in 2015 and 2019. All collected samples were preserved in ethanol >96% and stored at 4°C or below.

Voucher specimens of each Azorean lineage were dried and bleached overnight in 10% diluted household bleach to remove soft tissues for SEM inspection. This took place at the University of Vienna (Austria) with a JEOL JCM-6000Plus Benchtop, at the Natural History Museum London (NHMUK) with a LEO 1455VP and a JEOL IT500, at Senckenberg am Meer in Wilhelmshaven, Germany (SaM) and the Muséum National d'Histoire Naturelle in Paris, France (MNHN) with a Tescan VEGA SEM, and at CIBIO-Açores, Portugal with a Phenom Pro X. Dried reference material is kept at the institutes mentioned above as well as at the Biologiezentrum of the Oberösterreichische Landes-Kultur GmbH in Linz (OLL; collection Invertebrata except Insecta).

The morphology of historical type and topotypic material of Azorean *Reteporella* species was examined at the NHMUK and the MNHN. Putative Atlantic and Mediterranean species and lineages, as defined with molecular approaches and used for comparison, were identified using published literature (e.g. Hayward & McKinney, 2002; Hayward & Ryland, 1996, 1999). Species descriptions are not given here in detail but will be provided in future works in which the taxa are revised or newly introduced. The recently described *Reteporella azorensis* Souto, 2019 is not considered here, as its morphology (e.g. ovicell with a proximal fissure) suggests a placement in the genus *Schizoretepora* Gregory, 1893.

Molecular work

Total genomic DNA (gDNA) was extracted from clean colony fragments with the column-based commercial kit PureLink® Genomic DNA (Invitrogen™), following the manufacturer's instructions after homogenization of the mineralized skeleton with metallic pestles. DNA samples, in a final elution of 40 µL, were analysed using a Nanodrop® 2000 to assess DNA quality and quantity. DNA integrity was checked using agarose gel electrophoresis (0.8% w/v). PCR amplification of COI was performed in 25 µL volumes, using 3 µL of gDNA (some samples required 1:2 dilution), 10 x MgCl₂ free buffer, 2.5 mM MgCl₂, 0.2 mM dNTP, 10 µM of each primer, 0.1 µg/µL bovine serum albumin (BSA, Promega) and 0.3 U Platinum Taq DNA polymerase. Cycling conditions were as follows: 94°C for 5 minutes; 35 x (94°C for 30 seconds, 53°C for 45 seconds, 72°C for 1 minute); 72°C for 10 minutes. 16S and 28S markers were amplified in 20 µL

volumes, using 2 µl of gDNA, 10 µl of QIAGEN Multiplex PCR Master Mix (Qiagen, CA, USA), and 4 µl of each 2 µM primer. Cycling conditions were as follows: 95°C for 15 minutes; 35 x (95°C for 30 seconds, annealing for 1 minute, 72°C for 30 seconds); 72°C for 10 minutes. Details on the primers and annealing temperatures are presented at Table 5.1. A reverse COI primer (coiR_ret) was newly designed with Primer3 as implemented in Geneious 8.1.9 (Kearse et al., 2012) and checked for secondary structures with the web-based tool Netprimer (<http://www.premierbiosoft.com/netprimer/>). PCR products were evaluated using 2% (w/v) agarose gels with GelRed (DNA fluorescent dye, BioTarget™). Purification and bi-directional Sanger sequencing were performed by Genewiz (Azenta), Leipzig, Germany.

Table 5.1. Primers used for PCR amplification and sequencing of the markers COI, 16S, and 28S.

	Name	Sequence	T _{ann}	Reference
COI	cox1F_prifi	TTGRTTYTTTGGWCAYCCHGAAG	53°C	(Waeschenbach et al., 2012b)
	cox1R_prifi	TCHGARTAHCGNCGNGGTATHCC	53°C	(Waeschenbach et al., 2012b)
	coiR_ret	GCTAGHCCTAGGAARTGTTGA	53°C	This study
16S	16Sar-L	CGCCTGTTTATCAAAAACAT	52– 58°C *	(Palumbi et al., 1991)
	16Sbr-H	CCGGTCTGAACTCAGATCACGT	52– 58°C *	(Palumbi et al., 1991)
28S	28SC1	ACCCGCTGAATTTAAGCAT	55°C	(Dayrat et al., 2001)
	28SC2	TGAACTCTCTCTCAAAGTTCTTTTC	55°C	(Le et al., 1993)

* T_{ann} (annealing temperature) varied in this range for different species.

All chromatograms were inspected with Geneious v. 8.1.9. COI data were checked for premature stop codons and frameshift mutations using AliView v1.26 (Larsson, 2014). Living in close association with other biota complicates the production of *bona fide* genetic data for the target bryozoan species, inhibiting PCRs and/or causing amplification of non-target contaminant DNA (Orr et al., 2022; Waeschenbach et al., 2012b). Thus, contaminant sequences of dubious origin were identified and excluded from our datasets, based on nucleotide alignments and sequence identity searches with BLASTN (Zhang et al., 2000). All verified *bona fide* *Reteporella* sequences generated during this study were deposited on GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>; COI: OP070973 to OP071039, 16S: OP070973 to OP071039; 28S: OP070836 to OP070924). Publicly available mitogenomes and 28S sequences of several phidoloporids – *Reteporella beaniana* (King, 1846), *R. cf. grimaldii* (Jullien, 1903), *R. cf. graeffei* (Kirchenpauer, 1869), *R. tuberosa* Hayward, 2000, *Iodictyum yaldwyni* Powell, 1967, *Iodictyum violaceum* Hayward, 2004, *Hippellozoon novaezelandiae* (Waters, 1895), *Phidolopora avicularis* (MacGillivray, 1883), and *Triphylozoon arcuatum* (MacGillivray, 1889) – were added to the dataset; details are available in Supplementary Table A5.1.

Phylogenetic reconstructions

COI and 16S boundaries of unannotated mitogenome GenBank records were identified using the MITOS WebServer (Bernt et al., 2013) (<https://mitos.bioinf.uni-leipzig.de/index.py>). Alignments of rRNA genes were constructed using the MAFFT web server (Kuraku et al., 2013) with the Q-INS-I model, which considers secondary RNA structure (Kato & Toh, 2008), whereas for the protein-coding COI we used the Clustal Omega algorithm from Web Services by EMBL-EBI (McWilliam et al., 2013). MEGA11 (Tamura et al., 2021) was used to reduce alignments to single haplotypes, and to calculate uncorrected (p) distances. The best-fit partitioning schemes and models of molecular evolution were assessed with the software PartitionFinder v1.1.1 (Lanfear et al., 2012), following the Akaike Information Criterion (Akaike, 1973). To minimize the saturation effects of codon positions and to account for codon position-specific rates of molecular evolution, COI data were partitioned by codon position (Pond et al., 2009; Salemi, 2009). Poorly aligned positions in the rRNA gene alignments were removed with GBlocks v0.91b with default settings, available at <http://phylogeny.lirmm.fr/> (Castresana, 2000; Dereeper et al., 2008). The models of molecular evolution for the COI data were set as GTR+I (1st codon position), F81+I (2nd codon position), and GTR+G (3rd codon position), whereas GTR+I+G was the model chosen for the 16S and 28S markers. We used published data for *Celleporaria* cf. *fusca* (Busk, 1854) as the outgroup, as *Celleporaria* Lamouroux, 1821 was found to be the sister taxon to a derived clade of cheilostomes that included phidoloporids (Waeschenbach, unpublished data).

Phylogenetic analyses were conducted on single-gene partitions and the concatenated dataset (COI+16S+28S). Analyses were carried out using Bayesian inference (BI) and maximum likelihood (ML) methodologies. BI analyses were conducted in MrBayes v3.2.7 software (Ronquist et al., 2012). Two independent runs, each with four chains, were completed for 2×10^7 Markov Chain Monte Carlo (MCMC) generations. The heating chains parameter was set to 0.25 and the burn-in was 25%. Convergence between runs was determined once the average standard deviation of split frequencies was below 0.01. Trees and parameters were sampled every 1,000 generations. For the concatenated dataset, only BI analysis was performed. ML analyses were performed in W-IQ-Tree web server (Trifinopoulos et al., 2016), set for 1,000 ultrafast bootstraps (UFBoot) (Hoang et al., 2018), SH-aLRT branch tests (Guindon et al., 2010) for 1,000 replicates, and partitioned models (Chernomor et al., 2016) for the individual gene partitions. Trees were visualized, rooted, and edited with FigTree v1.4.3 software.

Species delimitation analyses using Bayesian implementation of the Poisson Tree Process model (bPTP; <https://species.h-its.org>) (Zhang et al., 2013) and a multi-rate PTP

(mPTP; <https://mptp.h-its.org/#/tree>) (Kapli et al., 2017) were carried out on the single locus COI maximum-likelihood tree. The bPTP analysis was carried out over 100,000 MCMC generations with a thinning of 100 and a 25% burn-in, whereas default settings were applied for the mPTP analysis. PTP approaches model speciation in terms of number of substitutions, such that more substitutions are expected in interspecific branching events (Zhang et al., 2013). The mPTP process is a recent improvement of PTP that incorporates intraspecific divergence caused by different evolutionary histories or sampling biases of the species (Kapli et al., 2017). To further evaluate the relationships among COI haplotypes and delineate putative species, a statistical parsimony haplotype network at the 95% connection limit was estimated with the software TCS v1.21 (Clement et al., 2000) and edited with tcsBU web-based program (Santos et al., 2016).

Results

Sequence data

The following *Reteporella* sequences were obtained: 109 COI sequences, ranging from 552-575 bp, comprising 41 haplotypes; 69 16S sequences, ranging from 372-458 bp, comprising 21 haplotypes; 92 28S sequences 334 bp long, comprising 6 haplotypes. Lineage-specific mutations were detected in both the 16S and 28S data, including indels in the former. The delimitation of putative species was based on the comparison of results from the analyses of molecular data (phylogenies, haplotype network, and genetic distances). Morphological characters (see morphological analysis' section below) allowed the validation of molecular lineages, identified as groups with COI genetic distances of over 3% and forming separated haplotype networks (indicated in Figures 5.1 and 5.2).

The non-monophyly of the genus *Reteporella* is evident in the phylogenetic reconstructions, with *R. tuberosa* being more closely related to other phidoloporiid genera, although its placement was variable or poorly supported (Figure 5.1; Supplementary Figures A5.1 and A5.2). The concatenated phylogenetic reconstruction recovers the Azorean taxa as a paraphyletic assemblage to the inclusion of the Mediterranean, Australian, and North Atlantic taxa (Figure 5.1). A clade composed of the Azorean taxa *R. tristis* (Jullien, 1903) and *Reteporella* sp. 1 forms the sister group to the remaining taxa. *Reteporella tristis* is represented by samples from Santa Maria and São Miguel islands, as well as Sabrina seamount (eastern group of islands) and Terceira (central group of islands). Its sister lineage, *Reteporella* sp. 1 from Terceira, which is distinguished from other *Reteporella* species by genetic distances ranging from 9.7–21%

(COI), 2.6–20.8% (16S) and 0.3–1.5% (28S), is regarded as a putative new species (see also morphological analysis).

Among the remaining *Reteporella* taxa, the largest clade is composed of representatives of *R. atlantica* (Busk, 1884) that originated from all three island groups and four seamounts. Despite relatively low levels of genetic divergence within this group [2.1% (COI), 0.2% (16S), 0.3% (28S); Supplementary Figures A5.1–3], samples from the easternmost islands of Santa Maria and São Miguel form a distinct clade amongst samples from more western localities (Figure 5.1, Supplementary Figure A5.1). *Reteporella oceanica* (Jullien, 1903) from Condor Bank and *Reteporella* sp. 5 from São Miguel form a poorly-supported and well-supported clade in the concatenated (Figure 5.1) and COI analyses (Supplementary Figure A5.1), respectively. These two taxa are part of a polytomy with *R. atlantica*, *Reteporella* sp. 7 from Santa Maria, São Miguel, Formigas (eastern island group) and Gigante Bank, and *Reteporella* sp. 6 from Terceira and Gigante Bank. The COI sequence divergence between *Reteporella* spp. 6 and 7 is 5.7–6.3%.

Among the non-Azorean *Reteporella* taxa (Figure 5.1), a clade is recovered composed of the North Atlantic *R. beaniana* (King, 1846) and three Mediterranean taxa, from Spain (*R. cf. grimaldii*), Corsica (*Reteporella* sp. 2), and Croatia (*Reteporella* sp. 3). Genetic divergence between *R. cf. grimaldii* and *Reteporella* spp. 2 and 3 vary according to the marker [4.2–6.2% (COI), 0.4–0.8% (16S), and 0% (28S)]. The Australian *R. cf. graeffei* clusters with the Mediterranean *Reteporella* sp. 4 with near-zero branch lengths (but see Discussion), forming a sister clade to the larger Azorean clade composed of *R. atlantica*, *R. oceanica*, and *Reteporella* spp. 5–7. The interrelationships of the non-*Reteporella* phidoloporid taxa are mostly well-supported, except for the sister-group relationships between *R. tuberosa* and *Triphyllozoon arcuatum* (MacGillivray, 1889), and between *Hippellozoon novaezelandiae* (Waters, 1895) and (*Phidolopora avicularis* (MacGillivray, 1883) + *Schizoretepora* sp.).

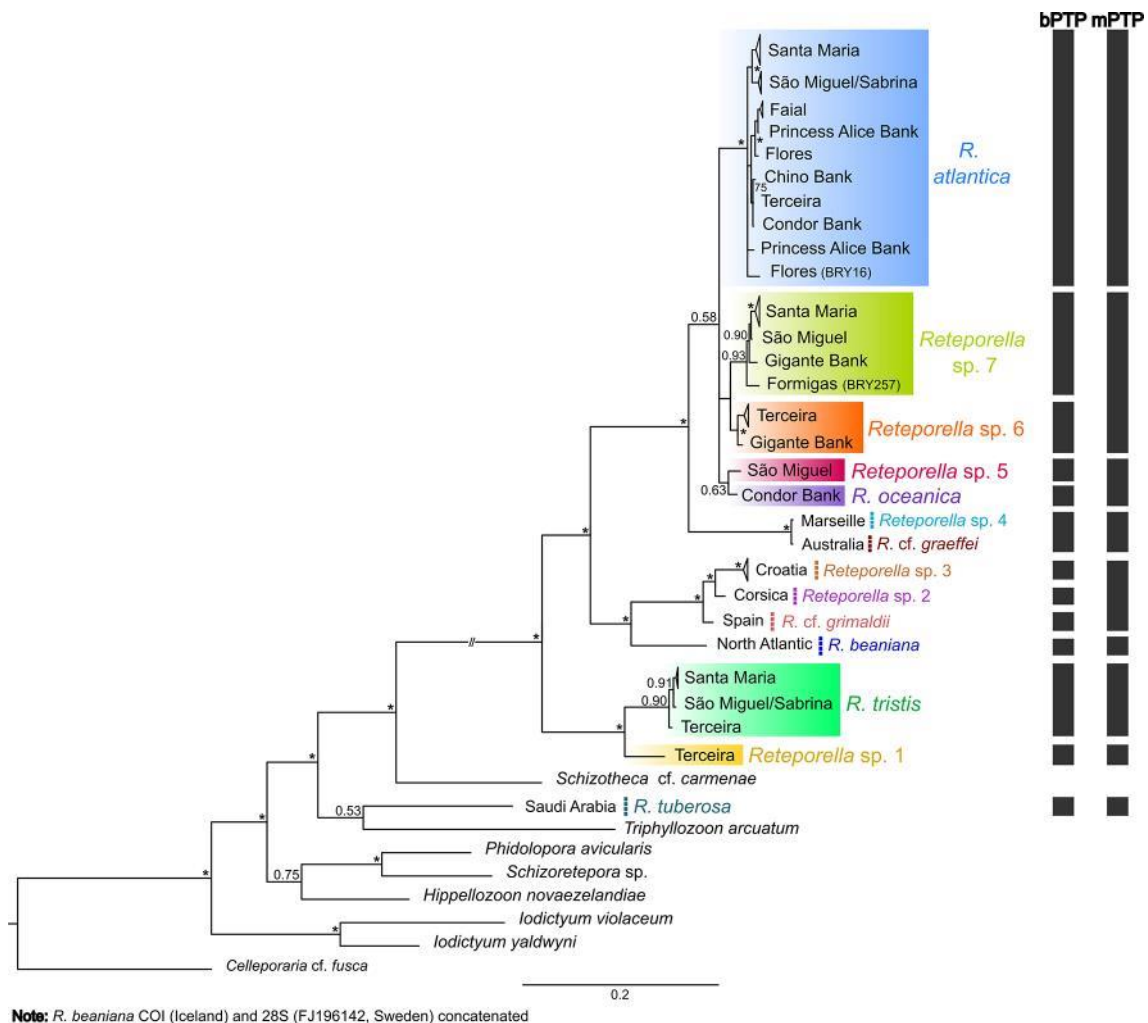


Figure 5.1. Bayesian inference analysis of the concatenated dataset (COI+16S+28S). Constructed using MrBayes v3.2.7 software (Ronquist et al., 2012) under the following models of nucleotide evolution: GTR+I, F81+I+G, GTR+G for the 1st, 2nd, and 3rd codons of the COI, and GTR+I+G for both 16S and 28S markers. Values at the nodes correspond to posterior probability (PP); asterisk (*) indicates well-supported nodes (PP ≥ 95%). Known and putative new Azorean *Reteporella* species are indicated with different coloured shading. Non-Azorean *Reteporella* species are indicated by dashed coloured lines. Geographical origins of *Reteporella* samples are given as terminal labels. Other phidoloporids included in the analyses are identified with colour coding. The scale bar represents substitutions per site. *Reteporella* species boundaries inferred with bPTP and mPTP approaches, conducted on the single locus COI maximum-likelihood tree, are plotted on the right-hand side

Genetic divergence between the three large Azorean/European clades [(*Reteporella* sp. 1 + *R. tristis*), (*R. beaniana* + *R. cf. grimaldii* + *Reteporella* sp. 2 + *Reteporella* sp. 3), (*R. atlantica* + *Reteporella* spp. 5-7)] is high in all the markers studied [maximum of 21% (COI), 10% (16S), 1.5% (28S)]. The maximum COI divergences among *Reteporella* species are of the same magnitude as those among other phidoloporid genera, ranging from 17% (*Schizoretopora* vs. *Phidolopora*) to 24.6% (*Schizoretopora* vs. *Iodictyum*). Among non-*Reteporella* genera, 16S divergences ranged from 5.5% (*Hippellozoon* vs.

Phidolopora) to 13.2% (*Triphyllozoon* vs. *Iodictyum*). The 28S divergences ranged from 3.6% (*Triphyllozoon* vs. *Iodictyum*) to 7.3% (*Phidolopora* vs. *Iodictyum*) (Supplementary Table A5.2).

The two tree-based species delimitation methods produced conflicting results, with bPTP inferring 13 *Reteporella* species and mPTP only 9 (Figure 5.1). The mPTP analysis considers the following taxa as one species: *Reteporella* sp. 6 + sp. 7; *R. oceanica* + *Reteporella* sp. 5; *Reteporella* sp. 4 + *R. cf. graeffei*; *R. cf. grimaldii* + *Reteporella* sp. 2 + *Reteporella* sp. 3. However, the bPTP results matched the morphological distinction of species (see below), except for *Reteporella* sp. 4 and *R. cf. graeffei*, which are regarded as the same taxon (see Discussion).

Single markers vary in their performance in recovering genetic lineages, although no major conflicts are found in the relationships inferred (Supplementary Figures A5.1–S3). The mitochondrial COI phylogeny has 23 well-supported nodes in both ML and BI inferences among the *Reteporella* clades, whereas 16S retrieves only five splits supported by ML and BI and two just in the ML reconstruction. Regarding the 28S, BI failed to resolve the tree and ML reconstructions were poorly supported for most splits. Despite the overall high support, COI does not fully resolve the polytomy of the crown group, formed by *R. atlantica*, *R. oceanica*, and *Reteporella* spp. 5–7. The specimen from Formigas (BRY257) diverges, on average, by 3.4% from *Reteporella* sp. 7 and by 6.3% from *Reteporella* sp. 6 in the COI marker (Supplementary Table A5.2 and Figure A5.1). In the 16S dataset, the similarity is reversed, with a closer relationship with *Reteporella* sp. 6 (0.9%) than with *Reteporella* sp. 7 (1.2%) (Supplementary Table A5.2 and Figure A5.2).

Haplotype network

The TCS network of COI haplotypes (Figure 5.2) was used to delineate putative species and evaluate intraspecific haplotype diversity (Hart & Sunday, 2007), as it was the most informative marker. Overall, haplotypes are distributed linearly and a high proportion of unsampled haplotypes occur in the dataset. In accordance with the terminal branches of the phylogenetic reconstruction, each cluster is considered to be a putative species, such that the analysis distinguishes five formal species (*R. atlantica*, *R. tristis*, *R. oceanica*, *R. beaniana*, *R. cf. grimaldii*) and seven species that cannot be attributed to any known species (Figure 5.1, Supplementary Figures A5.1–3).

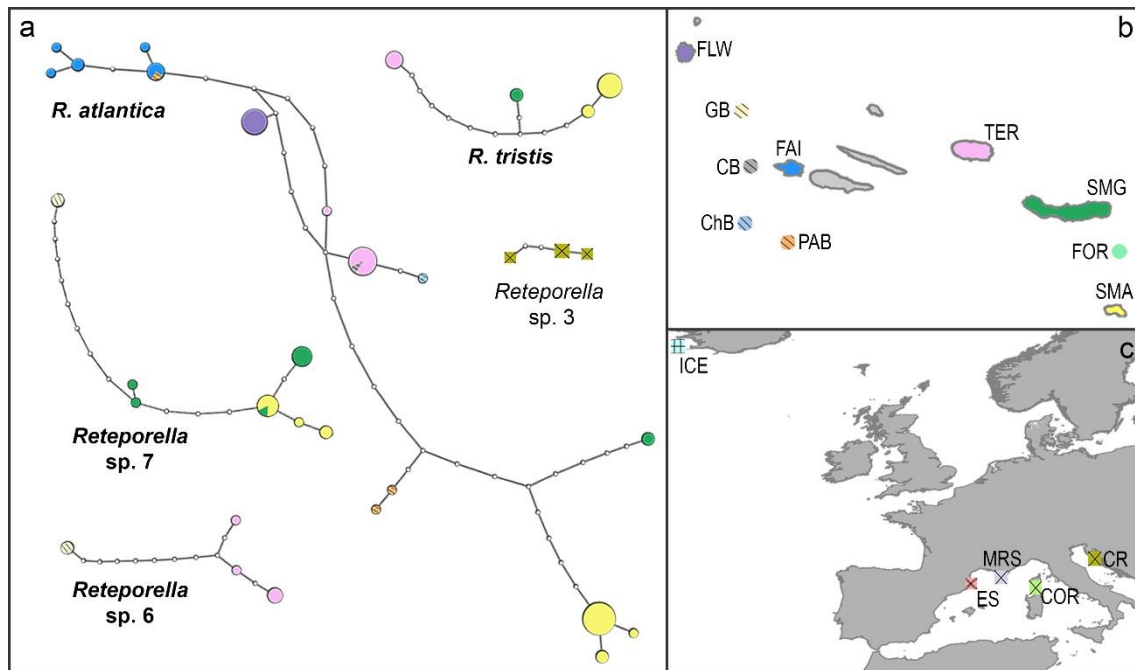


Figure 5.2. COI haplotype network and geographic distribution of *Reteporella* haplotypes. **a.** Network reconstruction at 95% parsimony connection limit based on 97 COI sequences and 33 haplotypes, representing two known *Reteporella* species and three putative new species. BRY16 and BRY257 are recognised as isolated clusters. Species represented by a single haplotype and/or individual [*R. oceanica*, *R. beaniana*, *Reteporella* spp. 1 and 5 (NE Atlantic), *R. cf. grimaldii*, *Reteporella* spp. 2 and 4 (Mediterranean), *Reteporella cf. graeffei* (Australia) and *R. tuberosa* (Saudi Arabia)] are not included in the dataset. Azorean haplotypes are represented by circles and taxon names in bold, whereas other Mediterranean haplotypes are represented by squares. Size of the circles and squares is proportional to the frequency of each haplotype; small uncoloured circles represent non-observed haplotypes; each line connecting haplotypes represents a single mutational change. Network obtained with TCS v1.21 (Clement et al., 2000) and tcsBU (Santos et al., 2016); **b.** Populations in the Azores are colour coded according to their geographical origin (map not to scale); **c.** Populations in the North Atlantic and Mediterranean Sea are colour coded according to their origin. Populations labelled as follows: [SMA (Santa Maria), SMG (São Miguel/Sabrina), TER (Terceira), FAI (Faial), FLW (Flores), FOR (Formigas), PAB (Princess Alice Bank), ChB (Chino Bank), CB (Condor Bank), GB (Gigante Bank), ICE (Iceland), ES (Spain), MRS (Marseille), COR (Corsica), CR (Croatia)]

In general, a geographical pattern is observed in each cluster, with most haplotypes restricted to a single sample site (private haplotypes). Exceptions are found in two *R. atlantica* haplotypes, shared by Faial and Princess Alice Bank as well as by Terceira and Condor Bank, respectively. Furthermore, one haplotype in *Reteporella* sp. 7 is present in both Santa Maria and São Miguel/ Sabrina). The genetic diversity within *R. atlantica* is depicted in a broad network, with eastern localities (Santa Maria and São Miguel/ Sabrina) at one end and samples from Faial at the other. The haplotypes represented by BRY16 (*R. atlantica*, Flores) and BRY257 (*Reteporella* sp. 7, Formigas; see Identification of Morphospecies section below) form isolated clusters, denoting their differentiation from

putative conspecifics. Finally, the Mediterranean samples are differentiated in four distinct clusters, according to their geographical origin and assignment to putative species (*R. cf. grimaldii* and *Reteporella* spp. 2–4).

Morphological investigation of *Reteporella* species

The identification of the newly collected and sequenced *Reteporella* specimens is based on an analysis of historical type and comparative material collected in the Azores during the late 19th century (e.g. Busk, 1884; Calvet, 1931; Jullien & Calvet, 1903), as well as during the second half of the 20th century (e.g. d'Hondt, 1975). A full revision of the established species (*R. atlantica*, *R. gracilis*, *R. oceanica*, *R. rara*, and *R. tristis*) is beyond the scope of the present paper and will be given elsewhere.

Reteporella tristis

Reteporella tristis was described from eastern Pico and is characterised by exclusively forming oval avicularia in the suboral position, as well as on the frontal surface (though very rarely) and on the abfrontal side of the colony (Figure 5.3a-c). The most conspicuous character, however, is a large spatulate avicularium that is positioned in the proximal axis of some of the fenestrae (Figure 5.3b). Sequenced specimens from Santa Maria and Terceira, which form one of the two *Reteporella* clades that are sister to the remaining Atlantic, Mediterranean and Azorean ones (Figure 5.1), are attributed to this species, as are additional colonies from São Miguel and Princess Alice Bank that were examined using SEM. All of the specimens, which were recovered from depths between 150 and 1,300 m, are morphologically very similar.

Reteporella oceanica

The identity and morphology of *Reteporella oceanica*, which was originally described for specimens from both the Azores (around Pico) and the Bay of Biscay, is unclear at present. Its type material could not be studied as syntypes are not present at the MNHN, while those available at the Musée Oceanographique de Monaco are not permitted to be taken on loan. Topotypic material from Pico subsequently identified as *R. oceanica* by Calvet (1931) was analysed instead. The species only forms small oval suboral avicularia (Figure 5.3d), while this avicularium type is also occasionally present on the abfrontal colony surface, albeit of slightly larger size. Specifically characteristic is a triangular frontal and abfrontal avicularium that is distinctly larger than in the other species described below, and in which the palate is extensively calcified so that the opesia distal to the crossbar is reduced to three small, subrounded foramina (Figure 5.3e), whereas

in all other species it is Y-shaped. This avicularium morphotype is in agreement with two sequenced specimens from Condor Bank that forms a clade together with *Reteporella* sp. 5 in the phylogenetic tree (Figure 5.1). Pending a revision of the species and designation of a lectotype, we here tentatively assign the sequenced specimens and the topotypic material to *R. oceanica*, which is recorded from Pico and Condor Bank in depths between 350 and 455 m.

Reteporella atlantica

Reteporella atlantica, originally described from off northern Pico, is characterised by the presence of three different morphotypes of suboral avicularia: a small oval one, a triangular one of intermediate size (Figure 5.3f), and occasionally a giant avicularium with its cystid covering the entire frontal shield of the zooid. While the two smaller types are most frequently observed, the zooids of large parts of a colony may form only one of the types. The giant avicularium apparently occurs only in colony regions with ovicellate zooids, but not all ovicellate zooids form the giant avicularium, and it is also not present in all fertile colony regions or even in entire colonies, such as the ones sequenced herein. Small oval as well as larger elongate triangular avicularia may additionally be budded on the zooid's frontal surface, particularly during later ontogeny. These two types also occur on the abfrontal side of the colony, while the size of the triangular avicularia is generally slightly larger there (Figure 5.3g). *Reteporella gracilis* from off eastern Pico is morphologically very similar to, and cannot be distinguished from, *R. atlantica* at present. The species are likely synonymous.

Most of the analysed specimens of the crown group of Azorean *Reteporella* belong to this morphospecies (Figures 5.1 and 5.2), and the species was found in all three groups of islands and on several seamounts, ranging in depths between 11 m (in Santa Maria) and 820 m (São Jorge Channel, downslope transport cannot be discarded). The single specimen from Flores (BRY16), which shows a higher genetic differentiation, does not display any apparent morphological deviations from the remaining colonies.

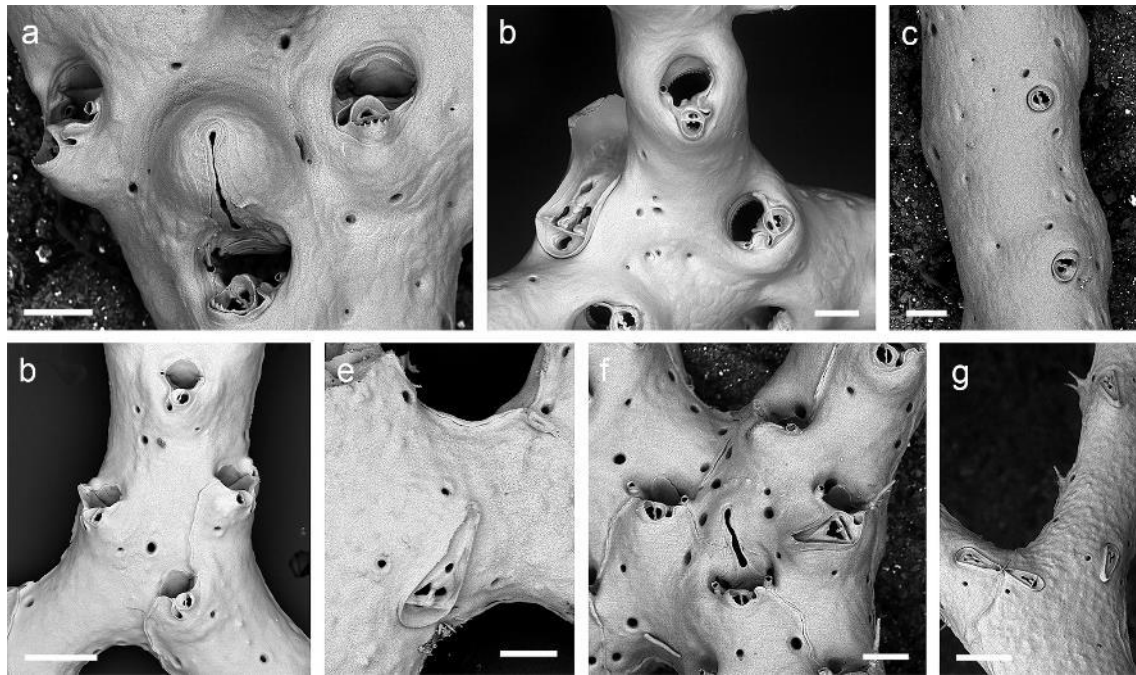


Figure 5.3. SEM images of sequenced *Reteporella* species from the Azores. **a.** *Reteporella tristis*, BRY127, Terceira, 152 m, scale bar 100 μ m; **b.** *Reteporella tristis*, M151-50341, São Miguel, 314 m, scale bar 100 μ m; **c.** *Reteporella tristis*, BRY127, Terceira, 152 m, scale bar 100 μ m; **d.** *Reteporella oceanica*, DOP9869, Condor Bank, 455 m, scale bar 200 μ m; **e.** *Reteporella oceanica*, DOP9869, Condor Bank, 455 m, scale bar 100 μ m; **f.** *Reteporella atlantica*, MB6, Santa Maria, 25 m, scale bar 100 μ m; **g.** *Reteporella atlantica*, BRY139, Terceira, 152 m, scale bar 200 μ m

Reteporella rara

Reteporella rara is easily distinguished from the other above-mentioned Azorean species by its reticulate surface structure and a significantly larger oval suboral avicularium, among several other differences. None of the recently sampled and sequenced specimens exhibit these features.

Thus, four of the Azorean lineages in the concatenated tree are morphotypes that are distinct from the established species and are regarded as putative new species (*Reteporella* spp. 1, 5, 6 and 7).

***Reteporella* sp. 1**

The single sequenced specimen from Terceira that is sister clade to *R. tristis* (Figure 5.1) produces an open-branched colony, i.e. it is not reteporiform as in other *Reteporella* species (Figure 5.4a-b). Its branches are also rather cylindrical while in most *Reteporella* colonies the abfrontal kenozooidal side is somewhat flattened, and the oval suboral avicularium is pointing proximomedially, not proximolaterally as in all others. In contrast to *R. tristis*, the Terceira specimen also forms triangular avicularia on its frontal and abfrontal surfaces. One character in common with that species, however, is the very

short pseudosinus next to the suboral avicularium. The open-branched mode of growth observed in the specimen is unique among other Azorean and European *Reteporella* species. Increased sampling effort in the Azores, and particularly in the type locality, is needed to obtain more individuals for a more complete morphological characterization and evaluation of the geographical and bathymetrical distribution of the new taxon, which was recovered from 277 m depth.

***Reteporella* sp. 5**

Specimens of *Reteporella* sp. 5 (Figure 5.4c-d), which form a clade together with *R. oceanica* (Figure 5.1), were only found off São Miguel at 310 m depth. The colonies are characterised by producing swollen cystids on the abfrontal side on which a large triangular avicularium is obliquely placed (Figure 5.4d). Frontal and abfrontal avicularium morphology is similar to the one in *R. oceanica*, i.e. with three small, subrounded foramina in the palate, while being slightly smaller in general and the suboral avicularia are also exclusively of the small oval type (Figure 5.4c).

***Reteporella* sp. 6**

The clade comprises populations from Terceira and Gigante Bank, sampled from 150 m and 450 m depth, respectively (Figures 5.1 and 5.2). The colonies almost exclusively form oval suboral avicularia as well as small oval and larger triangular avicularia on the frontal and abfrontal surfaces (Figure 5.4e-f). However, between the populations there are some differences in, among others, the morphology of the triangular avicularia (they are smaller and the opesia distal to the crossbar is wider and rounder in Gigante Bank specimen, whereas it's narrower and V-shaped in the colonies from Terceira), and in the position of the ovicell relative to the peristome (the labellum is positioned well above the proximal peristomial margin in the Gigante Bank colonies whereas in the Terceira specimens the labellum is longer and ends below the level of the peristome). The COI divergence levels of 1.5–2% between the two populations thus seem to be reflected in their morphologies.

On the other hand, *Reteporella* sp. 6 is distinguished from *R. atlantica* only by the lack of giant avicularia (as far as we can say from the available material). As entire colonies of *R. atlantica*, or at least large parts thereof, are also missing this type of avicularium, the two species may, despite the considerable divergence of 6.2–10.1% in the COI marker, be morphologically indistinguishable if only non-fertile colonies are available for study.

***Reteporella* sp. 7**

The specimens are morphologically very close to *R. atlantica*. Both oval and triangular suboral avicularia are, however, generally smaller in this group, while pseudosinus length and fenestra size are distinctly greater than in *R. atlantica* (Figure 5.4g-h). Giant avicularia are apparently absent in *Reteporella* sp. 7, and the triangular frontal and abfrontal avicularia are further characterised by a wider uncalcified palatal area distal to the crossbar, whereas in *R. atlantica* its shape is distinctly trifoliate. Most of the analysed specimens were found off São Miguel (310 m) and Santa Maria (150 m), while a single specimen is from Gigante Bank (495 m). Paralleling the molecular results, specimen BRY257 from Formigas (150 m) stands out in having a pseudosinus that is approximately only half the length than in the other colonies, and also in having distinctly smaller fenestrae. In contrast, the triangular frontal and abfrontal avicularia are larger.

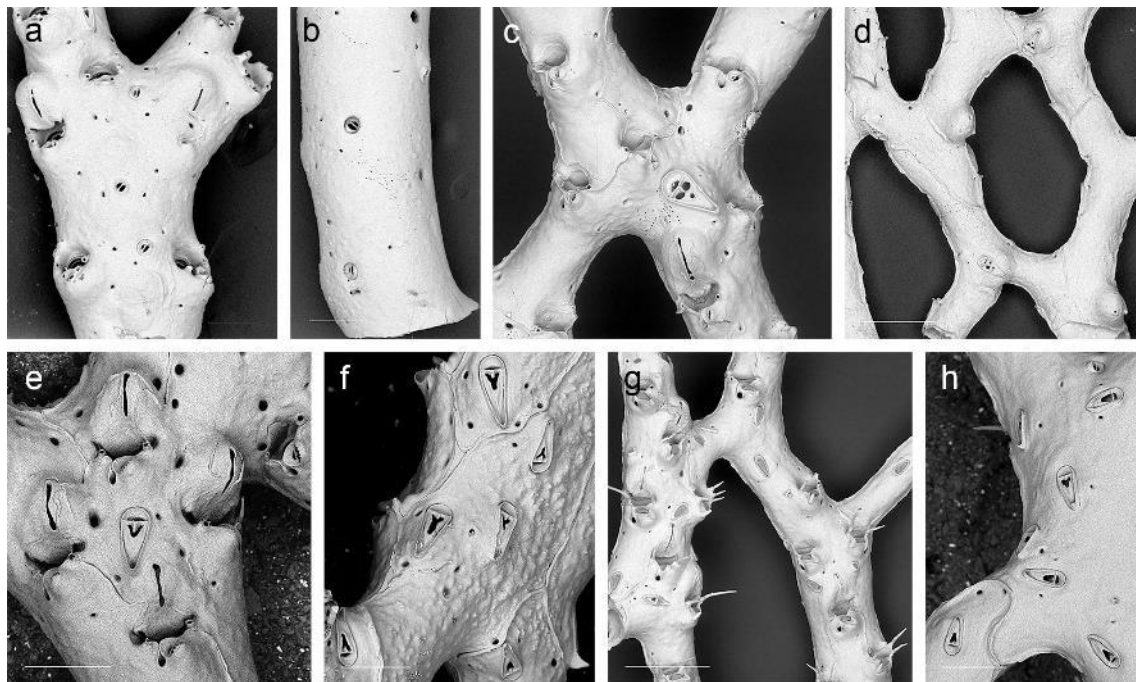


Figure 5.4. SEM images of sequenced *Reteporella* species from the Azores. a-b. *Reteporella* sp. 1, BRY116, Terceira, 277 m, scale bars 200 µm; c. *Reteporella* sp. 5, M151-50333, São Miguel, 311 m scale bar: 200 µm; d. *Reteporella* sp. 5, M151-50333, São Miguel, 311 m scale bar: 500 µm; e-f. *Reteporella* sp. 6, BRY146, Terceira, 152 m, scale bars 200 µm; g. *Reteporella* sp. 7, M151-50332, São Miguel, 310 m, scale bar 500 µm; h. *Reteporella* sp. 7, M151-50332, São Miguel, 310 m, scale bar 200 µm

Non-Azorean species

The specimen from Iceland is identified as *Reteporella beaniana* (King, 1846) based on comparisons with the description and images provided by Hayward & Ryland (1996) (Figure 5.5a).

Sister clade of *R. beaniana* is a Mediterranean group of three species that are characterised by triangular suboral avicularia and the occasional presence of a giant suboral avicularium similar to *R. atlantica* whereas small, oval, suboral avicularia are absent. This morphotype has previously been assigned to *R. grimaldii* (e.g. Hayward & McKinney, 2002), which was originally described from the Bay of Biscay. The specimen from Spain was sequenced by Orr et al. (2022) as *R. cf. grimaldii* and we have here adopted that assignment. SEM images of the specimen (BLEED 1851), and all others sequenced by Orr et al. (2022) that are included in our analysis, are available from Zenodo (<https://zenodo.org/record/5721078#.YZz39VMofY>). It is further characterised by the presence of small triangular frontal avicularia. The specimen from Corsica (*Reteporella* sp. 2) is distinguished by having rounded frontal avicularia that are usually slightly narrowing distally (Figure 5.5b). Differences between this species and the Croatian colonies (*Reteporella* sp. 3, Figure 5.5c) are less obvious: for instance, in the latter the small frontal avicularia are usually widening distally and the ovicells produce a larger labellum. All Mediterranean specimens are, however, very likely specifically distinct from the nominal species. The peculiar thickening at the base of the ovicell, which is well visible in the holotype of *R. grimaldii* (see Reverter-Gil & Fernández-Pulpeiro, 1999: fig. 5D, E), is missing in the Mediterranean colonies or at least not visible in frontal view.

The voucher specimen from Marseille (*Reteporella* sp. 4) lacks any frontal avicularia, and has, besides oval suboral avicularia and relatively large areolar pores of variable shape, no distinct features (Figure 5.5d). It cannot be attributed to any known species at present. It is genetically almost identical with, and morphologically very similar to, the specimen sequenced and identified as *Reteporella cf. graeffei* by Orr et al. (2022) (BLEED 1059), which is, however, supposedly from Australia. *Reteporella tuberosa* from Australia (BLEED 420B) is genetically significantly different from other species of the genus and in need of revision.

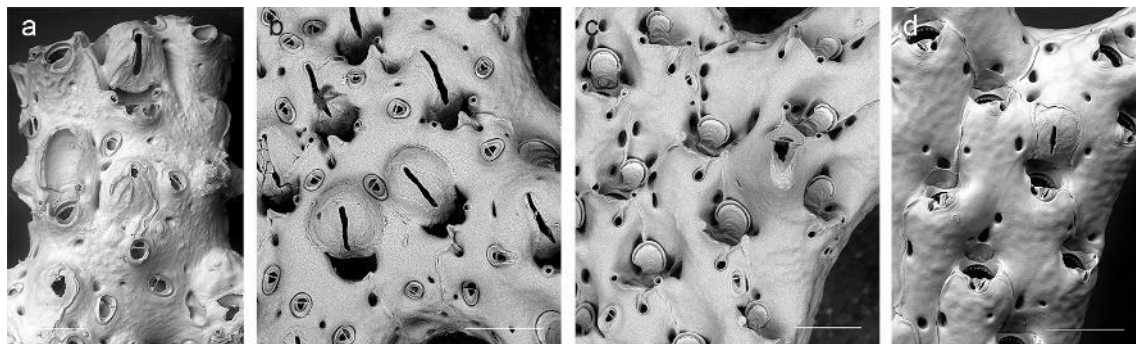


Figure 5.5. SEM images of sequenced *Reteporella* species from the NE Atlantic and Mediterranean Sea. a. *Reteporella beaniana*, NHMUK 2022.5.17.1, SSW Iceland, scale bar 200 μ m; b. *Reteporella* sp. 2,

DBUA-BRY 68, Gulf of Galéria, Corsica, 80 m, scale bar 200 μm ; **c.** *Reteporella* sp. 3, DBUA-BRY 64, Pula, Croatia, 5.2 m, scale bar 200 μm ; **d.** *Reteporella* sp. 4, AW006, Marseille (France), 10-12 m, scale bar 200 μm

Discussion

Oceanic islands that originate *de novo* from volcanic eruptions, which allow for a precise estimate of their origin via radiometric dating, are ideal places to study patterns and processes of evolution ever since Darwin. Depending on their distance to other islands or the continental shelf, colonisation of oceanic islands may prove difficult, not just for terrestrial but also for marine organisms, particularly those with short-lived larvae (Scheltema, 1989). Among the many dispersal strategies of organisms with non-planktotrophic larvae (for a review see Winston, 2012), which include all brooding bryozoans, rafting is the most relevant for epibenthic shallow-water taxa in temperate Atlantic regions (Ávila et al., 2012, 2019; Scheltema, 1986). Adding to this difficult quest, the Azores Archipelago sits in a particular geographic and oceanographic setting in the middle of the North Atlantic, with prevailing eastwards-flowing sea-surface currents (see Baptista et al., 2021a for details). It is remarkable that, despite the Gulf Stream and similarly to other marine and terrestrial taxa, non-planktotrophic Azorean bryozoans have their closest relatives in the NE Atlantic or the Mediterranean Sea, providing another example of the so-called “Azorean Biogeographical Paradox” (Ávila, 2006, 2013; Micael et al., 2019). Dispersal limitations imposed by distance and short-lived larvae would be expected to cause a low bryodiversity in the Azores, although if isolated long enough insular populations may evolve into new species. The Azores Archipelago, with its numerous islands and varying distances to each other, may be regarded as being particularly prone to adaptive radiations. One would, therefore, expect that the islands had originally been colonized by a few shallow-water taxa, more prone to chance events of dispersal, which have then diversified and spread into deeper waters, producing a larger number of endemic species. Its proximity to the mid-Atlantic ridge as well as to older seamounts in the region (e.g. the Meteor-Atlantis seamount complex in the south), may offer additional source regions for bathyal taxa.

Therefore, by extending the previously limited sampling of bryozoans in the Azores, and by the application of genetic data coupled with state-of-the-art taxonomic criteria and imaging methodologies, more species than hitherto recognised might be revealed, including unknown native and endemic taxa (Micael et al., 2019).

Marker performance and concordance with morphospecies

To examine the diversity of the genus *Reteporella* in the Azores we performed a multi-marker phylogenetic reconstruction complemented with a morphological characterization of the putative species defined. The mitochondrial COI is the marker performing best in the phylogenetic reconstructions and contributes to most of the phylogenetic signal of *Reteporella* species. The region of the 28S marker analysed appears to have a low differentiation power of *Reteporella* diversity, and has previously been pointed out as poorly informative (Fuchs et al., 2009; Orr et al., 2019). The genetic groups identified with 28S are consistent with the major mitochondrial lineages and morphospecies of *Reteporella*, with diagnostic point mutations across its 334 bp. The poor performance of this marker has been noted in other genera, such that another region of 28S or a different nuclear marker is required to increase the phylogenetic signal and resolve the nuclear phylogeny of *Reteporella* (Eberle et al., 2020; Knight et al., 2011; Orr et al., 2019).

Except for *Reteporella* sp. 6, which is morphologically extremely similar to *R. atlantica* and may be regarded as a cryptic species, species boundaries recovered with bPTP match the morphospecies defined in this study, whereas mPTP underestimates the number of lineages by clustering taxa that have been distinguished by morphological characters and mitochondrial divergence (e.g. *R. cf. grimaldii* from Spain, *Reteporella* sp. 2 from Corsica, and *Reteporella* sp. 3 from Croatia). Incongruent performance of these tree-based species delimitation methods has been previously linked to higher substitution rates, unevenness of sampling, different population sizes among species, ongoing gene flow, or unresolved nodes, leading to over-splitting or over-lumping of the taxa (Wang et al., 2022).

In understudied organisms, such as bryozoans and many other marine phyla, DNA barcoding is an important tool to reveal hidden biodiversity, particularly cryptic species and sister taxa with overlapping geographic ranges. It can also be used to test the extent of phenotypic variability among individuals genetically assigned to the same taxon and the species status (Gómez et al., 2007a), as is the case of *R. atlantica* in this study. Ultimately, it reveals overlooked taxa in repositories, such as in Macaronesian marine macroinvertebrates (Desiderato et al., 2019; Vieira et al., 2021), indicating the necessity for taxonomic revisions. In these poorly known groups, however, the barcoding task can be hampered by unknown levels of DNA sequence divergence, which vary greatly among taxa. Defining this “barcode gap” is challenging and implies a priori knowledge of morphology and phylogenetic relationships of the studied group, otherwise it can lead to over- or underestimation of the real diversity present in the dataset (Gómez et al., 2007b; Meyer & Paulay, 2005; Orr et al., 2019, 2021).

Of the markers analysed, the mitochondrial COI seems to be the best at delimiting genetic lineages and putative species. In the haplotype networks observed in our dataset, the separation of clusters at the 95% connection limit has been suggested by some to indicate deep differences and putative new species (e.g. Hart & Sunday, 2007). Such interpretations have to be applied cautiously, as the overall molecular divergence levels in bryozoans are high and often do not obey the traditional clustering threshold of 97% identity for species (Gómez et al., 2007a; Lee et al., 2011; Orr et al., 2021). Among *Reteporella*, the overall concordance between molecular analyses, species delimitation analyses with the bPTP algorithm, and morphospecies inferred in this study suggests the potential adequacy of the 3% COI cut-off in this group (Hebert et al., 2003, 2004; Meyer & Paulay, 2005).

In recent years a wave of criticism has grown regarding the improper use of DNA barcoding approaches alone to formally recognize species without further evidence from morphology or ecology (see Ahrens et al., 2021; Carstens et al., 2013; Eberle et al., 2020). Therefore, an integrative approach that encompasses molecular, morphological, and even ecological data is regarded as the future of taxonomical descriptions (Ahrens et al., 2021; Knight et al., 2011). This is especially important for cheilostome bryozoans, considering that this speciose order is the result of a rapid radiation starting in the mid-Cretaceous (Taylor, 1988), which can obscure the phylogenetic signal of extant divergences (Knight et al., 2011). Due to the high diversity of phenotypes and genotypes, relying on molecular analysis as a backbone to identify genetic lineages for subsequent morphological comparisons and characterization has been shown to be a fruitful approach in cheilostome bryozoans (Dick & Mawatari, 2005; Gómez et al., 2007b; Orr et al., 2019, 2021; Waeschenbach et al., 2012a, 2012b). In this study, we report the mutual support between morphospecies and genetic species in seven lineages and four established *Reteporella* species, following findings in other cheilostomes, namely *Stylopoma*, *Rhynchozoon*, *Celleporella*, among others (Dick & Mawatari, 2005; Gómez et al., 2007b; Hughes et al., 2008; Jackson & Cheetham, 1990; Orr et al., 2021). On the other hand, within certain *Reteporella* species, some well-supported branches do show morphological character differences that distinguish populations, while other genetically distinct populations are morphologically identical (see below). Therefore, we reinforce that molecular approaches have the potential to reveal unknown diversity, although morphological characterization and taxonomic validation by experts is mandatory. The idea that new morphospecies tend to be overlooked by classical taxonomy is often not true in understudied organisms, but rather a consequence of the low number of taxonomists specialized in these groups. Nowadays it certainly seems that molecular

analyses provide faster and cheaper results to uncover biodiversity, but it is the unbalance between the number of molecular biologists and classical taxonomists that hamper the complete description of diversity in these poorly studied groups (Wägele et al., 2011).

Phylogeny of the Phidoloporidae

Despite the low resolution of 16S and 28S markers, no major conflicts are found between the gene trees, each marker contributing to the overall topology of the concatenated tree. Inconsistent placement of taxa and low support of node split among the gene trees are expected, as the markers are likely to have distinct evolutionary rates (Knight et al., 2011). The low accuracy of the reconstruction can also be related to the outgroup choice (*Celleporaria* cf. *fusca*), which is often problematic as it is limited to the existing phylogenetic frameworks and sequence data available in GenBank. Nevertheless, the inclusion of phylogenetically closer phidoloporids did not further improve the support of the phylogenies. Albeit a detailed interpretation of the relationships with other phidoloporid taxa is out of the scope of this study and will be given elsewhere, we remark that the clades inferred here are in accordance with Orr et al. (2022).

The addition of non-*Reteporella* phidoloporid taxa to the dataset allowed the estimation of high intergeneric genetic distances within the family (17-25% in COI). Similar divergence levels were inferred between members of the genus *Reteporella*. This is especially true for the Atlantic and Mediterranean *Reteporella* lineages [(*Reteporella* sp. 1 + *R. tristis*), (*R. beaniana* + *R. cf. grimaldii* + *Reteporella* sp. 2 + *Reteporella* sp. 3), (*R. atlantica*, *Reteporella* spp.5-7)], with divergence levels ranging from 16 to 20% that suggest a considerable independent history. Such high levels of divergence hint either at the need to split *Reteporella* clades into several new genera, as generically distinct yet morphologically similar taxa seem to be currently classified under the same rank, or to lump other phidoloporids into the same genus. Phylogenetic reconstructions of the family Phidoloporidae, as well as morphological and morphometric analyses, are needed to support the reclassification and define diagnostic characters.

The genus *Reteporella* in the Azores

Diversity

Of the four established Azorean *Reteporella* species, three were studied – *R. atlantica*, *R. oceanica*, and *R. tristis* – being *R. gracilis* likely a junior synonym of *R. atlantica* and the *R. rara* morphotype not among the sampled specimens. Besides the

record of several other nominal species that have their type locality outside the Azores, which need to be revised, this study adds another four putative new species to the archipelago's bryodiversity, supported by phylogenetic reconstructions, haplotype networks, and distinctive morphological characters. The underestimation of bryozoan diversity in the Azores has been previously pointed out to be a consequence of sporadic sampling, focused mostly on the islands of Faial, Pico and São Miguel, and the lack of taxonomic revisions using modern imaging techniques (Micael et al., 2019). The sampling effort for this study was enhanced, with a considerable extension of the geographic and bathymetric ranges, allowing to reduce this bias and revealing new species in the region. Genetic diversity levels higher than reported are frequently estimated in taxonomic revisions that include molecular studies, often corroborating distinctions previously suggested based on morphological differences (Desiderato et al., 2019; Gómez et al., 2007b; Hughes et al., 2008; Orr et al., 2019). Intra-specific morphological variation and genetic divergence are not only associated with genetic drift but also with geographical and ecophenotypic factors (Dick & Mawatari, 2005; Voje et al., 2020). The observation of the full range of morphological variation in *Reteporella* and other erect bryozoans is limited by destructive sampling methods (Dick & Mawatari, 2005; Hayward, 2000, 2004). Thus, in order to record the full range of intraspecific variability and relate it with genetic divergence levels, further morphometric work is needed in the morphospecies of *Reteporella* here distinguished, with an increased number of colonies and colony sections to be scrutinized with SEM.

The predominantly linear haplogroups in the haplotype networks suggest stable, isolated lineages with infrequent gene flow (Baptista et al., 2021b). As in *Celleporella hyalina* in the NE Atlantic (Gómez et al., 2007b), most COI haplotypes across *Reteporella* species are classified as private, i.e., they are restricted to a single locality. In most cases, this is either an artefact of low sampling density [e.g., in the seamounts Chino Bank, Gigante Bank, Condor Bank) or a result the inclusion of rare species (e.g., *R. oceanica*), but even in the widely distributed *R. atlantica* (Busk, 1884) most haplotypes are private. The high incidence of unsampled haplotypes in the Azorean *Reteporella* lineages from this study indicates high diversity richness that has not yet been sampled or analysed (Clement et al., 2000), whereas the robustness of poorly supported nodes in the phylogenetic reconstruction can probably be increased by including crucial unsampled taxa (Orr et al., 2021). Both observations highlight the importance of a broader sampling geographically and particularly bathymetrically.

Overall, there is a marked phylogeographical pattern in each Azorean *Reteporella* clade, congruent with the low dispersal abilities of a non-planktotrophic developer

(Baptista et al., 2021b; Casoli et al., 2020b; Gómez et al., 2007b). Owing to the short duration the larvae of *Reteporella* spend in the water column before settlement, strong philopatry and isolation by distance patterns are common (Casoli et al., 2020a, 2020b; Deudero et al., 2010; Jackson & Coates, 1986; Mariani, 2003), particularly in an archipelago such as the Azores in which the habitable islands (hard substrata) are separated by uninhabitable deep-sea stretches (sandy to muddy sediments). A pattern of isolation by distance (i.e., increase of differentiation with geographical distance between the islands) is noticeable in the species sampled (*R. atlantica*, *R. tristis*, *Reteporella* spp. 6 and 7).

As aforementioned, all the species proposed in this study are supported by morphological and molecular data but some relationships are noteworthy. The phylogenetic position of the clade formed by *R. oceanica* and *Reteporella* sp. 5 is not resolved in any of the phylogenies, suggesting a missing link in the dataset. Still, considering the distinct morphological differences between the clades, their geographic separation (central group of island in the former, eastern group in the latter), and results of the species delimitation analyses with the bPTP algorithm, we believe these sister species to be a good example of the 3% barcode gap (Hebert et al., 2003, 2004; Meyer & Paulay, 2005), although more samples are necessary to assess the real levels of molecular differentiation between them. *Reteporella* sp. 6 and sp. 7 display high mitochondrial divergence levels (5.7-6.3%), which have been linked to reproductive incompatibility (Gómez et al., 2007a; Hughes et al., 2008), and should thus be considered different species. One sample of *Reteporella* sp. 7 from Formigas (BRY257) displays morphological distinctions in most of the parameters evaluated (see Results section “Identification of morphospecies”) and is inconsistently assigned to different species depending on the markers analysed. Erroneous placement of this sample within *Reteporella* sp. 7 cannot be discarded, although the unavailability of more samples from Formigas hampers identity testing. Despite a significant genetic distance, *Reteporella* sp. 6 differs from *R. atlantica* only in the absence of giant frontal avicularia. Colonies or colony fragments of *R. atlantica* without these avicularia are thus indistinguishable from *Reteporella* sp. 6, which may therefore be regarded as a cryptic species. Future work and a richer dataset of specimens from *Reteporella* sp. 6 and sp. 7 are needed for a detailed characterization based on molecular and morphological characters.

R. atlantica is the most abundant species included in the dataset and it is likely found in the Azores Archipelago between 11 and 820 m in all island groups. Despite extensive scuba diving efforts in islands other than Santa Maria, shallow-water *Reteporella* colonies were not found, being the next shallowest record of *R. atlantica* reported at 60

m depth off Faial by Wisshak et al. (Wisshak et al., 2015). Notwithstanding the absence of morphological distinctions, one specimen from off Flores (BRY16) shows, to some extent, mitochondrial differentiation to the remaining *R. atlantica*. The veracity of these genetic divergence levels was verified by two independent rounds of sequencing for both mitochondrial markers, all resulting in good quality sequences, confirming the genetic distinctiveness of this specimen. Ecological surveys to characterize the habitat at 150 m around Flores and to obtain more samples similar to BRY16 are necessary to clarify whether this constitutes a distinct lineage of *R. atlantica* or if it is just a product of natural genetic drift in the population (Voje et al., 2020). Population genetic studies are needed to clarify the diversity and distribution of *R. atlantica* in the Azores.

Biogeography and bathymetric distribution

The Azorean taxa are divided into two clades: *R. tristis* and *Reteporella* sp. 1 originate from an earlier diverging ancestor of unclear geographic origin, whereas *R. atlantica*, *R. oceanica*, and *Reteporella* spp. 5-7 have split more recently. The latter, closely related to the North Atlantic *R. beaniana* and the Mediterranean *R. cf. grimaldii* and *Reteporella* spp. 2-4, constitutes the crown clade and hints at the occurrence of a radiation in the archipelago. The moderate support of the clade might be due to the existence of other related species in the archipelago, which have not been sampled or that went extinct over time. The existence of two Azorean clades argues for at least two colonization events of *Reteporella* taxa in the remote Azores Archipelago. The close relationship between Mediterranean and Azorean taxa constitutes yet another example of the so-called “Azorean Biogeographical Paradox” (Ávila, 2006, 2013), but inferences cannot be made regarding the direction and timing of past colonization events. The inclusion of more of the c. 20 known species from the Mediterranean Sea and North Atlantic in future phylogenies of the genus or the family Phidoloporidae (Hayward & Ryland, 1999; Zabala & Maluquer, 1988) will allow a better understanding of this complex evolutionary history. Moreover, the lack of samples from the western North Atlantic has certainly affected the results of our analysis, although the genus is rare and species-poor in this region.

Brooding bryozoans such as *Reteporella* are unlikely to reach remote islands during their short planktonic larval stage, thus rafting of shallow water populations on floating algae and other suitable substrata has to be regarded as the most plausible means of transport (Porter et al., 2002; Scheltema, 1986; Watts & Thorpe, 2006; Winston, 2012). One would therefore expect the shallow-water populations/species to be ancestral but, except for *R. atlantica*, *Reteporella* species in the Azores mostly inhabit ecosystems deeper than the shallow euphotic zone where the potential rafts (algae and seagrass)

occur. For this reason, source populations from older seamounts near the Azores or the mid-Atlantic ridge may need to be taken into consideration.

Considering the geographical and oceanographical setting of the Azores in the central North Atlantic, isolated by over 1,000 kilometres from the nearest land, we believe that most (if not all) *Reteporella* species are endemic to the archipelago. It is very likely to also apply to the taxa historically referred to nominal species with type localities outside the archipelago [e.g. *R. mediterranea* by Calvet (1931) or *R. cf. sparteli* by d'Hondt (1975)]. Single island marine endemic species (SIMEs) are, however, rare in oceanic islands (Ávila et al., 2019; Freitas et al., 2019). Although our dataset suggests *Reteporella* sp. 1 (Terceira) and *Reteporella* sp. 5 (São Miguel) as potential SIMEs, this might be due to sampling bias and further work is necessary to clarify their status. The high degree of endemism of Azorean bryozoans at species-level (see also Berning et al., 2017, 2021; Berning & Kuklinski, 2008), and even at generic-level (seven genera, including the newly proposed genus derived from *Reteporella* sp. 1 and *R. tristis*, contributes to the biogeographic uniqueness of the Azores ecoregion (Freitas et al., 2019).

In some localities across the Azores Archipelago (Terceira, Santa Maria, São Miguel), different taxa co-occur in sympatry, especially below 150 m depth. Terceira was the locality with the highest number of species identified (four): *R. atlantica*, *R. tristis*, *Reteporella* spp. 1 and 6 in depths between 150 and 280 m. The geographic distribution of *Reteporella* sp. 7 suggests that the species may also be present in the island but remained unsampled. Located in the Central Group, this island is in a privileged location to be colonized and receive larvae from both the western and eastern localities (Sala et al., 2016). Off Santa Maria and São Miguel islands, a number of *Reteporella* species comparable to Terceira also occur in sympatry (*R. atlantica*, *R. tristis*, *Reteporella* sp. 7 in both islands, plus *Reteporella* sp. 5 in São Miguel). Being the easternmost islands of the archipelago, it is not surprising that high diversity is encountered here, as under the eastbound sea-surface circulation, bryozoan larvae/rafts are likely to divert to this area of the archipelago (Baptista et al., 2021b; Lima et al., 2020; Sala et al., 2013, 2016).

In Flores only one species (*R. atlantica*) was present, but this low diversity can be an artefact of the sampling at only one site and depth (150 m). More species are likely to occur at other bathymetries, although an overall lower diversity would not be surprising considering the westernmost position of this island in the archipelago, which, under the eastbound circulation regime, does not favour retention of particles, and thus larvae, in the area (Baptista et al., 2021b; Lima et al., 2020; Sala et al., 2016).

The greatly varying distances between islands, seamounts and groups of islands (e.g. 6 km between Faial and Pico within the central group; 220 km between Flores in the western group and the nearest island of Faial in the central group) promotes isolation of populations in some of the islands during normal times, i.e. under the prevailing east to southeast flowing Azores Current. *Reteporella* populations in distant islands that exceed the range that larvae can travel in the water column prior to successful settlement and metamorphosis, may thus be subject to prolonged evolution in isolation. Rare chance events such as strong storms or hurricanes (Ávila et al., 2020; Johnson et al., 2017), or the formation of eddies (Lima et al., 2020; Sala et al., 2016), may enhance sea-surface current speed or even induce a change in current direction, and thus connect separated populations or bring together distinct species (Baptista et al., 2021a).

In addition, during glacial periods the distance between islands and seamounts (the shallower ones of which turning into islands, e.g., Princess Alice Bank) was slightly reduced owing to the sea-level low stand, theoretically favouring an exchange of populations between islands then (Ávila et al., 2018). Changes in the latitudinal position, strength and direction of the Azores Current between glacial and interglacial periods may have contributed to the complex genetic and geographic pattern observed in some of the species today (Ávila et al., 2019; Melo et al., 2022).

The Azorean seamounts (Chino Bank, Condor Bank, Gigante Bank, and Princess Alice Bank) also seem to yield a considerable diversity of *Reteporella*. The deep waters separating the seamounts and islands have traditionally been regarded to constitute vast, stable, and homogeneous habitats without barriers to dispersal (McClain & Hardy, 2010). Today, it is known that even deep-water ecosystems are influenced and partitioned by currents and physico-chemical characteristics of the water masses, whereas seamounts are complex geomorphological structures under multi-scale dynamics, related to circulation and upwelling-downwelling patterns (Bashmachnikov et al., 2013; Puerta et al., 2020; and references therein).

Moreover, some of the seamounts in the vicinity of the Azores, such as the Great Meteor Bank to the south, are considerably older than the islands (Geldmacher et al., 2006), and may have been islands themselves during the late Miocene when the oldest Azorean island, Santa Maria, originated. It is unknown when *Reteporella* first occurred in the Azores. The early Pliocene colonies from Santa Maria reported as *Reteporella* sp. (Ávila et al., 2015) turned out to belong to the genus *Schizoretepora* upon closer inspection (B. Berning, pers. obs.).

As all these factors greatly affect biodiversity and biogeographical patterns, a full characterization of the islands' and seamounts' assemblages and biogeographical

affinities of *Reteporella* and other marine invertebrates would benefit from detailed future studies in the area from a multi-disciplinary perspective.

Non-Azorean *Reteporella* species

In addition to *R. tuberosa* and *R. beaniana*, three putative new species of *Reteporella* from the NE Atlantic and the Mediterranean are detected in this study, while *R. cf. graeffei* and *R. cf. grimaldii* are presumably also specifically distinct from their nominal species. The well-supported phylogenetic position of *R. tuberosa* in the reconstructions, clustering with *Triphyllozoon* as in the analyses of Orr et al. (2022), calls for the need to revise its generic classification. Two clades of Atlantic and Mediterranean *Reteporella* arise in the reconstructions: 1) *Reteporella* sp. 4 and *R. cf. graeffei*, which are regarded as the same taxon and are closely related to the Azorean crown group, and 2) *R. beaniana*, *R. cf. grimaldii*, and *Reteporella* spp. 2 and 3.

The presence of the same species in Mediterranean waters (*Reteporella* sp. 4) and the tropical waters of Camden Sound (Western Australia; *R. cf. graeffei*) is surprising and requires further investigation. *Reteporella graeffei* is not well defined, as its types have never been revised, and the SEM images provided by Orr et al. (2022) show that the sequenced specimen differs significantly from the morphotype that is usually regarded as *R. graeffei* (cf. Tilbrook, 2006; Tilbrook et al., 2001). Although the colony imaged by Orr et al. (2022) has two types of frontal avicularia, its overall morphology, including the presence of large areolar pores, agrees with *Reteporella* sp. 4 from Marseille. The absence of frontal avicularia in the small fragment available of *Reteporella* sp. 4 may, as in *R. atlantica*, may be due to intracolony variability. This taxon, represented by *Reteporella* sp. 4 and *R. cf. graeffei*, is thus either non-indigenous or one of the specimens may have been mislabelled.

Within the second clade, differences between the Mediterranean *R. cf. grimaldii* and *Reteporella* spp. 2 and 3 are not observed in the nuclear marker. We tentatively suggest that *Reteporella* spp. 2 and 3 are distinct species due to the divergence levels in COI and the presence of morphological character differences that may indicate potential reproductive isolation. *Reteporella* spp. 2 and 3 were sampled on opposite sides of the Italian Peninsula, respectively in the Adriatic and Ligurian Seas. These localities are separated not only by emerged land but also by complex sea-surface circulation patterns and oceanographic fronts (Sicily Channel and Otranto Strait), which are known to increase genetic differentiation in marine invertebrates with short-lived larvae and reduced adult mobility (Pascual et al., 2017). Nonetheless, the decision of whether these lineages constitute different morphospecies requires additional SEM analysis and

sequencing of colonies from geographically intermediate localities (i.e., southern Italy). The recognition of putative new Mediterranean *Reteporella* species highlights the need to revise them, as more species than hitherto acknowledged are present in the region.

Conclusions

This study constitutes the first detailed genetic analysis of the genus *Reteporella*, contributing to the catalogue of genetic data of this often-overlooked phylum. Over 100 samples collected in a wide geographical and bathymetrical range in the Azores revealed a higher diversity than previously thought, with the identification of four putative new *Reteporella* species in the Azores and three in the Mediterranean. An overall concordance between the morphospecies and COI data, with a threshold of c. 3%, is demonstrated for *Reteporella*. To prove the status of *Reteporella* morphospecies as good biological species would require mating experiments (Dick & Mawatari, 2005; Gómez et al., 2007a), which is, however, not an easy task in this genus.

The samples included in this study already grant considerable geographical coverage in the Azores, but further sampling in more regions and over greater depth ranges is necessary to uncover the real diversity of the genus *Reteporella* in the Azores. Of high importance is the study of seamounts, with the potential to yield unique biodiversity and areas of sympatric occurrence, as observed in Gigante Bank. The clarification of biogeographical questions would benefit from the application of genome-wide markers such as microsatellites to evaluate connectivity and differentiation at different temporal and spatial scales. An integrative approach with several lines of evidence – morphometrics, molecular inferences drawn from different markers, life-history traits, and ecology – is the future of taxonomy and species delimitation, and should be followed in future studies to properly characterise the diversity of *Reteporella*.

5.2 Ecological differentiation in the bryozoan genus *Reteporella* suggested by microsatellite data

Introduction

Bryozoans are among the most overlooked invertebrate phyla and many aspects of their ecology, biology, and evolution remain a mystery. Whilst phylogenetic studies are mostly conducted at higher levels in an attempt to better understand the evolution of the phylum (e.g., Orr et al., 2019, 2022; Waeschenbach et al., 2012b), most population genetic studies in bryozoans are focused on species' complexes, non-indigenous and/or invasive species such as *Celleporella hyalina* (Linnaeus, 1767) and *Amathia verticillata* (delle Chiaje, 1822) (Gómez et al., 2007b; Nascimento et al., 2021) or genera such as *Bugula* Oken, 1815 and *Watersipora* Neviani, 1896 (Johnson et al., 2012; Mackie et al., 2006). Although the bryozoan diversity in some areas of the globe seems to be well documented (see Denisenko & Blicher, 2021; Figuerola et al., 2017; Gordon, 1999; Rosso & Di Martino, 2016; Wood et al., 2012; Wood & Probert, 2013), modern taxonomic revisions of allegedly well-known genera or species usually result in a doubling of species numbers, even in well-studied regions such as the Mediterranean Sea (e.g. Berning & Kuklinski, 2008; Di Martino & Rosso, 2021; Harmelin et al., 2019). In less well-studied ecosystems, such as seamounts or the oceanic Azores Archipelago in the central North Atlantic, limited sampling usually impedes a thorough diversity assessment, and taxonomic revisions of the few historical records using modern imaging and molecular techniques are scarce or lacking altogether (Berning & Spencer Jones, 2023; Micael et al., 2019). In an attempt to tackle these problems, the Azorean species of the genus *Reteporella* Busk, 1884, the most widespread of the 23 currently recognized genera within the family Phidoloporidae, were recently studied following an integrative taxonomic approach, unravelling a distinctly greater diversity than previously documented and clarifying the phylogenetic relationships among the different species in the archipelago (Baptista et al., 2022).

The Azores Archipelago is considered a hotspot of biodiversity in the North Atlantic, thriving due to its isolation, geological evolution, complex geomorphology, temperate climate, varied environments, and oceanographical setting (Sala et al., 2016; Santos et al., 1995). The uniqueness of the Azores ecoregion (sensu Freitas et al., 2019) is expressed in the increasing number of endemic species reported, as well as taxa previously regarded as widely distributed that are potential cryptic or sibling species

restricted to the archipelago [e.g., microgastropods of the family Rissoidae (Baptista et al., 2021b; Cordeiro & Ávila, 2015) or deep water corals of several genera (Carreiro-Silva et al., 2017)]. Azorean bryozoans are particularly rich in endemic taxa, with most of the genera containing endemic species, while seven monospecific genera, for instance *Nimbella* Jullien, 1903, *Pulpeirina* Reverter-Gil & Souto, 2015, or *Harmelinus* Rosso, 2018, are endemic to the archipelago. Intra-archipelagic gene flow and connectivity of marine species with non-planktotrophic larvae in the Azores has seen a renewed interest with the development of new molecular techniques and integrative approaches. At least for intertidal species, several factors such as habitat type, sea-surface circulation regimes, and dispersal ability, seem to act concomitantly to shape population structure (Baptista et al., 2021b). For benthic inhabitants of remote islands, however, the full range of processes influencing the population dynamics remains poorly studied.

In light of a recent review of their insular diversity (Baptista et al., 2022), *Reteporella* species become an interesting proxy to explore genetic structure and population dynamics of benthic marine invertebrates in the Azores Archipelago. Occurring from 10 to 820 m depths on several islands and seamounts, *Reteporella* spp. occupy wide bathymetrical and geographical ranges (Baptista et al., 2022). This study, based on morphological and molecular data, retrieved two clades of Azorean *Reteporella* species (Figure 5.6): Clade I comprises five closely related species – *R. atlantica* (Busk, 1884), *R. oceanica* (Jullien in Jullien & Calvet, 1903), and *Reteporella* spp. 5–7 – whilst Clade II comprises *R. tristis* (Jullien in Jullien & Calvet, 1903) and *Reteporella* sp. 1. *Reteporella* species possess non-planktotrophic larval development, thus lack a feeding stage and are relatively short-lived. Accordingly, their larvae are thought to have restricted dispersal abilities, thus genetic structure should be pronounced across the archipelago, reflecting geographical distance (Scheltema, 1986; Watts & Thorpe, 2006). In the temperate Azorean waters, rafting is expected to constitute the main strategy for species with non-planktotrophic larvae to reach the archipelago, whilst intra-archipelagic dispersal is mainly achieved by drifting with the currents or chance events such as storms (Ávila, 2013; Ávila et al., 2019; Winston, 2012).

Aiming to estimate connectivity and explore the factors shaping population dynamics across Azorean islands and seamounts, the population genetics of five *Reteporella* species – *R. atlantica*, *R. tristis*, *R. oceanica*, and *Reteporella* spp. 6 and 7 (*sensu* Baptista et al., 2022) – was studied. *Reteporella* sp. 5 was not included, as only one locality was sampled. SSR-GBAS and COI haplotyping were used to account for reticulate patterns of nuclear and mitochondrial variation, respectively. Congruence of genetic structure to species boundaries, sampling locality, and habitat were evaluated.

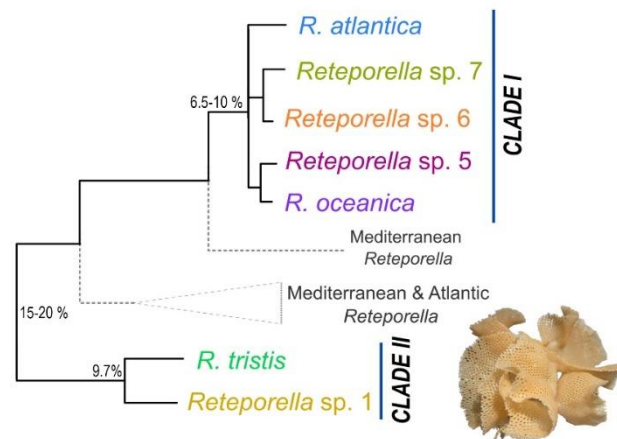


Figure 5.6. Phylogenetic relationships of *Reteporella* species in the Azores. Two clades are distinguished (I and II), diverging over 15% in the COI marker; mitochondrial divergence ranges from 6.5 to 10% within Clade I. Grey dotted branches represent Mediterranean and non-Azorean Atlantic *Reteporella* species. *Reteporella* colony is merely illustrative. Adapted from Baptista et al. (2022).

Material and Methods

Sampling and DNA extraction

Reteporella specimens from several locations around the Azores Archipelago were sub-sampled from material deposited at biological collection (DBUA – Department of Biology of the University of the Azores and DOP – Department of Oceanography and Fisheries of the University of the Azores) or collected during the scientific cruise M150 BIODIV “Controls in benthic and pelagic BIODiversity of the AZores BIODIAZ” with the German RV *Meteor* in 2018 (George et al., 2018) (cf. Supplementary Table A6.1 for further details). All collected samples were preserved in ethanol > 96 % and stored at 4°C or lower. Total genomic DNA was extracted with the column-based commercial kit PureLink® Genomic DNA (Invitrogen™), after maceration of clean colony fragments with metallic pestles. The quality and integrity of the DNA extracted were evaluated with electrophoresis of agarose gels (0.8%).

Molecular work for SSR-GBAS genotyping

Genetic variation of *Reteporella* was assessed by short sequence repeat genotyping by amplicon sequencing (SSR-GBAS) that has been shown to be highly effective in discriminating genetic structure in a variety of non-model organisms including marine invertebrates (Baptista et al., 2021b; Curto et al., 2019; Tibihika et al., 2019).

De novo SSR markers were developed from shot-gun sequencing data from two low-coverage Illumina MiSeq runs using two *Reteporella* individuals. Library preparation with the Nextera XT DNA Library Preparation Kit (Illumina, Inc.) and paired-end sequencing

(2 x 300 bp reads) was conducted at the Genomics Service Unit, Ludwig-Maximilian's-Universität (LMU, München, Germany) and marker development followed established workflows (see Baptista et al., 2021b; Curto et al., 2019; Lanner et al., 2021; Tibihika et al., 2019). The quality control step kept the sequences containing five repeats for penta- and tetranucleotides; eight repeats for trinucleotides; and 10 repeats for dinucleotides. Primer design and initial testing followed the workflow previously established (cf. Baptista et al., 2021b and references therein for details). The 36 successful primers (i.e., those that amplified a fragment of the expected size) were combined at a final concentration of 1 µM in four multiplex mixes, each with nine primer pairs (Supplementary Table A6.2). Primers Bry1_CATA and Bry2_TATG were designed from BRY16 – *R. atlantica* from Flores; the remaining primers were designed from BRY141 – *Reteporella* sp. 6 from Terceira (Supplementary Table A6.2). Amplicon library preparation was conducted following Curto et al.'s (2019) protocol, consisting on a first multiplex PCR followed by the pooling of all reactions per sample, PCR cleanup and index PCR using dual barcode. Details on the workflow can be consulted in Baptista et al. (2021b). Illumina MiSeq sequencing (2 x 300 bp) of indexed amplicon pools was carried out at the Genomics Service Unit at the LMU. Quality control of demultiplexed reads and merging of overlapping forward and reverse reads followed the workflow by Baptista et al. (2021b). Genotype calling was achieved using the SSR_GBAS_pipeline scripts (Curto et al., 2019; Tibihika et al., 2019), available at <https://github.com/mcurto/SSR-GBS-pipeline>, generating a codominant matrix with alleles based on whole sequence information and not only amplicon length.

Population structure analyses

The codominant matrix resulting from the SSR-GBAS genotyping was used as input for descriptive population genetic analyses and assessment of genetic structure patterns. To avoid biases in the analyses, individuals and loci with more than 60% missing data were excluded from the dataset. The frequency of null alleles in each marker per species was determined with FreeNA (Chapuis & Estoup, 2007).

Genetic structure of the newly developed SSR markers was first assessed with a principal coordinate analysis (PCoA), colour coded by species and geographical localities, as implemented in GenAlEx v6.51 (Peakall & Smouse, 2006, 2012) and allowing for an evaluation of genetic structure without assumption of the Hardy-Weinberg equilibrium (HWE). Genetic structure was further investigated with STRUCTURE v2.3.4 (Hubisz et al., 2009; Pritchard et al., 2000) that assigns individuals to hypothetical populations and subpopulations by optimizing for HWE (Porrás-Hurtado et al., 2013). A

total of 500,000 MCMC generations were run in STRUCTURE followed by an initial burn-in of 100,000 generations that were discarded. Simulations considering a cluster number (K) between 2 and 15 were conducted producing 10 iterations per K, keeping the default settings from the admixture model and allele frequencies correlated. Processing of the results and inference of the K-value that best suits the dataset was performed using the delta-K (Δk) method implemented in the online program Structure Harvester (<http://taylor0.biology.ucla.edu/structureHarvester/>). Based on the natural log posterior probability of the data (LnPD) or the Δk value, the software evaluates K values for optimal detection of the suitable assumed population size for the dataset (Earl & VonHoldt, 2012). The online pipeline CLUMPAK (Kopelman et al., 2015), available at <http://clumpak.tau.ac.il/>, was used to summarize replicates per K-value and obtain a graphical representation of the STRUCTURE results. Hierarchical assignment of microsatellite genotypes, based on the SSR-GBAS dataset, and further evaluation of genetic structure was achieved with the reconstruction of a network using Splitstree4 v4.18.3 (Huson & Bryant, 2006), following the UPGMA and Consensus Network methods, based on pairwise genetic distance for codominant datasets as implemented in GenAEx v6.51.

Genetic diversity indices

Genetic diversity patterns across the archipelago was evaluated using both mitochondrial and SRA-GBAS loci. The mitochondrial dataset consisted on *Reteporella* sequences of the marker Cytochrome c oxidase subunit 1 (COI) publicly available at GenBank (www.ncbi.nlm.nih.gov/genbank) (Supplementary Table A6.1). The COI alignment was constructed using the Clustal Omega algorithm hosted by EMBL-EBI web services (McWilliam et al., 2013). For each *Reteporella* species (*sensu* Baptista et al., 2022), genetic diversity indices in the COI dataset – number of haplotypes, haplotype and nucleotide diversity – and the SSR-GBAS dataset – number of alleles and heterozygosity levels – were estimated in DnaSP v6 (Rozas et al., 2017) and GenAEx v6.51, respectively. GenAEx v6.51 was also used to estimate deviations from HWE in the SSR-GBAS dataset, as well as to conduct Mantel tests. This allowed to evaluate the role of distance in shaping genetic using both COI and SSR-GBAS datasets, by comparing geographical and genetic distance matrices for all localities with more than two individuals: Condor Bank, Gigante Bank, Princess Alice Bank, Sabrina, São Miguel Island, and Santa Maria Island (the latest separated in shallow and deep water groups). Hierarchical analyses of molecular variance (AMOVA; Excoffier et al., 1992), implemented in GenAEx v6.51, were conducted to simultaneously assess pairwise

differentiation among species and sampling localities, regardless of the species assignment (ϕ_{ST} in the COI dataset, F_{ST} in the SSR-GBAS dataset).

Population genetic analyses in *R. atlantica*

As *R. atlantica* is the most widespread species in the archipelago (cf. Baptista et al., 2022), its population structure was assessed in detail. Analyses were conducted on the COI dataset to evaluate the intra-specific diversity in the archipelago for localities represented by two or more individuals: Flores, Princess Alice Bank, Faial, Terceira, Sabrina/São Miguel, and shallow waters around Santa Maria. Fu's F_s (Fu, 1997) and R_2 (Ramos-Onsins & Rozas, 2002) neutrality tests, powerful and suitable for small sample sizes, were performed using DnaSP v6 to assess the occurrence of demographic expansion of *R. atlantica* in the Azores. Statistical significance was based on 1,000 coalescent simulations under a Standard Neutral Model conditioned on Theta. As multiple pairwise comparisons were conducted, Bonferroni correction was used to adjust the level of significance ($p < 0.033$; Rice, 1989). AMOVA analyses were also conducted to test the congruence of genetic variation with population delimitation of *R. atlantica*, based on both the SSR-GBAS and COI datasets.

Results

SSR-GBAS dataset

The MiSeq runs produced 18,172 and 435,151 paired reads BRY16 (*R. atlantica*) and BRY141 (*Reteporella* sp. 6), respectively, after quality control. A total of 16,444 and 355,080 merged reads, respectively, were screened for SSR motifs. Of these, respectively, 202 and 5,684 reads contained SSR motifs that complied with the criteria defined for the SSR search pipeline, resulting in: 89 di-, 38 tri-, 11 tetra-, and 0 pentanucleotide repeats for BRY16; 2,461 di-, 1,115 tri-, 313 tetra-, and 10 pentanucleotide repeats for BRY141. A total of 42 primer pairs for *Reteporella* were designed from sequences containing penta-, tetra-, and trinucleotide repeats. Six of these failed to amplify in the single PCR test phase. Information of the remaining 36 primers, included in the four multiplex mixes is provided in Supplementary Table A6.2.

For SSR-GBAS genotyping, a total of 16,125,228 paired reads were produced, which were reduced to 2,614,915 after quality control, merging and primer demultiplex steps. The number of reads per marker ranged from 3,792 (Bry1_CATA) to 303,491 (Bry32_TTG), whereas the number of sequences generated per sample varied between 159 (BRY15 – *R. atlantica*, and DR2B2C – *Reteporella* sp. 7) to 102,359 (P8 – *R. atlantica*). The matrix was filtered for the removal of samples with more than 60% of

missing data across all markers, reducing it to 48 samples (out of 83), as follows: 22 *R. atlantica* (out of 42), four *R. tristis* (out of 14), two *R. oceanica* (out of two), seven *Reteporella* sp. 6 (out of seven), ten *Reteporella* sp. 7 (out of 16), and one unidentified for which it was not possible to obtain their COI sequence. Although successful in the single PCR test, the markers Bry18_ATAC, Bry25_TGT, Bry27_ATA, Bry34_AAT, and Bry40_ACT yielded more than 60% missing data and were thus removed from the SSR-GBAS dataset. A total of 31 markers in the SSR-GBAS dataset were suitable for population genetic analyses, with the number of alleles ranging from 15 (Bry26_TATG, Bry26_ATA) to 66 (Bry8_TGTC) (Supplementary Table A6.2).

Marker efficiency varied across the five *Reteporella* species studied (Figure 5.7). Overall, the SSR-GBAS primers performed better in species assigned to Clade I: all samples of *Reteporella* sp. 6 and *R. oceanica*, 63% of *Reteporella* sp. 7 samples. Only 29% of *R. tristis* (Clade II) specimens were successfully amplified (Figure 5.7a). Discrepancy in the amplification success of *R. atlantica* from shallow and deep-waters was observed, with higher success in the former (53%). Missing data per sample was widely variable in *Reteporella* sp. 7 and *R. atlantica*; high levels of missing data were inferred for *R. tristis*; marker failure was low in *Reteporella* sp. 6, *R. oceanica*, and shallow water *R. atlantica* (Figure 5.7b).

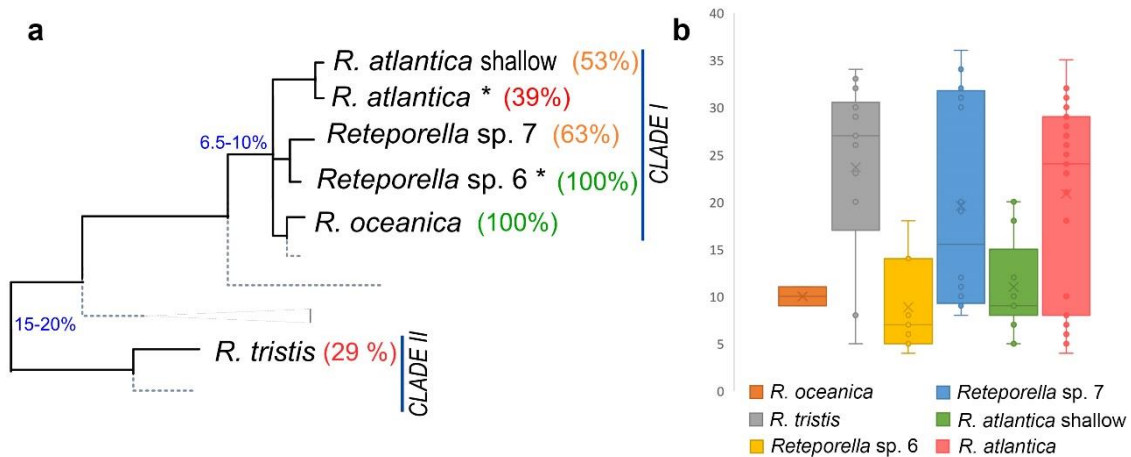


Figure 5.7. Efficiency of the SSR-GBAS markers designed for Azorean *Reteporella* species. a. Proportion of samples successfully amplified (< 60% missing data) in the working dataset per *Reteporella* species: red (< 50%), orange (50-75%), green (> 75%). Asterisk (*) indicates the species used in primer design (*R. atlantica* – two primer pairs, *Reteporella* sp. 6 – 29 primer pairs). Two clades are distinguished (I and II), diverging over 15% in the COI marker. Grey dotted branches represent *Reteporella* species not included in the SSR-GBAS analysis. Phylogeny adapted from Baptista et al. (2022). **b.** Missing data, in absolute number of primer pairs that failed amplification, in the SSR-GBAS dataset. Internal points represent missing data per individual sample; *Reteporella* species are colour-coded.

Population structure of *Reteporella* species across the Azores Archipelago

PCoA analysis of the complete SSR-GBAS dataset with samples colour coded by species (Figure 5.8a,b) showed a clear differentiation of a *R. atlantica* group composed exclusively by shallow waters (< 25 m) samples around Santa Maria. Although some overlap was detected between *Reteporella* sp. 7 and the remaining groups, most of the *Reteporella* sp. 7 individuals were genetically distinct with various degrees of divergence. The remaining species/populations did not form identifiable clusters in any of the dimensions. The PCoA analysis was repeated after removal of *R. atlantica* of shallow water, aiming to clarify the relationships among the remaining taxa, but no further clustering was obtained (data not shown). The PCoA, plotted by geographical locations (Figure 5.8c,d), also distinguished samples from shallow waters (< 25 m) around Santa Maria as a well-defined cluster in every dimension. Overall, the patterns were not clear but to some extent samples of the same locality clustered closely. Still, when comparing axes 1 and 3, some geographical clustering can be distinguished: mostly eastern (Santa Maria and São Miguel) localities in the bottom left corner, Terceira samples were distributed in a linear pattern in the top left quadrant of the plot, and the seamounts tended to cluster in the centre of the plot.

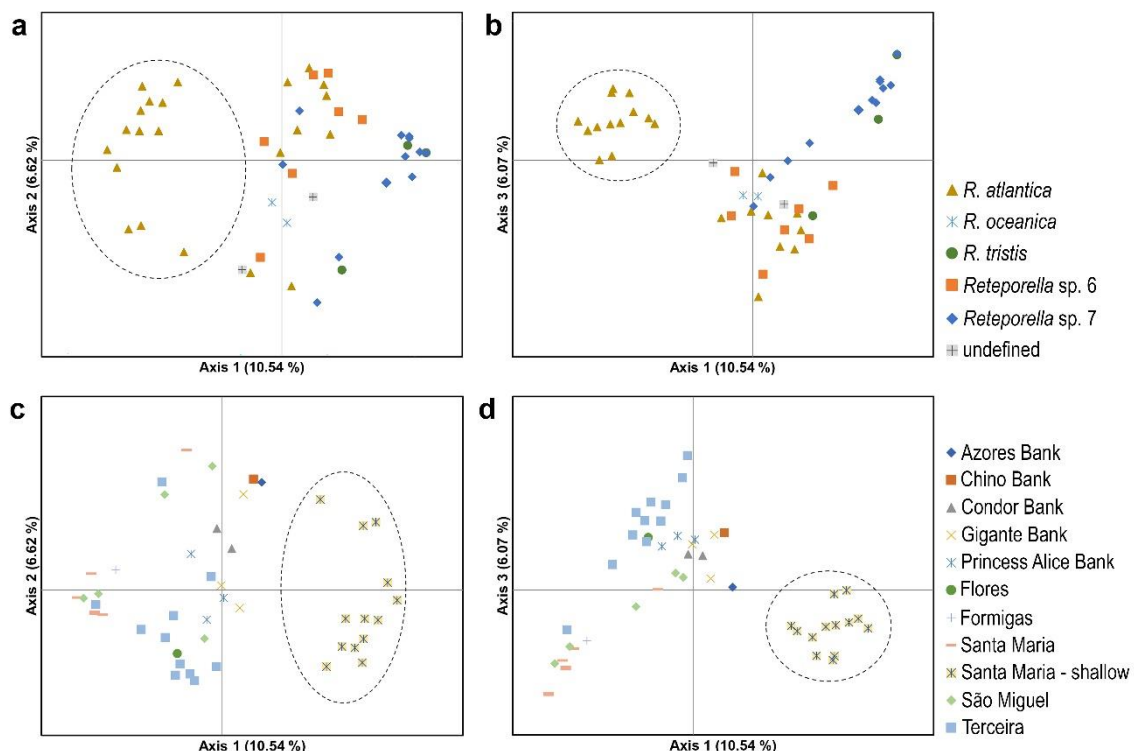


Figure 5.8. Principal coordinates analyses from complete SSR-GBAS dataset of *Reteporella* from the Azores, with samples colour coded according to the taxa (a-b) and geographical localities (c-d) sampled. **a.** Variance explained by axis 1 (10.54 %) and axis 2 (6.62%) in the analysis by species; **b.** Variance explained by axis 1 (10.54 %) and axis 3 (6.07 %) in the analysis by species; **c.** Variance explained by axis

1 (10.54 %) and axis 2 (6.62 %) in the analysis by locality; **d.** Variance explained by axis 1 (10.54 %) and axis 3 (6.07 %) in the analysis by locality. Cluster of *R. atlantica* from shallow waters of Santa Maria Island (<25 m) delimited with dash-lines. PCoAs conducted in GenAlEx v6.5 (Peakall & Smouse, 2006, 2012); groups in study are coded by colour and icon shape.

In the STRUCTURE reconstruction (Figure 5.9), four genetic clusters were defined as optimal and shared by different *Reteporella* species that co-exist in the same deep-water localities, as all specimens were collected from depths over 150 m (cf. Supplementary Table A6.1) with the exception of shallow water samples around Santa Maria. Only three samples of *R. tristis* were successfully genotyped by the SSR-GBAS approach (two from Terceira, one from Santa Maria), and assigned to two genetic groups. Within *R. atlantica*, a genetic group comprising only the shallow water specimens of Santa Maria emerged at K=2 and was consistent throughout the K-values tested. In general, the major clusters matched the geographical distribution of the samples: shallow water *R. atlantica* from Santa Maria, *R. atlantica* from Terceira, one predominant in deep waters of Santa Maria and São Miguel (eastern group), and another from the seamounts (central group). For K=4, two clusters were inferred in São Miguel (*Reteporella* sp. 7 and *R. atlantica*), whilst in Terceira we can distinguish three genetic groups (*Reteporella* sp. 6, *R. tristis*, and *R. atlantica*). Population structure became more complex with higher K-values, as each locality was subdivided into clusters mainly corresponding to the species occurring there, however with limited admixture with the clusters of the same species in other localities. *Reteporella oceanica*, from Condor Bank, formed an independent cluster at K=6. At K=8, a subdivision in Santa Maria was observed, mostly corresponding to the separation of *Reteporella* sp. 7 and *R. tristis*; a cluster of *R. atlantica* was shared among São Miguel, Princess Alice Bank, and Chino Bank. The clustering analyses were not biologically informative for K-values over 9 (data not shown). The reconstruction of a genetic distance network (Figure 5.10) supported the hierarchical assignment of microsatellite clusters first by showing deeper splits based on geographical localities and shallower by species present in the area.

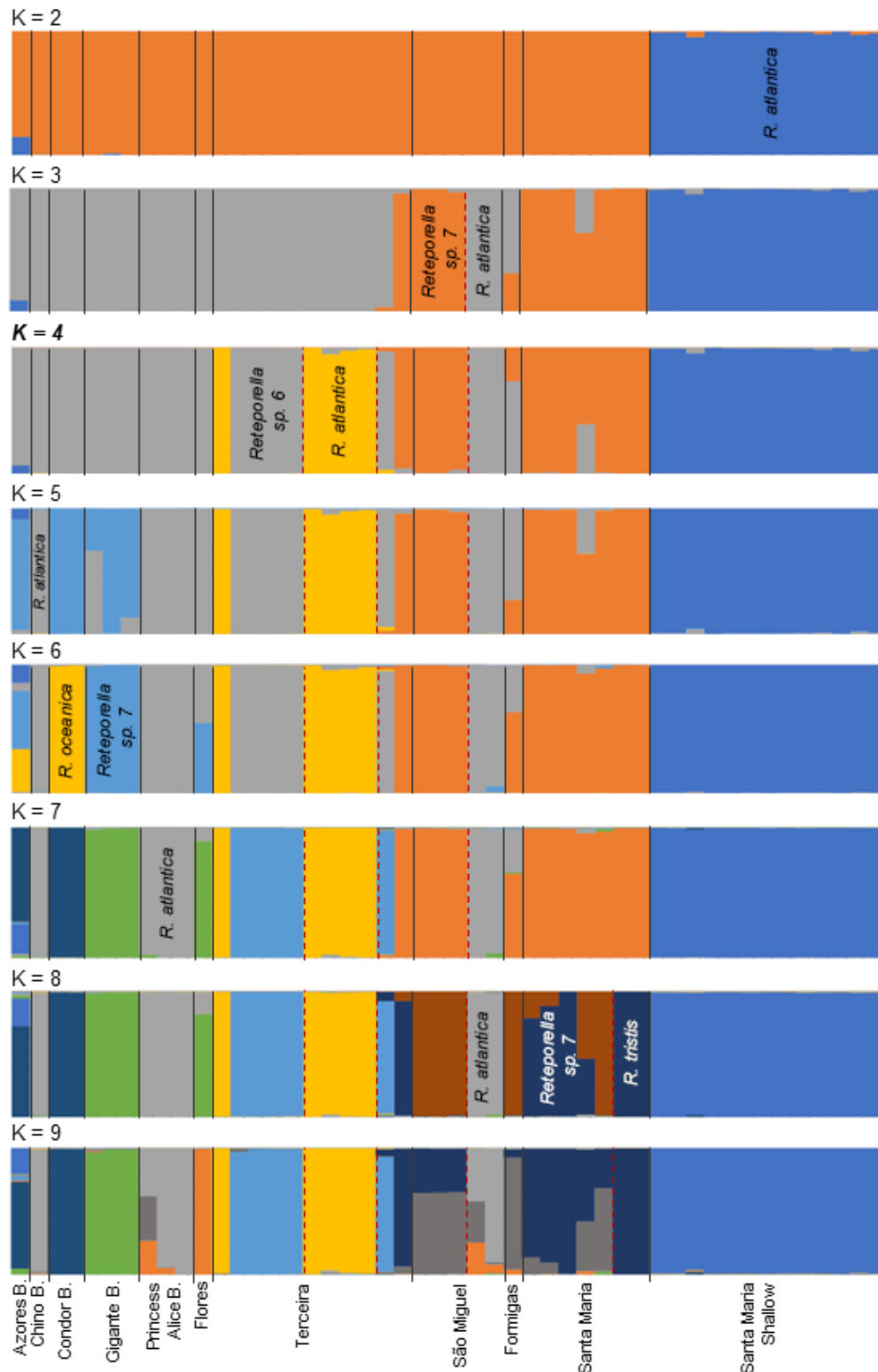


Figure 5.9. Genetic structure analysis of the complete SSR-GBAS dataset of *Reteporella*, plotted by geographical localities. Inferred with STRUCTURE v2.3.4 (Hubisz et al., 2009; Pritchard et al., 2000) and reporting the results from K-values between 2 and 9, with an optimal K = 4. As K increases, the software progressively assigns individuals of the same species to a single cluster (within each locality). Species name is shown in the plot when it is distinguished as a single cluster within a locality for the first time. Black lines divide the localities, whereas the dashed red lines indicate the subdivision into taxon-specific clusters, within each locality, as K increases.

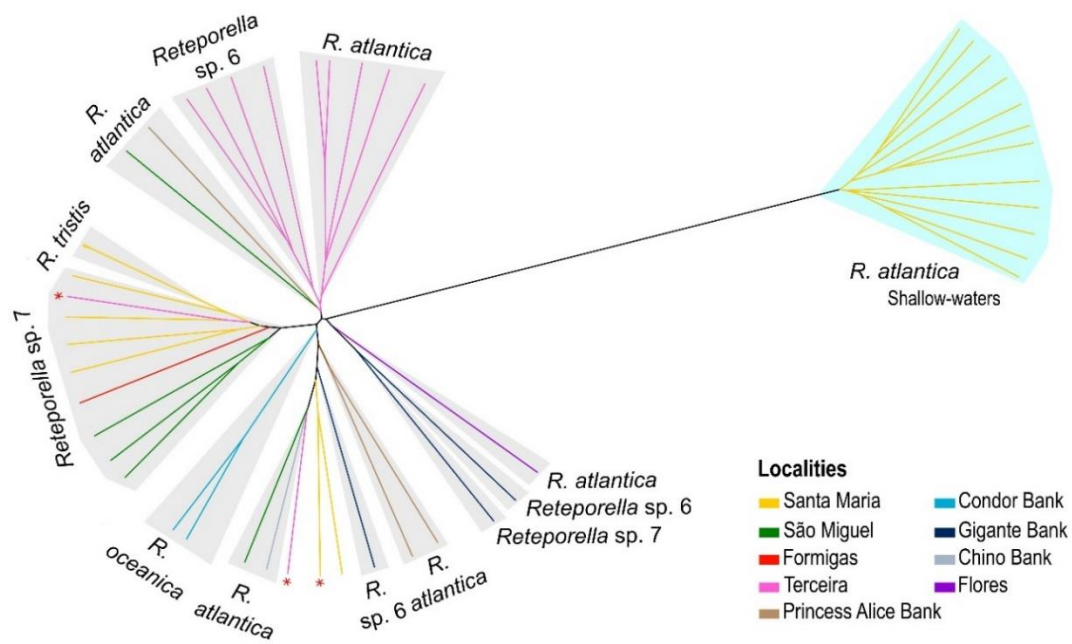


Figure 5.10. Genetic distance network of Azorean *Reteporella* species, based on the SSR-GBAS dataset, representing the hierarchical assignment by geographical locality and further subdivision by species. Inferred with Splitstree4 v4.18.3 (Huson & Bryant, 2006) under the UPGMA method. Branches are color-coded by geographical locality, according to the label depicted in the bottom right corner. Clusters of the same species are identified and delimited in light grey. Red asterisks (*) indicate outlier specimens in species or locality assignment. Shallow water specimens from Santa Maria form a distinct cluster.

By plotting STRUCTURE cluster assignment into a map, a longitudinal gradient of shared clusters in the Azores is observed (Figure 5.11). For example, for $K=4$, a cluster (grey), despite being found all over the archipelago, dominates in the west being shared by all individuals of Flores and the central seamounts; a cluster (yellow) is only found in the eastern group and Terceira island being more prevalent in the east; the remaining clusters are island specific comprising the shallow waters of Santa Maria island (blue) and some individuals of Terceira island (orange).

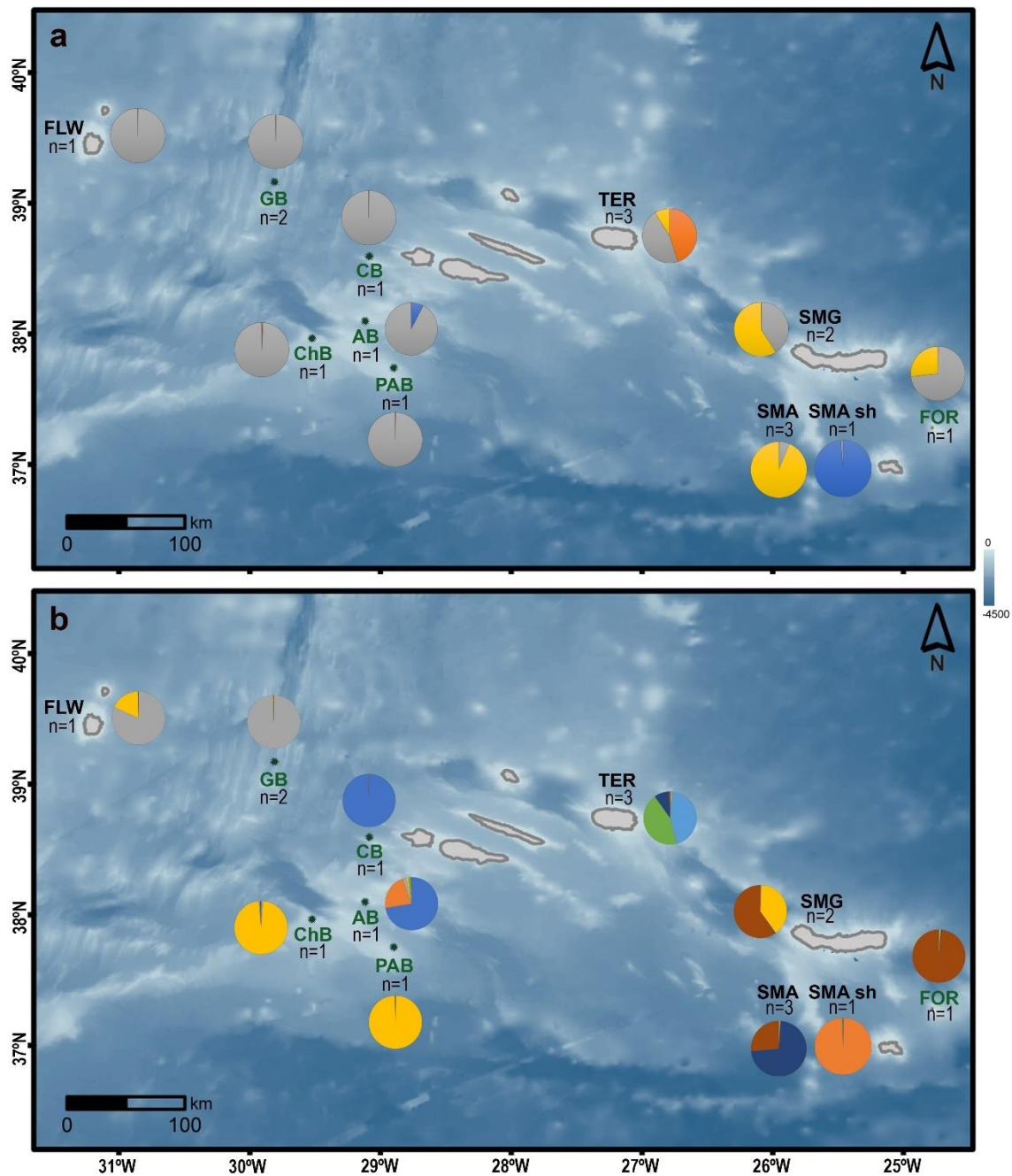


Figure 5.11. Directional gradient of the frequency of each genetic cluster per geographical locality, estimated in the STRUCTURE reconstruction for: a. the optimal K=4; b. the maximum informative K=8. Number of species (n) known to occur in each locality is indicated under the locality designation. Abbreviations are depicted in black for islands (SMA – Santa Maria, SMG – São Miguel, TER – Terceira, FAI – Faial, FLW – Flores) and in green for seamounts (FOR – Formigas, PAB – Princess Alice Bank, AB – Azores Bank, ChB – Chino Bank, CB – Condor Bank, GB – Gigante Bank). Coastline shape retrieved from the Portuguese Hydrographic Institute (<https://www.hidrografico.pt/op/33>) and bathymetry, indicated at the right margin, derived from GEBCO 2020 (https://www.gebco.net/data_and_products/gridded_bathymetry_data/).

Genetic diversity indices

Genetic diversity indices were estimated for species using both the COI and SSR-GBAS datasets and are summarized in Supplementary Table A6.3. Overall, in the COI dataset, haplotype and nucleotide diversity were high (≥ 0.700 and ≥ 0.00848 , respectively) across the four species analysed. *Reteporella atlantica* was represented by the largest number of individuals (54) and yielded the highest haplotype diversity (0.857). Similar levels of haplotype diversity were obtained for *Reteporella* spp. 6 and 7, which were represented by fewer individuals: 7 and 18, respectively. *Reteporella tristis* had the lowest haplotype diversity (0.7), with only four haplotypes occurring across 16 individuals. Overall, haplotype diversity was high across taxa, except in *R. atlantica* of shallow waters (0.00058). Heterozygosity levels in the SSR-GBAS dataset were estimated without accounting for monomorphic markers. Observed heterozygosity was higher than expected for *Reteporella tristis*, whereas it was lower than the expected heterozygosity for the remaining species.

Deviations from HWE per microsatellite locus were estimated for each species. The cause of the deviations was assessed by estimating the frequency of null alleles and checking the original matrix for homozygote excess. HWE deviation results are summarized alongside the frequency of null alleles at Supplementary Table A6.4. High incidence of null alleles was detected at several loci, although never consistently across all taxa. Significant deviation from HWE was shown at seven loci in *Reteporella* sp. 6 and in 11 loci of *Reteporella* sp. 7. However, the highest level of deviation from HWE was observed in *R. atlantica* (27 out of 31) with high frequency of null alleles associated with most of them. The number of loci deviating from HWE decreased by separate deep and shallow water *R. atlantica* samples but the shallow water cluster still revealed a large number of deviations (14).

Differentiation amongst *Reteporella* species and localities in the Azores

The AMOVA by species, based on the SSR-GBAS dataset, revealed that only 9% of the genetic variation can be explained by the current classification scheme (Supplementary Table A6.5a). The AMOVA analysis by geographical localities (Supplementary Table A6.5b), regardless of species assignment, attributed only 15% of the genetic variation among localities, a value that was reduced to 11% after the removal of shallow water samples from Santa Maria (Supplementary Table A6.5c). In all cases, over 40% of the variation in the SSR-GBAS dataset occurred among individuals. The AMOVA analyses were repeated by each locus per species and localities to assess whether the structure pattern retrieved by the SSR-GBAS dataset was an artefact of a

single or few loci (Supplementary Figure A6.1). For most of the markers (>18), up to 25% of the genetic variation was consistently explained by partition in species or localities; outliers were not detected. For markers with over 25% of genetic variation in the dataset, matrices were checked and it was verified that the levels of variation are likely related to the marker genotyping success, especially in shallow water *R. atlantica* (data not shown). Regarding F-statistics, significant ($p < 0.05$) F_{ST} values of 0.153 and 0.110 were estimated for the overall SSR-GBAS dataset by locality with and without shallow water samples, respectively. In the COI dataset, the variation among species accounts for 83%, whilst among geographical localities this value decreases to 43% (Supplementary Table A6.6). Regarding ϕ -statistics, significant ($p < 0.05$) ϕ_{PT} values of 0.825 and 0.433 were obtained per species and geographical localities, respectively. The correlation between genetic similarities and geographic distance of localities sampled was evaluated with Mantel tests. The results retrieved were positive, non-significant and low in magnitude ($r = 0.082$, $p = 0.255$ in the SSR-GBAS dataset; $r = -0.177$, $p = 0.240$ in the COI dataset).

Differentiation within *Reteporella atlantica*

Due to its wide geographical and bathymetrical distribution, additional population genetic analyses were conducted for *R. atlantica* to evaluate its population structure and intra-specific diversity in the Azores. Pairwise F_{ST} estimates based on the SSR-GBAS dataset were mostly significant (Table 5.2), except among shallow water populations around Santa Maria (Gruta Azul, Pedrinha, and Mar da Barca), and among Princess Alice Bank, Terceira, and Gruta Azul. Nevertheless, differentiation level estimated between Princess Alice Bank and the shallow water Gruta Azul was moderate but non-significant (0.776). Most pairwise ϕ_{ST} (Table 5.2) comparisons among *R. atlantica* populations were non-significant. The easternmost localities of Santa Maria and São Miguel revealed no significant divergence from each other, whereas shallow water localities around Santa Maria were significantly different from the westernmost Flores locality. Significant differentiation among several localities in the central group were also retrieved. Overall, differentiation levels increase with geographical distance among populations, reaching levels over 90% in some comparisons. If not accounting for the pairwise comparisons among shallow water populations, the lower ϕ_{ST} involves Flores (23.7–71.9%).

Table 5.2. Matrix of pairwise among population differentiation: F_{ST} values for the SSR-GBAS dataset (below diagonal) and ϕ_{ST} values for the COI dataset (above diagonal) for populations represented by more than one individual. Hyphen (-) indicate pairwise comparisons not performed due to missing data of that population in the SSR-GBAS dataset. Bold asterisk (*) indicates significance for the adjusted critical p-value after Bonferroni correction ($p = 0.005$ in the SSR-GBAS dataset; $p = 0.00179$ in the COI dataset). Conducted as implemented in GenAlEx v6.5 (Peakall & Smouse, 2006, 2012). Gruta Azul, Mar da Barca, and Pedrinha are all (sub)localities from shallow waters of Santa Maria.

	Flores	P. Alice Bank	Faial	Terceira	São Miguel	Gruta Azul	Mar da Barca	Pedrinha
Flores		0.237	0.427 *	0.542	0.612	0.719 *	0.668 *	0.662 *
Princess Alice B.	-		0.585	0.689	0.440 *	0.776	0.679	0.688
Faial	-	-		0.891 *	0.863	0.938 *	0.916	0.921 *
Terceira	0.071	-	-		0.923	0.973	0.962	0.968 *
São Miguel	-	-	-	-		0.885	0.817	0.825
Gruta Azul	0.194	0.231 *	-	-	-		0.233	0.020
Mar da Barca	0.183 *	0.226 *	0.086	-	-	-		0.167
Pedrinha	0.169 *	0.201 *	0.024	0.041	-	-	-	

The AMOVA analysis (Supplementary Table A6.7) attributes 15% of the overall genetic variation to the populations based on the SSR-GBAS dataset and 42% due to among individuals. The overall pairwise F_{ST} of 0.146 is significant ($p = 0.001$). For the COI dataset, 76% of the overall variation of *R. atlantica* is found among populations. Neutrality tests in the COI dataset discard the occurrence of demographic expansion of *R. atlantica* in the Azores, with non-significant results in both parameters inferred (Fu's $F_s = 1.548$, $p = 0.716$; $R_2 = 0.123$, $p = 0.739$). Mantel test for populations of *R. atlantica* in the SSR-GBAS dataset revealed strong non-significant correlation between genetic and geographical distances ($r = 0.835$, $p = 0.084$), whereas in the COI dataset this value is significant and negative ($r = -0.300$, $p = 0.039$).

Discussion

In this work, we aimed to narrow the knowledge gap in the dynamics shaping the evolution of bryozoans in the remote archipelago of the Azores, shedding some light into processes and patterns that are probably more widespread in the marine realm than previously thought. Phylogenetic reconstructions by Baptista et al. (2022) support the occurrence of at least eight *Reteporella* species in the Azores. A cryptic lineage of *R. atlantica* seems to occur only in shallow waters of Santa Maria Island, presumably as a consequence of ecologically-driven differentiation across its bathymetrical range. Incongruent patterns of genetic structure were inferred with the two classes of markers, which is attributed to methodological constraints in the application of the SSR-GBAS approach to five different species or, alternatively, potential interspecific gene flow. These topics will be further discussed in the next sections.

SSR-GBAS marker performance

Disparity in performance and success of the SSR-GBAS primers is expected in a situation of cross-species amplification (Curto et al., 2019; Turini et al., 2014). It is related to the phylogenetic relationships of the studied taxa due to the so-called “ascertainment bias” (Brandström & Ellegren, 2008), as better rates of success were obtained for taxa closely related to those used to design the primers (*R. atlantica* and *Reteporella* sp. 6). As only two primers were designed from *R. atlantica*, the success rates in this species are lower than those estimated for *Reteporella* sp. 6. Cross-species amplification can also be locus-dependant and affected by admixture (Tibihika et al., 2019), as detected in this study. Previous tests of cross-species amplification using SSR-GBAS primer sets showed that the development of markers based on several species minimises the ascertainment bias within each of the study species, whilst using only one marker set from one species would contribute to biases in estimated variability values (Curto et al., 2019). Designing primers for each species was, however, not possible within the framework of this study.

Of the two primer pairs designed from *R. atlantica* (BRY16), both showed significant deviation from HWE only in the target species. Mitochondrial and morphological data (not shown) suggest that this specimen might constitute a potentially divergent lineage of deep-water *R. atlantica*. If this is confirmed in future integrative analyses, lower specificity of primers even for closely related *R. atlantica* populations can explain the deviations observed (Carlsson, 2008; Turini et al., 2014). Overall, observed heterozygosity was higher than expected in each species (except *R. tristis*). Most loci amplified for *R. atlantica* deviate from HWE due to homozygote excess as mentioned above or, particularly, higher incidence of null alleles in the shallow waters of Santa Maria. The lower efficiency of the SSR-GBAS primers in the shallow water cluster was expected as these populations appear to constitute a genetically distinct lineage of *R. atlantica*. Most *R. atlantica* specimens from Flores failed to amplify with the SSR-GBAS primer battery due to poor quality of the DNA or high sequence divergence in the flanking regions, if these constitute a divergent deep-water lineage.

Other factors and life-traits can have contributed to the deviation to HWE and confounded the population genetic signals. Bryozoan colonies are not distinguished by sex, producing both male and female gametes, thus sex-biased dispersal (DiBattista et al., 2012) is unlikely to affect HWE in the genus *Reteporella*. In *Reteporella* species and other cheilostomes, dispersal occurs either via the short-lived, non-planktotrophic larvae or by rafting of adult, bisexual colonies. The limited dispersal capacity of the larvae, together with the hydrographic conditions in the archipelago, likely contribute to the

increasing isolation and divergence of distant populations (Baptista et al., 2021b; Goldson et al., 2001; Watts & Thorpe, 2006; Zouros & Foltz, 1984). This subpopulation structure contributes to an expected deficiency of heterozygotes detected, which is attributed to the Wahlund effect (Wahlund, 1928). Alternatively, heterozygote genotypes erroneously genotyped as homozygotes can have the same effect, although it is unlikely considering the workflow and respective checkpoints. High effective population sizes have also been shown to cause deviations from the HWE (DiBattista et al., 2012), namely in species with clonal reproduction (Balloux et al., 2003; Campbell & Husband, 2005). *Reteporella* colonies display clonal growth, but reproduce sexually or, rather rarely, multiply via regrowth of a colony from a colony fragment. Estimates of effective population sizes are not available for cheilostomes bryozoans, such that its effect on population structure cannot be accounted for.

Discordance between marker classes

The adjoined use of COI and SSR-GBAS datasets is a powerful tool to evaluate different inheritance dynamics and processes that shape phylogeographic and demographic patterns (DiBattista et al., 2012). Mitochondrial markers provide insights into the initial colonization and phylogenetic evolution, whereas microsatellite markers allow to assess fine-scale genetic structure, population fragmentation, and detect signatures of reticulate events as hybridization in recent times (DiBattista et al., 2012; Galtier et al., 2009; Ruiz-García et al., 2020). All species studied (*R. atlantica*, *R. oceanica*, *R. tristis*, and *Reteporella* spp. 6 and 7) are morphologically distinguishable, in accordance with COI divergence levels over 6% (Baptista et al., 2022) and high genetic diversity levels that suggest clear interspecific divergence. However, discordant cyto-nuclear patterns were inferred in this study for the bryozoan genus *Reteporella* in the Azores. Such discordance was unexpected, as that the faster rate of evolution of microsatellite markers should result in strong population structure in *Reteporella* spp..

As mentioned before, primer pairs designed from *R. atlantica* and *Reteporella* sp. 6 were applied to a dataset comprising five *Reteporella* spp., in a typical case of cross-species amplification. Mutations in the primer-binding region are expected across different taxa, with the potential to cause allelic dropout and prevent amplification of more divergent alleles. For different species, the same loci will behave and be amplified at different rates, causing disparity in the performance of the primer battery (Carlsson, 2008; Curto et al., 2019; Turini et al., 2014). Null alleles will arise differently across different species, conversely, the amplification of alleles shared across species should not be affected. This might result in an underestimation of genetic differentiation and

genetic structure patterns that resembles null allele distribution rather than genetic similarity in the microsatellite evolution. The weak genetic structure inferred for the genus *Reteporella* (except for shallow water *R. atlantica*; Figures 5.8-5.11) with the SSR-GBAS is proposed to be due to methodological constraints, as a consequence of null allele distribution and disparity in the marker performance in the five species studied.

Nevertheless, the congruence detected between genetic patterns and the geographical distribution of specimens was striking. The SSR-GBAS dataset suggests a first subdivision by geographical locality and then by species assignment within each locality (cf. $K > 4$ in Figures 5.9-5.11), with biological significance. As an alternative hypothesis to null alleles, other dynamic processes as interspecific introgression in areas of sympatry, can be driving the evolution of Azorean *Reteporella*. Incongruence between mitochondrial and microsatellite/nuclear markers has been reported in other marine sessile taxa and suggested to be a consequence of semipermeable species boundaries or incomplete sorting of ancestral polymorphisms (Frade et al., 2010; Harrison & Larson, 2014; Nydam et al., 2017; Quattrini et al., 2019). Occasional gene flow, involving mostly neutral nuclear regions, favours locally dependant exchanges between closely related species co-occurring in one locality, rather than exchange between conspecific colonies located far apart (Frade et al., 2010; Gillespie et al., 2020). Such exchanges might be kept in an interplay with the selective forces acting upon the mitochondrial DNA, maintaining the morphological integrity and phylogenetic identity of each species (Baiz et al., 2018; Capblancq et al., 2020; Frade et al., 2010; Galtier et al., 2009; Larson et al., 2013). Testing this hypothesis is, unfortunately, not possible with the available dataset and low sample size for each species and localities. Future phylogenomic studies with a comprehensive *Reteporella* dataset might be useful to test the hybridization hypothesis as a non-neutral process in this bryozoan genus, with the identification of putative areas of congruence among species.

Factors shaping genetic structure of *Reteporella* species in the Azores

Our results suggest complex interrelationships among *Reteporella* species inhabiting Azorean islands and seamounts. Water temperature decreases as depth increases, reaching near-constant temperatures of $\sim 15^{\circ}\text{C}$ and $\sim 12^{\circ}\text{C}$ at 150 m and 500 m throughout the year, respectively (Wisshak et al., 2010). In the first 150 m of the water column, currents flow predominantly in a southeast direction under the influence of the Gulf Stream and associated eddies (Sala et al., 2016), while changes in flow direction may occasionally occur (e.g. Santos et al., 1995). At 500 m depth, a directional change of the predominant currents occurs as a consequence of a weakened Gulf Stream and

Azores Current, promoting westward transport of particles under the influence of eddies or the Mediterranean Outflow Water (Sala et al., 2016). Thus, major currents and associated eddies, as well as occasional storms are expected to influence the direction of larval exchange differently at different depths within the Azores.

Another level of complexity is introduced by habitat fragmentation, i.e. the distance between suitable habitats, and the geological evolution of the archipelago. As *Reteporella* species grow on hard substrata in depths shallower than 1000 m, the distance between islands and seamounts offering this kind of substratum at these depths plays a crucial role in the successful dispersal and recruitment of their short-lived larvae, besides the speed and direction of currents. Thus, the short distances between islands within groups of islands (western, central, eastern) may allow for relatively unrestricted genetic connectivity whereas the distance between groups of islands may, under normal conditions, act as a barrier to gene flow, promoting isolation and independent evolution of populations (see the special case of Santa Maria below) as well as, possibly, adaptive radiations. In this case, only chance or short-lived events, such as current reversals during strong storms or latitudinal shifts in the Gulf Stream, may result in a range expansion beyond the critical threshold. In addition, with the easternmost Santa Maria being the oldest island (c. 6 Ma; Ramalho et al., 2017) and Pico the youngest (c. 270,000 yrs; Demand et al., 1982), the *de novo* origin of the islands during the Pliocene and Pleistocene offered new habitats, and reduced the distance between existing suitable habitats, thus potentially promoting the sympatric occurrence of formerly geographically separated (incipient) species. These historical factors complicate matter but have to be taken into account when interpreting recent phylogeographic and population genetic patterns.

The cryptic *Reteporella atlantica* lineage in shallow waters

The analysis of the COI dataset from eight populations of *R. atlantica* revealed a strong population structure, with most of the genetic variation explained by geographical partitioning. In the SSR-GBAS dataset only 15% of the variation is assigned to geographical populations, however caution is advised when interpreting this value as only five populations (three of which are shallow water populations) were successfully represented in the analysis. Still, marked population structure of *R. atlantica* across the archipelago, and to a certain point correlated to the geographical distance between localities, is suggested by the pairwise F_{ST} and ϕ_{ST} comparisons, in accordance with previous studies and as expected for species with non-planktotrophic larval development (Baptista et al., 2022; Watts & Thorpe, 2006). Shallow water populations of *R. atlantica*

from the easternmost island of Santa Maria stand out in all the analyses as different from conspecifics, especially in the SSR-GBAS dataset in which a shallow water cluster is constantly retrieved across the inferences.

Reteporella atlantica are found around Santa Maria at diving depths (< 25 m), mostly in low energy conditions on the ceilings and uppers sidewalls of submerged caves or tunnels, protected from wave action and with low luminosity (LB and BB, pers. obs.). Sampling was conducted in July in Pedrinha, Mar da Barca, and Gruta Azul, at depths ranging from 11 to 28 m and seawater temperatures of 18-19°C. Although similar environments occur in other Azorean islands, *R. atlantica* colonies were not observed in shallow waters elsewhere, according to reports of experienced and knowledgeable divers, as well as field observations by LB (July and August, 2017-2022). The next shallowest record is from a settlement panel deployed at 60 m depth off Faial (Wisshak et al., 2015), which is slightly over the storm wave base at around 100 m in the archipelago. In the settlement panel experiment by Wisshak et al. (2015), *Reteporella* colonies grew to a height and width of c. 2 cm within a year of submersion at 60 m depth and to about 1 cm at 150 m (BB, pers. obs.). As colonies may reach a size of over 10 cm even at 150 m depth, they may live for over 10 years and are prone to abrasion in high-energy environments, such that larvae preferentially settle in calm conditions. Thus, the greatest diversity of *Reteporella* species in the Azores and elsewhere is found below the storm wave base. None of the other Azorean species occur in depths shallower than 150 m (Baptista et al., 2022). In contrast, numerous *Reteporella* species occur in various shallow water environments in the Mediterranean Sea, in which the fair-weather and storm wave bases are considerably shallower (e.g., Gautier, 1962).

Nevertheless, the absence of *R. atlantica* from sheltered, shallow water environments in Azorean islands other than Santa Maria is peculiar. Santa Maria is the easternmost island of the archipelago where sea-surface currents flow in a prevailing eastward direction (Sala et al., 2016), such that larvae of the shallow water *R. atlantica* are likely being lost to the open ocean, hampering the colonization of the more westerly positioned islands. If the group remains reproductively isolated and surface circulation patterns established in the Azores are unchanged, populations of *R. atlantica* in shallow waters might persist around the island and are expected to continue to differentiate, with the potential to give rise to a single-island marine endemic species, which are rarely recorded in the marine realm (Ávila et al., 2019; Okamura & Hatton-Ellis, 1995).

Morphologically, specimens from shallow waters of Santa Maria match the taxonomical concept of *R. atlantica* (Busk, 1884), but results from the COI and SSR-GBAS datasets showed a clear distinction of the group with no evidence of introgression

with other *Reteporella* taxa from deeper waters. Differentiation was found between *R. atlantica* from shallow waters and most other localities, but not among the three shallow water localities sampled. Unfortunately, *R. atlantica* is not represented among the 17 deep-water specimens collected off Santa Maria (see Supplementary Table S1), thus it was not possible to ascertain the levels of divergence between shallow and deep-water lineages in this area, nor the occurrence of gene flow with other localities at the same depth. The shallow water *R. atlantica* populations might constitute an example of potentially cryptic diversity among Azorean bryozoans, with ecological differentiation preceding phylogenetic splits yet to be detected (Teske et al., 2019; Prada & Hellberg, 2021). Bathymetrical zonation might be playing a role in promoting the differentiation of the shallow water lineage of *R. atlantica* (Gillespie et al., 2020; Jackson & McKinney, 1990), as already reported in several other marine sessile organisms [e.g., corals in Frade et al. (2010), Prada & Hellberg (2013, 2021), and Stefanoudis et al. (2019); review by Knowlton, 1993]. The low tendency for vertical migration of larvae and/or adults contributes for divergence of lineages to prevail over the vertical rather than horizontal axis in other marine sessile invertebrates (Frade et al., 2010; Taboada et al., 2022; Taylor & Roterman, 2017). Regional and local hydrographic patterns are also known to affect gene flow and species distribution in sessile marine invertebrates (Goldson et al., 2001; Radice et al., 2016; Teske et al., 2019). Thermocline position, estimated between 40 and 100 m in the Azores region (Santos et al., 1995), often acts as barrier to populations at different depths due to a light, nutrient, and temperature gradient (cf. Wisshak et al., 2010), with changes in predation pressure and force of waves and currents as depth increases (Frade et al., 2010; Prada & Hellberg, 2013). Reproductive isolation between deep- and shallow water colonies can be maintained by pleiotropic interactions, such as mismatching reproductive timings reported for *Eunicea flexuosa* (Potkamp & Fransen, 2019; Prada & Hellberg, 2013, 2021).

Two alternative scenarios, which await testing in the future, are proposed to explain the finding of a potentially cryptic ecotype of *R. atlantica* restricted to shallow waters around Santa Maria. For the “bottom-up” scenario we propose that the archipelago was colonised from other nearby deep-sea source regions such as the Mid-Atlantic Ridge (e.g., Souto & Albuquerque, 2019) or the cluster of seamounts around the Great Meteor Bank south of the Azores where the genus is present (BB, pers. obs.), and that, at some time, an opportunity arose for *R. atlantica* to invade shallower niches. As demographic expansion was not detected in *R. atlantica*, we suggest that shallow water specimens might constitute a reproductively isolated sibling group (Mayr, 1963) that recently diverged, explaining the low nucleotide and haplotype diversities (Supplementary Table

A6.3). As Santa Maria is the oldest island in the archipelago ample time was potentially available for the island's population to adapt to adverse shallow water conditions (higher current energy and abrasion rate, competition with faster growing photoautotrophs, among others). This scenario, however, would have likely taken place also in other Azorean islands given their geological age and because *Reteporella* species seem to be successful across wide bathymetrical and environmental ranges, with different species occurring in sympatry (Baptista et al., 2022).

Alternatively, in the “top-down” scenario, the shallow water *R. atlantica* represents the ancestral lineage (at least of Clade I) that first colonized the Azores. The most plausible mechanism of transport of brooding bryozoans, such as *Reteporella*, to the remote Azores Archipelago is by rafting of external shallow water source populations on floating substrata (Porter et al., 2002; Scheltema, 1986; Watts & Thorpe, 2006; Winston, 2012). Following the origins of the other islands, and the onset of more erratic bathyal currents, the deeper population of *R. atlantica* may have established itself throughout the remaining archipelago.

Whether the shallow water lineage constitutes a recent divergence restricted to Santa Maria or a relic of an ancestral shallow water lineage remains to be tested. A better understanding of the invasion history of the Azores would greatly benefit from a phylogenetic reconstruction of the family Phidoloporidae in the North Atlantic. With the currently available dataset, we cannot make any further inferences on the direction of evolution of *R. atlantica* species or their ancestral states.

Conclusions

The biogeographical analyses performed retrieved incongruent patterns between mitochondrial and microsatellite markers being both, at different levels, congruent with geographical distance and species boundaries. The detection of a shallow water lineage of *R. atlantica*, likely undergoing ecologically-driven incipient cryptic speciation, raises new questions regarding the biogeography of sessile marine organisms across their bathymetrical range. The weak genetic structure in the SSR-GBAS was unexpected, as the five *Reteporella* taxa are distinguished based on morphological characters and mitochondrial differentiation. Although this is attributed to methodological constraints of the SSR-GBAS approach applied to multiple species, an alternative hypothesis is proposed related to interspecific introgression in areas of sympatry. With the current dataset this hypothesis cannot, unfortunately, be tested, and future phylogenomic approaches are needed to identify areas of congruence. We also do not discard the

possibility of loci duplications in the *Reteporella* genome, nor unreported polyploidy with the potential to mask phylogeographic and population genetic patterns.

The genetic architecture and mechanisms acting on bryozoan genomes are far from being fully comprehended. Recently, the largest number of mitochondrial introns ever recorded from bilaterian animals were described in four bryozoan species (Jenkins et al., 2022) and large-scale phylogenies at the phylum-level are only now becoming available from next-generation sequencing techniques (Orr et al., 2019, 2022). That being said, the patterns reported might be more widespread than previously thought across the phylum Bryozoa, and the extent of ecological differentiation and hybridization in marine animal species is now starting to be unveiled, with important evolutionary consequences. A better understanding and interpretation of these processes in cheilostomes thus depend upon advances in basic molecular knowledge of bryozoans and application of phylogenomic approaches to assess to what extent the evolution of *Reteporella* is shaped by dynamic genomic processes.

References

- Ahrens, D., Ah Yong, S. T., Ballerio, A., Barclay, M. V. L., Eberle, J., Espeland, M., Huber, B. A., Mengual, X., Pacheco, T. L., Peters, R. S., Rulik, B., Vaz-de-Mello, F., Wesener, T., & Krell, F.-T. (2021). Is it time to describe new species without diagnoses? - A comment on Sharkey et al. (2021). *Zootaxa*, 5027(2), 151–159. <https://doi.org/10.11646/zootaxa.5027.2.1>
- Akaike, H. (1973). Information Theory and an Extension of the Maximum Likelihood Principle. *Proceedings of the 2nd International Symposium on Information Theory*. Akademiai Kiado, Budapest, 267–281.
- Ávila, S. P. (2006). Oceanic islands, rafting, geographical range and bathymetry: a neglected relationship. In *Proceedings of the 5th International Symposium on the Fauna and Flora of Atlantic Islands*, Vol. 9, 22–39. Irish Biogeographical Society.
- Ávila, S. P. (2013). Unravelling the patterns and processes of evolution of marine life in oceanic islands: a global framework. In *Fernández-Palacios, JM, Nascimento, L, Hernández, JC, Clemente, S., González, A & Díaz-González, JP (Eds.) Climate Change perspectives from the Atlantic: past, present and future*. Universidad de La Laguna, Tenerife, 95–125.
- Ávila, S. P., Cordeiro, R., Madeira, P., Silva, L., Medeiros, A., Rebelo, A. C., Melo, C., Neto, A. I., Haroun, R., Monteiro, A., Rijdsdijk, K., & Johnson, M. E. (2018). Global change impacts on large-scale biogeographic patterns of marine organisms on Atlantic oceanic islands. *Marine Pollution Bulletin*, 126, 101–112. <https://doi.org/10.1016/j.marpolbul.2017.10.087>
- Ávila, S. P., Goud, J., & Martins, A. M. F. (2012). Patterns of Diversity of the Rissoidae (Mollusca: Gastropoda) in the Atlantic and the Mediterranean Region. *The Scientific World Journal*, Article ID, 164890. <https://doi.org/10.1100/2012/164890>
- Ávila, S. P., Johnson, M. E., Rebelo, A. C., Baptista, L., & Melo, C. S. (2020). Comparison of modern and Pleistocene (MIS 5e) coastal boulder deposits from Santa Maria Island (Azores Archipelago, NE Atlantic Ocean). *Journal of Marine Science and Engineering*, 8(6), 386. <https://doi.org/10.3390/JMSE8060386>
- Ávila, S. P., Melo, C., Berning, B., Nuno, S., Quartau, R., Rijdsdijk, K. F., Ramalho, R. S., Cordeiro, R., De Sá, N. C., Pimentel, A., Baptista, L., Medeiros, A., Gil, A., & Johnson, M. E. (2019). Towards a 'Sea-Level Sensitive' dynamic model: impact of island ontogeny and glacio-eustasy on global patterns of marine island biogeography. *Biological Reviews*, 94(3), 1116–1142. <https://doi.org/10.1111/brv.12492>
- Ávila, S. P., Ramalho, R. S., Habermann, J. M., Quartau, R., Kroh, A., Berning, B., Johnson, M., Kirby, M. X., Zanon, V., Titschack, J., Goss, A., Rebelo, A. C., Melo, C., Madeira, P., Cordeiro, R., Meireles, R., Bagaço, L., Hipólito, A., Uchman, A., da Silva, C. M., Cachão, M., & Madeira, J. (2015). Palaeoecology, taphonomy, and preservation of a lower Pliocene shell bed (coquina) from a volcanic oceanic island (Santa Maria Island, Azores). *Palaeogeography, Palaeoclimatology, Palaeoecology*, 430, 57–73. <https://doi.org/10.1016/j.palaeo.2015.04.015>
- Baiz, M. D., Tucker, P. K., & Cortés-Ortiz, L. (2019). Multiple forms of selection shape

- reproductive isolation in a primate hybrid zone. *Molecular Ecology*, 28(5), 1056-1069. <https://doi.org/10.1111/mec.14966>
- Balloux, F., Lehmann, L., & De Meeûs, T. (2003). The Population Genetics of Clonal and Partially Clonal Diploids. *Genetics*, 164, 1635–1644. <https://doi.org/10.1093/genetics/164.4.1635>
- Baptista, L., Berning, B., Waeschenbach, A., Curto, M., Meimberg, H., Santos, A. M., & Ávila, S. P. (2022) Morphospecies and molecular diversity of ‘lace corals’: the genus *Reteporella* (Bryozoa: Cheilostomatida) in the central North Atlantic Azores Archipelago. *BMC Ecology and Evolution*, 22, Article number: 128. <https://doi.org/10.1186/s12862-022-02080-z>
- Baptista, L., Meimberg, H., Ávila, S. P., Santos, A. M., & Curto, M. (2021b). Dispersal ability, habitat characteristics, and sea-surface circulation shape population structure of *Cingula trifasciata* (Gastropoda: Rissoidae) in the remote Azores Archipelago. *BMC Ecology and Evolution*, 21(1), 128. <https://doi.org/10.1186/s12862-021-01862-1>
- Baptista, L., Santos, A. M., Melo, C. S., Rebelo, A. C., Madeira, P., Cordeiro, R., Botelho, A. Z., Hipólito, A., Pombo, J., Voelker, A. H. L., & Ávila, S. P. (2021a). Untangling the origin of the newcomer *Phorcus sauciatatus* (Mollusca: Gastropoda) in a remote Atlantic archipelago. *Marine Biology*, 168(1), Article number: 9. <https://doi.org/10.1007/s00227-020-03808-5>
- Bashmachnikov, I., Loureiro, C. M., & Martins, A. (2013). Topographically induced circulation patterns and mixing over Condor seamount. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 98, 38-51. <https://doi.org/10.1016/j.dsr2.2013.09.014>
- Berning, B., & Kuklinski, P. (2008). North-east Atlantic and Mediterranean species of the genus *Buffonellaria* (Bryozoa, Cheilostomata): implications for biodiversity and biogeography. *Zoological Journal of the Linnean Society*, 152, 537–566. <https://doi.org/10.1111/j.1096-3642.2007.00379.x>
- Berning, B. & Spencer Jones, M. E. (2023). Revision of the Cheilostomatida (Bryozoa) collected during the HMS ‘Challenger’ Expedition (1872–1876) in the central North Atlantic. *Journal of the Marine Biological Association of the United Kingdom*, 103, e32, 1-22. <https://doi.org/10.1017/S0025315423000231>
- Berning, B., Achilleos, K., & Wisshak, M. (2019). Revision of the type species of *Metroperiella* (Bryozoa, Cheilostomatida), with the description of two new species. *Journal of Natural History*, 53(3–4), 141–158. <https://doi.org/10.1080/00222933.2019.1582725>
- Berning, B., Harmelin, J.-G., & Bader, B. (2017). New Cheilostomata (Bryozoa) from NE Atlantic seamounts, islands, and the continental slope: evidence for deep-sea endemism. *European Journal of Taxonomy*, 347, 1–51. <https://doi.org/10.5852/ejt.2017.347>
- Berning, B., Jones, M. E. S., & Vieira, L. M. (2021). Revision of the European species of the genus *Hincksina* Norman, 1903 (Bryozoa, Cheilostomatida, Flustridae). *Zootaxa*, 5081(3), 333–352. <https://doi.org/10.11646/zootaxa.5081.3.2>
- Bernt, M., Donath, A., Jühling, F., Externbrink, F., Florentz, C., Fritzsche, G., Pütz, J., Middendorf, M., & Stadler, P. F. (2013). MITOS: Improved de novo metazoan mitochondrial genome annotation. *Molecular Phylogenetics and Evolution*, 69(2), 312-319. <https://doi.org/10.1016/j.ympev.2012.08.023>

- Bock, P. (2022). *World List of Bryozoa. Phidoloporidae Gabb & Horn, 1862*. Accessed through: World Register of Marine Species at: <https://www.marinespecies.org/Aphia.php?P=taxdetails&id=110768> on 2022-01-05.
- Brandström, M., & Ellegren, H. (2008). Genome-wide analysis of microsatellite polymorphism in chicken circumventing the ascertainment bias. *Genome Research*, 18(6), 881-887. <https://doi.org/10.1101/gr.075242.107>
- Busk, G. (1884). *Report on the Polyzoa collected by H.M.S. Challenger during the years 1873-1876. Part 1. The Cheilostomata*. Report on the Scientific Results of the Voyage of H.M.S. Challenger during the years 1873-76. *Zoology*, 10, 1-216.
- Calvet, L. (1907). *Bryozoaires. Expéditions scientifiques du Travailleur et du Talisman pendant les années 1880, 1881, 1882 et 1883*. Imprimerie de Monaco.
- Calvet, L. (1931). Bryozoaires provenant des campagnes scientifiques du Prince Albert 1^{er} de Monaco. In M. Richard (Ed.), *Résultats des Campagnes Scientifiques du Prince Albert 1^{er} de Monaco* 83. Imprimerie de Monaco. 1-188.
- Campbell, L. G., & Husband, B. C. (2005). Impact of clonal growth on effective population size in *Hymenoxys herbacea* (Asteraceae). *Heredity*, 94(5), 526-532. <https://doi.org/10.1038/sj.hdy.6800653>
- Capblancq, T., Després, L., & Mavárez, J. (2020). Genetic, morphological and ecological variation across a sharp hybrid zone between two alpine butterfly species. *Evolutionary Applications*, 13(6), 1435-1450. <https://doi.org/10.1111/eva.12925>
- Carlsson, J. (2008). Effects of microsatellite null alleles on assignment testing. *Journal of Heredity*, 99(6), 616-623. <https://doi.org/10.1093/jhered/esn048>
- Carreiro-Silva, M., Ocaña, O., Stankovic, D., Sampaio, Í., Porteiro, F. M., Fabri, M. C., & Stefanni, S. (2017). Zoantharians (Hexacorallia: Zoantharia) associated with cold-water corals in the Azores region: New species and associations in the deep sea. *Frontiers in Marine Science*, 4, 88. <https://doi.org/10.3389/FMARS.2017.00088/BIBTEX>
- Carstens, B. C., Pelletier, T. A., Reid, N. M., & Satler, J. D. (2013). How to fail at species delimitation. *Molecular Ecology*, 22(17), 4369-4383. <https://doi.org/10.1111/mec.12413>
- Casoli, E., Mancini, G., Ventura, D., Pace, D. S., Belluscio, A., & Ardizzone, G. D. (2020b). *Reteporella* spp. success in the re-colonization of bare coralligenous reefs impacted by Costa Concordia shipwreck: The pioneer species you did not expect. *Marine Pollution Bulletin*, 161. <https://doi.org/10.1016/j.marpolbul.2020.111808>
- Casoli, E., Piazzzi, L., Nicoletti, L., Jona-Lasinio, G., Cecchi, E., Mancini, G., Belluscio, A., & Ardizzone, G. (2020a). Ecology, distribution and demography of erect bryozoans in Mediterranean coralligenous reefs. *Estuarine, Coastal and Shelf Science*, 235(January), 106573. <https://doi.org/10.1016/j.ecss.2019.106573>
- Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, 17(4), 540-552. <http://mbe.oxfordjournals.org/>

- Chapuis, M. P. M., & Estoup, A. (2007). Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution*, 24(3), 621–631. <https://doi.org/10.1093/molbev/msl191>
- Chernomor, O., Von Haeseler, A., & Minh, B. Q. (2016). Terrace aware data structure for phylogenomic inference from supermatrices. *Systematic Biology*, 65(6), 997–1008. <https://doi.org/10.1093/sysbio/syw037>
- Clement, M., Posada, D., & Crandall, K. A. (2000). TCS: A computer program to estimate gene genealogies. *Molecular Ecology*, 9(10), 1657–1659. <https://doi.org/10.1046/j.1365-294X.2000.01020.x>
- Cordeiro, R., & Ávila, S. P. (2015). New species of Rissoiidae (Mollusca, Gastropoda) from the Archipelago of the Azores (northeast Atlantic) with an updated regional checklist for the family. *ZooKeys*, 480, 1–19. <https://doi.org/10.3897/zookeys.480.8599>
- Curto, M., Winter, S., Seiter, A., Schmid, L., Scheicher, K., Barthel, L. M. F., Plass, J., & Meimberg, H. (2019). Application of a SSR-GBS marker system on investigation of European Hedgehog species and their hybrid zone dynamics. *Ecology and Evolution*, 9(5), 2814–2832. <https://doi.org/10.1002/ece3.4960>
- d'Hondt, J.-L. (1975). Bryozoaires Cténostomes et Cheilostomes (Cribrimorphes et Escharellidae exceptés) provenant des dragages de la campagne océanographique Bjaçores du "Jean-Charcot". *Bulletin Du Muséum National d'Histoire Naturelle*, 3, 553–600.
- Dayrat, B., Tillier, A., Lecointre, G., & Tillier, S. (2001). New clades of euthyneuran gastropods (Mollusca) from 28S rRNA sequences. *Molecular Phylogenetics and Evolution*, 19(2). <https://doi.org/10.1006/mpev.2001.0926>
- Demand, J., Fabriol, R., Gerard, F., Lundt, F., & Chovelon, P. (1982) Prospection géothermique, îles de Faial et de Pico (Açores). *Rapport Géologique, Geochemique et Gravimétrique*. Technical Report BRGM 82 SGN 003 GTH
- Denisenko, N. V. (2022). New species and new records of *Reteporella* (Bryozoa: Cheilostomatida) from Greenland waters. *Zootaxa*, 5129(4), 530–542. <https://doi.org/10.11646/ZOOTAXA.5129.4.3>
- Denisenko, N. V., & Blicher, M. E. (2021). Bryozoan diversity, biogeographic patterns and distribution in Greenland waters. *Marine Biodiversity*, 51(5), 1–15. <https://doi.org/10.1007/S12526-021-01213-9/FIGURES/11>
- Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J. F., Guindon, S., Lefort, V., Lescot, M., Claverie, J. M., & Gascuel, O. (2008). Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research*, 36(Web Ser, W465-9. <https://doi.org/10.1093/nar/gkn180>
- Desiderato, A., Costa, F. O., Serejo, C. S., Abbiati, M., Queiroga, H., & Vieira, P. E. (2019). Macaronesian islands as promoters of diversification in amphipods: The remarkable case of the family Hyalidae (Crustacea, Amphipoda). *Zoologica Scripta*, 48(3), 359–375. <https://doi.org/10.1111/zsc.12339>
- Deudero, S., Blanco, A., Box, A., Mateu-Vicens, G., Cabanellas-Reboredo, M., & Sureda, A.

- (2010). Interaction between the invasive macroalga *Lophocladia lallemandii* and the bryozoan *Reteporella grimaldii* at seagrass meadows: Density and physiological responses. *Biological Invasions*, 12(1), 41–52. <https://doi.org/10.1007/s10530-009-9428-1>
- DiBattista, J. D., Rocha, L. A., Craig, M. T., Feldheim, K. A., & Bowen, B. W. (2012). Phylogeography of two closely related Indo-Pacific butterflyfishes reveals divergent evolutionary histories and discordant results from mtDNA and microsatellites. *Journal of Heredity*, 103(5), 617–629. <https://doi.org/10.1093/jhered/ess056>
- Di Martino, E. & Rosso, A. (2021). Seek and ye shall find: new species and new records of *Microporella* (Bryozoa, Cheilostomatida) in the Mediterranean. *Zookeys*, 1053, 1–42. <https://doi.org/10.3897/zookeys.1053.65324>
- Dick, M. H., & Mawatari, S. F. (2005). Morphological and molecular concordance of *Rhynchozoon* clades (Bryozoa, Cheilostomata) from Alaska. *Invertebrate Biology*, 124(4), 344–354. <https://doi.org/10.1111/j.1744-7410.2005.00032.x>
- Earl, D. A., & VonHoldt, B. M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4(2), 359–361. <https://doi.org/10.1007/s12686-011-9548-7>
- Eberle, J., Ahrens, D., Mayer, C., Niehuis, O., & Misof, B. (2020). A plea for standardized nuclear markers in Metazoan DNA taxonomy. *Trends in Ecology and Evolution*, 35(4), 336–345. <https://doi.org/10.1016/j.tree.2019.12.003>
- Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of Molecular Variance Inferred from Metric Distances Among DNA Haplotypes: Application. *Genetics*, 491, 479–491
- Figuerola, B., Barnes, D. K. A., Brickle, P., & Brewin, P. E. (2017). Bryozoan diversity around the Falkland and South Georgia Islands: Overcoming Antarctic barriers. *Marine Environmental Research*, 126, 81–94. <https://doi.org/10.1016/J.MARENRES.2017.02.005>
- Frade, P. R., Reyes-Nivia, M. C., Faria, J., Kaandorp, J. A., Luttikhuisen, P. C., & Bak, R. P. M. (2010). Semi-permeable species boundaries in the coral genus *Madracis*: Introgression in a brooding coral system. *Molecular Phylogenetics and Evolution*, 57(3), 1072–1090. <https://doi.org/10.1016/j.ympev.2010.09.010>
- Freitas, R., Romeiras, M., Silva, L., Cordeiro, R., Madeira, P., (...) & Ávila, S. P. (2019). Restructuring of the ‘Macaronesia’ biogeographic unit: A marine multi-taxon biogeographical approach. *Scientific Reports*, 9(1), 1–18. <https://doi.org/10.1038/s41598-019-51786-6>
- Fu, Y. X. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, 147, 915–925.
- Fuchs, J., Obst, M., & Sundberg, P. (2009). The first comprehensive molecular phylogeny of Bryozoa (Ectoprocta) based on combined analyses of nuclear and mitochondrial genes. *Molecular Phylogenetics and Evolution*, 52(1), 225–233. <https://doi.org/10.1016/j.ympev.2009.01.021>
- Gautier, Y.V. (1962). Recherches écologiques sur les Bryozoaires chilostomes en Méditerranée occidentale. *Recueil des travaux de la Station marine d'Endoume*, 38(24), 1-434.
- Geldmacher, J., Hoernle, K., Klügel, A., van den Bogaard, P., & Duggen, S. (2006). A

- geochemical transect across a heterogeneous mantle upwelling: Implications for the evolution of the Madeira hotspot in space and time. *Lithos*, 90(1–2), 131–144. <https://doi.org/10.1016/J.LITHOS.2006.02.004>
- George, K. H., Arndt, H., Wehrmann, A., Baptista, L., Berning, B., Bruhn, M., Carvalho, F., Cordeiro, R., Creemers, M., Defise, A., Domingues, A., Hermanns, K., Hohlfeld, M., Iwan, F., Janßen, T., Jeskulke, K., Kagerer, M., Kaufmann, M., Kieneke, A., ... Wilsenack, M. (2018). *Controls in benthic and pelagic BIODiversity of the AZores BIODIAZ, Cruise No. M150, 27.08.2018 - 02.10.2018, Cádiz (Spain) - Ponta Delgada, São Miguel (Azores)*. https://doi.org/10.2312/cr_m150
- Gillespie, R. G., Bennett, G. M., De Meester, L., Feder, J. L., Fleischer, R. C., Harmon, L. J., Hendry, A. P., Knape, M. L., Mallet, J., Martin, C., Parent, C. E., Patton, A. H., Pfennig, K. S., Rubinoff, D., Schluter, D., Seehausen, O., Shaw, K. L., Stacy, E., Stervander, M., Stroud, J. T., Wagner, C., & Wogan, G. O. U. (2020). Comparing Adaptive Radiations Across Space, Time, and Taxa. *Journal of Heredity*, 111(1), 1–20. <https://doi.org/10.1093/jhered/esz064>
- Goldson, A. J., Hughes, R. N., & Gliddon, C. J. (2001). Population genetic consequences of larval dispersal mode and hydrography: A case study with bryozoans. *Marine Biology*, 138(5), 1037–1042. <https://doi.org/10.1007/s002270000511>
- Gómez, A., Hughes, R. N., Wright, P. J., Carvalho, G. R., & Lunt, D. H. (2007b). Mitochondrial DNA phylogeography and mating compatibility reveal marked genetic structuring and speciation in the NE Atlantic bryozoan *Celleporella hyalina*. *Molecular Ecology*, 16(10), 2173–2188. <https://doi.org/10.1111/j.1365-294X.2007.03308.x>
- Gómez, A., Wright, P. J., Lunt, D. H., Cancino, J. M., Carvalho, G. R., & Hughes, R. N. (2007a). Mating trials validate the use of DNA barcoding to reveal cryptic speciation of a marine bryozoan taxon. *Proceedings of the Royal Society B: Biological Sciences*, 274(1607), 199–207. <https://doi.org/10.1098/rspb.2006.3718>
- Gordon, D. P. (1999). Bryozoan diversity in New Zealand and Australia. In W. F. Ponder & D. Lunney (Eds.), *The Other 99%: The Conservation and Biodiversity of Invertebrates* (pp. 199–204). Transactions of the Royal Zoological Society of New South Wales. <https://doi.org/10.7882/rzsns.1999.033>
- Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Systematic Biology*, 59(3), 696–704. <https://doi.org/10.1093/sysbio/syq010>
- Harmelin, J.-G. (1976). Sur un Reteporidae nouveau, *Reteporella elegans* n. sp. (Bryozaires Cheilostomes), découvert aux Iles d'Hyères. *Travaux Scientifiques Du Parc National de Port-Cros*, 2, 99–104.
- Harmelin, J.-G. (1978). Sur quelques cribrimorphes (Bryozoa, Cheilostomata) de l'Atlantique oriental. *Téthys*, 8(2), 173–192.
- Harmelin, J. G., Bishop, J. D. D., Madurell, T., Souto, J., Spencer Jones, M. E., & Zabala, M. (2019). Unexpected diversity of the genus *Collarina* Jullien, 1886 (Bryozoa, Cheilostomatida)

- in the NE Atlantic-Mediterranean region: New species and reappraisal of *C. balzaci* (Audouin, 1826) and *C. fayalensis* Harmelin, 1978. *Zoosystema*, 40, 385–418. <https://doi.org/10.5252/zoosystema2019v41a21>
- Harrison, R. G., & Larson, E. L. (2014). Hybridization, introgression, and the nature of species boundaries. *Journal of Heredity*, 105(S1), 95–809. <https://doi.org/10.1093/jhered/esu033>
- Hart, M. W., & Sunday, J. (2007). Things fall apart: Biological species form unconnected parsimony networks. *Biology Letters*, 3(5), 509–512. <https://doi.org/10.1098/rsbl.2007.0307>
- Hayward, P. J. (2000). Lace corals (Bryozoa: Phidoloporidae) from Australia and the tropical south-west Pacific. *Journal of the Zoological Society of London*, 252, 109–136.
- Hayward, P. J. (2004). Taxonomic studies on some Indo-West Pacific Phidoloporidae (Bryozoa: Cheilostomata). *Journal of the Zoological Society of London*, 1, 305–326. <https://doi.org/10.1017/S1477200003001191>
- Hayward, P. J., & McKinney, F. K. (2002). Northern Adriatic Bryozoa from the vicinity of Rovinj, Croatia. *Bulletin of the American Museum of Natural History*, 270, 1–139.
- Hayward, P. J., & Ryland, J. S. (1996). Some British Phidoloporidae (Bryozoa: Cheilostomatida). *Zoological Journal of the Linnean Society*, 117, 103–112.
- Hayward, P. J., & Winston, J. E. (2011). Bryozoa collected by the United States Antarctic Research Program: new taxa and new records. *Journal of Natural History*, 45, 2259–2338. <https://doi.org/10.1080/00222933.2011.574922>
- Hayward, P., & Ryland, J. (1999). Cheilostomatous Bryozoa. Part 2. Hippothooidea–Celleporoidea. In R. Barnes & J. Crothers (Eds.), *Synopses of the British Fauna (New Series)* (Vol. 14). Field Studies Council, London. pp. 1–416
- Hebert, P. D. N., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, 270(1512), 313–332. <https://doi.org/10.1098/rspb.2002.2218>
- Hebert, P. D. N., Stoeckle, M. Y., Zemlak, T. S., & Francis, C. M. (2004). Identification of birds through DNA barcodes. *PLoS Biology*, 2(10), e312. <https://doi.org/10.1371/journal.pbio.0020312>
- Hoang, D. T., Chernomor, O., Von Haeseler, A., Minh, B. Q., & Vinh, L. S. (2018). UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, 35(2), 518–522. <https://doi.org/10.1093/molbev/msx281>
- Hubisz, M., Falush, D., Stephens, M., & Pritchard, J. K. (2009). Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources*, 9, 1322–1332. <https://doi.org/10.1111/j.1755-0998.2009.02591.x>
- Hughes, R. N., Gómez, A., Wright, P. J., Moyano, H. I., Cancino, J. M., Carvalho, G. R., & Lunt, D. H. (2008). Molecular phylogeny supports division of the “cosmopolitan” taxon *Celleporella* (Bryozoa; Cheilostomata) into four major clades. *Molecular Phylogenetics and Evolution*, 46(1), 369–374. <https://doi.org/10.1016/j.ympev.2007.08.014>
- Huson, D. H., & Bryant, D. (2006). Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution*, 23(2), 254–267. <https://doi.org/10.1093/MOLBEV/MSJ030>

- Jackson, J. B. C., & Cheetham, A. H. (1990). Evolutionary significance of morphospecies: A test with cheilostome Bryozoa. *Science*, 248(4955), 579–583. <https://doi.org/10.1126/science.248.4955.579>
- Jackson, J. B. C., & Coates, A. (1986). Life cycles and evolution of clonal (modular) animals. *Philosophical Transactions - Royal Society of London, Series B*, 313, 7–22. <https://doi.org/10.1098/rstb.1986.0022>
- Jackson, J. B., & McKinney, F. K. (1990). Macroevolution of Marine Clonal Benthos. *Causes of Evolution: a Paleontological Perspective*, 173.
- Jenkins, H. L., Graham, R., Porter, J. S., Vieira, L. M., Almeida, A. C. D. S., Hall, A., O'Dea, A., Coppard, S. E., & Waeschenbach, A. (2022). Unprecedented frequency of mitochondrial introns in colonial bilaterians. *Scientific Reports*, 12, 10889. <https://doi.org/10.1038/s41598-022-14477-3>
- Johnson, M. E., Uchman, A., Costa, P. J. M., Ramalho, R. S., & Ávila, S. P. (2017). Intense hurricane transports sand onshore: Example from the Pliocene Malbusca section on Santa Maria Island (Azores, Portugal). *Marine Geology*, 385, 244–249. <https://doi.org/10.1016/J.MARGE.2017.02.002>
- Jullien, J., & Calvet, L. (1903). Bryozoaires provenant des campagnes de l'Hirondelle (1886–1888). In M. Richard (Ed.), *Résultats des campagnes scientifiques accomplies sur son yacht par Albert 1^{er}, Prince Souverain de Monaco* (Vol. 23). Imprimerie de Monaco. pp. 1–188.
- Kapli, P., Lutteropp, S., Zhang, J., Kobert, K., Pavlidis, P., Stamatakis, A., & Flouri, T. (2017). Multi-rate Poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo. *Bioinformatics*, 33(11), 1630–1638. <https://doi.org/10.1093/bioinformatics/btx025>
- Katoh, K., & Toh, H. (2008). Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT-based framework. *BMC Bioinformatics*, 9, Article number: 212. <https://doi.org/10.1186/1471-2105-9-212>
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., & Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28(12), 1647–1649. <https://doi.org/10.1093/bioinformatics/bts199>
- Knight, S., Gordon, D. P., & Lavery, S. D. (2011). A multi-locus analysis of phylogenetic relationships within cheilostome bryozoans supports multiple origins of ascophoran frontal shields. *Molecular Phylogenetics and Evolution*, 61(2), 351–362. <https://doi.org/10.1016/j.ympev.2011.07.005>
- Knowlton, N. (1993). Sibling species in the sea. *Annual Review of Ecology and Systematics*, 24, 189–216. <https://doi.org/10.1146/annurev.es.24.110193>
- Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, 15(5), 1179–1191. <https://doi.org/10.1111/1755->

[0998.12387](https://doi.org/10.1093/nar/gkt389)

- Kuraku, S., Zmasek, C. M., Nishimura, O., & Katoh, K. (2013). aLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. *Nucleic Acids Research*, 41(Web Server Issue), W22-8. <https://doi.org/10.1093/nar/gkt389>
- LANFAR, R., CALCOTT, B., HO, S. Y. W., & GUINDON, S. (2012). PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, 29(6), 1675–1701. <https://doi.org/10.1093/molbev/mss020>
- LANNER, J., GSTÖTTENMAYER, F., CURTO, M., GESLIN, B., HUCHLER, K., ORR, M. C., PACHINGER, B., SEDIVY, C., & MEIMBERG, H. (2021). Evidence for multiple introductions of an invasive wild bee species currently under rapid range expansion in Europe. *BMC Ecology and Evolution*, 21, 71. <https://doi.org/10.1186/s12862-020-01729-x>
- LARSON, E. L., ANDRÉS, J. A., BOGDANOWICZ, S. M., & HARRISON, R. G. (2013). Differential introgression in a mosaic hybrid zone reveals candidate barrier genes. *Evolution*, 67(12), 3653-3661. <https://doi.org/10.1111/evo.12205>
- LARSON, E. L., ANDRÉS, J. A., BOGDANOWICZ, S. M., & HARRISON, R. G. (2013). Differential introgression in a mosaic hybrid zone reveals candidate barrier genes. *Evolution*, 67(12), 3653-3661. <https://doi.org/10.1111/evo.12205>
- LARSSON, A. (2014). AliView: A fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics*, 30(22), 3276–3278. <https://doi.org/10.1093/bioinformatics/btu531>
- LE, H. L. VAN, LECOINTRE, G., & PERASSO, R. (1993). A 28S rRNA-based phylogeny of the gnathostomes: First steps in the analysis of conflict and congruence with morphologically based cladograms. *Molecular Phylogenetics and Evolution*, 2(1), 31–51. <https://doi.org/10.1006/mpev.1993.1005>
- LEE, H., KWAN, Y., KONG, S., MIN, B. S., SEO, J. E., & WON, Y. (2011). DNA Barcode Examination of Bryozoa (Class: Gymnolaemata) in Korean Seawater. *Korean Journal of Systematic Zoology*, 27, 159–163.
- LIMA, M. J., SALA, I., & CALDEIRA, R. M. A. (2020). Physical Connectivity Between the NE Atlantic Seamounts. *Frontiers in Marine Science*, 7. <https://doi.org/10.3389/fmars.2020.00238>
- LÓPEZ DE LA CUADRA, C. M., & GARCÍA GÓMEZ, J. C. (2001). New and little-known ascophoran bryozoans from the Western Mediterranean, collected by “Fauna Ibérica” expeditions. *Journal of Natural History*, 35(11), 1717–1732. <https://doi.org/10.1080/002229301317092414>
- LU, G., BASLEY, D. J., & BERNATCHEZ, L. (2001). Contrasting patterns of mitochondrial DNA and microsatellite introgressive hybridization between lineages of lake whitefish (*Coregonus clupeaformis*); relevance for speciation. *Molecular Ecology*, 10, 965-985.
- MARIANI, S. (2003). Recruitment in invertebrates with short-lived larvae: The case of the bryozoan *Disporella hispida* (Fleming). *Helgoland Marine Research*, 57(1), 47–53. <https://doi.org/10.1007/s10152-002-0131-1>
- MACKIE, J. A., KEOUGH, M. J., & CHRISTIDIS, L. (2006). Invasion patterns inferred from cytochrome oxidase I sequences in three bryozoans, *Bugula neritina*, *Watersipora subtorquata*, and

- Watersipora arcuata*. *Marine Biology*, 149, 285–95. <https://doi.org/10.1007/s00227-005-0196-X>
- McClain, C. R., & Hardy, S. M. (2010). The dynamics of biogeographic ranges in the deep sea. *Proceedings of the Royal Society B: Biological Sciences*, 277(1700), 3533–3546. <https://doi.org/10.1098/rspb.2010.1057>
- McWilliam, H., Li, W., Uludag, M., Squizzato, S., Park, Y. M., Buso, N., Cowley, A. P., & Lopez, R. (2013). Analysis tool web services from the EMBL-EBI. *Nucleic Acids Research*, 41(W1), W597–W600.
- Mayr, E. (1963). *Animal Species and Evolution*. Cambridge, MA and London, England: Harvard University Press. <https://doi.org/10.4159/harvard.9780674865327>
- Meireles, R. P., Keyser, D., & Ávila, S. P. (2014). The Holocene to Recent ostracods of the Azores archipelago (NE Atlantic): Systematics and biogeography. *Marine Micropaleontology*, 112, 13–26. <https://doi.org/10.1016/j.marmicro.2014.08.002>
- Melo, C. S., Martín-González, E., da Silva, C. M., Galindo, I., González-Rodríguez, A., Baptista, L., Rebelo, A. C., Madeira, P., Voelker, A. H. L., Johnson, M. E., Arruda, S. A., & Ávila, S. P. (2022). Range expansion of tropical shallow-water marine molluscs in the NE Atlantic during the last interglacial (MIS 5e): Causes, consequences and utility of ecostratigraphic indicators for the Macaronesian archipelagos. *Quaternary Science Reviews*, 278, 107377. <https://doi.org/10.1016/j.quascirev.2022.107377>
- Meyer, C. P., & Paulay, G. (2005). DNA barcoding: Error rates based on comprehensive sampling. *PLoS Biology*, 3(12), 1–10. <https://doi.org/10.1371/journal.pbio.0030422>
- Micael, J., Marina, J. G., Costa, A. C., & Occhipinti-Ambrogi, A. (2014). The non-indigenous *Schizoporella errata* (Bryozoa: Cheilostomatida) introduced into the Azores Archipelago. *Marine Biodiversity Records*, 7, 1–6. <https://doi.org/10.1017/S1755267214001298>
- Micael, J., Tempera, F., Berning, B., López-Fé, C. M., Occhipinti-Ambrogi, A., & Costa, A. C. (2019). Shallow-water bryozoans from the Azores (central North Atlantic): native vs. non-indigenous species, and a method to evaluate taxonomic uncertainty. *Marine Biodiversity*, 49(1), 469–480. <https://doi.org/10.1007/s12526-017-0833-x>
- Nascimento, K. B., Migotto, A. E., & Fehlaue-Ale, K. H. (2021) Molecular data suggest the worldwide introduction of the bryozoan *Amathia verticillata* (Ctenostomata, Vesiculariidae). *Marine Biology*, 168, 1–9. <https://doi.org/10.1007/S00227-021-03837-8/TABLES/2>
- Nydam, M. L., Yanckello, L. M., Bialik, S. B., Giesbrecht, K. B., Nation, G. K., & Peak, J. L. (2017). Introgression in two species of broadcast spawning marine invertebrate. *Biological Journal of the Linnean Society*, 120(4), 879–890. <https://doi.org/10.1093/BIOLINNEAN/BLW012>
- Okamura, B., & Hatton-Ellis, T. (1995). Population biology of bryozoans: correlates of sessile, colonial life histories in freshwater habitats. *Experientia*, 51(5), 510–525. <https://doi.org/10.1007/BF02143202>
- Orr, R. J. S., Di Martino, E., Gordon, D. P., Ramsfjell, M. H., Mello, H. L., Smith, A. M., & Liow, L. H. (2021). A broadly resolved molecular phylogeny of New Zealand cheilostome bryozoans as a framework for hypotheses of morphological evolution. *Molecular Phylogenetics and*

- Evolution*, 161. <https://doi.org/10.1016/j.ympev.2021.107172>
- Orr, R. J. S., Haugen, M. N., Berning, B., Bock, P., Cumming, R. L., Florence, W. K., Hirose, M., Di Martino, E., Ramsfjell, M. H., Sannum, M. M., Smith, A. M., Vieira, L. M., Waeschenbach, A., & Liow, L. H. (2019). A genome-skimmed phylogeny of a widespread bryozoan family, Adeonidae. *BMC Evolutionary Biology*, 19(1), 1–10. <https://doi.org/10.1186/s12862-019-1563-4>
- Orr, R. J. S., Martino, E. Di, Ramsfjell, M. H., Gordon, D. P., Berning, B., Chowdhury, I., Craig, S., Cumming, R. L., Figuerola, B., Florence, W., Harmelin, J.-G., Hirose, M., Huang, D., Jain, S. S., Jenkins, H. L., Kotenko, O. N., Kuklinski, P., Lee, H. E., Madurell, T., ... Liow, L. H. (2022). Paleozoic origins of cheilostome bryozoans and their parental care inferred by a new genome-skimmed phylogeny. *Science Advances*, 8. <https://doi.org/10.1126/sciadv.abm7452>
- Palumbi, S. R., Martin, A., Romano, S., McMillan, W. O., Stice, L., & Grabowski, G. (1991). *The Simple Fool's Guide to PCR*. University of Hawaii, Honolulu. <https://doi.org/10.1186/s13620-015-0060-3>
- Pascual, M., River, B., Schunter, C., & Macpherson, E. (2017). Impact of life history traits on gene flow: A multispecies systematic review across oceanographic barriers in the Mediterranean Sea. *PLoS One*, 12(5). <https://doi.org/10.1371/journal.pone.0176419>
- Peakall, R., & Smouse, P. E. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6, 288–295. <https://doi.org/10.1111/j.1471-8286.2005.01155.x>
- Peakall, R., & Smouse, P. E. (2012). GenALEX 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics*, 28, 2537–2539. <https://doi.org/10.1111/j.1471-8286.2005.01155.x>
- Pond, S. L. K., Poon, A. F. Y., & Frost, S. D. W. (2009). Estimating selection pressures on alignments of coding sequences. In P. Lemey, M. Salemi, & A. Vandamme (Eds.), *The Phylogenetic Handbook: A Practical Approach to Phylogenetic Analysis and Hypothesis*. 2nd ed. Cambridge University Press. pp. 419–451.
- Porras-Hurtado, L., Ruiz, Y., Santos, C., Phillips, C., Carracedo, Á., Lareu, M. V, Gao, G., & Zhang, Q. (2013). An overview of STRUCTURE: applications, parameter settings, and supporting software. *Frontiers in Genetics*, 4. <https://doi.org/10.3389/fgene.2013.00098>
- Porter, J. S., Ryland, J. S., & Carvalho, G. R. (2002). Micro- and macrogeographic genetic structure in bryozoans with different larval strategies. *Journal of Experimental Marine Biology and Ecology*, 272(2), 119–130. [https://doi.org/10.1016/S0022-0981\(02\)00042-4](https://doi.org/10.1016/S0022-0981(02)00042-4)
- Potkamp, G., & Fransen, C. H. J. M. (2019). Speciation with gene flow in marine systems. *Contributions to Zoology*, 88(2), 133–172. <https://doi.org/10.1163/18759866-20191344>
- Prada, C., & Hellberg, M. E. (2013). Long prereproductive selection and divergence by depth in a Caribbean candelabrum coral. *Proceedings of the National Academy of Sciences*, 110(10), 3961–3966. <https://doi.org/10.1073/pnas.1208931110>

- Prada, C., & Hellberg, M. E. (2021). Speciation-by-depth on coral reefs: Sympatric divergence with gene flow or cryptic transient isolation?. *Journal of Evolutionary Biology*, 34(1), 128–137. <https://doi.org/10.1073/pnas.1208931110>
- Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155, 945–959.
- Puerta, P., Johnson, C., Carreiro-Silva, M., Henry, L.-A., Kenchington, E., Morato, T., Kazanidis, G., Rueda, J. L., Urra, J., Ross, S., Wei, C.-L., González-Irusta, J. M., Arnaud-Haond, S., & Orejas, C. (2020). Influence of water masses on the biodiversity and biogeography of deep-sea benthic ecosystems in the North Atlantic. *Frontiers in Marine Science*, 7, 239. <https://doi.org/10.3389/fmars.2020.00239>
- Quattrini, A. M., Wu, T., Soong, K., Jeng, M. S., Benayahu, Y., & McFadden, C. S. (2019). A next generation approach to species delimitation reveals the role of hybridization in a cryptic species complex of corals. *BMC Evolutionary Biology*, 19(1), 116. <https://doi.org/10.1186/s12862-019-1427-y>
- Ramalho, L. V., López-Fé, C. M., & Rueda, J. L. (2018). Three species of *Reteporella* (Bryozoa: Cheilostomata) in a diapiric and mud volcano field of the Gulf of Cádiz, with the description of *Reteporella victori* n. sp. *Zootaxa*, 4375(1), 90–104. <https://doi.org/10.11646/zootaxa.4375.1.4>
- Ramalho, R. S., Helffrich, G., Madeira, J., Cosca, M., Thomas, C., Quartau, R., Hipólito, A., Rovere, A., Hearty, P. J., & Ávila, S. P. (2017). Emergence and evolution of Santa Maria Island (Azores) – The conundrum of uplifted islands revisited. *Bulletin of the Geological Society of America*, 129(3–4), 372–391. <https://doi.org/10.1130/B31538.1>
- Ramos-Onsins, S. E., & Rozas, J. (2002). Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution*, 19(12), 2092–2100. <https://doi.org/10.1093/oxfordjournals.molbev.a004034>
- Rebelo, A. C., Johnson, M. E., Rasser, M. W., Silva, L., Melo, C. S., & Ávila, S. P. (2021). Global biodiversity and biogeography of rhodolith-forming species. *Frontiers of Biogeography*, 13, e50646. <https://doi.org/10.21425/F5FBG50646>
- Reverter-Gil, O., Berning, B., & Souto, J. (2015). Diversity and systematics of *Schizomavella* species (Bryozoa: Bitectiporidae) from the bathyal NE Atlantic. *PLoS ONE*, 10(10). <https://doi.org/10.1371/journal.pone.0139084>
- Reverter-Gil, O., & Fernández-Pulpeiro, E. (1999). Some records of Bryozoans from NW Spain. *Cahiers de Biologie Marine*, 40(1), 35–45.
- Rice, W. R. (1989). Analysing tables of statistical tests. *Evolution*, 43, 223–225.
- Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61(3), 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Rosso, A., & Di Martino, E. (2016). Bryozoan diversity in the Mediterranean Sea: An update. *Mediterranean Marine Science*, 17(2), 567–607. <https://doi.org/10.12681/mms.1474>
- Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J., Guirao-Rico, S., Librado, P., Ramo-Onsins, S.

- E., & Sanchez Gracia, A. (2017). DnaSP v6: DNA Sequence Polymorphism Analysis of Large Datasets. *Molecular Biology and Evolution*, 34, 3299–3302. <https://doi.org/10.1093/molbev/msx248>
- Ruiz-García, M., Vásquez, J. Y. A., Restrepo, H., Cáceres-Martínez, C. H., & Shostell, J. M. (2020). The genetic structure of the spectacled bear (*Tremarctos ornatus*; Ursidae, Carnivora) in Colombia by means of mitochondrial and microsatellite markers. *Journal of Mammalogy*, 101(4), 1072–1090. <https://doi.org/10.1093/JMAMMAL/GYAA082>
- Sala, I., Caldeira, R. M. A., Estrada-Allis, S. N., Froufe, E., & Couvelard, X. (2013). Lagrangian transport pathways in the northeast Atlantic and their environmental impact. *Limnology and Oceanography: Fluids and Environments*, 3(1), 40–60. <https://doi.org/10.1215/21573689-2152611>
- Sala, I., Harrison, C. S., & Caldeira, R. M. A. (2016). The role of the Azores Archipelago in capturing and retaining incoming particles. *Journal of Marine Systems*, 154, 146–156. <https://doi.org/10.1016/j.jmarsys.2015.10.001>
- Salemi, M. (2009). Genetic distances and nucleotide substitution models. In P. Lemey, M. Salemi, & A. Vandamme (Eds.), *The Phylogenetic Handbook: A Practical Approach to Phylogenetic Analysis and Hypothesis*. 2nd ed. Cambridge University Press. pp. 126–140.
- Santos, A. M., Cabezas, M. P., Tavares, A. I., Xavier, R., & Branco, M. (2016). tcsBU: a tool to extend TCS network layout and visualization. *Bioinformatics*, btv636. <https://doi.org/10.1093/bioinformatics/btv636>
- Santos, R. S., Hawkins, S., Monteiro, L. R., Alves, M., & Isidro, E. J. (1995). Marine research, resources and conservation in the Azores. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 5(4), 311–354. <https://doi.org/10.1002/aqc.3270050406>
- Scheltema, R. S. (1986). On dispersal and planktonic larvae of benthic invertebrates: An eclectic overview and summary of problems. *Bulletin of Marine Science*, 39(2), 290–322.
- Scheltema, R. S. (1989). Planktonic and non-planktonic development among prosobranch gastropods and its relationship to the geographic range of species. In J. S. Ryland & P. A. Tyles (Eds.), *Reproduction, Genetics and Distribution of Marine Organisms*. Fredensborg: Olsen and Olsen. pp. 183–188.
- Souto, J. & Albuquerque, M. (2019). Diversity and community structure of Cheilostomata (Bryozoa) from the Hayes Fracture Zone (Mid-Atlantic ridge). *Deep Sea Research, Part I* 147, 32–53. <https://doi.org/10.1016/j.dsr.2019.03.006>
- Stefanoudis, P. V., Rivers, M., Smith, S. R., Schneider, C. W., Wagner, D., Ford, H., Rogers, A. D., & Woodall, L. C. (2019). Low connectivity between shallow, mesophotic and rariphotic zone benthos. *Royal Society Open Science*, 6(9). <https://doi.org/10.1098/rsos.190958>
- Taboada, S., Ríos, P., Mitchell, A., Cranston, A., Busch, K., (...) & Riesgo, A. (2022). Genetic diversity, gene flow and hybridization in fan-shaped sponges (*Phakellia* spp.) in the North-East Atlantic deep sea. *Deep-Sea Research Part I: Oceanographic Research Papers*, 181, 103685. <https://doi.org/10.1016/j.dsr.2021.103685>
- Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis

- Version 11. *Molecular Biology and Evolution*, 38(7), 3022–3027.
<https://doi.org/10.1093/molbev/msab120>
- Taylor, P. D. (1988). Colony growth pattern and astogenetic gradients in the Cretaceous cheilostome bryozoan *Herpetopora*. *Palaeontology*, 31, 519–549.
- Taylor, M. L., & Roterman, C. N. (2017). Invertebrate population genetics across Earth's largest habitat: The deep-sea floor. *Molecular Ecology*, 26, 4872–4896.
<https://doi.org/10.1111/mec.14237>
- Teske, P. R., Sandoval-Castillo, J., Golla, T. R., Emami-Khoyi, A., Tine, M., Von Der Heyden, S., & Beheregaray, L. B. (2019). Thermal selection as a driver of marine ecological speciation. *Proceedings of the Royal Society B*, 286(1896), 20182023.
<https://doi.org/10.1098%2Frspb.2018.2023>
- Tibihika, P. D., Curto, M., Dornstauder-Schrammel, E., Winter, S., Alemayehu, E., Waidbacher, H., & Meimberg, H. (2019). Application of microsatellite genotyping by sequencing (SSR-GBS) to measure genetic diversity of the East African *Oreochromis niloticus*. *Conservation Genetics*, 20(2), 357–372. <https://doi.org/10.1007/s10592-018-1136-x>
- Tilbrook, K. J. (2006). *Cheilostomatous Bryozoa from the Solomon Islands*. Santa Barbara Museum of Natural History Monographs Number 4 - Studies in Biodiversity Number 3.
- Tilbrook, K. J., Hayward, P. J., & Gordon, D. P. (2001). Cheilostomatous Bryozoa from Vanuatu. *Zoological Journal of the Linnean Society*, 131(1), 35–109.
<https://doi.org/10.1006/zjls.2000.0249>
- Trifinopoulos, J., Nguyen, L. T., von Haeseler, A., & Minh, B. Q. (2016). W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research*, 44(W1), W232–5.
<https://doi.org/10.1093/NAR/GKW256>
- Turini, F. G., Steinert, C., Heubl, G., Bringmann, G., Lombe, B. K., Mudogo, V., & Meimberg, H. (2014). Microsatellites facilitate species delimitation in Congolese Ancistrocladus (Ancistrocladaceae), a genus with pharmacologically potent naphthylisoquinoline alkaloids. *Taxon*, 63, 329–341.
- Vieira, P. E., Lavrador, A. S., Parente, M. I., Parretti, P., Costa, A. C., Costa, F. O., & Duarte, S. (2021). Gaps in DNA sequence libraries for Macaronesian marine macroinvertebrates imply decades till completion and robust monitoring. *Diversity and Distributions*, 27(10), 2003–2015.
<https://doi.org/10.1111/ddi.13305>
- Voje, K. L., Martino, E. Di, & Porto, A. (2020). Revisiting a landmark study system: No evidence for a punctuated mode of evolution in *Metrarabdotos*. *American Naturalist*, 195(5).
<https://doi.org/10.1086/707664>
- Waeschenbach, A., Porter, J. S., & Hughes, R. N. (2012a). Molecular variability in the *Celleporella hyalina* (Bryozoa; Cheilostomata) species complex: Evidence for cryptic speciation from complete mitochondrial genomes. *Molecular Biology Reports*, 39(9), 8601–8614.
<https://doi.org/10.1007/s11033-012-1714-9>
- Waeschenbach, A., Taylor, P. D., & Littlewood, D. T. J. (2012b). A molecular phylogeny of bryozoans. *Molecular Phylogenetics and Evolution*, 62(2), 718–735.

<https://doi.org/10.1016/j.ympev.2011.11.011>

- Wägele, H., Klussmann-Kolb, A., Kuhlmann, M., Haszprunar, G., Lindberg, D., Koch, A., & Wägele, J. W. (2011). The taxonomist - an endangered race. A practical proposal for its survival. *Frontiers in Zoology*, 8(1), 1–7. <https://doi.org/10.1186/1742-9994-8-25/TABLES/1>
- Wahlund, S. (1928). Zusammensetzung von Populationen und Korrelationserscheinungen von Stadtpunkt der Vererbungslehre aus Betrachtet. *Hereditas*, 11. <https://doi.org/10.1111/J.1601-5223.1928.TB02483.X>
- Wang, R., Tsui, C. K. M., & You, C. (2022). Cryptic species diversity and phylogenetic relationship in the rust genus *Chrysomyxa* from China. *Journal of Fungi*, 8(1). <https://doi.org/10.3390/jof8010083>
- Watts, P. C., & Thorpe, J. P. (2006). Influence of contrasting larval developmental types upon the population-genetic structure of cheilostome bryozoans. *Marine Biology*, 149(5), 1093–1101. <https://doi.org/10.1007/s00227-006-0288-2>
- Winston, J. E. (2012). Dispersal in marine organisms without a pelagic larval phase. *Integrative and Comparative Biology*, 52(4), 447–457. <https://doi.org/10.1093/icb/ics040>
- Wisshak, M., Berning, B., Jakobsen, J., & Freiwald, A. (2015). Temperate carbonate production: biodiversity of calcareous epiliths from intertidal to bathyal depths (Azores). *Marine Biodiversity*, 45(1), 87–112. <https://doi.org/10.1007/s12526-014-0231-6>
- Wisshak, M., Form, A., Jakobsen, J., & Freiwald, A. (2010). Temperate carbonate cycling and water mass properties from intertidal to bathyal depths (Azores). *Biogeosciences*, 7, 2379–2396. <https://doi.org/10.5194/bg-7-2379-2010>
- Wood, A. C. L., & Probert, P. K. (2013). Bryozoan-dominated benthos of Otago shelf, New Zealand: Its associated fauna, environmental setting and anthropogenic threats. *Journal of the Royal Society of New Zealand*, 43(4), 231–249. <https://doi.org/10.1080/03036758.2012.756819>
- Wood, A. C. L., Probert, P. K., Rowden, A. A., & Smith, A. M. (2012). Complex habitat generated by marine bryozoans: A review of its distribution, structure, diversity, threats and conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 22(4), 547–563. <https://doi.org/10.1002/aqc.2236>
- Zabala, M., & Maluquer, P. (1988). *Illustrated keys for the classification of Mediterranean Bryozoa*. Treballs - Museu de Zoologia, Barcelona.
- Zhang, J., Kapli, P., Pavlidis, P., & Stamatakis, A. (2013). A general species delimitation method with applications to phylogenetic placements. *Bioinformatics*, 29(22), 2869–2876. <https://doi.org/10.1093/bioinformatics/btt499>
- Zhang, Z., Schwartz, S., Wagner, L., & Miller, W. (2000). A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology*, 7(1–2), 203–214. <https://doi.org/10.1089/10665270050081478>
- Zouros, E., & Foltz, D. W. (1984). Possible explanations of heterozygote deficiency in bivalve molluscs. *Malacologia*, 25, 583–591.

Chapter 6

General Discussion and Future Perspectives

General Discussion

This project aimed to address the marine biodiversity and biogeographic mechanisms in the remote Azores Archipelago, focusing on the processes and patterns inferred from gastropod and bryozoan species with different types of larval development. Three main goals were defined: 1) test biogeographical hypotheses and questions using marine invertebrates as model species; 2) compare the population structure between species with different modes of larval development (planktotrophic *versus* non-planktotrophic) and between two organismal groups (gastropods *versus* bryozoans); 3) evaluate the factors shaping connectivity, evolution, and biogeographical patterns in NE Atlantic marine populations, at intra-archipelago and ocean basin scales. Furthermore, the studies conducted during this thesis have uncovered new diversity and unanticipated relationships in the Azores Archipelago and NE Atlantic area. Throughout this chapter, the key findings of this thesis relative to each of the aforementioned goals will be summarized and discussed. Based on the current knowledge caveats, some of these unravelled with this thesis, I present prospects for future research on population genetics and biogeography of marine invertebrate populations in the Azores. The current datasets can be further complemented with the application of different molecular methodologies, granting new perspectives to the questions posed. Finally, a series of concluding remarks are presented.

6.1 Key findings

6.1.1 Testing biogeographical hypotheses with marine invertebrates

The type and duration of larval development of marine invertebrates in their dispersal ability has long been suggested to have crucial biogeographical and evolutionary implications (Levin & Bridges, 1995; Scheltema, 1986, 1989, 1995). Many biogeographical hypotheses try to explain how marine invertebrate species, especially those with sessile adults or limited dispersal ability, were able to colonize remote areas and how connectivity is maintained between populations. Species with long-lived planktotrophic larvae are expected to exhibit high levels of connectivity across their distribution range, resulting in (sometimes almost total) absence of population structure especially at smaller geographical scales and making them rather unsuitable to test such biogeographical hypotheses. Species with non-planktotrophic larvae and limited dispersal abilities are, however, expected to show strong genetic differentiation and population structure even between close localities, constituting a useful tool to infer the

role of demographic history and evolution on current biogeographical patterns. These two theoretical expectations, relating modes of larval development and population genetic structure, were studied in this thesis resorting to gastropod species: the non-planktotrophic species of the family Rissoidae Gray, 1847 (**Chapters 3.1 and 4.1**) and the planktotrophic species *Stramonita haemastoma* (Linnaeus, 1767) (**Chapter 3.2**). The successful testing of biogeographical hypotheses and inference of strong population structure with non-planktotrophic marine invertebrates reinforce their usefulness as ideal subjects in the field of (palaeo)biogeography.

Genetic data for species of the microgastropod family Rissoidae was used to test a biogeographical hypothesis proposed by Ávila (2013) based on palaeontological and ecological observations (**Chapter 3.1**). The hypothesis predicts that if two congeneric species, both with non-planktotrophic larval development, currently co-occur in two remote archipelagos, then the one inhabiting deeper waters should be evolutionarily older than the intertidal relative (Ávila, 2013). Theoretically, and due to their limited dispersal abilities, benthic, non-planktotrophic, shallow-water gastropod species should be unable to cross important biogeographical barriers and establish themselves in distant archipelagos (Ávila 2013; Scheltema, 1986, 1989, 1995). Nevertheless, exceptions are found [e.g. *Alvania sleursi* (Amati, 1987) in Azores, Madeira, and Selvagens; *Anachis avaroides* F. Nordsieck, 1975 in all the NE Atlantic Archipelagos except Cabo Verde; see Freitas et al., 2019)]. The most plausible explanation for the occurrence in two remote, oceanic archipelagos (e.g. Azores/Madeira, 840 km apart) would be the speciation in a remote archipelago and later range expansion (e.g. by rafting) by chance events to another distant archipelago. Ecological zonation affects the success of rafting, as intertidal species are more prone to be disturbed and thus, subjected to rafting, by constant wave action than subtidal congeners. Therefore, the subtidal/circalittoral lineage is expected to have appeared earlier in the geological scale, granting it enough time to successfully expand its distributional range to the same extent as the intertidal relative (Ávila, 2006, 2013). Using sequence data, a species tree of the family Rissoidae in the Azores was reconstructed and the older divergence of the subtidal *Alvania sleursi* (Amati, 1987) was inferred, providing molecular support to the aforementioned hypothesis (Baptista et al., 2019). Population genetics analyses for the rissoid species *Cingula trifasciata* (J. Adams, 1800) (**Chapter 4.1**) revealed strong genetic structure within the Azores Archipelago, supporting the expectations for organisms with non-planktotrophic larvae. This study allowed to evaluate the factors influencing the dispersal of intertidal species with non-planktotrophic larvae in remote islands (see next section; Baptista et al., 2021b).

To evaluate the usefulness of marine invertebrates with a long planktotrophic larval stage, which facilitates dispersal and broad geographical distributions, population genetic inferences were conducted in the gastropod *Stramonita haemastoma* (Linnaeus, 1767) (**Chapter 3.2**). Evidences of panmixia across the NE Atlantic archipelagos and the Mediterranean Sea were obtained, suggesting the great survival capacity of teleplanic larvae across expected biogeographical barriers. Complete absence of population structure in the NE Atlantic, without further distinguishing of archipelagic ecoregions as defined by Freitas et al. (2019), was inferred and similar patterns have previously been reported for other NE Atlantic species with planktotrophic larvae (Baptista et al., 2021a; Quinteiro et al., 2015; Xavier et al., 2010). The frequent long-distance genetic exchanges hinder the inference of processes affecting planktotrophic species in remote archipelagos, making them rather unsuitable to test biogeographical predictions in the marine realm.

Further work in other marine invertebrate species with non-planktotrophic larval development (**Chapters 4 and 5**) provided insights into the population structure and evolutionary history, contributing to a better understanding of the processes and factors shaping marine biogeography in the remote Azores Archipelago.

6.1.2 Biogeographical patterns and factors shaping the population structure in Azorean marine invertebrates

Population dynamics of marine invertebrate species with non-planktotrophic larvae inhabiting Azorean waters were studied in this thesis for three biological systems – the gastropods *Cingula trifasciata* and *Phorcus sauciatus* (Koch, 1845), and the sessile bryozoan genus *Reteporella* Busk, 1884 (comprising at least seven species in the archipelago) – each providing different insights on biogeographical patterns in volcanic oceanic islands (**Chapters 4 and 5**). Studies on the gastropods allowed a better understanding of the factors shaping the frequency and timing of dispersal events and gene flow, whilst the study of the bryozoan genus provided insights into the processes of diversification and speciation in a remote archipelago.

Non-planktotrophic larval development, as is the case of *C. trifasciata* and *P. sauciatus*, hinders dispersal during early-life stages, although circulation patterns, rafting, or extreme weather events might promote occasional exchange of adults or larvae (Ávila, 2013; Ávila et al., 2015b; Baptista et al., 2021a,b; Winston, 2012). This is expected to result in high population structure at small geographical scales (e.g. intra-archipelagic or even within an island), with occasional signatures of gene flow. The study of *C. trifasciata* in the Azores (**Chapter 4.1**) matched these expectations, revealing a

highly structured archipelagic population (Baptista et al., 2021b). Intra-archipelagic connectivity of *P. sauciatus* was not reconstructed in detail (**Chapter 4.2**), as this gastropod constitutes a new addition to the Azorean intertidal assemblages (Ávila et al., 2015b) and was only present in two islands (Santa Maria and São Miguel) when sampling was conducted for this study. Nevertheless, and considering its accelerated range expansion in the archipelago (it is now present in São Jorge, Pico, and Terceira islands as well; pers. obs.), strong genetic structure is expected to be observed once the Azorean population stabilizes. The marked genetic structure inferred for *C. trifasciata* between populations of the same island (São Miguel) indicates unforeseen barriers to the dispersal operating at a small spatial scale that could constitute the starting point of population differentiation. Several factors seem to drive population structure of *C. trifasciata* within the Azores – dispersal ability (life stages in which rafting can occur), habitat type (algal mat *versus* bare rocky substrates), and small-scale oceanographic currents (Baptista et al., 2021b). These factors likely act on other intertidal species, shaping the structure of communities in the remote Azores Archipelago, and probably play a role in the dispersal and colonization of new localities (e.g. the colonization of the Azores by *P. sauciatus*).

Unexpected signatures of occasional long-distance gene flow (e.g. between island groups or NE Atlantic archipelagos) were detected for both gastropod species in study. In *C. trifasciata*, unexpected connectivity between different island groups is probably related to unusual local-circulation patterns (Baptista et al., 2021b). The study on *P. sauciatus* (**Chapter 4.2**) in the NE Atlantic aimed to determine the most plausible source population of the Azorean colonizers, highlighting the role of random chance events in long-distance dispersal events (Baptista et al., 2021a). Madeira Archipelago was retrieved as an important contributor for the Azorean gene pool, which is unexpected under the predominant current regime established in the NE Atlantic and usual biogeographical affinities of Azorean taxa (see Freitas et al., 2019). A mechanism of dispersal and colonization was proposed, based on association of *P. sauciatus* juveniles to rafts of the pelagic crustacean barnacle *Dosima fascicularis* (Ellis & Solander, 1786), which were assembled close to Madeira Archipelago and advected northwards due to exceptional changes in the circulation regime and periodic storms. The interpretations of the population structure patterns of *C. trifasciata* and *P. sauciatus*, after discarding human-mediated transport (for details see Chapter 4, Baptista et al., 2021a,b), highlight the influence of unconsidered transient oceanographic changes in the connectivity and population dynamics of intertidal non-planktotrophic invertebrates.

The population structure of the bryozoan genus *Reteporella* in the Azores was also evaluated (**Chapter 5.2**), using as a backbone the phylogenetic relationships inferred between Azorean species supported by the concordance between mitochondrial and morphological data (**Chapter 5.1**; Baptista et al., 2022). Bathymetry and ecologically-driven differentiation may play a crucial role in shaping the population dynamics of species with non-planktotrophic larvae and broad bathymetrical ranges, as the results point at the occurrence of a divergent of *R. atlantica* (Busk, 1884) inhabiting shallow-waters. Knowledge of deep-water ecosystem functioning and factors affecting population structure is still in its infancy. Signatures of interspecific introgression and hybridization events were detected in the microsatellite dataset of *Reteporella* species co-existing in several localities of the Azores Archipelago. Incongruent patterns of population structure of five *Reteporella* species were retrieved with two molecular datasets. Semipermeable boundaries are suggested to allow for local-dependent exchange of nuclear non-coding regions between sympatric congeners, whilst the maternally inherited mitochondrial dataset retains the phylogenetic identity of each species. These phenomena have been described in other marine taxa, namely some coral, ascidian, and fish species [e.g. Frade et al. (2010); Quattrini et al. (2019); Nydam et al. (2017); Belaiba et al. (2019); Laakkonen et al. (2015); Roques et al. (2001)]. Further testing of the hybridization hypothesis as a non-neutral process, acting differently across the genome, requires further testing with phylogenomic approaches to identify regions of congruence among *Reteporella* species (Frade et al., 2010). Interspecific introgression can promote further radiations and speciation events by generating new genetic and morphological combinations in the populations, with important biogeographical implications as it can mask phylogeographic patterns (Frade et al., 2010; Gillespie et al., 2020). This is the first observation of a potential case of interspecific introgression in a bryozoan group.

6.1.3 Contributes to the marine biodiversity of the Azores

Compared to other NE Atlantic archipelagos, the marine biota in the Azores is impoverished, with some guilds missing (Hawkins et al., 2000). While the knowledge on native species is far from complete (Borges et al., 2010), the interest on non-native and on invasive fauna and flora of the Azores is currently growing (see Castro et al., 2022). A recent project – Azorean Biodiversity Portal (<https://azoresbiportal.uac.pt/>) – compiles the current known taxa occurring in the Azores, counting ~13,000 species of which ~31% (~3,600) are marine species. This represents almost a 2-fold increase in the number of marine species reported by Borges et al. (2010), and yet the real number of species inhabiting Azorean marine ecosystems is underestimated. Increased sampling effort for

basic inventories and taxonomic reviews are needed to get a better picture of the marine biodiversity in this remote archipelago (Cordeiro & Ávila, 2015).

Most of the studies conducted in the scope of this thesis have uncovered new marine diversity in the Azores Archipelago, contributing to its view as a unique biogeographical unit in support to Freitas et al. (2019) and references therein. For the first time, a species tree for Azorean species of the microgastropod family Rissoidae was obtained, hinting on their evolution in the archipelago with diversification sometime during the Early Miocene (**Chapter 3.1**). Potentially cryptic diversity was detected between the gastropod *C. trifasciata* in the Azores and the Iberian Peninsula, as they constitute divergent evolutionary lineages (**Chapter 4.1**). The top-shell *P. sauciatus* is a recent, natural, arrival to the Azores – reported in Santa Maria for the first time in 2013, sporadic sightings in São Miguel in 2013 and established populations by 2015, in Pico by 2021, and in São Jorge and Terceira by 2022 (update on the current distribution of *P. sauciatus* in the Azores following observations during fieldtrips) (**Chapter 4.2**).

Two genetically distinct Azorean *Reteporella* clades were retrieved in the phylogenetic analysis, paraphyletic to eastern Atlantic and Mediterranean taxa (**Chapter 5.1**). Three of the historically reported species were included in the dataset, and four putative new *Reteporella* species were inferred, all of which likely endemic to the archipelago. There is the potential for further adaptive evolution of the genus *Reteporella* in the Azores and new local diversity to be discovered due to the effects of hybridization in the deep-waters of the Azores (**Chapter 5.2**). Furthermore, a divergent lineage of *R. atlantica* was identified and, to the best of our knowledge, restricted to the shallow-waters around Santa Maria Island, representing a potential case of ecologically-driven incipient cryptic speciation.

6.2 Remarks on the chosen molecular markers

The molecular inferences presented in this thesis were based on mitochondrial and nuclear (including microsatellites) datasets, providing different temporal scales for the biogeographical interpretations and decisions on species boundaries. Genetic data is nowadays considered an independent source of evolutionary data (Ahrens et al., 2021; Collins et al., 2019). The mitochondrial, protein-coding Cytochrome C Oxidase subunit I gene (COI) is the barcode of choice in animals due to its high interspecific variability, predictable sequence variation, and availability of extensive reference databases for comparisons (e.g. GenBank, Barcode of Life Database) (Blaxter, 2016; Collins et al., 2019; Hebert et al., 2003; Hebert & Gregory, 2005). Considering its fastest evolution and higher saturation rates, mitochondrial markers are useful to provide insights into the initial

colonization and phylogenetic evolution (Baptista et al., 2019). In the gastropod and bryozoan species studied in this thesis, the COI marker provided reliable phylogeographic and population structure signals, allowing to identify putative new species, some of which might be cryptic (Chapter 5.1; Baptista et al., 2022). For the microgastropod *C. trifasciata* and the bryozoan genus *Reteporella* in the Azores (Chapters 4.1 and 5.1), 3% divergence levels seem to distinguish species (Hebert et al., 2003, 2004; Meyer & Paulay, 2005), with the potential to be used as a cut-off in these groups. The high number of COI sequences generated in this thesis ultimately contributed to the representation of overlooked marine invertebrates in repositories (Vieira et al., 2021). In GenBank, the number of COI sequences of *C. trifasciata* increased from three to 75; *P. sauciatus* is now represented by 36 sequences from the Azores and 16 new sequences from Madeira; 109 new COI sequences of *Reteporella* species, until then represented only by a single sequence of *R. beaniana* (King, 1846), are now publicly available. The mitochondrial 16S rRNA marker was also used in some studies, although providing a lower phylogenetic resolution than the COI.

Due to their slower mutation rate, nuclear coding regions take longer time to coalesce and are useful to evaluate evolutionary relationships at higher taxonomic levels (Baptista et al., 2019; Zheng et al., 2011). Nevertheless, in most of the cases tested in this thesis, the relationships inferred with nuclear markers were rarely informative of past split events (e.g. *Tricolia* in Baptista et al., subm). The adequacy of the nuclear markers chosen is also hindered by the current molecular knowledge on the target group. For the bryozoan genus *Reteporella*, studies based on nuclear markers are limited to the 18S rRNA, useful for deep phylogenies (Eberle et al., 2020; Orr et al., 2019), and a short fragment of the 28S rRNA. An unsuccessful attempt was made to design primers for a larger 28S region, likely due to the high variability of the few sequences of closely related cheilostomatid bryozoans currently available in databases.

Microsatellite data was generated following the cost-effective SSR-GBAS workflow (Baptista et al., 2021b; Curto et al., 2019; Lanner et al., 2021; Tibihika et al., 2019), which allows to retrieve the traditional allele length information, plus whole amplicon sequence information. De novo SSR primer sets, mostly located in neutral regions of the genome, were obtained for three non-model organisms without a reference genome available: two gastropod (*S. haemastoma* in Chapter 3.2 and *C. trifasciata* in Chapter 4.1, Baptista et al., 2021b) and a bryozoan species (genus *Reteporella* in Chapter 5.2). Higher statistical power is possible even with a reduced number of markers and/or specimens, but the further inclusion of either can build upon the already existing SSR-GBAS datasets (Curto et al., 2019; Farrell et al., 2016; Vartia et al., 2016). The analyses were performed with

a minimum of five individuals per population, although patterns inferred in these cases were cautiously interpreted (see Chapter 4.1; Baptista et al., 2021b). Cross-species amplification might be problematic due to the ascertainment bias and variability in the flanking regions, leading to null alleles in the non-specific targets (Baptista et al., 2021a; Ruiz-García et al., 2020; Tibihika et al., 2019; Turini et al., 2014). Nevertheless, even with a suboptimal performance in some cases, the SSR primer sets developed for this thesis were informative and allowed to identify the occurrence of unreported patterns of introgression and hybridization (see example for the genus *Reteporella* in Chapter 5.2). Overall, an excess of homozygotes was detected in several loci of the species in study. However, these have been previously shown to be prevalent in other marine invertebrates, without affecting the inferences of population structure (Cahill & Viard, 2014; Kemppainen et al., 2009; Zouros & Foltz, 1984). The SSR-GBAS pipeline developed by Curto et al. (2019), followed in the studies comprised in this thesis, is suitable for the study of non-model organisms' systems at reduced temporal and geographical scales. At its present form, it has the potential to be integrated in long-term research projects and meta-analyses, and the datasets generated can easily be combined with data from other sources (Baptista et al., 2021b; Curto et al., 2019). Due to all its advantages and results obtained throughout this thesis, the SSR-GBAS workflow seems appropriate for exploratory analyses of populational, evolutionary, and biogeographical questions on marine invertebrates for which, so far, studies have been mostly based in traditional molecular markers (Cahill et al., 2018; Donald et al., 2020; Weersing & Toonen, 2009). Its application to three marine invertebrate species in this thesis represents a starting point to the broad use of a methodology to recover comparable datasets and analyses in the same study system, crucial for a better understanding of the connectivity in the marine realm (Baptista et al., 2021b; Teske et al., 2018).

Multi-loci genotyping approaches allow to evaluate different inheritance dynamics and phylogeographic patterns at different temporal and spatial scales (DiBattista et al., 2012). The combination of mitochondrial and nuclear sequences, either concatenated or as independent markers (e.g. in the software BEAST), allow to account for their different evolutionary rates (Degnan & Rosenberg, 2006; Knowles, 2009; Ogilvie et al., 2017; Stefanni et al., 2018). In this thesis, the reconstruction of a calibrated species tree for the gastropod family Rissoidae in the Azores allowed to test a biogeographical theory (Chapter 3.1; Baptista et al., 2019). The fossil record of Santa Maria Island, dating from the Pliocene (Zanclean) and Pleistocene (Last interglacial; Ávila et al., 2016; Raposo et al., 2018), constitutes the best source of calibration points for reconstructions of the

evolutionary history of Azorean marine invertebrates, setting a minimum age for the speciation or arrival to the archipelago (Baptista et al., 2019). Also in this thesis, multi-marker phylogenetic analyses allowed the definition of species boundaries (Chapters 4.1 and 5.1; Baptista et al., 2021b, 2022, subm), which can be tested with the observation of morphological characters. Once validated, species boundaries are crucial for biogeographic theories testing and assessments of population dynamics (Baptista et al., 2021b, 2022; Palumbi, 1996). Population genetic analyses based on combined sequence and SSR-GBAS data revealed recent connectivity dynamics and fine-scale patterns within the archipelago (Chapters 3.2 and 4; Baptista et al., 2021b). The occurrence of introgression between *Reteporella* species was detected, suggesting that reticulated patterns of evolution also exist in the marine realm. Incongruence between markers is indicative of potential hybridization, although it can be difficult to distinguish from incomplete lineage sorting (Chapter 5; Degnan and Rosenberg, 2006; Frade et al., 2010; Puppo et al., 2016). Hence, the combined analyses of multiple markers scattered in the genome allows for a wide panoply of inferences in different contexts, ranging for phylogenetics to population genetics, and granting a broader perspective of evolutionary and biogeographical trends of the systems in study.

6.3 Biogeography of marine invertebrates in the Azores

With the development of biogeography as a scientific discipline, Ball (1975) distinguished three phases of a biogeographic study: descriptive, narrative, and analytical (Sluys, 2020). Often, biogeographical studies follow an inductive framework to provide a narrative that is based on assumptions about present-day patterns, biasing the proposal of explanatory historical events that cannot be observed nor manipulated directly (Crisp et al., 2011). The inductive approach, which has been deemed as “storytelling and unscientific”, is overcome by framing testable hypotheses to rule out alternative interpretations (Crisp et al., 2011). Testing these hypotheses, however, is not an easy task, especially in the understudied marine realm.

Ávila (2013) proposed a theoretical biogeographical hypothesis to address the simultaneous existence of two congeneric non-planktotrophic species with different bathymetric ranges in two nearby Atlantic archipelagos. Following the analytical phase (Ball, 1975; Sluys, 2020), this biogeographical hypothesis was tested under a phylogenetic framework and interpreted based on palaeontology, ecology, and molecular data (Baptista et al., 2019, Chapter 3.1), gathering advised observations in independent scientific fields (Crisp et al., 2011). On the other hand, it has long been defended that a good knowledge of species boundaries and diversity is crucial and

should be obtained prior to any biogeographical testing (Palumbi, 1996). In this thesis, the importance of having a phylogenetic backbone prior to population genetic analyses was highlighted in the studies on the bryozoan genus *Reteporella*, revealing the existence of several morphologically similar species that could have confounded populational interpretations if considered as the same taxon, as initially thought (Chapter 5; Baptista et al., 2022). Therefore, integrative approaches allow for a better understanding of marine biogeography.

The remaining chapters of this thesis follow mostly the biogeographical narrative phase (Ball, 1975; Sluys, 2020). Although some hypotheses are proposed (see Baptista et al., 2021a, Chapter 4.2 for the arrival of *P. sauciatus* to the Azores), these are difficult to test due to limitations in today's scientific knowledge of external processes shaping biogeographical patterns in remote archipelagos (e.g. oceanographic circulation, deep-water habitats). Marine biogeographical patterns tend to be taxon-specific (Ávila et al., 2018, 2019; Hachich et al., 2015, 2020), and even within the same taxon, species with similar ecological requirements are affected differently by biotic and abiotic factors (Ávila et al., 2012; Ávila, 2013; Palumbi, 1994; Watts & Thorpe, 2006), thus differently shaping their genetic patterns (e.g. population structure inferred for the congeneric *Phorcus sauciatus* and *P. lineatus* by Baptista et al., 2021a; Chapter 4.2). As stated by Quinteiro et al. (2020), “diverse genetic patterns have been described for Azorean marine invertebrates”, as some are highly differentiated from other NE Atlantic populations (e.g. Chapters 4.1 and 5, Baptista et al., 2021a; Desiderato et al., 2019; Faria et al., 2017; Quinteiro et al., 2020; Riesgo et al., 2019; Vieira et al., 2019, 2022), others show close relationships with Madeira and/or Canaries (e.g. Baptista et al., 2021a; Quinteiro et al., 2015; Xavier et al., 2010), whilst some are widely distributed and apparently panmitic throughout the Atlanto-Mediterranean area (e.g. Chapter 3.2, Fourdrilis & Backeljau, 2019; Patarnello et al., 2007).

The multidisciplinary study of different marine invertebrates in the Azores Archipelago and NE Atlantic provided new insights into their evolution and biogeographical expectations in such unique areas. Based on the results and interpretations conducted in this thesis, it certainly seems that the dispersal capacity of marine invertebrates in early or late-life stages influences the differentiation levels and, consequently, speciation events. High differentiation levels often result in higher levels of endemism (see Chapter 5.1; Baptista et al., 2022), contributing to the biogeographic uniqueness of the Azores ecoregion (*sensu* Freitas et al., 2019). This scenario is more often observed in species with non-planktotrophic larvae, which frequently depend on the combination of random chance events to successfully increase their distribution range or exchange individuals

with other populations. Contrariwise, planktotrophic species are usually widely distributed and genetic homogenization across wide geographical ranges is facilitated during the larval stage (Goldson et al., 2001; Scheltema, 1971, 1986; Watts & Thorpe, 2006). The absence of genetic structure inferred for *S. haemastoma* (Chapter 3.2) and other planktotrophic invertebrates (e.g. Crocetta et al., 2020; Quinteiro et al., 2020; Riesgo et al., 2019) suggests they should not be the preferred target for biogeographical inferences in remote islands. Instead, species with non-planktotrophic larvae are the key to better understand the processes and patterns behind the evolution of marine life in remote insular ecosystems. Expanding phylogeographic and population genetics approaches to more taxa will certainly provide an even more complete history of marine evolution in remote archipelagos, uncovering the mechanisms of colonization and subsequent diversification in these areas.

Biogeographical narratives become even more complex in remote islands as the Azores, as distance, unsuitable habitats, and/or historical and contemporary limitations imposed by the deep-waters can constitute formidable obstacles for the dispersal of marine invertebrates (Ávila, 2013). Even within the archipelago, distance between islands ascend to ~200 km of deep-waters but occasional exchanges of individuals can occur due to extreme weather events (Johnson et al., 2017; Waters & Craw, 2018), periodic disruptions of the normal sea-surface circulation patterns (Hawkins et al., 2000; Lima et al., 2020; Sala et al., 2016; Santos et al., 1995), and other chance events (Baptista et al., 2021b). In the temperate Atlantic waters, rafting is the dominant process allowing the exchange of non-planktotrophic shallow-water species (Ávila et al., 2012; Ávila, 2013; Thiel & Haye, 2006). Rafting occurs in different life stages – mostly as juveniles in big-sized species or during their entire lifetime for small-sized species – facilitating gene flow at a broad temporal scale (Ávila, 2013; Baptista et al., 2021a; 2021b; Kojima et al., 2004; Winston, 2012). The preferential intertidal habitat will also promote or hinder gene flow, as populations associated with algal patches are more prone to initiate rafting than conspecifics in bare rocky substrata (Baptista et al., 2019; Wort et al., 2019). Likewise, populations inhabiting enclosed and protected intertidal areas are less likely to be disturbed by mechanical action (e.g. waves) that induces breakage of the algae coverage sheltering juveniles and small-sized adults (Baptista et al., 2021b). From an historical perspective, the likelihood of rafting events can also be related to the littoral area of the island, which varies during its ontogeny, according to recent marine island biogeographical models – the Vitória-Trindade Chain Model (VTCM; Pinheiro et al., 2017) and the Sea-Level Sensitive dynamic model (SLS; Ávila et al., 2019).

Although circulation within the Azores region is not fully understood, it can create permanent or transient pathways for inter-island dispersal (Baptista et al., 2019; Lima et al., 2020; Sala et al., 2016). Rafts and larvae are expected to be advected eastwards, following the predominant direction of the current regime in the archipelago (Lima et al., 2020; Sala et al., 2013, 2016). Localities in the Central group are expected to receive migrants from both Western and Eastern groups, increasing their connectivity and diversity. These localities can act as important centres of distribution in the archipelago. On the other hand, larvae released in eastern localities are more likely to be lost to the open-ocean and not disperse westwards, unless exceptional conditions are established (Baptista et al., 2021a, 2022; Sala et al., 2016, 2013; Chapter 5). The gastropod *P. sauciatus* is one of the exceptions in the expected eastward dispersal, as it expands its distribution in the Azores in the opposite direction, having first colonized Santa Maria Island (Baptista et al., 2021a). Besides emerged islands, more than 400 seamounts exist in the Azorean economic exclusive zone (EEZ) (Morato et al., 2008). These geomorphological structures, mostly in deep-water habitats, are affected by multi-scale complex of oceanographic and ecological dynamics (Bashmachnikov et al., 2013; Puerta et al., 2020). High retention capacity, weak current flow, and expected slower larval metabolism due to cold waters turn seamounts into interesting areas for the emerge of new lineages (McClain and Hardy, 2010; Puerta et al., 2020; Taylor and Roterman, 2017; Chapter 5.2). The knowledge on these unique habitats advances at a slow pace, often limited by technology available, but their importance and protection as vulnerable marine ecosystems and centres of biodiversity is fast becoming evident (Puerta et al., 2020; Watling & Auster, 2017; Zeng et al., 2020), and Azorean seamounts are no exception.

Hydrographic features along the water column affect gene flow and species distribution of sessile marine invertebrates (Baptista et al., 2021b; Goldson et al., 2001; Radice et al., 2016; Chapter 5.2). Ecological changes occur with bathymetry, which can result in 1) the establishment of barriers (e.g. thermocline) that promote ecologically-driven differentiation (Frade et al., 2010; Gillespie et al., 2020; Stefanoudis et al., 2019; Teske et al., 2019; Chapter 5.2); 2) homogeneous habitats in deep-waters, promoting secondary contact between closely related lineages and potential hybridization (Frade et al., 2010; Chapter 5.2). The finding of a divergent lineage of *Reteporella atlantica* in shallow-waters goes towards the expectations of the biogeographical models for marine insular habitats (Ávila et al., 2019), as ontogenetic changes in the island create new niches in the littoral area. In the understudied Bryozoa phylum, interspecific introgression and ecologically-driven differentiation seem to be driving opportunities for the emergence

of new lineages (Chapter 5), following an evolutionary pathway that might be more widespread than previously thought especially in remote areas.

A biogeographical paradox was formulated by Ávila (2005) based on the high similarity of Azorean and European/Mediterranean taxa, both in recent and fossil (Last Interglacial) assemblages, despite the predominant eastward sea-surface currents (Ávila, 2006, 2013). Due to the geographical proximity, it could be more likely to have rafts arriving to the Azores with origin in NE Atlantic coasts (e.g. Iberian Peninsula or North Africa). However, as currents flow predominantly in an eastward direction and rafts are usually advected following the predominant wind and sea-surface currents, westward dispersal events (as reported for *P. sauciatus* in Baptista et al., 2021a) would imply temporary current reversals, storms, or mechanism yet unknown. The so-called “Azorean Biogeographical Paradox” (Ávila, 2005, 2006, 2013) is far from being well-understood but new studies developed for marine communities in the archipelago, aiming to test such similarities and biogeographical scenarios, slowly provide invaluable contributes to comprehend it. With this thesis, it becomes clear that several factors interact differently to shape the diversity and mechanisms of evolution of marine invertebrate species with non-planktotrophic larvae within and outside the Azores, giving rise to new, unique patterns of genetic structure, many of which still unknown. Over geological time, all these factors might become important promoters of divergence and speciation in remote islands. Random chance events, as storms, have the power to facilitate long-distance dispersal to and from remote islands as the Azores. If such events are rare but recurrent through times, they will likely have a cumulative effect through geological times, with tremendous impacts in the biodiversity and evolution of marine life in the Azores and contributing to the key role of these islands as centres of diversification in the middle of the ocean.

6.4 Future prospects

Overall, the studies conducted mostly achieved the main goals proposed in the scope of this thesis. Nevertheless, due to unanticipated patterns inferred and different perspectives of the factors shaping the evolution and connectivity in the islands, new questions arise to be addressed in future studies.

Despite the resolution already granted by SSR loci, the understanding of population structure and diversification of the target species can be enhanced with the study of the phylogenetic information provided by SNPs datasets, already generated with the SSR-GBAS protocols for *C. trifasciata*, *S. haemastoma* and *Reteporella* spp., but not explored in the scope of this thesis. SNPs provide unprecedented resolution for population

genomics and better reproducibility than SSRs (Curto et al., 2019), although their use is still arduous in non-model organisms as the bryozoan genus *Reteporella* (Helyar et al., 2011; Yu et al., 2020). More genome-wide SNPs for non-model organisms can be obtained with the application of other cost-effective next-generation sequencing techniques, namely restriction-site associated DNA sequencing (RADseq; Baird et al., 2008; Davey & Blaxter, 2010) which uncovers hundreds to thousands of markers to infer fine-scale population parameters with as little as 10-20 specimens (Andrews et al., 2016). Notwithstanding the potential to address knowledge gaps on phylogenomics, populational, and ecological questions in closely related species (Andrews et al., 2016; da Fonseca et al., 2016), the application of RADseq to marine invertebrates is still reduced (e.g. Iguchi et al., 2021; Leiva et al., 2019; Quattrini et al., 2019; Reitzel et al., 2013; Taboada et al., 2022; Xuereb et al., 2018) and limited to resolve confounding situations of interspecific gene flow and incomplete lineage sorting (da Fonseca et al., 2016). In this thesis, RADseq was not applied to the genus *Reteporella*, as the number of samples and geographical coverage were prioritized in relation to the number of markers obtained. Moreover, missing data in RAD loci often occurs due to mutation-disruption in the cut-sites, such that fewer RAD loci are shared among distant relatives (Eaton et al., 2017). When working with multiple species, as is the case in the analyses conducted in the genus *Reteporella*, this could lead to biased inferences. Alternative approaches can, instead, be considered for future phylogenomic analyses in the genus *Reteporella*, such as HiMAP (Dupuis et al., 2018) and Target-Capture (Manthey et al., 2016). HiMAP allows to inexpensively build phylogenomic datasets with large numbers of taxa using single-tube, highly multiplexed amplicon sequencing. Besides the applicability to a high number of taxa at a single time, data analysis can be accomplished without the need for read mapping or assembly (Dupuis et al., 2018). Target-capture of particular regions of the genome (e.g. ultraconserved elements) has been developed to reconstruct phylogenetic relationships at higher taxonomic levels, with good performance providing that loci with little information content are carefully analysed (Manthey et al., 2016).

Ávila's (2006, 2013) biogeographical hypothesis is expected to be extensible to marine phyla other than gastropods under the same premisses. As an extension, Baptista et al.'s (2019) suggested that, for congeneric species occurring in the NE Atlantic archipelagos with similar ecological traits, those with broader distributions are expected to be evolutionarily older than the ones restricted to one or two archipelagos. This prediction can be tested with molecular phylogenetics in several marine invertebrate

groups, contributing to clarify inter-archipelagic connectivity patterns and speciation of shallow-water taxa in remote islands.

Among the factors shaping population genetics of marine invertebrates in the remote Azores, probably the least understood are the oceanographic circulation and Lagrangian pathways at the archipelagic and NE Atlantic scales. More studies on the physical and chemical oceanography in the area, both at shallow and deep-waters, would surely allow for a better comprehension of potential rafting routes and directional connectivity, especially when fine-scale patterns seem to constitute barriers to the genetic exchange between intertidal populations located a few kilometres apart within the same island (Baptista et al., 2021b). Long-term tracking of temporal disruptions in the normal circulation pattern in the NE Atlantic, as well as formation, composition, and advection of rafts, would allow to test the theoretical mechanism proposed for the arrival of *P. sauciatus* to the Azores (Baptista et al., 2021a).

New diversity was reported for the Azores Archipelago in intertidal and deep-waters habitats. Seamounts arose as areas of special interest and are expected to yield unique biodiversity (Chapter 5.2). Specially for habitats in deep-waters, negative bias in the knowledge of the real marine diversity of the archipelago still exists due to sporadic sampling and lack of specialists to characterize the samples (Madeira et al., 2019; Micael et al., 2019; Baptista et al., 2022; Chapter 5.1). Increased sampling effort in the Azores, as well as a detailed characterization of specimens deposited in collections and development of integrative studies, are thus needed to further characterize often overlooked organisms of Azorean waters.

The study of the newcomer *P. sauciatus* pointed to Madeira as a potential origin of the first colonizers of the Azores. Nevertheless, the role of Iberian populations as sources should be assessed in the future. With its geographical range rapidly expanding in the Azores, the perfect setting is established to track the natural dispersal process by a marine gastropod in real time and study its population genetic dynamics in a remote archipelago. This would provide crucial insights into the arrival of new intertidal invertebrate species to remote areas and northern latitudes, a process that is becoming more frequent due to climate change (Afonso et al., 2013; Ávila et al., 2018, 2019; Canning-Clode & Carlton, 2017).

The studies in the bryozoan genus *Reteporella* revealed unanticipated patterns of diversity and population dynamics, with signatures of interspecific introgression in Azorean deep-waters and incipient cryptic speciation of a *R. atlantica* lineage in shallow-waters (Chapter 5). The biology, ecology, and evolution of bryozoans, in general, is yet to be fully understood. Considering the exciting new findings regarding molecular

mechanisms of evolution, backed up by morphological characters (see Chapter 5.1, Baptista et al., 2022), *Reteporella* and other phidoloporids are suggested as interesting study-models to clarify evolutionary processes on this under-looked phylum. A better understanding of the reticulate evolution that appears to be taking place in the genus *Reteporella* in the Azores would benefit from phylogenomic genome-wide approaches, aiming to assess the incongruent phylogenetic patterns inferred with different markers and identify regions of the genome under selective pressures (Helyar et al., 2011; Reitzel et al., 2013; Rosser et al., 2017; Taylor & Roterman, 2017). This methodology is also promising to clarify *Reteporella* species delimitation and evaluate the extent of differentiation detected in shallow-water *R. atlantica* (Herrera & Shank, 2016; Leiva et al., 2019; Pante et al., 2015). Moreover, morphological characterization of molecular lineages, based on modern imaging techniques, is crucial to fully describe the diversity of poorly studied groups, relying upon the expertise by classical taxonomists. The relationships and evolutionary history of the family Phidoloporidae, including samples from the Mediterranean and North Atlantic, as well as from older seamounts near the Azores or the mid-Atlantic ridge, should soon be explored to put into perspective the arrival of ancestral lineages to the remote Azores.

Ultimately, the SSR-GBAS approach, combined with traditional markers as well as morphological and ecological data, can be expanded to other marine invertebrates, generating comparative datasets. Such studies will allow to assess how transversal is the role played by the complex interaction between functional traits, ecological features, sea-surface circulation, and episodic disruptions in intertidal communities, as well as the role of bathymetry as promoter of differentiation in species with wide bathymetrical ranges.

6.5 Concluding remarks

Studies on population structure of some gastropods and bryozoans revealed a multitude of factors shaping connectivity in the Azores Archipelago and NE Atlantic, even in species with the same larval development mode and occupying similar environments. Despite the expected influence of early life stages in the dispersal ability, it becomes clear that population dynamics and evolution are ruled by intrinsically complex interactions among biotic and abiotic factors, especially in remote areas. Therefore, there is no “one size fits all” biogeographical pattern expected to be retrieved for marine invertebrates inhabiting these unique ecosystems. Unprecedented patterns of connectivity and potential modes of diversification (e.g. introgressive evolution, ecological differentiation driven by bathymetry, isolation of populations within the same

island) were uncovered in this thesis, and others likely remain to be discovered. The remote Azores region, with its outstandingly dynamic marine habitats ranging from the coasts to seamounts, arise as a centre of diversification for marine invertebrates, particularly in species whose exchanges with other NE Atlantic areas are limited.

Marine ecosystems of remote islands as the Azores Archipelago are not yet fully comprehended and, although we are far from accomplishing a “complete picture”, it improves as new data arise from distinct scientific disciplines. Multi-disciplinary, integrative approaches are key to catalogue genetic and morphological diversity and to attain a better knowledge of marine life on remote islands, especially on understudied taxa. Only with strong foundations on distinct areas as oceanography, climatology, geology, palaeontology, genetics, ecology, and biology, will we clarify which and how factors shape population structure and speciation, making room for complete biogeographical studies and hypothesis testing.

The oceanic volcanic Azores Archipelago is highlighted as a promising case-study for marine biogeographical research, considering its geographical isolation in the NE Atlantic, biological richness, and ever-growing knowledge in complementary scientific areas. Addressing biogeographical questions in the Azores, focusing on gastropods and bryozoans in the present thesis, supports their usefulness in advancing this research field as a proxy for other remote oceanic volcanic archipelagos in the world. The hypotheses proposed, as well as the patterns retrieved, might be more widespread than previously thought and the analyses can be expanded to other marine invertebrate taxa. Therefore, this thesis constitutes the starting point for unravelling the processes and patterns of marine life as a study system, setting the pace for the formulation of an even more complete theory of biogeography that, grounded on Ávila et al.'s (2019) SLS dynamic model, further explores the evolution of marine invertebrates in remote volcanic oceanic islands.

References

- Afonso, P., Porteiro, F. M., Fontes, J., Tempera, F., Morato, T., Cardigos, F., & Santos, R. S. (2013). New and rare coastal fishes in the Azores islands: occasional events or tropicalization process? *Journal of Fish Biology*, 83, 272–294. <https://doi.org/10.1111/jfb.12162>
- Ahrens, D., Ah Yong, S. T., Ballerio, A., Barclay, M. V. L., Eberle, J., Espeland, M., Huber, B. A., Mengual, X., Pacheco, T. L., Peters, R. S., Rulik, B., Vaz-de-Mello, F., Wesener, T., & Krell, F.-T. (2021). Is it time to describe new species without diagnoses? - A comment on Sharkey et al. (2021). *Zootaxa*, 5027(2), 151–159. <https://doi.org/10.11646/zootaxa.5027.2.1>
- Andrews, K. R., Good, J. M., Miller, M. R., Luikart, G., & Hohenlohe, P. A. (2016). Harnessing the power of RADseq for ecological and evolutionary genomics. *Nature Reviews Genetics*, 17(2), 81–92. <https://doi.org/10.1038/nrg.2015.28>
- Ávila, S. P. (2005). *Processos e Padrões de Dispersão e Colonização nos Rissoidae (Mollusca: Gastropoda) dos Açores*, x+329 pp. Unpublished PhD thesis, Universidade dos Açores, Ponta Delgada.
- Ávila, S. P. (2006). Oceanic islands, rafting, geographical range and bathymetry: a neglected relationship? *Occasional Publication of the Irish Biogeographical Society*, 9, 22–39.
- Ávila, S. P. (2013). Unravelling the patterns and processes of evolution of marine life in oceanic islands: a global framework. In J. M. Fernández-Palacios, L. Nascimento, J. C. Hernández, S. Clemente, A. González, & J. P. Díaz-González (Eds.), *Climate Change Perspectives From the Atlantic: Past, Present and Future* (pp. 99–125). Universidad de La Laguna.
- Ávila, S. P., Cachão, M., Ramalho, R. S., Botelho, A. Z., Madeira, P., Rebelo, A. C., Cordeiro, R., Melo, C., Hipólito, A., Ventura, M. A., & Lipps, J. H. (2016). The Palaeontological Heritage of Santa Maria Island (Azores: NE Atlantic): a Re-evaluation of Geosites in GeoPark Azores and Their Use in Geotourism. *Geoheritage*, 8(2), 155–171. <https://doi.org/10.1007/s12371-015-0148-x>
- Ávila, S. P., Cordeiro, R., Madeira, P., Silva, L., Medeiros, A., Rebelo, A. C., Melo, C., Neto, A. I., Haroun, R., Monteiro, A., Rijdsdijk, K., & Johnson, M. E. (2018). Global change impacts on large-scale biogeographic patterns of marine organisms on Atlantic oceanic islands. *Marine Pollution Bulletin*, 126, 101–112. <https://doi.org/10.1016/j.marpolbul.2017.10.087>
- Ávila, S. P., Goud, J., & De Frias Martins, A. M. (2012). Patterns of diversity of the Rissoidae (Mollusca: Gastropoda) in the Atlantic and the Mediterranean region. In *The Scientific World Journal* (Vol. 2012). <https://doi.org/10.1100/2012/164890>
- Ávila, S. P., Madeira, P., Rebelo, A. C., Melo, C., Hipólito, A., Pombo, J., Botelho, A. Z., & Cordeiro, R. (2015b). *Phorcus sauciatu*s (Koch, 1845) (Gastropoda: Trochidae) in Santa Maria, Azores archipelago: the onset of a biological invasion. *Journal of Molluscan Studies*, 81(4), 516–521. <https://doi.org/10.1093/mollus/eyv012>
- Ávila, S. P., Madeira, P., Zazo, C., Kroh, A., Kirby, M., da Silva, C. M., Cachão, M., & Martins, A. M. F. (2009). Palaeoecology of the Pleistocene (MIS 5.5) outcrops of Santa Maria Island

- (Azores) in a complex oceanic tectonic setting. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 274(1–2), 18–31. <https://doi.org/10.1016/j.palaeo.2008.12.014>
- Ávila, S. P., Melo, C., Berning, B., Nuno, S., Quartau, R., Rijdsdijk, K. F., Ramalho, R. S., Cordeiro, R., De Sá, N. C., Pimentel, A., Baptista, L., Medeiros, A., Gil, A., & Johnson, M. E. (2019). Towards a 'Sea-Level Sensitive' dynamic model: impact of island ontogeny and glacio-eustasy on global patterns of marine island biogeography. *Biological Reviews*, 94(3), 1116–1142. <https://doi.org/10.1111/brv.12492>
- Azorean Biodiversity Portal | Portal da Biodiversidade dos Açores. Retrieved October 27, 2022, from <https://azoresbiportal.uac.pt/>
- Baird, N. A., Etter, P. D., Atwood, T. S., Currey, M. C., Shiver, A. L., Lewis, Z. A., Selker, E. U., Cresko, W. A., & Johnson, E. A. (2008). Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PloS One*, 3(10). <https://doi.org/10.1371/JOURNAL.PONE.0003376>
- Ball, I. R. (1975). Nature and Formulation of Biogeographical Hypotheses. *Systematic Biology*, 24(4), 407–430. <https://doi.org/10.1093/SYSBIO/24.4.407>
- Baptista, L., Berning, B., Waeschenbach, A., Curto, M., Meimberg, H., Santos, A. M., & Ávila, S. P. (2022) Morphospecies and molecular diversity of 'lace corals': the genus *Reteporella* (Bryozoa: Cheilostomatida) in the central North Atlantic Azores Archipelago. *BMC Ecology and Evolution*, 22, Article number: 128. <https://doi.org/10.1186/s12862-022-02080-z>
- Baptista, L., Fassio, G., Gofas, S., Santos, A. M., Oliverio, M., & Ávila, S. P. (subm). Evaluating the taxonomic status of *Tricolia* Risso, 1826 in the Northeast Atlantic and Mediterranean Sea. *Molecular Phylogenetics and Evolution*.
- Baptista, L., Meimberg, H., Ávila, S. P., Santos, A. M., & Curto, M. (2021b). Dispersal ability, habitat characteristics, and sea-surface circulation shape population structure of *Cingula trifasciata* (Gastropoda: Rissoidae) in the remote Azores Archipelago. *BMC Ecology and Evolution*, 21(1), 128. <https://doi.org/10.1186/s12862-021-01862-1>
- Baptista, L., Santos, A. M., Cabezas, M. P., Cordeiro, R., Melo, C., & Ávila, S. P. (2019). Intertidal or subtidal/circalittoral species: which appeared first? A phylogenetic approach to the evolution of non-planktotrophic species in Atlantic Archipelagos. *Marine Biology*, 166, 1–16. <https://doi.org/10.1007/s00227-019-3536-y>
- Baptista, L., Santos, A. M., Melo, C. S., Rebelo, A. C., Madeira, P., Cordeiro, R., Botelho, A. Z., Hipólito, A., Pombo, J., Voelker, A. H. L., & Ávila, S. P. (2021a). Untangling the origin of the newcomer *Phorcus sauciatus* (Mollusca: Gastropoda) in a remote Atlantic archipelago. *Marine Biology*, 168(1), Article number: 9. <https://doi.org/10.1007/s00227-020-03808-5>
- Bashmachnikov, I., Loureiro, C. M., & Martins, A. (2013). Topographically induced circulation patterns and mixing over Condor seamount. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 98. <https://doi.org/10.1016/j.dsr2.2013.09.014>
- Belaiba, E., Marrone, F., Vecchioni, L., Bahri-Sfar, L., & Arculeo, M. (2019). An exhaustive phylogeny of the combtooth blenny genus *Salaria* (Pisces, Blenniidae) shows introgressive hybridization and lack of reciprocal mtDNA monophyly between the marine species *Salaria*

- basilisca* and *Salaria pavo*. *Molecular Phylogenetics and Evolution*, 135, 210–221. <https://doi.org/10.1016/J.YMPEV.2019.02.026>
- Blaxter, M. (2016). Imagining sisypheus happy: DNA barcoding and the unnamed majority. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 371(1702). <https://doi.org/10.1098/rstb.2015.0329>
- Borges, P.A.V., Costa, A., Cunha, R., Gabriel, R., Gonçalves, V., Martins, A.F., Melo, I., Parente, M., Raposeiro, P., Rodrigues, P., Santos, R.S., Silva, L., Vieira, P., & Vieira, V. (Eds.) (2010). *A list of the terrestrial and marine biota from the Azores*, 9-33. Princípia, Cascais, 432 pp.
- Cahill, A. E., Pearman, J. K., Borja, A., Carugati, L., Carvalho, S., Danovaro, R., Dashfield, S., David, R., Féral, J. P., Olenin, S., Šiaulys, A., Somerfield, P. J., Trayanova, A., Uyarra, M. C., & Chenuil, A. (2018). A comparative analysis of metabarcoding and morphology-based identification of benthic communities across different regional seas. *Ecology and Evolution*, 8(17), 8908–8920. <https://doi.org/10.1002/ece3.4283>
- Cahill, A. E., & Viard, F. (2014). Genetic structure in native and non-native populations of the direct-developing gastropod *Crepidula convexa*. *Marine Biology*, 161(10), 2433–2443. <https://doi.org/10.1007/s00227-014-2519-2>
- Canning-Clode, J., & Carlton, J. T. (2017). Refining and expanding global climate change scenarios in the sea: Poleward creep complexities, range termini, and setbacks and surges. *Diversity and Distributions*, 23(5), 463–473. <https://doi.org/10.1111/ddi.12551>
- Castro, N., Carlton, J. T., Costa, A. C., Marques, C. S., Hewitt, C. L., Cacabelos, E., Lopes, E., Gizzi, F., Gestoso, I., Monteiro, J. G., Costa, J. L., Parente, M., Ramalhosa, P., Fofonoff, P., Chainho, P., Haroun, R., Santos, R. S., Herrera, R., Marques, T. A., Ruiz, G. M., & Canning-Clode, J. (2022). Diversity and patterns of marine non-native species in the archipelagos of Macaronesia. *Diversity and Distributions*, 28, 667-684. <https://doi.org/10.1111/ddi.13465>
- Collins, R. A., Bakker, J., Wangenstein, O. S., Soto, A. Z., Corrigan, L., Sims, D. W., Genner, M. J., & Mariani, S. (2019). Non-specific amplification compromises environmental DNA metabarcoding with COI. *Methods in Ecology and Evolution*, 10(11). <https://doi.org/10.1111/2041-210X.13276>
- Cordeiro, R., & Ávila, S. P. (2015). New species of Rissoiidae (Mollusca, Gastropoda) from the Archipelago of the Azores (northeast Atlantic) with an updated regional checklist for the family. *ZooKeys*, 480, 1–19. <https://doi.org/10.3897/zookeys.480.8599>
- Crisp, M. D., Trewick, S. A., & Cook, L. G. (2011). Hypothesis testing in biogeography. *Trends in Ecology and Evolution*, 26(2), 66–72. <https://doi.org/10.1016/j.tree.2010.11.005>
- Crocetta, F., Caputi, L., Paz-Sedano, S., Tanduo, V., Vazzana, A., & Oliverio, M. (2020). High genetic connectivity in a gastropod with long-lived planktonic larvae. *Journal of Molluscan Studies*, 86(1), 42–55. <https://doi.org/10.1093/MOLLUS/EYZ032>
- Curto, M., Winter, S., Seiter, A., Schmid, L., Scheicher, K., Barthel, L. M. F., Plass, J., & Meimberg, H. (2019). Application of a SSR-GBS marker system on investigation of European Hedgehog species and their hybrid zone dynamics. *Ecology and Evolution*, 9(5), 2814–2832. <https://doi.org/10.1002/ece3.4960>

- da Fonseca, R. R., Albrechtsen, A., Themudo, G. E., Ramos-Madrugal, J., Sibbesen, J. A., Marett, L., Zepeda-Mendoza, M. L., Campos, P. F., Heller, R., & Pereira, R. J. (2016). Next-generation biology: Sequencing and data analysis approaches for non-model organisms. *Marine Genomics*, 30, 3–13. <https://doi.org/10.1016/j.margen.2016.04.012>
- Davey, J. L., & Blaxter, M. W. (2010). RADSeq: next-generation population genetics. *Briefings in Functional Genomics*, 9(5–6), 416. <https://doi.org/10.1093/BFGP/ELQ031>
- Degnan, J. H., & Rosenberg, N. A. (2006). Discordance of species trees with their most likely gene trees. *PLoS Genetics*, 2(5), 762–768. <https://doi.org/10.1371/journal.pgen.0020068>
- Desiderato, A., Costa, F. O., Serejo, C. S., Abbiati, M., Queiroga, H., & Vieira, P. E. (2019). Macaronesian islands as promoters of diversification in amphipods: The remarkable case of the family Hyalidae (Crustacea, Amphipoda). *Zoologica Scripta*, 48(3), 359–375. <https://doi.org/10.1111/zsc.12339>
- DiBattista, J. D., Rocha, L. A., Craig, M. T., Feldheim, K. A., & Bowen, B. W. (2012). Phylogeography of two closely related Indo-Pacific butterflyfishes reveals divergent evolutionary histories and discordant results from mtDNA and microsatellites. In *Journal of Heredity*, 103, 617–629. <https://doi.org/10.1093/jhered/ess056>
- Donald, K. M., McCulloch, G. A., Dutoit, L., & Spencer, H. G. (2020). Population structure of the New Zealand whelk, *Cominella glandiformis* (Gastropoda: Buccinidae), suggests sporadic dispersal of a direct developer. *Biological Journal of the Linnean Society*, 130(1), 49–60. <https://doi.org/10.1093/biolinnean/blaa033>
- Dupuis, J. R., Bremer, F. T., Kauwe, A., San Jose, M., Leblanc, L., Rubinoff, D., & Geib, S. M. (2018). HiMAP: Robust phylogenomics from highly multiplexed amplicon sequencing. *Molecular Ecology Resources*, 18(5), 1000–1019. <https://doi.org/10.1111/1755-0998.12783>
- Eaton, D. A. R., Spriggs, E. L., Park, B., & Donoghue, M. J. (2017). Misconceptions on missing data in RAD-seq phylogenetics with a deep-scale example from flowering plants. *Systematic Biology*, 66(3), 399–412. <https://doi.org/10.1093/sysbio/syw092>
- Eberle, J., Ahrens, D., Mayer, C., Niehuis, O., & Misof, B. (2020). A plea for standardized nuclear markers in Metazoan DNA taxonomy. *Trends in Ecology and Evolution*, 35(4), 336–345. <https://doi.org/10.1016/j.tree.2019.12.003>
- Faria, J., Martins, G. M., Pita, A., Ribeiro, P. A., Hawkins, S. J., Presa, P., & Neto, A. I. (2017). Disentangling the genetic and morphological structure of *Patella candei* complex in Macaronesia (NE Atlantic). *Ecology and Evolution*, 7(16), 6125–6140. <https://doi.org/10.1002/ECE3.3121>
- Farrell, E. D., Carlsson, J. E. L., & Carlsson, J. (2016). Next gen pop gen: Implementing a high-throughput approach to population genetics in boarfish (*Capros aper*). *Royal Society Open Science*, 3(12), 160651. <https://doi.org/10.1098/rsos.160651>
- Forest, F. (2009). Calibrating the tree of life: Fossils, molecules and evolutionary timescales. *Annals of Botany*, 104(5), 789–794. <https://doi.org/10.1093/aob/mcp192>
- Fourdrilis, S., & Backeljau, T. (2019). Highly polymorphic mitochondrial DNA and deceiving haplotypic differentiation: Implications for assessing population genetic differentiation and

- p>connectivity.
- BMC Evolutionary Biology*
- , 19(1).
- <https://doi.org/10.1186/s12862-019-1414-3>
- Frade, P. R., Reyes-Nivia, M. C., Faria, J., Kaandorp, J. A., Luttikhuisen, P. C., & Bak, R. P. M. (2010). Semi-permeable species boundaries in the coral genus *Madracis*: Introgression in a brooding coral system. *Molecular Phylogenetics and Evolution*, 57(3), 1072–1090. <https://doi.org/10.1016/j.ympev.2010.09.010>
- Freitas, R., Romeiras, M., Silva, L., Cordeiro, R., Madeira, P., González, J. A., Wirtz, P., Falcón, J. M., Brito, A., Floeter, S. R., Afonso, P., Porteiro, F., Viera-Rodríguez, M. A., Neto, A. I., Haroun, R., Farminhão, J. N. M., Rebelo, A. C., Baptista, L., Melo, C. S., Martínez, A., Núñez, J., Berning, B., Johnson, M. E., Ávila, S. P. (2019). Restructuring of the ‘Macaronesia’ biogeographic unit: A marine multi-taxon biogeographical approach. *Scientific Reports*, 9(1), 1–18. <https://doi.org/10.1038/s41598-019-51786-6>
- Gillespie, R. G., Bennett, G. M., De Meester, L., Feder, J. L., Fleischer, R. C., Harmon, L. J., Hendry, A. P., Knape, M. L., Mallet, J., Martin, C., Parent, C. E., Patton, A. H., Pfennig, K. S., Rubinoff, D., Schluter, D., Seehausen, O., Shaw, K. L., Stacy, E., Strevander, M., Stroud, J. T., Wagner, C., & Wogan, G. O. U. (2020). Comparing Adaptive Radiations Across Space, Time, and Taxa. *Journal of Heredity*, 111(1), 1–20. <https://doi.org/10.1093/jhered/esz064>
- Goldson, A. J., Hughes, R. N., & Gliddon, C. J. (2001). Population genetic consequences of larval dispersal mode and hydrography: A case study with bryozoans. *Marine Biology*, 138(5), 1037–1042. <https://doi.org/10.1007/s002270000511>
- Hachich, N. F., Bonsall, M. B., Arraut, E. M., Barneche, D. R., Lewinsohn, T. M., & Floeter, S. R. (2015). Island biogeography: Patterns of marine shallow-water organisms in the Atlantic Ocean. *Journal of Biogeography*, 42(10), 1871–1882. <https://doi.org/10.1111/jbi.12560>
- Hachich, N. F., Ferrari, D. S., Quimbayo, J. P., Pinheiro, H. T., & Floeter, S. R. (2020). Island biogeography of marine shallow-water organisms. In *Encyclopedia of the World’s Biomes* (Vols. 1–5, pp. 61–75). Elsevier. <https://doi.org/10.1016/B978-0-12-409548-9.11947-5>
- Hawkins, S. J., Corte-Real, H. B. S. M., Pannacciulli, F. G., Weber, L. C., & Bishop, J. D. D. (2000). Thoughts on the ecology and evolution of the intertidal biota of the Azores and other Atlantic islands. *Hydrobiologia*, 440, 3–17. <https://doi.org/10.1023/A:1004118220083>
- Hebert, P. D. N., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, 270(1512), 313–332. <https://doi.org/10.1098/rspb.2002.2218>
- Hebert, P. D. N., & Gregory, T. R. (2005). The promise of DNA barcoding for taxonomy. *Systematic Biology*, 54, 852–859. <https://doi.org/10.1080/10635150500354886>
- Hebert, P. D. N., Stoeckle, M. Y., Zemlak, T. S., & Francis, C. M. (2004). Identification of birds through DNA barcodes. *PLoS Biology*, 2(10), e312. <https://doi.org/10.1371/journal.pbio.0020312>
- Helyar, S. J., Hemmer-Hansen, J., Bekkevold, D., Taylor, M. I., Ogden, R., Limborg, M. T., Cariani, A., Maes, G. E., Diopere, E., Carvalho, G. R., & Nielsen, E. E. (2011). Application of SNPs for population genetics of nonmodel organisms: new opportunities and challenges. *Molecular Ecology Resources*, 11(SUPPL. 1), 123–136. <https://doi.org/10.1111/J.1755->

[0998.2010.02943.X](#)

- Herrera, S., & Shank, T. M. (2016). RAD sequencing enables unprecedented phylogenetic resolution and objective species delimitation in recalcitrant divergent taxa. *Molecular Phylogenetics and Evolution*, 100, 70–79. <https://doi.org/10.1016/J.YMPEV.2016.03.010>
- Iguchi, A., Tada, I., Nagano, A. J., & Yasuda, N. (2021). Genetic structure of Pacific crown-of-thorns starfish (*Acanthaster cf. solaris*) in southern Japan based on genome-wide RADseq analysis. *Coral Reefs*, 40, 1379–1385. <https://doi.org/10.1007/s00338-021-02145-3>
- Johnson, M. E., Uchman, A., Costa, P. J. M., Ramalho, R. S., & Ávila, S. P. (2017). Intense hurricane transports sand onshore: Example from the Pliocene Malbusca section on Santa Maria Island (Azores, Portugal). *Marine Geology*, 385, 244–249. <https://doi.org/10.1016/J.MARGEO.2017.02.002>
- Kemppainen, P., Panova, M., Hollander, J., & Johannesson, K. (2009). Complete lack of mitochondrial divergence between two species of NE Atlantic marine intertidal gastropods. *Journal of Evolutionary Biology*, 22(10), 2000–2011. <https://doi.org/10.1111/j.1420-9101.2009.01810.x>
- Knowles, L. L. (2009). Estimating species trees: methods of phylogenetic analysis when there is incongruence across genes. *Systematic Biology*, 58, 463–467. <https://doi.org/https://doi.org/10.1093/sysbio/syp061>
- Kojima, S., Hayashi, I., Kim, D., Iijima, A., & Furota, T. (2004). Phylogeography of an intertidal direct-developing gastropod *Batillaria cumingi* around the Japanese Islands. *Marine Ecology Progress Series*, 276, 161–172. <https://doi.org/10.3354/meps276161>
- Laakkonen, H. M., Strelkov, P., Lajus, D. L., & Väinölä, R. (2015). Introgressive hybridization between the Atlantic and Pacific herrings (*Clupea harengus* and *C. pallasii*) in the north of Europe. *Marine Biology*, 162(1), 39–54. <https://doi.org/10.1007/S00227-014-2564-X/FIGURES/5>
- Lanner, J., Gstöttenmayer, F., Curto, M., Geslin, B., Huchler, K., Orr, M. C., Pachinger, B., Sedivy, C., & Meimberg, H. (2021). Evidence for multiple introductions of an invasive wild bee species currently under rapid range expansion in Europe. *BMC Ecology and Evolution*, 21, 71. <https://doi.org/10.1186/s12862-020-01729-x>
- Leiva, C., Taboada, S., Kenny, N. J., Combosch, D., Giribet, G., Jombart, T., & Riesgo, A. (2019). Population substructure and signals of divergent adaptive selection despite admixture in the sponge *Dendrilla antarctica* from shallow waters surrounding the Antarctic Peninsula. *Molecular Ecology*, 28(13), 3151–3170. <https://doi.org/10.1111/MEC.15135>
- Lima, M. J., Sala, I., & Caldeira, R. M. A. (2020). Physical connectivity between the NE Atlantic Seamounts. *Frontiers in Marine Science*, 7. <https://doi.org/10.3389/fmars.2020.00238>
- Madeira, P., Kroh, A., Cordeiro, R., Martins, A.M.F. & Ávila, S.P. (2019). The echinoderm fauna of the Azores (NE Atlantic Ocean). *Zootaxa*, 4639(1), 1–231. <https://doi.org/10.1098/10.11646/zootaxa.4639.1>
- McClain, C. R., & Hardy, S. M. (2010). The dynamics of biogeographic ranges in the deep sea. *Proceedings of the Royal Society B: Biological Sciences*, 277, 3533–3546.

<https://doi.org/10.1098/rspb.2010.1057>

- Meyer, C. P., & Paulay, G. (2005). DNA barcoding: Error rates based on comprehensive sampling. *PLoS Biology*, 3(12), 1–10. <https://doi.org/10.1371/journal.pbio.0030422>
- Micael, J., Tempera, F., Berning, B., López-Fé, C. M., Occhipinti-Ambrogi, A., & Costa, A. C. (2019). Shallow-water bryozoans from the Azores (central North Atlantic): native vs. non-indigenous species, and a method to evaluate taxonomic uncertainty. *Marine Biodiversity*, 49(1), 469–480. <https://doi.org/10.1007/s12526-017-0833-x>
- Morato, T., Machete, M., Kitchingman, A., Tempera, F., Lai, S., Menezes, G., Pitcher, T. J., & Santos, R. S. (2008). Abundance and distribution of seamounts in the Azores. *Marine Ecology Progress Series*, 357, 17–21. <https://doi.org/10.3354/meps07268>
- Nydam, M. L., Yancello, L. M., Bialik, S. B., Giesbrecht, K. B., Nation, G. K., & Peak, J. L. (2017). Introgression in two species of broadcast spawning marine invertebrate. *Biological Journal of the Linnean Society*, 120(4), 879–890. <https://doi.org/10.1093/BIOLINNEAN/BLW012>
- Ogilvie, H. A., Bouckaert, R. R., & Drummond, A. J. (2017). StarBEAST2 brings faster species tree inference and accurate estimates of substitution rates. *Molecular Biology and Evolution*, 34(8), 2101–2114. <https://doi.org/10.1093/molbev/msx126>
- Orr, R. J. S., Haugen, M. N., Berning, B., Bock, P., Cumming, R. L., Florence, W. K., Hirose, M., Di Martino, E., Ramsfjell, M. H., Sannum, M. M., Smith, A. M., Vieira, L. M., Waeschenbach, A., & Liow, L. H. (2019). A genome-skimmed phylogeny of a widespread bryozoan family, Adeonidae. *BMC Evolutionary Biology*, 19(1), 1–10. <https://doi.org/10.1186/s12862-019-1563-4>
- Palumbi, S. R. (1994). Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics*, 25, 547–572. <https://doi.org/10.1146/annurev.es.25.110194.002555>
- Palumbi, S. R. (1996). What can molecular genetics contribute to marine biogeography? An urchin's tale. *Journal of Experimental Marine Biology and Ecology*, 203(1), 75–92. [https://doi.org/10.1016/0022-0981\(96\)02571-3](https://doi.org/10.1016/0022-0981(96)02571-3)
- Pante, E., Abdelkrim, J., Viricel, A., Gey, D., France, S. C., Boisselier, M. C., & Samadi, S. (2015). Use of RAD sequencing for delimiting species. *Heredity*, 114(5), 450–459. <https://doi.org/10.1038/hdy.2014.105>
- Patarnello, T., Volckaert, F. A. M. J., & Castilho, R. (2007). Pillars of Hercules: Is the Atlantic-Mediterranean transition a phylogeographical break? *Molecular Ecology*, 16, 4426–4444. <https://doi.org/10.1111/j.1365-294X.2007.03477.x>
- Puerta, P., Johnson, C., Carreiro-Silva, M., Henry, L.-A., Kenchington, E., Morato, T., Kazanidis, G., Rueda, J. L., Urra, J., Ross, S., Wei, C.-L., González-Irusta, J. M., Arnaud-Haond, S., & Orejas, C. (2020). Influence of water masses on the biodiversity and biogeography of deep-sea benthic ecosystems in the North Atlantic. *Frontiers in Marine Science*, 7, 239. <https://doi.org/10.3389/fmars.2020.00239>
- Puppo, P., Curto, M., & Meimberg, H. (2016). Genetic structure of Micromeria (Lamiaceae) in Tenerife, the imprint of geological history and hybridization on within-island diversification.

- Ecology and Evolution*, 6(11), 3443–3460. <https://doi.org/10.1002/ECE3.2094>
- Quattrini, A. M., Wu, T., Soong, K., Jeng, M. S., Benayahu, Y., & McFadden, C. S. (2019). A next generation approach to species delimitation reveals the role of hybridization in a cryptic species complex of corals. *BMC Evolutionary Biology*, 19(1), 116. <https://doi.org/10.1186/s12862-019-1427-y>
- Quinteiro, J., Manent, P., Pérez-Diéguez, L., González, J. A., Almeida, C., Lopes, E., Araújo, R., Carreira, G. P., Rey-Méndez, M., & González-Henríquez, N. (2015). Phylogeography of a marine insular endemic in the Atlantic Macaronesia: the Azorean barnacle, *Megabalanus azoricus* (Pilsbry, 1916). *PLOS ONE*, 10(4), e0124707. <https://doi.org/10.1371/JOURNAL.PONE.0124707>
- Quinteiro, J., Rodríguez-Castro, J., Rey-Méndez, M., & Henríquez, N. G. (2020). Phylogeography of the insular populations of common octopus, *Octopus vulgaris* Cuvier, 1797, in the Atlantic Macaronesia. *PLoS ONE*, 15(3). <https://doi.org/10.1371/journal.pone.0230294>
- Radice, V. Z., Quattrini, A. M., Wareham, V. E., Edinger, E. N., & Cordes, E. E. (2016). Vertical water mass structure in the North Atlantic influences the bathymetric distribution of species in the deep-sea coral genus *Paramuricea*. *Deep Sea Research Part I: Oceanographic Research Papers*, 116, 253–263. <https://doi.org/10.1016/J.DSR.2016.08.014>
- Raposo, V. B., Melo, C. S., Silva, L., Ventura, A., Câmara, R., Pombo, J., Johnson, M. E., & Ávila, S. P. (2018). Comparing methods of evaluation of geosites: The fossiliferous outcrops of Santa Maria Island (Azores, NE Atlantic) as a case study for sustainable Island tourism. *Sustainability (Switzerland)*, 10(10). <https://doi.org/10.3390/su10103596>
- Reitzel, A. M., Herrera, S., Layden, M. J., Martindale, M. Q., & Shank, T. M. (2013). Going where traditional markers have not gone before: Utility of and promise for RAD sequencing in marine invertebrate phylogeography and population genomics. *Molecular Ecology*, 22(11), 2953–2970. <https://doi.org/10.1111/mec.12228>
- Riesgo, A., Taboada, S., Pérez-Portela, R., Melis, P., Xavier, J. R., Blasco, G., & López-Legentil, S. (2019). Genetic diversity, connectivity and gene flow along the distribution of the emblematic Atlanto-Mediterranean sponge *Petrosia ficiformis* (Haplosclerida, Demospongiae). *BMC Evolutionary Biology*, 19. <https://doi.org/10.1186/S12862-018-1343-6>
- Roques, S., Sévigny, J. M., & Bernatchez, L. (2001). Evidence for broadscale introgressive hybridization between two redfish (genus *Sebastes*) in the North-west Atlantic: a rare marine example. *Molecular Ecology*, 10(1), 149–165. <https://doi.org/10.1046/J.1365-294X.2001.01195.X>
- Rosser, N. L., Thomas, L., Stankowski, S., Richards, Z. T., Kennington, W. J., & Johnson, M. S. (2017). Phylogenomics provides new insight into evolutionary relationships and genealogical discordance in the reef-building coral genus *Acropora*. *Proceedings of the Royal Society B: Biological Sciences*, 284(1846). <https://doi.org/10.1098/RSPB.2016.2182>
- Ruiz-García, M., Vásquez, J. Y. A., Restrepo, H., Cáceres-Martínez, C. H., & Shostell, J. M. (2020). The genetic structure of the spectacled bear (*Tremarctos ornatus*; Ursidae, Carnivora) in Colombia by means of mitochondrial and microsatellite markers. *Journal of Mammalogy*,

- 101(4), 1072–1090. <https://doi.org/10.1093/JMAMMAL/GYAA082>
- Sala, I., Caldeira, R. M. A., Estrada-Allis, S. N., Froufe, E., & Couvelard, X. (2013). Lagrangian transport pathways in the northeast Atlantic and their environmental impact. *Limnology and Oceanography: Fluids and Environments*, 3(1), 40–60. <https://doi.org/10.1215/21573689-2152611>
- Sala, I., Harrison, C. S., & Caldeira, R. M. A. (2016). The role of the Azores Archipelago in capturing and retaining incoming particles. *Journal of Marine Systems*, 154, 146–156. <https://doi.org/10.1016/j.jmarsys.2015.10.001>
- Santos, R. S., Hawkins, S., Monteiro, L. R., Alves, M., & Isidro, E. J. (1995). Marine research, resources and conservation in the Azores. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 5(4), 311–354. <https://doi.org/10.1002/aqc.3270050406>
- Scheltema, R. S. (1971). Larval dispersal as a means of genetic exchange between geographically separated populations of shallow-water benthic marine gastropods. *Biological Bulletin*, 140, 284–322.
- Scheltema, R. S. (1986). On dispersal and planktonic larvae of benthic invertebrates: An eclectic overview and summary of problems. *Bulletin of Marine Science*, 39(2), 290–322.
- Sluys, R. (2020). Reflections on the Nature, Framing, and Testing of Historical Biogeographic Hypotheses. *Species Diversity*, 25(2), 361–367. <https://doi.org/10.12782/SPECDIV.25.361>
- Stefanni, S., Stanković, D., Borme, D., de Olazabal, A., Juretić, T., Pallavicini, A., & Tirelli, V. (2018). Multi-marker metabarcoding approach to study mesozooplankton at basin scale. *Scientific Reports 2018 8:1*, 8(1), 1–13. <https://doi.org/10.1038/s41598-018-30157-7>
- Stefanoudis, P. V., Rivers, M., Smith, S. R., Schneider, C. W., Wagner, D., Ford, H., Rogers, A. D., & Woodall, L. C. (2019). Low connectivity between shallow, mesophotic and rariphotic zone benthos. *Royal Society Open Science*, 6(9). <https://doi.org/10.1098/rsos.190958>
- Taboada, S., Ríos, P., Mitchell, A., Cranston, A., Busch, K., Tonzo, V., Cárdenas, P., Sánchez, F., Leiva, C., Koutsouveli, V., Cristobo, J., Xavier, J. R., Hentschel, U., Rapp, H. T., Morrow, C., Drewery, J., Romero, P. E., Arias, M. B., Whiting, C., & Riesgo, A. (2022). Genetic diversity, gene flow and hybridization in fan-shaped sponges (*Phakellia* spp.) in the North-East Atlantic deep sea. *Deep-Sea Research Part I: Oceanographic Research Papers*, 181, 103685. <https://doi.org/10.1016/j.dsr.2021.103685>
- Taylor, M. L., & Roterman, C. N. (2017). Invertebrate population genetics across Earth's largest habitat: The deep-sea floor. *Molecular Ecology*, 26, 4872–4896. <https://doi.org/10.1111/mec.14237>
- Teske, P. R., Golla, T. R., Sandoval-Castillo, J., Emami-Khoyi, A., Van Der Lingen, C. D., Von Der Heyden, S., Chiazari, B., Jansen Van Vuuren, B., & Beheregaray, L. B. (2018). Mitochondrial DNA is unsuitable to test for isolation by distance. *Scientific Reports*, 8(1), 1–9. <https://doi.org/10.1038/s41598-018-25138-9>
- Teske, P. R., Sandoval-Castillo, J., Golla, T. R., Emami-Khoyi, A., Tine, M., Von Der Heyden, S., & Beheregaray, L. B. (2019). Thermal selection as a driver of marine ecological speciation. *Proceedings of the Royal Society B*, 286(1896), 20182023.

<https://doi.org/10.1098/rspb.2018.2023>

- Thiel, M., & Haye, P. A. (2006). The ecology of rafting in the marine environment. III. Biogeographical and evolutionary consequences. *Oceanography and Marine Biology*, 44, 323–429.
- Tibihika, P. D., Curto, M., Dornstaeder-Schrammel, E., Winter, S., Alemayehu, E., Waidbacher, H., & Meimberg, H. (2019). Application of microsatellite genotyping by sequencing (SSR-GBS) to measure genetic diversity of the East African *Oreochromis niloticus*. *Conservation Genetics*, 20(2), 357–372. <https://doi.org/10.1007/s10592-018-1136-x>
- Turini, F. G., Steinert, C., Heubl, G., Bringmann, G., Lombe, B. K., Mudogo, V., & Meimberg, H. (2014). Microsatellites facilitate species delimitation in Congolese *Ancistrocladus* (*Ancistrocladaceae*), a genus with pharmacologically potent naphthylisoquinoline alkaloids. *Taxon*, 63, 329–341.
- Vartia, S., Villanueva-Cañas, J. L., Finarelli, J., Farrell, E. D., Collins, P. C., Hughes, G. M., Carlsson, J. E. L., Gauthier, D. T., McGinnity, P., Cross, T. F., Fitzgerald, R. D., Mirimin, L., Crispie, F., Cotter, P. D., & Carlsson, J. (2016). A novel method of microsatellite genotyping-by-sequencing using individual combinatorial barcoding. *Royal Society Open Science*, 3, 150565. <https://doi.org/10.1098/rsos.150565>
- Vieira, P. E., Desiderato, A., Azevedo, S. L., Esquete, P., Costa, F. O., & Queiroga, H. (2022). Molecular evidence for extensive discontinuity between peracarid (Crustacea) fauna of Macaronesian islands and nearby continental coasts: over fifty candidate endemic species. *Marine Biology*, 169(5), 1–13. <https://doi.org/10.1007/S00227-022-04051-W/FIGURES/3>
- Vieira, P. E., Desiderato, A., Holdich, D. M., Soares, P., Creer, S., Carvalho, G. R., Costa, F. O., & Queiroga, H. (2019). Deep segregation in the open ocean: Macaronesia as an evolutionary hotspot for low dispersal marine invertebrates. *Molecular Ecology*, 28(7), 1784–1800. <https://doi.org/10.1111/mec.15052>
- Vieira, P. E., Lavrador, A. S., Parente, M. I., Parretti, P., Costa, A. C., Costa, F. O., & Duarte, S. (2021). Gaps in DNA sequence libraries for Macaronesian marine macroinvertebrates imply decades till completion and robust monitoring. *Diversity and Distributions*, 27(10), 2003–2015. <https://doi.org/10.1111/ddi.13305>
- Waters, J. M., & Craw, D. (2018). Cyclone-driven marine rafting: storms drive rapid dispersal of buoyant kelp rafts. *Marine Ecology Progress Series*, 602, 77–85. <https://doi.org/10.3354/meps12695>
- Watling, L., & Auster, P. J. (2017). Seamounts on the high seas should be managed as vulnerable marine ecosystems. *Frontiers in Marine Science*, 4(JAN), 1–4. <https://doi.org/10.3389/fmars.2017.00014>
- Watts, P. C., & Thorpe, J. P. (2006). Influence of contrasting larval developmental types upon the population-genetic structure of cheilostome bryozoans. *Marine Biology*, 149(5), 1093–1101. <https://doi.org/10.1007/s00227-006-0288-2>
- Weersing, K., & Toonen, R. J. (2009). Population genetics, larval dispersal, and connectivity in marine systems. *Marine Ecology Progress Series*, 393, 1–12.

<https://doi.org/10.3354/meps08287>

- Winston, J. E. (2012). Dispersal in marine organisms without a pelagic larval phase. *Integrative and Comparative Biology*, 52(4), 447–457. <https://doi.org/10.1093/icb/ics040>
- Wort, E. J. G., Chapman, M. A., Hawkins, S. J., Henshall, L., Pita, A., Rius, M., Williams, S. T., & Fenberg, P. B. (2019). Contrasting genetic structure of sympatric congeneric gastropods: Do differences in habitat preference, abundance and distribution matter? *Journal of Biogeography*, 46(2), 369–380. <https://doi.org/10.1111/jbi.13502>
- Xavier, J. R., Van Soest, R. W. M., Breeuwer, J. A. J., Martins, A. M. F., & Menken, S. B. J. (2010). Phylogeography, genetic diversity and structure of the poecilosclerid sponge *Phorbas fictitius* at oceanic islands. *Contributions to Zoology*, 79(3), 119–129. <https://doi.org/10.1163/18759866-07903004>
- Xuereb, A., Kimber, C. M., Curtis, J. M. R., Bernatchez, L., & Fortin, M. J. (2018). Putatively adaptive genetic variation in the giant California sea cucumber (*Parastichopus californicus*) as revealed by environmental association analysis of restriction-site associated DNA sequencing data. *Molecular Ecology*, 27(24), 5035–5048. <https://doi.org/10.1111/mec.14942>
- Yu, L., Boström, C., Franzenburg, S., Bayer, T., Dagan, T., & Reusch, T. B. H. (2020). Somatic genetic drift and multilevel selection in a clonal seagrass. *Nature Ecology and Evolution*, 4(7), 952–962. <https://doi.org/10.1038/s41559-020-1196-4>
- Zeng, C., Rowden, A. A., Clark, M. R., & Gardner, J. P. A. (2020). Species-specific genetic variation in response to deep-sea environmental variation amongst Vulnerable Marine Ecosystem indicator taxa. *Scientific Reports*, 10(1), 0–15. <https://doi.org/10.1038/s41598-020-59210-0>
- Zheng, Y., Peng, R., Kuro-o, M., & Zeng, Z. (2011). Exploring Patterns and Extent of Bias in Estimating Divergence Time from Mitochondrial DNA Sequence Data in a Particular Lineage: A Case Study of Salamanders (Order Caudata). *Molecular Biology and Evolution*, 28, 2521–2535. <https://doi.org/10.1093/molbev/msr072>
- Zouros, E., & Foltz, D. W. (1984). Possible explanations of heterozygote deficiency in bivalve molluscs. *Malacologia*, 25, 583–591.

Appendix A

Supplementary Information

Supplementary Information for Section 3.1.

Intertidal or subtidal/circalittoral species: who appeared first? A phylogenetic approach to the evolution of non-planktotrophic species in Atlantic Archipelagos

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Figure A1.1. Bayesian Inference tree obtained for mtDNA COI gene, resorting to MrBayes v3.2.6 software.

Figure A1.2. Bayesian Inference tree obtained for nDNA 28S gene with MrBayes v3.2.6 software.

Table A1.1. Checklist and distribution of the shallow-water Rissoidae from the North Atlantic Ocean and adjacent seas. Distribution areas modified from ecoregions described by Spalding et al. (2007): CAR, Caribbean Sea; BAH, Bahamian; GME, Gulf of Mexico; FLO, Floridian; BER, Bermuda Islands; CRL, Carolinian; VIR, Virginian; NSC, Scotian; WGR, West Greenland Shelf; EGR, East Greenland Shelf; ICE, Iceland; FAR, Faroe Islands; SNO, Southern Norway; NOR, North Sea; CEL, Celtic Seas; SEA, South European Atlantic Shelf; MED, Mediterranean Sea; AZO, Azores Islands; MAD, Madeira Islands; SEL, Selvagens Islands; CAN, Canary Islands; SAH, Saharan Upwelling; SEN, Sahelian Upwelling; and CAB, Cabo Verde Islands. Species present in a single area when their distribution extends out of the considered areas are marked with an asterisk. Prefix “e” followed by an acronym of a distribution area means that the species is endemic to that area.

Species	CAR	BAH	GME	FLO	BER	CRL	VIR	NSC	WGR	EGR	ICE	FAR	SNO	NOR	CEL	SEA	MED	AZO	MAD	SEL	CAN	SAH	SEN	CAB
<i>Alvania aartseni</i> Verduin, 1986																	1							
<i>Alvania abstersa</i> van der Linden & van Aartsen, 1994																		1						
<i>Alvania aeoliae</i> Palazzi, 1988																	1							
<i>Alvania africana</i> Gofas, 1999																						1	1	1
<i>Alvania algeriana</i> (Monterosato, 1877)																	1							
<i>Alvania aliceae</i> Amati, 2014																	1							
<i>Alvania amatii</i> Oliverio, 1986																1	1							
<i>Alvania angioyi</i> van Aartsen, 1982																		1						
<i>Alvania arubensis</i> De Jong & Coomans, 1988	1																							
<i>Alvania aspera</i> (Philippi, 1844)																	1							
<i>Alvania auberiana</i> (d'Orbigny, 1842)	1	1	1	1	1	1																		
<i>Alvania aurantiaca</i> (R. B. Watson, 1873)																			1					
<i>Alvania balearica</i> Oliver & Templado, 2009																	1							
<i>Alvania bartolinorum</i> Amati & Smriglio, 2019																	1							
<i>Alvania basteriae</i> (Moolenbeek & Faber, 1986)																					1			
<i>Alvania beanii</i> (Hanley in Thorpe, 1844)													1	1	1	1	1							
<i>Alvania bermudensis</i> Faber & Moolenbeek, 1987					1																			
<i>Alvania bozcaadensis</i> Tisselli & Giunchi, 2013																	1							
<i>Alvania campanii</i> Tisselli & Giunchi, 2013																	1							
<i>Alvania canariensis</i> (d'Orbigny, 1840)																			1	1	1			
<i>Alvania cancellata</i> (da Costa, 1778)													1	1	1	1	1	1	1	1	1	1	1	1
<i>Alvania carinata</i> (da Costa, 1778)													1	1	1	1	1							
<i>Alvania cimex</i> (Linnaeus, 1758)																1	1					1	1	
<i>Alvania clarae</i> Nofroni & Pizzini, 1991																	1							
<i>Alvania clathrella</i> L. Seguenza, 1903																	1							
<i>Alvania claudioi</i> Buzzurro & Landini, 2007																	1							
<i>Alvania colombiana</i> Romer & Moore, 1988	1	1	1	1																				
<i>Alvania colossophilus</i> Oberling, 1970																	1							
<i>Alvania corona</i> Nordsieck, 1972																	1							
<i>Alvania curacaoensis</i> De Jong & Coomans, 1988	1																							
<i>Alvania dalmatica</i> Buzzurro & Prkić, 2007																	1							
<i>Alvania datchaensis</i> Amati & Oliverio, 1987																	1							
<i>Alvania debruynei</i> Faber & Moolenbeek, 2004	1																							
<i>Alvania dejongi</i> Faber & Moolenbeek, 2004	1	1																						
<i>Alvania denhartogi</i> Hoenselaar & Goud, 1998																								1
<i>Alvania desabatae</i> Amati & Smriglio, 2016																	1							
<i>Alvania dianiensis</i> Oliverio, 1988																1	1							
<i>Alvania dictyophora</i> (Philippi, 1844)																	1							
<i>Alvania discors</i> (T. Allan, 1818)																	1							
<i>Alvania elisae</i> Margelli, 2001																	1							
<i>Alvania euchila</i> (R. B. Watson, 1886)																			1		1			

[illegible]

Species	CAR	BAH	GME	FLO	BER	CRL	VIR	NSC	WGR	EGR	ICE	FAR	SNO	NOR	CEL	SEA	MED	AZO	MAD	SEL	CAN	SAH	SEN	CAB
<i>Alvania scuderi</i> Villari, 2017																	1							
<i>Alvania sculptilis</i> (Monterosato, 1877)																	1							
<i>Alvania settepassii</i> Amati & Nofroni, 1985																	1							
<i>Alvania sleursi</i> (Amati, 1987)																		1	1	1				
<i>Alvania spinosa</i> (Monterosato, 1890)*																	1							
<i>Alvania stocki</i> Moolenbeek & Rolán, 1988																								1
<i>Alvania subareolata</i> Monterosato, 1869																	1							
<i>Alvania subcalathus</i> Dautzenberg & H. Fischer, 1906																				1	1			
<i>Alvania subcrenulata</i> (Bucquoy, Dautzenberg & Dollfus, 1884)																1	1							
<i>Alvania tarsodes</i> (R. B. Watson, 1886)																		1						
<i>Alvania tenera</i> (Philippi, 1844)																1	1				1			
<i>Alvania tessellata</i> Schwartz in Weinkauff, 1868																	1							
<i>Alvania turkensis</i> Faber & Moolenbeek, 2004	1	1																						
<i>Alvania unica</i> Amati & Quaggitto, 2019																	1							
<i>Alvania vermaasi</i> van Aartsen, 1975																1	1							
<i>Alvania villarii</i> Micali, Tisselli & Giunchi, 2005																	1							
<i>Alvania watsoni</i> (Schwartz in R. B. Watson, 1873)																			1	1	1			
<i>Alvania weinkauffi jacobusi</i> Oliverio, Amati & Nofroni, 1986																	1							
<i>Alvania zaraensis</i> Amati & Appolloni, 2019																	1							
<i>Boreocingula castanea</i> (Møller, 1842)									1	1	1													
<i>Boreocingula globulus</i> (Møller, 1842)								1	1															
<i>Botryphallus epidauricus</i> (Brusina, 1866)																1	1		1	1	1			
<i>Botryphallus ovummuscae</i> (Gofas, 1990)																		1						
<i>Botryphallus tuber</i> (Rolán, 1991)																								1
<i>Cingula stewardsoni</i> (Vanatta, 1909)					1																			
<i>Cingula trifasciata</i> (J. Adams, 1800)													1	1	1	1	1	1						
<i>Crisilla aartseni</i> (Verduin, 1984)																	1							
<i>Crisilla alvarezi</i> Templado & Rolán, 1994																								1
<i>Crisilla beniamina</i> (Monterosato, 1884)																	1				1			
<i>Crisilla callosa</i> (Manzoni, 1868)																				1	1			
<i>Crisilla chiarellii</i> (Cecalupo & Quadri, 1995)																	1							
<i>Crisilla depicta</i> (Manzoni, 1868)																			1	1	1			
<i>Crisilla galvagni</i> (Aradas & Maggiore, 1844)																	1							
<i>Crisilla graxai</i> Templado & Rolán, 1994																								1
<i>Crisilla innominata</i> (R. B. Watson, 1897)																			1	1	1			
<i>Crisilla iunoniae</i> (Palazzi, 1988)																		1	1	1	1			
<i>Crisilla luquei</i> Templado & Rolán, 1994																								1
<i>Crisilla maculata</i> (Monterosato, 1869)																1	1							
<i>Crisilla marioni</i> (Fasulo & Gaglioli, 1987)																	1							
<i>Crisilla morenoi</i> Templado & Rolán, 1994																								1
<i>Crisilla ortei</i> Templado & Rolán, 1994																								1
<i>Crisilla perminima</i> (Manzoni, 1868)																	1		1	1	1			
<i>Crisilla picta</i> (Jeffreys, 1867)																			1	1	1			
<i>Crisilla postrema</i> (Gofas, 1990)																		1	1					
<i>Crisilla quisquiliarum</i> (R. B. Watson, 1886)																		1			1			
<i>Crisilla ramosorum</i> Oliver, Templado & Kersting, 2012																	1							
<i>Crisilla semistriata</i> (Montagu, 1808)													1	1	1	1	1							
<i>Crisilla senegalensis</i> Rolán & Hernández, 2006																							1	
<i>Crisilla transitoria</i> Gofas, 1999*																							1	
<i>Crisilla ugesae</i> (Verduin, 1988)																					1			

[illegible]

Species	CAR	BAH	GME	FLO	BER	CRL	VIR	NSC	WGR	EGR	ICE	FAR	SNO	NOR	CEL	SEA	MED	AZO	MAD	SEL	CAN	SAH	SEN	CAB
<i>Peringiella denticulata</i> Ponder, 1985																	1							
<i>Peringiella elegans</i> (Locard, 1891)																1	1							
<i>Plagyostila asturiana</i> P. Fischer in de Folin, 1872																1	1					1		1
<i>Plagyostila senegalensis</i> Rolán & Pelorce, 2002																							1	
<i>Pusillina benzi</i> (Aradas & Maggiore, 1844)																1	1							
<i>Pusillina inconspicua</i> (Alder, 1844)													1	1	1	1	1	1			1	1	1	
<i>Pusillina lineolata</i> (Michaud, 1830)																1	1							
<i>Pusillina marginata</i> (Michaud, 1830)																1	1							
<i>Pusillina munda</i> (Monterosato, 1884)																	1							
<i>Pusillina philippi</i> (Aradas & Maggiore, 1844)																	1							
<i>Pusillina radiata</i> (Philippi, 1836)																1	1		1			1		
<i>Pusillina sarsii</i> (Lovén, 1846)											1		1	1	1	1	1							
<i>Pusillina testudae</i> (Verduin, 1979)																	1					1		
<i>Pusillina tumidula</i> (G. O. Sars, 1878)											1	1												
<i>Quarkia sculpturata</i> Faber, 2009	1																							
<i>Rissoa aartseni</i> Verduin, 1985																	1							
<i>Rissoa albugo</i> R. B. Watson, 1873																			1	1	1			
<i>Rissoa alleryi</i> (Nordsieck, 1972)																	1							
<i>Rissoa auriformis</i> Pallary, 1904																	1							
<i>Rissoa auriscalpium</i> (Linnaeus, 1758)																1	1							
<i>Rissoa decorata</i> Philippi, 1846																1	1							
<i>Rissoa frauenfeldiana</i> Brusina, 1866																	1							
<i>Rissoa gemmula</i> P. Fischer in de Folin, 1869*																	1							
<i>Rissoa gomerica</i> (Nordsieck & Talavera, 1979)																					1			
<i>Rissoa guerinii</i> Récluz, 1843														1	1	1	1							
<i>Rissoa guernei</i> Dautzenberg, 1889																		1						
<i>Rissoa italiensis</i> Verduin, 1985																	1							
<i>Rissoa janusi</i> (Nordsieck, 1972)																			1	1				
<i>Rissoa lia</i> (Monterosato, 1884)																1	1		1	1	1			
<i>Rissoa lilacina</i> Récluz, 1843													1	1	1	1	1				1	1		
<i>Rissoa membranacea</i> (J. Adams, 1800)													1	1	1	1	1							
<i>Rissoa mirabilis</i> Manzoni, 1868																		1	1	1	1			
<i>Rissoa monodonta</i> Philippi, 1836																1	1							
<i>Rissoa multicincta</i> Smriglio & Mariottini, 1995																	1							
<i>Rissoa multicostata</i> (Nordsieck & Talavera, 1979)																					1			
<i>Rissoa panhormensis</i> Verduin, 1985																	1							
<i>Rissoa paradoxa</i> (Monterosato, 1884)																	1							
<i>Rissoa parva</i> (da Costa, 1778)												1	1	1	1	1	1					1		
<i>Rissoa pseudoguerini</i> (Nordsieck & Talavera, 1979)																					1			
<i>Rissoa rodhensis</i> Verduin, 1985																	1							
<i>Rissoa scurra</i> (Monterosato, 1917)																1	1							
<i>Rissoa similis</i> Scacchi, 1836																1	1				1			
<i>Rissoa splendida</i> Eichwald, 1830																	1							
<i>Rissoa torquilla</i> Pallary, 1912																	1							
<i>Rissoa variabilis</i> (Megerle von Mühlfeld, 1824)																1	1				1			
<i>Rissoa ventricosa</i> Desmarest, 1814																1	1							
<i>Rissoa verdensis</i> Rolán & Oliveira, 2008																								1
<i>Rissoa violacea</i> Desmarest, 1814																1	1							
<i>Setia alexandrae</i> Ávila & Cordeiro, 2015																		1						
<i>Setia amabilis</i> (Locard, 1886)																	1							

[illegible]

Table A1.2. Target gene, primer name and sequence (5'-3'), length of the fragments amplified, reference and cycling profile (temperature, time and number of cycles).

Gene	Primer	Sequence (5'–3')	Fragment length (bp)	Reference	Cycling profile
COI	LCO1490	F: GGTCAACAAATCATAAAGATATTGG	619-658	Folmer et al. (1994)	94°C (4'); [x35]* 94°C (1'), 55°C (40"/1'), 72°C (2'); 72°C (4')
	HCO2198	R: TAAACTTCAGGGTGACCAAAAAATCA			
	COR722b	R: TAAACTTCAGGGTGACCAAAAAATYA		Wilke and Davis (2000)	94°C (4'); [x35]* 94°C (30"-1'), 45°C/ 48°C/ 55°C (30"-50") or 55°C (50"-1'), 72°C (45"-2'); 72°C (5')
	jgLCO1490	F: TITCIACIAAYCAYAARGAYATTGG		Geller et al. (2013)	
	jgHCO2198	R: TAIACYTCIGGRTGICCRAARAAYCA			
28S	28SDKF	F: GATCGGACGAGATTACCCGCTGAA	462-496	Strong et al. (2011)	94°C (5'); [x35]* 94°C (1'), 55°C (1'), 72°C (2'); 72°C (10')
	LSU1600R	R: AGCGCCATCCATTTTCAGG		Williams et al. (2003)	
	Rd1a	F: CCCSCGTAAYTTAGGCATAT		Edgecombe and Giribet (2006)	94°C (4'); [x41]* 94°C (20"), 56°C/ 58°C/ 61°C (30"), 72°C (40"); 72°C (10')
	28Sb	R: TCGGAAGGAACCAGCTAC		Whiting (2002)	

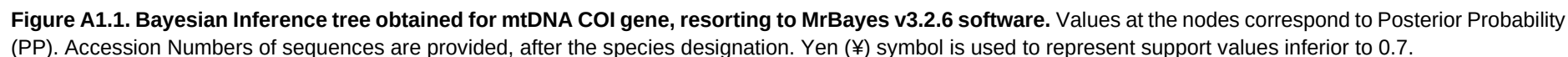
* Some samples required 40 cycles to achieve an acceptable amplification level.

Table A1.3. Sequences of mitochondrial COI and nuclear 28S genes of Rissoidae species reported for the North Atlantic Ocean and of the outgroups *Anabathron contabulatum* and *Pisinna glabrata*, included in the molecular analyses. Accession numbers (AN), voucher details (if applicable), locality of sample collection and reference of the published sequences are provided. Accession numbers in bold refer to sequences obtained in this study. AM C – Malacological collections of the Australian Museum, Sydney. DBUA – Marine Molluscs Reference Collection of the Department of Biology of the University of the Azores. n.a. – not applicable.

	COI AN	28S AN	Voucher details	Locality	Reference
<i>Alvania</i> Risso 1826					
<i>A. aeoliae</i> Palazzi, 1988		KR698251	AM C.463789	Santa Tecla harbour, Sicily, Italy	Criscione et al. (2017)
<i>A. angioyi</i> van Aartsen, 1982	MG652373	MG663174	DBUA 1144	Baixa de João Lopes, Santa Maria, Azores, Portugal	This study
	MG652374-78	MG663173	DBUA 1146	Baixa da Pedrinha, Santa Maria, Azores, Portugal	
		MG663175-8			
<i>A. beanii</i> (Hanley in Thorpe, 1844)	FN650144-46		n.a.	Bjelkarøyna, Norway	Tverberg (unpub)
<i>A. cimex</i> (Linnaeus, 1758)		KC109987	AM C.463830	Ognina Catania fishery harbour, Sicily, Italy	Criscione & Ponder (2013)
<i>A. formicarum</i> Gofas, 1989	MG652379	MG663179	n.a.	Baixa do Vigário, Santa Maria Island, Azores Portugal	This study
	MG652380		DBUA 1145	Ilhéu de Vila do Porto, Santa Maria, Azores, Portugal	This study
<i>A. discors</i> (Allan, 1818)		KR698255	AM C.463833	Isola dei Porri, Ispica, Sicily, Italy	Criscione et al. (2017)

<i>A. geryonia</i> (Nardo, 1847)			AM C 463838	Faro Lake, Faro, Sicily, Italy	Criscione et al. (2017)
<i>A. lanciae</i> (Calcara, 1841)		KR698257	AM C.463841	Ganzirri Lake, Ganzirri, Sicily, Italy	Criscione et al. (2017)
<i>A. lineata</i> Risso, 1826		KR698258	AM C.463842	Santa Tecla harbour, Sicily, Italy	Criscione et al. (2017)
<i>A. mamillata</i> Risso, 1826			AM C.463794	Ganzirri Lake, Ganzirri, Sicily, Italy	Criscione et al. (2017)
<i>A. mediolittoralis</i> Gofas, 1989	MG652381-2 MG652385	MG663180	DBUA 755	Cerco-Caloura, São Miguel, Azores, Portugal	This study
	MG652383-4	MG663181	n.a.	Caloura, São Miguel Island, Azores, Portugal	This study
<i>A. punctura</i> (Montagu, 1803)	FN650147-49		n.a.	Bjelkarøyna, Norway	Tverberg (unpub)
<i>A. scabra</i> Philippi, 1844		KR698259	AM C.463798	Santa Tecla harbour, Sicily, Italy	Criscione et al. (2017)
<i>A. sleursi</i> (Amati, 1987)	MG652386-7	MG663182-3	DBUA 1146	Baixa da Pedrinha, Santa Maria, Azores, Portugal	
<i>A. subcrenulata</i> (Bucquoy et al., 1884)		KR698261	AM C.463799	Santa Tecla harbour, Sicily, Italy	Criscione et al. (2017)
<i>A. subsoluta</i> (Aradas, 1847)	FN650150		n.a.	unspecified, Norway	Tverberg (unpub)
<i>A. tenera</i> Philippi, 1844		KR698262	AM C.463800	Trapani, Sicily, Italy	Criscione et al. (2017)
<i>Cingula</i> Fleming, 1818					
<i>C. trifasciata</i> (J. Adams, 1800)	KU496634		n.a.	Caloura, São Miguel Island, Azores, Portugal	Borges et al. (2016)
		KR698266	AM C.466893	Portaferry, Ballyhenry Island, Northern Ireland	Criscione et al. (2017)
	MG652395-8	MG663189-90 MG663193-4	n.a.	Poça da Barra, Pico, Azores, Portugal	
	MG652399-402 MG652405		n.a.	Lajes, Pico, Azores, Portugal	This study
	MG652403-4 MG652406-7	MG663191-2 MG663195-6	n.a.	Caloura, São Miguel, Azores, Portugal	
<i>Crisilla</i> Monterosato, 1917					
<i>C. postrema</i> (Gofas, 1990)	MG652392-4	MG663185-8	DBUA 1145	Ilhéu de Vila do Porto, Santa Maria, Azores, Portugal	This study
<i>Onoba</i> H. Adams & A. Adams, 1852					
<i>O. semicostata</i> (Montagu, 1803)	FN650151 - 53		n.a.	Bjelkarøyna, Norway	Tverberg (unpub)
		KR698277	AM C.467203	Portaferry, Ballyhenry Island, Northern Ireland	Criscione et al. (2017)
<i>Pseudosetia</i> Monterosato, 1884					
<i>P. semipellucida</i> (Friele, 1879)	FN650154, 56		n.a.	unspecified, Norway	Tverberg (unpub)
<i>P. sp.</i>	FN650172-73		n.a.	unspecified, Norway	Tverberg (unpub)
<i>Pusillina</i> Monterosato, 1884					
<i>P. inconspicua</i> (Alder, 1844)	FN650157 - 59		n.a.	unspecified, Norway	Tverberg (unpub)
		KR698283	AM C.463810	Acitrezza fishery harbour, Sicily, Italy	Criscione et al. (2017)
<i>P. marginata</i> (Michaud, 1832)		KR698284	AM C.463816	Porto Vecchio pond, Oliveri, Sicily, Italy	Criscione et al. (2017)

<i>P. philippi</i> (Aradas & Maggiore, 1844)	KR698285	AM C.463821	Calabernardo, Sicily, Italy	Criscione et al. (2017)
<i>P. radiata</i> (Philippi, 1836)	KR698286	AM C.463828	Lacco Ameno, Ischia Island, Italy	Criscione et al. (2017)
<i>P. sarsii</i> (Lovén, 1846)	FN650160	n.a.	unspecified, Norway	Tverberg (unpub)
Rissoa Desmaret, 1814				
<i>R. auriscalpium</i> (Linnaeus, 1758)	HQ623175	n.a.	unspecified	(Wilke et al. 2013)
	KR698287	AM C.463845	Lacco Ameno, Ischia Island, Italy	Criscione et al. (2016)
<i>R. guerinii</i> Récluz, 1843	KR698288	AM C.463851	Santa Tecla harbour, Sicily, Italy	Criscione et al. (2016)
<i>R. guernei</i> Dautzenberg, 1889	MG652419-21	MG663201 MG663207 MG663209	DBUA 1145 Ilhéu de Vila do Porto, Santa Maria, Azores, Portugal	This study
	MG652408-13	MG663197-200 MG663202-3	n.a. Baixa do Vigário, Santa Maria, Azores, Portugal	This study
<i>R. cf. guernei</i>	MG652414-5 MG652417-8 MG652422	MG663204-6 MG663208	n.a. Baixa do Vigário, Santa Maria, Azores, Portugal	This study
	MG652416	DBUA 1144	Baixa de João Lopes, Santa Maria, Azores, Portugal	
<i>R. lia</i> (Monterosato, 1884)	KR698291	AM C.463858	Santa Tecla harbour, Sicily, Italy	Criscione et al. (2017)
<i>R. membranacea</i> (Montagu, 1803)	KR698290	AM C.463856	Portopalo di Menfi fishery harbour, Sicily, Italy	Criscione et al. (2017)
<i>R. monodonta</i> Philippi, 1836	KR698292	AM C.463859	Oliveri, Sicily, Italy	Criscione et al. (2017)
<i>R. ventricosa</i> Desmaret, 1814	KC110026	AM C.463868	Lacco Ameno, Ischia Island, Italy	Criscione & Ponder (2013)
Setia H. Adams & A. Adams, 1852				
<i>S. ambigua</i> (Brugnone, 1873)	KR698296	AM C.463876	Ganzirri, Sicily, Italy	Criscione et al. (2017)
<i>S. subvaricosa</i> Gofas, 1990	MG652429-31	MG663215-6	DBUA 1145 Ilhéu de Vila do Porto, Santa Maria, Azores, Portugal	
Anabathron Frauenfeld, 1867				
<i>A. contabulatum</i> (Frauenfeld, 1867)	KC109989	AM C.466922	Long Reef, Collaroy, New South Wales, Australia	Criscione & Ponder (2013)
Pisinna Monterosato, 1878				
<i>P. glabrata</i> (von Mühlfeld, 1824)	MG652432-36	MH047302-4	n.a. Baixa do Vigário, Santa Maria, Azores, Portugal	This study



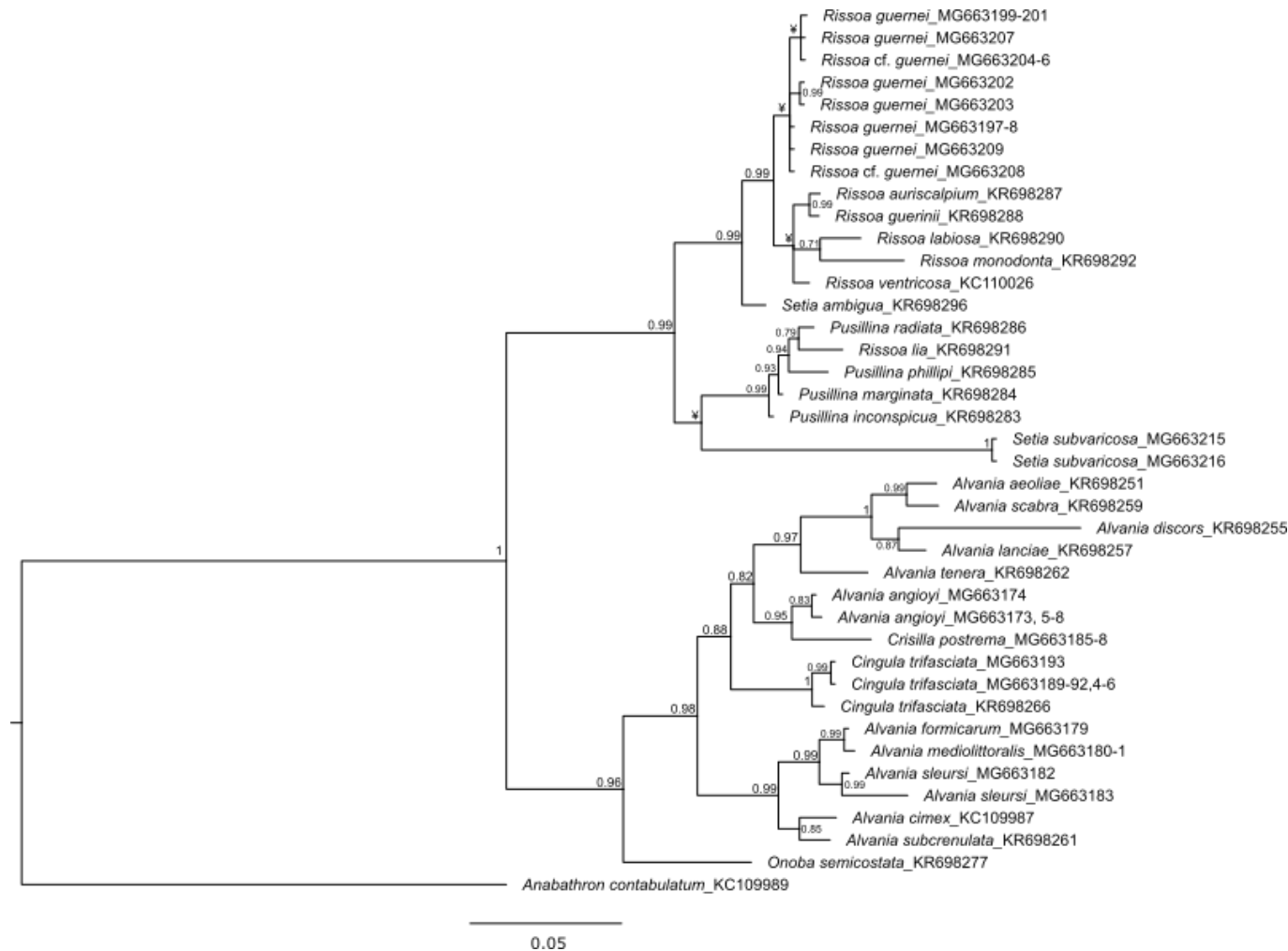


Figure A1.2. Bayesian Inference tree obtained for nDNA 28S gene with MrBayes v3.2.6 software. Values at the nodes correspond to Posterior Probability (PP). Accession Numbers of sequences are provided, after the species designation. Yen (¥) symbol is used to represent support values inferior to 0.7

References

- Borges, L. M. S., Hollatz, C., Lobo, J., Cunha, A. M., Vilela, A. P., Calado, G., Coelho, R., Costa, A. C., Ferreira, M. S. G., Costa, M. H., Costa, F. O. (2016). With a little help from DNA barcoding: Investigating the diversity of Gastropoda from the Portuguese coast. *Science Reports*. <https://doi.org/10.1038/srep20226>
- Criscione, F., & Ponder, W. F. (2013). A phylogenetic analysis of rissooidean and cingulopsoidean families (Gastropoda: Caenogastropoda). *Molecular Phylogenetics and Evolution*, 66(3), 1075–1082. <https://doi.org/10.1016/j.ympev.2012.11.026>
- Criscione, F., Ponder, W. F., Köhler, F., Takano, T., & Kano, Y. (2016). A molecular phylogeny of Rissoidae (Caenogastropoda: Rissooidea) allows testing the diagnostic utility of morphological traits. *Zoological Journal of the Linnean Society*, 179(1), 23–40. <https://doi.org/10.1111/zoj.12447>
- Edgecombe, G. D., & Giribet, G. (2006). A century later - a total evidence re-evaluation of the phylogeny of scutigeromorph centipedes (Myriapoda: Chilopoda). *Invertebrate Systematics*, 20, 503–525. <https://doi.org/10.1071/IS05044>
- Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial Cytochrome C Oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.
- Geller, J., Meyer, C., Parker, M., & Hawk, H. (2013). Redesign of PCR primers for mitochondrial Cytochrome C Oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Molecular Ecology Resources*, 13, 51–861. <https://doi.org/10.1111/1755-0998.12138>
- Spalding, M. D., Fox, H. E., Allen, G. R., Davidson, N., Ferdaña, Z. A., Finlayson, M., Halpern, B. S., Jorge, M. A., Lombana, A., Lourie, S. A., Martin, K. D., McManus, E., Molnar, J., Recchia, C. A., & Robertson, J. (2007). Marine Ecoregions of the World: A Bioregionalization of Coastal and Shelf Areas. *BioScience*, 57, 573–583. <https://doi.org/10.1641/B570707>
- Strong, E. E., Colgan, D. J., Healy, J. M., Lydeard, C., Ponder, W. F., & Glaubrecht, M. (2011). Phylogeny of the gastropod superfamily Cerithioidea using morphology and molecules. *Zoological Journal of the Linnean Society*, 162, 43–89. <https://doi.org/10.1111/j.1096-3642.2010.00670.x>
- Tverberg, J. (unpub) *Molecular phylogenetics of North-East Atlantic hydrothermal vent and wood fall associated Rissoidae (Mollusca: Gastropoda)*
- Whiting, M.F. (2002). Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera. *Zoologica Scripta*, 31, 93–104. <https://doi.org/10.1046/j.0300-3256.2001.00095.x>
- Wilke, T., & Davis, G. M. (2000). Intraspecific mitochondrial sequence diversity in *Hydrobia ulvae* and *Hydrobia ventrosa* (Hydrobiidae: Rissooidea: Gastropoda): Do their different life histories affect biogeographic patterns and gene flow? *Biological Journal of the Linnean Society*, 70, 89–105. <https://doi.org/10.1006/bjil.1999.0388>
- Wilke, T., Haase, M., Hershler, R., Liu, H. P., Misof, B., & Ponder, W. (2013). Pushing short DNA fragments to the limit: Phylogenetic relationships of “hydrobioid” gastropods (Caenogastropoda: Rissooidea). *Molecular Phylogenetics and Evolution*, 66, 715–736. <https://doi.org/10.1016/j.ympev.2012.10.025>
- Williams, S. T., Reid, D. G., & Littlewood, D. T. J. (2003). A molecular phylogeny of the Littorininae (Gastropoda: Littorinidae): unequal evolutionary rates, morphological parallelism, and biogeography of the Southern Ocean. *Molecular Phylogenetics and Evolution*, 28, 60–86. [https://doi.org/10.1016/S1055-7903\(03\)00038-1](https://doi.org/10.1016/S1055-7903(03)00038-1)

Supplementary Information for Section 3.2.

Population structure of *Stramonita haemastoma* (Linnaeus, 1767) in the NE Atlantic Ocean

List of Supplementary Tables

Table A2.1. Complete list of the SSR primers designed for *S. haemastoma* in this study.

Figure A2.1. Genetic structure analysis of the microsatellite dataset of *S. haemastoma*, reporting the results from $K = 2$ up to $K = 10$.

Table A2.1. Complete list of the SSR primers designed for *S. haemastoma* in this study. Includes sequence of forward and reverse primers (5'-3'), the repetition motif, number of repeats in the original sequence used for the primer design, the primer mix in which it was included for the Multiplex PCR, and notes on the failure and inclusion on the final analyses.

Primer	Forward (5'-3')	Reverse (5'-3')	Motif	Mix	Notes
SH1_TTTA	TCTTTCCCTACACGACGCTCTTCCGATCT	TACGTGATGGCTCCTCTT	(TTTA)11	1	Failed (>60% missing data)
SH2_GATA	AGGCATAAGGGAGAGCTAGA	TGACTTAACCTTCACGTGGT	(GATA)14		Failed in single PCR test
SH3_ATTT	GGGGAACCTTGGGAAATCAG	AGCACATAAGTTCTACATCCA	(ATTT)10	1	Failed (>60% missing data)
SH4_CTGT	GTGATTACAGTGAGCATGCT	AGTAGCCTCGGGAATTCTTT	(CTGT)9	1	
SH5_GATT	TGCCAACTTTAAATATTTCTG	CATTGTGGACTGTTTTCATGG	(GATT)6	2	Failed (>60% missing data)
SH6_TATT	GGTGCTTCCCTCTTCTGC	TAAAGTCCTTCAAACCGAGGT	(TATT)5	1	
SH7_ATGGT	TGAAAATGCACTCTGATGGAG	ACATCGAAATTCACAAATCA	(ATGGT)7		Failed in single PCR test
SH8_AATAC	CCTACAGCACCAGAGAGTAG	AGGAGAATATGATGCGAAAGA	(AATAC)12	1	
SH9_CTAT	TTCAACCTTTATTTGGACAGA	GGTACCAAATGAGCCACAAC	(CTAT)5	2	
SH10_TGTT	TTTTGCAAAATGTGGTTCAGA	TGTCGTAATGTGAACAGACAC	(TGTT)5		Failed in single PCR test
SH11_GTCAA	ACCACAAAATGCATGCGTG	CGGGACTAGGCTAGCTTAGT	(GTCAA)8		Failed in single PCR test
SH12_TCTG	TGGTGTGTTACTACAGGTGA	TGTCTCAGCGATGATGCATA	(TCTG)5	2	
SH13_AAAC	AGTATTAGAATGACATTCGGG	TCGCCATTATGACCGGAAG	(AAAC)6		Failed in single PCR test
SH14_ACTC	TCTCTTCAGATATCTGCGTAC	ACTTTAGGCAGTACGCAGAT	(ACTC)5		Failed in single PCR test
SH15_CCAGA	ACTTTAGGCAGTACGCAGAT	CAACTCGACCAAAGCAAAT	(CCAGA)5		Failed in single PCR test
SH16_ACAG	TTCTGTCATTTGAATATCACA	ATGCCTCGCTATTTTCATTTT	(ACAG)8	1	Failed (>60% missing data)
SH17_ACCT	ACAGGACAAGAAATGACAACG	TTCTGTCATTTGAATATCACA	(ACCT)7	2	Failed (>60% missing data)
SH18_TACA	CACATGTCGACCCTGAGG	ACAGGACAAGAAATGACAACG	(TACA)19	2	
SH19_TTTA	GAAACCATAAACCCTGTCC	CACATGTCGACCCTGAGG	(TTTA)5	2	
SH20_ATAA	AAGGTGGCAGTATGAACCA	CACATACTTTACCGCAAAAT	(ATAA)9	2	
SH21_AGAT	GCAGAGAAATCTTACGTGACA	GTGCGTGTATTCCGAATCTG	(AGAT)21		Failed in single PCR test
SH22_ATTG	TGTCTCACACCATGTGTTGA	ATGTCTACCTACCCCTCACA	(ATTG)8		Failed in single PCR test
SH23_TTGA	TGCATTACCGTGTCCCTAA	GAAACCATAAACCCTGTCC	(TTGA)5		Failed in single PCR test
SH24_TTTC	GTGCTCTTTTGTGACAAT	AAGGTGGCAGTATGAACCA	(TTTC)5	1	
SH25_CCAA	CGTTGTTTCAAGGTTGGTGT	GCAGAGAAATCTTACGTGACA	(CCAA)5		Failed in single PCR test
SH26_AAAT	TGATTCTGGGTGTACAGCTC	TGTCTCACACCATGTGTTGA	(AAAT)5		Failed in single PCR test
SH27_TATT	GAAATACTTGGACTCCAGC	TGCATTTACCGTGTCCCTAA	(TATT)5		Failed in single PCR test
SH28_GCAG	CCGGTCCCCATGTCAATATT	GTGCTCTTTTGTGACAAT	(GCAG)5	2	
SH29_AGAC	CCACACCCTCAGACAGTATC	CGTTGTTTCAAGGTTGGTGT	(AGAC)14		Failed in single PCR test
SH30_CCTG	ATATCATTATGCTTGGCGCC	TGATTCTGGGTGTACAGCTC	(CCTG)5		Failed in single PCR test
SH31_TCTG	GCTCTGCCAGTCTGTATCTT	GAAATACTTGGCACTCCAGC	(TCTG)5		Failed in single PCR test
SH32_AAAC	TCACCAATTACCTGGTCTAC	CCGGTCCCCATGTCAATATT	(AAAC)8		Failed in single PCR test
SH33_TTTG	GGGAGAGATGTCTTTGGAG	GCATGGCGTATGACAAAAGT	(TTTG)6		Failed in single PCR test
SH34_ATCC	ACCCTCAAGCTAAATGGTCA	ACTTTCTGTTTGCCTTGTG	(ATCC)8		Failed in single PCR test
SH35_AACA	ACAAAAGCAAAAGCAAAAGTGA	GCTTGCATCACCAATCTCTG	(AACA)6	1	
SH36_TATTT	CCTACCGACTTGACGCTTA	ACACAAAGCGAGGGTACATA	(TATTT)6		Failed in single PCR test
SH37_TACAA	AGTCTACCTTAGCCACAGA	GACGACTGGCGATTTAAAGG	(TACAA)11	1	
SH38_GTCAA	GCTGTACGATTTCAACCC	GTGAATTAAGGCATGGCAGG	(GTCAA)8		Failed in single PCR test
SH39_TCCAA	GAGTGTACGCAAAAGAGAGC	AATGGTTAGAGGCTTGTGCT	(TCCAA)6	2	Failed (>60% missing data)
SH40_AATAC	AATGTTGGCAGTAAGGGGAC	TTTTGTACTGTCTGGCCAGT	(AATAC)8	2	
SH41_CAAGG	CCGCACTGAGGACGTATAAA	CAAGCAAGTAGGAGAAGGGT	(CAAGG)6		Failed in single PCR test
SH42_CCTTG	GAACCTCATCCAGTCCCGC	CTCCCTTTCACAACATGCTG	(CCTTG)7		Failed in single PCR test
SH43_TTCTG	ACACAGTGAAAGCTAGGTTGA	CCACACCCTCAGACAGTATC	(TTCTG)7		Failed in single PCR test
SH44_GTACT	CGTGTTGCATTATGATGGCA	ATATCATTATGCTTGGCGCC	(GTACT)8		Failed in single PCR test
SH45_GTCAA	GCCAGACACTAACACACATG	GCTCTGCCAGTCTGTATCTT	(GTCAA)12		Failed in single PCR test
SH46_TATTG	TTGGATCGCATTATTTCCC	TCACCAATTACCCTGGTCAC	(TATTG)9		Failed in single PCR test
SH47_AGGTC	CCTATCTGTCTGGCCGAATT	GGGGAGAGATGTCTTTGGAG	(AGGTC)6	1	

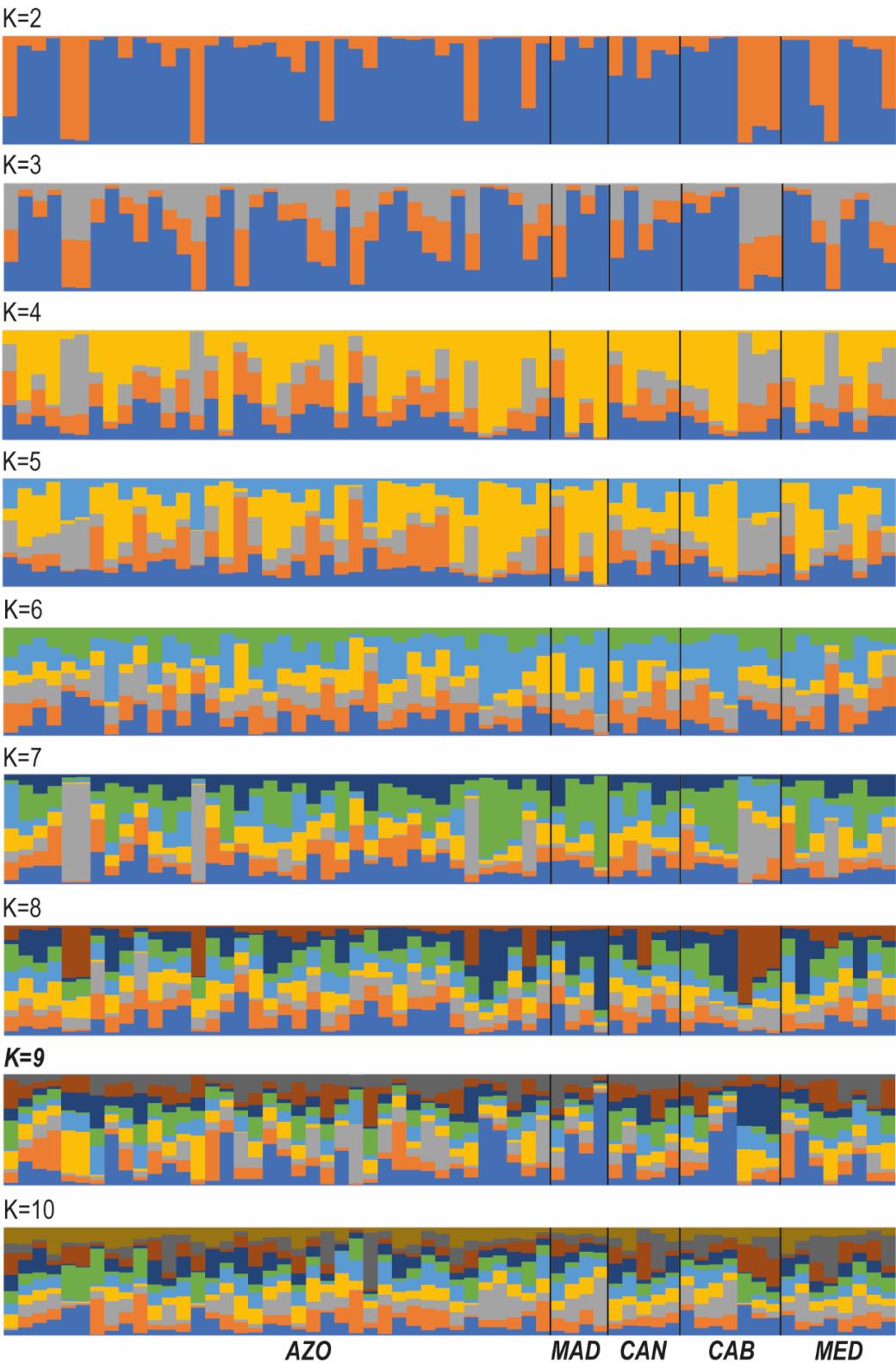


Figure A2.1. Genetic structure analysis of the microsatellite dataset of *S. haemastoma*, reporting the results from K = 2 up to K = 10, estimated with STRUCTURE v2.3.4 (Pritchard et al., 2000). The optimal K = 9 is highlighted in bold italic. Population acronyms: AZO – Azores; MAD – Madeira; CAN – Canaries; CAB – Cabo Verde; MED – Mediterranean.

Supplementary Information for Section 4.1.

Using SSR-GBAS to infer the population structure of *Cingula trifasciata* (Gastropoda: Rissoidae) in the Azores Archipelago (NE Atlantic Ocean)

List of Supplementary Tables and Figures

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Table A3.2. Estimates of evolutionary divergence (raw p-distances) between COI haplotypes of *Cingula trifasciata*.

Table A3.3. Pairwise F_{ST} values between populations of *Cingula trifasciata*, based on the COI dataset.

Table A3.4. Pairwise F_{ST} values between populations of *Cingula trifasciata*, based on the complete WAI dataset.

Table A3.5. Estimates of evolutionary divergence (raw p-distances) between COI sequences of several Rissoidae species.

Figure A3.1. Genetic diversity of the SSR dataset in study.

Figure A3.2. Genetic structure analysis of the complete WAI dataset of *Cingula trifasciata*.

Table A3.1. Complete list of all SSR primers designed in this study. Includes sequence of forward and reverse primers (5'-3'), the repetition motif, and number of repeats in the original sequence used for the primer design, the primer mix in which it was included for the Multiplex PCR, and allele length range (bp).

Primer/ Loci	Forward (5'-3')	Reverse (5'-3')	Repetition motif	Mix	Allele length range (bp)
CT1_ATGA	CCCTACTTTTCAGCAGGAGAA	TGCGATCCTTATACCGCTTT	(TGTT)5	4	Failed (>60% missing data)
CT3_GAATA	CCGGACAGTGTAATTATAGCA	ACGGCGGTTGAATTGACTA	(GAATA)7	3	Failed (>60% missing data)
CT5_TGAA	CCTGGTTAATTTTCAGCGCA	GGTGCCGGTATATTGAGCTA	(TGAA)6	3	
CT6_TTGT	TAGTGTTTCGCTTGCTTGTTG	TAGTGTTTCGCTTGCTTGTTG	(TTGT)5	1	Failed (non-specific marker)
CT10_GTTT	TCTATCGGTGATGTGTTGCA	GGACTAGACAGAATCCGAGC	(GTTT)5	2	Failed (>60% missing data)
CT11_GTTT	GATCTGCTCAGTGAACATGC	GGGTGAGACAGGTTGAGAAA	(GTTT)5	4	
CT13_GTTG	ATACTGGGATGCACAGGAAC	ACGGAATTAACAAGCGCA	(GTTG)5	2	
CT14_CAGA	AGAAACGAGCTCATTCTTGT	CGTGGCCTTGCTTTTCTAAA	(CAGA)9	4	
CT15_TTTG	AAGCTCCATATCAACAGCCA	TCCTGTTGTCGAGAAGATGG	(TTTG)5	1	
CT16_TTTG	TGACACGAGTCTGACGTAAG	CCCCACTTATACTGTGCCTT	(TTTG)5	3	
CT17_TTTG	AAGCGTTCTAGGACTGAACC	TTCTGAACATCGACACCACA	(TTTG)5	2	Failed (non-specific marker)
CT18_AAAG	GACAGAAGGTCCAGACATTTT	TGACACGAGTCTGACGTAAG	(AAAG)5	3	
CT19_GACA	ACTGGCAAAGTCCTTCTCAA	CCCAATCATCACGGTCAAAC	(GACA)6	4	
CT21_CAAA	GAACGAAAGGGAACGGATG	GAGACGGGTTCTCTGACATT	(CAAA)5	1	
CT22_ACAG	AACCTCATCCGCCATGTAAT	TTGCTCACATTTCACTGTGC	(ACAG)7	2	
CT23_TTTA	AACTTTTGATCTTGCTGCCG	TCTGAATTCGGGGTTTGTC	(TTTA)5	3	
CT24_CAAA	CAATGGTGCTAGCTTTCTGC	AGGCTTTGGATCTGGATCTG	(CAAA)6	2	
CT26_TGTT	CCTCACAGGCTCAAGATACA	GCGACAGAACAACGTTGTAA	(TGTT)5	4	
CT28_CTGT	ATTAACAGCTGGGTGGACC	TGAGCATAAGTGACCTGCAT	(CTGT)5	2	
CT29_AAAT	CCCAGAAAGAAAACCTTCACC	GCAAGCAACCTGTACTATGC	(AAAT)6	1	
CT30_TGTC	TGATCAGATGCCACATTCA	GCGCACTTTAAGTTGAGAGA	(TGTC)5	2	
CT31_AATA	GGGGTTCATTCAGTGTTTACA	TGACATCCACTGAACAGGAG	(AATA)5	1	
CT32_TTTG	GGCGAGCTTTAAGTGACATC	ACATTCAGCATTGAGACCAA	(TTTG)6	4	
CT33_CATT	CTCCATGCAAAATTCAGG	CCACCCTCGTCCATTTAGAA	(CATT)5	3	
CT34_ACCA	TCTTGGGTTTGATGTGTGGA	ATGGTTAGGTGCAAGGGATC	(ACCA)5	1	
CT35_TTTG	GTGCCACTTTAAGAAACGCT	CGAAATTTGGAATCCGATACG	(TTTG)12	1	
CT37_AAAT	ACAGCCACAAGTTTGTCAAC	GTCCTTCTGTCTCTGCCTG	(AAAT)5	2	
CT38_TCAT	GTCCTTTACACAGCAAAGCT	TAGGATCGCCATCGGAGTT	(TCAT)8	3	
CT39_CATT	ATACTTCAGGCCGGAAGAG	CTAAGCCTCAGCAGAGACTG	(CATT)6	1	
CT40_ATCC	GAGAACGAACCTCAGCTACT	CTTCAAAGCACAGTGTAGGG	(ATCC)5	3	
CT42_TAAA	GCCAGTCCTCATACGAACAT	CTGCGTCTCAGATGTTTCAC	(TAAA)5	4	

CT2_TGTT	TGGCGCAATGAATAGAATGTC	AAAGAGGGCGCTATGTACTC	(TGTT)5	-	Failed in single PCR test
CT4_TAGAA	TGCTTTTCATCAGTATCGTCA	TGATGCATTTGTGATCATTGA	(TAGAA)5	-	Failed in single PCR test
CT7_TTATG	CCCCTTAAACGATTTCTGAGA	CTGGTAGGGAGGTTTACGAA	(TTATG)6	-	Failed in single PCR test
CT8_AACAT	CTGCAGTGTCAAGTATGCAG	CGGTGGCAAGTTTCTGTATC	(AACAT)5	-	Failed in single PCR test
CT9_GTCAA	TGGATACATCACGAGATCGG	TCACCTGTTGCACAAATCAT	(GTCAA)5	-	Failed in single PCR test
CT12_GTTG	ATGGGTTACAAGATACGCGT	AGTGCACATTCAGTCCATCA	(GTTG)7	-	Failed in single PCR test
CT20_ATAC	ACATCTGTCGTTCTCTTCA	ACCGTTACCTCTCAATAGACT	(ATAC)5	-	Failed in single PCR test
CT25_AAGA	GCCAGATGTATGTTTTCCCG	GTTGGGATTCAGGTCAGCTA	(AAGA)6	-	Failed in single PCR test
CT27_CATT	GTGATGGAAGTACTCATGCG	TGGAATGGAAAGAGACGGAT	(CATT)10	-	Failed in single PCR test
CT36_AGAC	GTAGTGTGCATGCCTAAGTG	CACAATTGTTTATGCCTTGGT	(AGAC)7	-	Failed in single PCR test
CT41_TCAA	GCAAGGAGAGTGGAGGTTAC	ACGGAGTGATTATGGAAGGG	(TCAA)6	-	Failed in single PCR test

Table A3.2. Estimates of evolutionary divergence (raw p-distances) between COI haplotypes of *Cingula trifasciata*. The analysis, performed on MEGA v7 (Kumar et al., 2016), considered 44 haplotypes and 658 bp after excluding positions containing gaps and/or missing data for each sequence pair analysed. Populations of the sequences collapsed within each haplotype is referred in the “Pops” column, as follows: SMA-Santa Maria, CAL-Caloura, MOS-Mosteiros, GRW-Graciosa, PIX-Pico, FSC-São Jorge, FLW-Flores, VIG-Vigo.

Pops	Hap	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43			
SMA	1																																														
SMA	2	0.011																																													
SMA	3	0.002	0.009																																												
SMA	4	0.021	0.014	0.02																																											
SMA	5	0.011	0.003	0.009	0.011																																										
SMA	6	0.018	0.011	0.017	0.011	0.011																																									
CAL	7	0.014	0.006	0.012	0.011	0.006	0.009																																								
CAL	8	0.017	0.009	0.015	0.014	0.009	0.012	0.006																																							
CAL	9	0.015	0.008	0.014	0.012	0.008	0.011	0.005	0.008																																						
CAL	10	0.012	0.008	0.014	0.012	0.008	0.011	0.005	0.008	0.006																																					
CAL	11	0.021	0.014	0.02	0.003	0.014	0.011	0.011	0.014	0.012	0.012																																				
CAL	12	0.023	0.015	0.021	0.008	0.015	0.012	0.012	0.015	0.014	0.014	0.008																																			
CAL	13	0.014	0.009	0.015	0.014	0.009	0.012	0.006	0.009	0.008	0.005	0.014	0.015																																		
MOS, GRW	14	0.021	0.014	0.02	0.003	0.014	0.011	0.008	0.014	0.012	0.012	0.003	0.008	0.014																																	
MOS	15	0.018	0.011	0.017	0.015	0.011	0.012	0.008	0.008	0.009	0.006	0.015	0.017	0.011	0.015																																
MOS, FSC, PIX, GRW	16	0.02	0.012	0.018	0.002	0.012	0.009	0.009	0.012	0.011	0.011	0.002	0.006	0.012	0.002	0.014																															
MOS	17	0.026	0.018	0.024	0.008	0.018	0.015	0.015	0.018	0.017	0.017	0.008	0.012	0.018	0.008	0.02	0.006																														
MOS	18	0.02	0.012	0.018	0.017	0.012	0.014	0.008	0.009	0.011	0.008	0.017	0.018	0.012	0.017	0.002	0.015	0.021																													
MOS	19	0.02	0.012	0.018	0.008	0.012	0.009	0.008	0.012	0.011	0.011	0.008	0.009	0.012	0.008	0.014	0.006	0.012	0.015																												
MOS	20	0.021	0.014	0.02	0.003	0.014	0.011	0.009	0.014	0.012	0.012	0.003	0.008	0.014	0.003	0.015	0.002	0.008	0.017	0.005																											
MOS	21	0.011	0.003	0.009	0.014	0.003	0.011	0.006	0.009	0.008	0.008	0.014	0.015	0.009	0.014	0.008	0.012	0.018	0.009	0.012	0.014																										
PIX	22	0.024	0.017	0.023	0.006	0.017	0.014	0.014	0.017	0.015	0.015	0.006	0.011	0.017	0.006	0.018	0.005	0.011	0.02	0.008	0.006	0.017																									
PIX	23	0.021	0.014	0.02	0.003	0.014	0.011	0.011	0.014	0.012	0.012	0.003	0.008	0.014	0.003	0.015	0.002	0.008	0.017	0.008	0.003	0.014	0.006																								
PIX	24	0.021	0.014	0.02	0.003	0.014	0.011	0.011	0.014	0.012	0.012	0.003	0.008	0.014	0.003	0.015	0.002	0.008	0.017	0.008	0.003	0.014	0.006	0.003	0.003																						
PIX	25	0.017	0.009	0.015	0.014	0.009	0.012	0.006	0.009	0.008	0.008	0.014	0.015	0.009	0.014	0.011	0.012	0.015	0.012	0.012	0.014	0.009	0.017	0.014	0.014	0.009	0.017	0.014	0.014																		
FSC	26	0.021	0.014	0.02	0.003	0.014	0.011	0.011	0.014	0.012	0.012	0.003	0.008	0.014	0.003	0.015	0.002	0.008	0.017	0.008	0.003	0.014	0.006	0.003	0.003	0.014																					
FSC	27	0.021	0.014	0.02	0.003	0.014	0.011	0.011	0.014	0.012	0.012	0.003	0.008	0.014	0.003	0.015	0.002	0.008	0.017	0.008	0.003	0.014	0.006	0.003	0.003	0.014	0.003																				
FSC	28	0.018	0.011	0.017	0.015	0.011	0.014	0.008	0.008	0.009	0.006	0.015	0.017	0.011	0.015	0.006	0.014	0.02	0.008	0.014	0.015	0.011	0.018	0.015	0.015	0.011	0.015	0.015																			
GRW1	29	0.024	0.017	0.023	0.006	0.017	0.014	0.011	0.017	0.015	0.015	0.006	0.011	0.017	0.003	0.018	0.005	0.008	0.02	0.011	0.006	0.017	0.009	0.006	0.006	0.011	0.006	0.006	0.018																		
GRW1	30	0.015	0.011	0.017	0.012	0.011	0.014	0.008	0.009	0.009	0.003	0.012	0.014	0.008	0.012	0.009	0.011	0.017	0.011	0.014	0.012	0.011	0.015	0.012	0.012	0.011	0.012	0.012	0.009	0.015																	
GRW1	31	0.002	0.01	0.003	0.021	0.01	0.018	0.013	0.016	0.014	0.011	0.021	0.022	0.013	0.021	0.018	0.019	0.026	0.018	0.018	0.019	0.01	0.022	0.021	0.021	0.016	0.021	0.021	0.018	0.024	0.014																
GRW2	32	0.021	0.014	0.02	0.003	0.014	0.011	0.011	0.012	0.012	0.012	0.003	0.008	0.014	0.003	0.015	0.002	0.008	0.017	0.008	0.003	0.014	0.006	0.003	0.003	0.014	0.003	0.003	0.015	0.006	0.009	0.021															
GRW2	33	0.023	0.015	0.021	0.005	0.015	0.012	0.009	0.015	0.014	0.005	0.009	0.015	0.002	0.017	0.003	0.009	0.018	0.006	0.005	0.015	0.005	0.005	0.005	0.015	0.005	0.005	0.017	0.005	0.014	0.022	0.005															
GRW2	34	0.017	0.009	0.015	0.014	0.009	0.012	0.006	0.009	0.002	0.008	0.014	0.015	0.009	0.014	0.011	0.012	0.018	0.012	0.012	0.014	0.009	0.017	0.014	0.014	0.009	0.014	0.014	0.011	0.017	0.011	0.016	0.014	0.015													
GRW2	35	0.018	0.011	0.017	0.006	0.011	0.011	0.008	0.011	0.009	0.009	0.006	0.008	0.011	0.006	0.012	0.005	0.011	0.014	0.008	0.006	0.011	0.009	0.006	0.006	0.011	0.006	0.006	0.012	0.009	0.009	0.018	0.006	0.008	0.011												
FLW	36	0.015	0.008	0.014	0.012	0.008	0.011	0.005	0.005	0.006	0.003	0.012	0.014	0.008	0.012	0.003	0.011	0.017	0.005	0.011	0.012	0.008	0.015	0.012	0.012	0.008	0.012	0.012	0.003	0.015	0.006	0.014	0.012	0.014	0.008	0.009											
FLW	37	0.017	0.009	0.015	0.014	0.009	0.012	0.006	0.006	0.008	0.005	0.014	0.015	0.009	0.014	0.005	0.012	0.018	0.006	0.012	0.014	0.009	0.017	0.014	0.014	0.009	0.014	0.014	0.005	0.017	0.008	0.014	0.014	0.015	0.009	0.011	0.002										
FLW	38	0.02	0.012	0.018	0.017	0.012	0.015	0.009	0.009	0.011	0.008	0.017	0.018	0.012	0.017	0.005	0.015	0.021	0.006	0.015	0.017	0.009	0.02	0.017	0.017	0.012	0.017	0.017	0.008	0.02	0.011	0.019	0.017	0.018	0.012	0.014	0.005	0.006	0.003								
FLW	39	0.02	0.012	0.018	0.017	0.012	0.015	0.009	0.009	0.011	0.008	0.017	0.018	0.012	0.017																																

Table A3.3. Pairwise F_{ST} values between populations of *Cingula trifasciata*, based on the COI dataset.

	<i>Santa Maria</i>	<i>Caloura</i>	<i>Mosteiros</i>	<i>Graciosa</i>	<i>Pico</i>	<i>São Jorge</i>	<i>Flores</i>	<i>Vigo</i>
<i>Santa Maria</i>								
<i>Caloura</i>	0.222**							
<i>Mosteiros</i>	0.262**	0.048						
<i>Graciosa</i>	0.361**	0.153**	0.061					
<i>Pico</i>	0.527**	0.293	0.129	0.083				
<i>São Jorge</i>	0.419**	0.186	0.041	0.056	-0.014			
<i>Flores</i>	0.433**	0.308**	0.346**	0.712**	0.611**	0.529**		
<i>Vigo</i>	0.855**	0.852**	0.848**	0.869**	0.944**	0.929**	0.949**	

Significance tests derived from 1023 permutations; ** $p < 0.01$.

Table A3.4. Pairwise F_{ST} values between populations of *Cingula trifasciata*, based on the complete WAI dataset.

	<i>Santa Maria</i>	<i>Caloura</i>	<i>Mosteiros</i>	<i>Graciosa</i>	<i>Pico</i>	<i>São Jorge</i>	<i>Vigo</i>
<i>Santa Maria</i>							
<i>Caloura</i>	0.177**						
<i>Mosteiros</i>	0.198**	0.173**					
<i>Graciosa</i>	0.143**	0.142**	0.119**				
<i>Pico</i>	0.193**	0.222**	0.110**	0.122**			
<i>São Jorge</i>	0.166**	0.212**	0.169**	0.138**	0.178**		
<i>Vigo</i>	0.359**	0.382**	0.430**	0.393**	0.442**	0.365**	

Significance tests derived from 1000 permutations; ** $p < 0.01$.

Table A3.5. Estimates of evolutionary divergence (raw p-distances) between COI sequences of several Rissoidae species. The analysis, performed on MEGA v7 (Kumar et al., 2016), considered 18 sequences and 658 bp after excluding positions containing gaps and/or missing data for each sequence pair analysed. Sequences retrieved from GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>), accession Number (AN) and species identification are provided in the table. Highlighted in bold is the divergence level between the recognized species *Alvania formicarum* and *A. mediolittoralis*, and between *Cingula trifasciata* from the Azores and Vigo.

Species	AN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
<i>Alvania angioyi</i>	MG652373																	
<i>Alvania beani</i>	FN650144	0.139																
<i>Alvania formicarum</i>	MG652380	0.144	0.125															
<i>Alvania mediolittoralis</i>	MG652385	0.144	0.123	0.029														
<i>Alvania punctura</i>	FN650148	0.141	0.153	0.178	0.172													
<i>Alvania sleursi</i>	MG652386	0.15	0.116	0.106	0.096	0.176												
<i>Alvania subsoluta</i>	FN650150	0.151	0.146	0.15	0.148	0.168	0.15											
<i>Cingula trifasciata</i> Azores	MG652397	0.128	0.152	0.147	0.147	0.15	0.152	0.137										
<i>Cingula trifasciata</i> Vigo	MW518858	0.125	0.149	0.140	0.146	0.160	0.158	0.145	0.044									
<i>Crisilla postrema</i>	MG652392	0.141	0.207	0.202	0.198	0.158	0.207	0.188	0.152	0.149								
<i>Onoba semicostata</i>	FN650151	0.176	0.177	0.17	0.167	0.201	0.176	0.162	0.173	0.176	0.199							
<i>Pseudosetia</i> sp.	FN650172	0.178	0.155	0.169	0.166	0.196	0.17	0.132	0.167	0.172	0.191	0.176						
<i>Pseudosetia semipellucida</i>	FN650154	0.202	0.182	0.187	0.193	0.187	0.191	0.174	0.191	0.148	0.199	0.187	0.179					
<i>Pusillina inconspicua</i>	FN650157	0.176	0.188	0.188	0.181	0.195	0.185	0.164	0.181	0.179	0.205	0.185	0.188	0.216				
<i>Pusillina sarsii</i>	FN650160	0.176	0.191	0.195	0.187	0.198	0.188	0.167	0.181	0.179	0.208	0.185	0.187	0.219	0.009			
<i>Rissoa auriscalpium</i>	HQ623175	0.197	0.206	0.202	0.193	0.204	0.201	0.215	0.213	0.218	0.221	0.204	0.208	0.215	0.199	0.208		
<i>Rissoa guernei</i>	MG652409	0.19	0.21	0.202	0.207	0.219	0.196	0.217	0.205	0.195	0.213	0.21	0.213	0.229	0.196	0.199	0.199	
<i>Setia subvaricosa</i>	MG652429	0.144	0.161	0.16	0.157	0.184	0.182	0.157	0.164	0.167	0.179	0.173	0.179	0.201	0.170	0.173	0.210	0.188

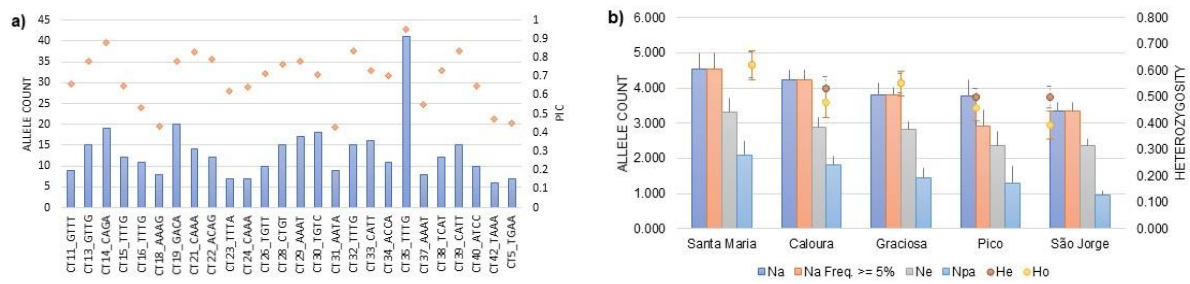


Figure A3.1. Genetic diversity of the SSR dataset in study. a) Variability measures estimated for each of the 26 SSR loci in study in complete whole amplicon information (WAI) dataset: number of alleles (Na) and polymorphic information content (PIC); b) Genetic diversity patterns across the five populations of *Cingula trifasciata* with more than five individuals: number of alleles (Na), number of different alleles with frequency over 5%, number of effective alleles (Ne), number of private alleles (Npa), expected (H_e) and observed (H_o) heterozygosity.

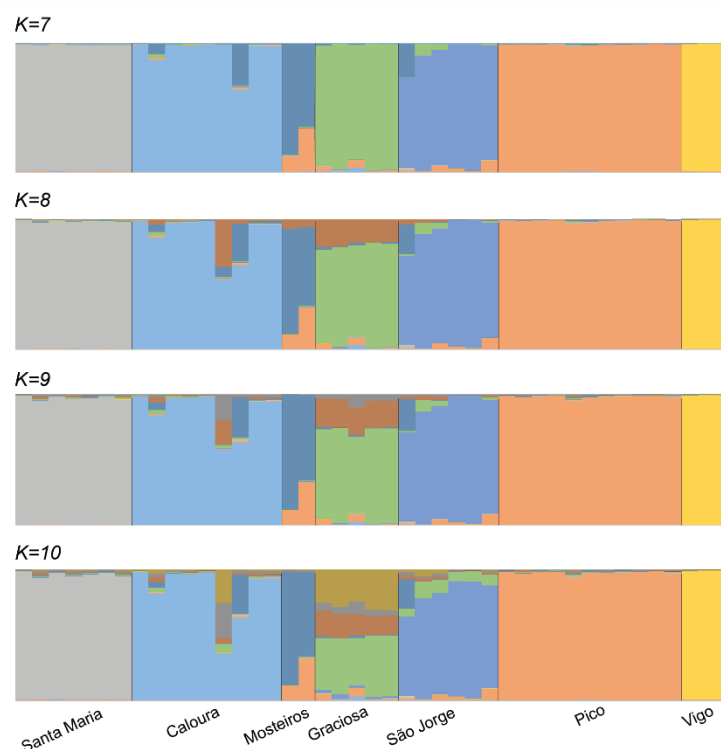


Figure A3.2. Genetic structure analysis of the complete WAI dataset of *Cingula trifasciata*. Inferred with STRUCTURE v2.3.4 (Hubisz et al., 2009; Pritchard et al., 2000), reporting the results from K=7 to K=10.

References

- Hubisz, M., Falush, D., Stephens, M., & Pritchard, J. K. (2009). Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources*, 9, 1322–32. <https://doi.org/10.1111/j.1755-0998.2009.02591.x>
- Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33, 1870–4. <https://doi.org/10.1093/molbev/msw054>
- Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155, 945–59.

Supplementary Information for Section 4.2.

Untangling the origin of the newcomer *Phorcus sauciatus* (Mollusca: Gastropoda) in a remote northeast Atlantic archipelago

List of Supplementary Tables

Table A4.1. Estimates of evolutionary divergence (raw p-distances) between COI haplotypes of *Phorcus sauciatus*.

Table A4.2. Estimates of evolutionary divergence (raw p-distances) between COI haplotypes of *Phorcus lineatus*.

Table A4.1. Estimates of evolutionary divergence (raw p-distances) between COI haplotypes of *Phorcus sauciatatus*. Conducted on MEGA version 7 (Kumar et al., 2016) and involving 20 haplotypes sequences after elimination of all positions containing gaps and missing data.

	HS1	HS2	HS3	HS4	HS5	HS6	HS7	HS8	HS9	HS10	HS11	HS12	HS13	HS14	HS15	HS16	HS17	HS18	HS19	HS20	HS21	HS22	HS23	HS24	HS25	HS26	HS27
HS1																											
HS2	0.003																										
HS3	0.011	0.008																									
HS4	0.005	0.002	0.01																								
HS5	0.006	0.003	0.011	0.005																							
HS6	0.005	0.002	0.01	0.003	0.005																						
HS7	0.006	0.003	0.011	0.005	0.003	0.005																					
HS8	0.005	0.002	0.01	0.003	0.002	0.003	0.002																				
HS9	0.008	0.005	0.013	0.006	0.008	0.006	0.008	0.006																			
HS10	0.011	0.011	0.019	0.013	0.015	0.013	0.015	0.013	0.016																		
HS11	0.013	0.013	0.021	0.015	0.016	0.011	0.016	0.015	0.018	0.002																	
HS12	0.003	0.006	0.015	0.008	0.01	0.008	0.01	0.008	0.011	0.015	0.016																
HS13	0.006	0.003	0.011	0.005	0.003	0.005	0.003	0.002	0.008	0.015	0.016	0.01															
HS14	0.018	0.015	0.023	0.016	0.018	0.013	0.018	0.016	0.019	0.016	0.015	0.021	0.018														
HS15	0.01	0.006	0.015	0.008	0.01	0.008	0.01	0.008	0.011	0.008	0.01	0.013	0.01	0.011													
HS16	0.006	0.006	0.015	0.008	0.01	0.008	0.01	0.008	0.011	0.005	0.006	0.01	0.01	0.011	0.003												
HS17	0.013	0.01	0.018	0.011	0.013	0.011	0.013	0.011	0.015	0.011	0.013	0.016	0.013	0.011	0.006	0.006											
HS18	0.011	0.008	0.016	0.01	0.011	0.01	0.011	0.01	0.013	0.01	0.011	0.015	0.011	0.013	0.005	0.005	0.008										
HS19	0.008	0.005	0.013	0.006	0.008	0.006	0.008	0.006	0.01	0.006	0.008	0.011	0.008	0.01	0.002	0.002	0.005	0.003									
HS20	0.011	0.008	0.016	0.01	0.011	0.01	0.011	0.01	0.013	0.01	0.011	0.015	0.011	0.013	0.002	0.005	0.008	0.003	0.003								
HS21	0.011	0.008	0.016	0.01	0.008	0.01	0.011	0.01	0.013	0.01	0.011	0.015	0.011	0.013	0.005	0.005	0.008	0.006	0.003	0.006							
HS22	0.01	0.006	0.015	0.008	0.01	0.008	0.01	0.008	0.011	0.008	0.01	0.013	0.01	0.011	0.003	0.003	0.006	0.005	0.002	0.005	0.005						
HS23	0.008	0.008	0.016	0.01	0.011	0.01	0.011	0.01	0.013	0.006	0.008	0.011	0.011	0.013	0.005	0.002	0.008	0.006	0.003	0.006	0.006	0.005					
HS24	0.01	0.006	0.015	0.008	0.01	0.008	0.01	0.008	0.011	0.008	0.01	0.013	0.01	0.011	0.003	0.003	0.006	0.005	0.002	0.005	0.005	0.003	0.005				
HS25	0.013	0.01	0.018	0.011	0.01	0.011	0.013	0.011	0.015	0.011	0.013	0.016	0.013	0.015	0.006	0.006	0.01	0.008	0.005	0.008	0.002	0.006	0.008	0.006			
HS26	0.01	0.006	0.015	0.008	0.01	0.008	0.01	0.008	0.011	0.006	0.008	0.013	0.01	0.011	0.003	0.003	0.006	0.005	0.002	0.005	0.005	0.003	0.005	0.003	0.006		
HS27	0.008	0.008	0.016	0.01	0.011	0.01	0.011	0.01	0.013	0.005	0.006	0.011	0.011	0.013	0.005	0.002	0.008	0.006	0.003	0.006	0.006	0.005	0.003	0.005	0.008	0.005	
HS28	0.007	0.003	0.012	0.005	0.003	0.005	0.003	0.002	0.008	0.013	0.015	0.01	0.003	0.018	0.01	0.01	0.013	0.012	0.008	0.012	0.012	0.01	0.012	0.01	0.013	0.01	0.012

Table A4.2. Estimates of evolutionary divergence (raw p-distances) between COI haplotypes of *Phorcus lineatus*. Conducted on MEGA version 7 (Kumar et al., 2016) and involving 23 haplotypes sequences after elimination of all positions containing gaps and missing data.

	HL1	HL2	HL3	HL4	HL5	HL6	HL7	HL8	HL9	HL10	HL11	HL12	HL13	HL14	HL15	HL16	HL17	HL18	HL19	HL20	HL21	HL22	HL23
HL1																							
HL2	0.003																						
HL3	0.009	0.009																					
HL4	0.004	0.004	0.011																				
HL5	0.004	0.008	0.011	0.007																			
HL6	0.015	0.015	0.004	0.015	0.015																		
HL7	0.013	0.013	0.002	0.013	0.013	0.002																	
HL8	0.013	0.013	0.002	0.013	0.013	0.006	0.004																
HL9	0.011	0.011	0.004	0.011	0.011	0.004	0.002	0.006															
HL10	0.006	0.002	0.013	0.006	0.009	0.013	0.011	0.015	0.009														
HL11	0.005	0.005	0.011	0.002	0.01	0.017	0.015	0.013	0.013	0.008													
HL12	0.011	0.011	0.015	0.013	0.013	0.021	0.019	0.017	0.017	0.015	0.013												
HL13	0.015	0.015	0.018	0.017	0.017	0.025	0.023	0.021	0.021	0.019	0.016	0.003											
HL14	0.005	0.005	0.011	0.002	0.01	0.017	0.015	0.013	0.013	0.008	0.003	0.013	0.016										
HL15	0.005	0.005	0.011	0.006	0.01	0.017	0.015	0.013	0.013	0.008	0.006	0.013	0.016	0.006									
HL16	0.003	0.006	0.013	0.008	0.008	0.019	0.017	0.015	0.015	0.01	0.008	0.015	0.018	0.008	0.008								
HL17	0.002	0.005	0.011	0.006	0.006	0.017	0.015	0.013	0.013	0.008	0.006	0.013	0.016	0.006	0.006	0.002							
HL18	0.005	0.002	0.01	0.006	0.01	0.015	0.013	0.011	0.013	0.004	0.006	0.013	0.016	0.006	0.006	0.008	0.006						
HL19	0.006	0.006	0.01	0.008	0.008	0.015	0.013	0.011	0.011	0.01	0.008	0.011	0.015	0.008	0.008	0.01	0.008	0.008					
HL20	0.005	0.008	0.015	0.008	0.008	0.019	0.017	0.015	0.015	0.01	0.01	0.016	0.019	0.01	0.01	0.008	0.006	0.01	0.011				
HL21	0.008	0.008	0.002	0.009	0.009	0.006	0.004	0.004	0.002	0.011	0.01	0.013	0.016	0.01	0.01	0.011	0.01	0.01	0.008	0.013			
HL22	0.003	0.006	0.013	0.006	0.006	0.017	0.015	0.015	0.013	0.007	0.008	0.015	0.018	0.008	0.008	0.006	0.005	0.008	0.01	0.005	0.011		
HL23	0.002	0.005	0.011	0.004	0.004	0.015	0.013	0.013	0.011	0.006	0.006	0.013	0.016	0.006	0.006	0.005	0.003	0.006	0.008	0.003	0.01	0.002	

Supplementary Information for Section 5.1.

Morphospecies and molecular diversity of ‘lace corals’: the genus *Reteporella* (Bryozoa: Cheilostomatida) in the central North Atlantic Azores Archipelago

List of Supplementary Tables and Figures

Table A5.1. Detailed information of *Reteporella* samples used in this work.

Table A5.2. Estimates of evolutionary divergence (mean raw p-distances) between *Reteporella* species and additional phidoloporids.

Figure A5.1. Maximum likelihood analysis of the COI data.

Figure A5.2. Maximum likelihood analysis of the 16S rRNA data.

Figure A5.3. Maximum likelihood analysis of the 28S rRNA data.

Table A5.1. Detailed information of *Reteporella* samples used in this work. Localities with coordinates in decimal degrees relative to WGS84 datum (whenever available), depth (in meters), collection voucher (if applicable: DBUA – Department of Biology of the University of the Azores; DOP – Department of Oceanography and Fisheries of the University of the Azores, Portugal; M150 – Research Cruise M150 BIODIAZ 2018; SaM – Senckenberg am Meer, Wilhelmshaven, Germany; NHMUK – Natural History Museum London, United Kingdom; Biolce – Benthic invertebrates of Icelandic waters collections at the Icelandic Institute of Natural History; SP – settlement panels of Wisshak et al. (2015). COI, 16S rRNA, and 28S rRNA accession numbers are provided; * = newly generated sequences. Collaborators have provided samples from Corsica, and Croatia as well as from additional locations in the Azores deriving from a settlement experiment (Wisshak et al., 2015) that are not assigned to a particular collection.

Species	Sample ID	Locality	Depth (m)	Coordinates DD	Collection voucher	COI Acc.	16S rRNA Acc.	28S rRNA Acc.
<i>R. atlantica</i>	P8	Santa Maria, Azores	24	36.935, -25.092	DBUA:BRY 009	* OP086115	* OP070995	* OP070884
	P10	Santa Maria, Azores	24	36.935, -25.092	DBUA:BRY 011	* OP086116	* OP070996	* OP070885
	P15	Santa Maria, Azores	24	36.935, -25.092	DBUA:BRY 016	* OP086117	* OP070997	* OP070886
	P16	Santa Maria, Azores	28	36.935, -25.092	DBUA:BRY 017	* OP086106	* OP070998	* OP070887
	P17	Santa Maria, Azores	28	36.935, -25.092	DBUA:BRY 018	* OP086118	* OP070999	* OP070888
	MB4	Santa Maria, Azores	27	36.995, -25.187	DBUA:BRY 023	* OP086107		* OP070879
	MB5	Santa Maria, Azores	27	36.995, -25.187	DBUA:BRY 024	* OP086104	* OP070993	* OP070880
	MB6	Santa Maria, Azores	27	36.995, -25.187	DBUA:BRY 025	* OP086105	* OP070994	* OP070881
	MB10	Santa Maria, Azores	27	36.995, -25.187	DBUA:BRY 029	* OP086108		* OP070882
	MB12	Santa Maria, Azores	27	36.995, -25.187	DBUA:BRY 031	* OP086109	* OP070992	* OP070883
	GA8	Santa Maria, Azores	11-13	36.982, -25.039	DBUA:BRY 031	* OP086110	* OP071002	* OP070874
	GA16	Santa Maria, Azores	11-13	36.982, -25.039	DBUA:BRY 055	* OP086111	* OP071003	* OP070875
	GA17	Santa Maria, Azores	11-13	36.982, -25.039	DBUA:BRY 057	* OP086112	* OP071004	* OP070876
	GA20	Santa Maria, Azores	11-13	36.982, -25.039	DBUA:BRY 059	* OP086113	* OP071001	* OP070877
	GA21	Santa Maria, Azores	11-13	36.982, -25.039	DBUA:BRY 060	* OP086114		* OP070878
	SM15-1-1	Santa Maria, Azores	10		Personal:BBerning	* OP086119	* OP071005	* OP070890
	SM15-6-1	Santa Maria, Azores	10		Personal:BBerning	* OP086120		
	SM15-6-2	Santa Maria, Azores	10		Personal:BBerning	* OP086121	* OP071000	* OP070891
	BRY15	Flores, Azores	151	39.570, -31.217	M150-BIOD 072	* OP086135	* OP071006	* OP070857
	BRY16	Flores, Azores	151	39.570, -31.217	M150-BIOD 073	* OP086099	* OP070991	* OP070858
	BRY18	Flores, Azores	151	39.570, -31.217	M150-BIOD 076	* OP086136		* OP070859
	BRY19	Flores, Azores	151	39.570, -31.217	M150-BIOD 077	* OP086137	* OP071009	
	BRY20	Flores, Azores	151	39.570, -31.217	M150-BIOD 078	* OP086138	* OP071007	* OP070860
	BRY21	Flores, Azores	151	39.570, -31.217	M150-BIOD 079	* OP086139	* OP071010	* OP070861
	BRY22	Flores, Azores	151	39.570, -31.217	M150-BIOD 080	* OP086140		* OP070862
	BRY23	Flores, Azores	151	39.570, -31.217	M150-BIOD 081	* OP086141	* OP071011	* OP070863
	BRY24	Flores, Azores	151	39.570, -31.217	M150-BIOD 082	* OP086142	* OP071012	* OP070864

Species	Sample ID	Locality	Depth (m)	Coordinates DD	Collection voucher	COI Acc.	16S rRNA Acc.	28S rRNA Acc.
	BRY25	Flores, Azores	151	39.570, -31.217	M150-BIOD 083	* OP086143	* OP071008	* OP070865
	BRY 100	Terceira, Azores	160	38.811, -27.335	M150-BIOD 434	* OP086126	* OP071017	* OP070866
	BRY 102	Terceira, Azores	160	38.811, -27.335	M150-BIOD 436	* OP086127		* OP070867
	BRY 104	Terceira, Azores	160	38.811, -27.335	M150-BIOD 438	* OP086128	* OP071018	* OP070868
	BRY 105	Terceira, Azores	160	38.811, -27.335	M150-BIOD 439	* OP086129	* OP071019	* OP070869
	BRY 130	Terceira, Azores	152	38.802, -27.071	M150-BIOD 532	* OP086130	* OP071020	* OP070870
	BRY 134	Terceira, Azores	152	38.802, -27.071	M150-BIOD 536	* OP086131	* OP071021	* OP070871
	BRY 139	Terceira, Azores	152	38.802, -27.07	M150-BIOD 543	* OP086132	* OP071016	* OP070872
	BRY-95-1	Terceira, Azores	151	38.811, -27.335	M150-BIOD 50	* OP086122		* OP070889
	BRY-95-2	Terceira, Azores	151	38.811, -27.335	M150-BIOD 504	* OP086125		
	SP-60m-2	Faial, Azores	60	38.520, -28.605	Personal:BBerning-SP		* OP071013	
	SP-60m-3	Faial, Azores	60	38.520, -28.605	Personal:BBerning-SP	* OP086145	* OP071015	
	SP-60m-5	Faial, Azores	60	38.520, -28.605	Personal:BBerning-SP	* OP086150		* OP070892
	SP-60m-6	Faial, Azores	60	38.520, -28.605	Personal:BBerning-SP	* OP086146		* OP070893
	SP-60m-7	Faial, Azores	60	38.520, -28.605	Personal:BBerning-SP	* OP086147		* OP070894
	SP-60m-8	Faial, Azores	60	38.520, -28.605	Personal:BBerning-SP	* OP086144		* OP070895
	SP-150m-1	Faial, Azores	150	38.509, -28.610	Personal:BBerning-SP	* OP086151		
	SP-150m-3	Faial, Azores	150	38.509, -28.610	Personal:BBerning-SP	* OP086148		
	SP-150m-6	Faial, Azores	150	38.509, -28.610	SP-150m	* OP086152		* OP070896
	DR5.B9	Sabrina, Azores	150	37.869, -25.902	DBUA:SAB2011	* OP086101		* OP070873
	A02	Princess Alice Bank, Azores	265	37.753, -28.991	DOP-4193	* OP086102		* OP070852
	A08	São Miguel, Azores	117	37.77, -25.116	DOP-5300	* OP086100		
	A10	Chino Bank, Azores	371	37.987, -29.53	DOP-5892	* OP086123		* OP070853
	A11	Princess Alice Bank, Azores	256	37.694, -28.935	DOP-6132	* OP086103		* OP070854
	A13	Princess Alice Bank, Azores	367.59	37.975, -29.531	DOP-6627			* OP070856
	A12	Princess Alice Bank, Azores	393.2	37.990, -29.565	DOP-6622	* OP086149	* OP071014	* OP070855
	A19	Terceira, Azores	86	38.815, -27.302	DOP-5325	* OP086124		
	A20	Terceira, Azores	86	38.815, -27.302	DOP-5325	* OP086134		
	9860B	Condor Bank, Azores	455	38.555, -29.044	DOP-9860	* OP086133		
<i>R. tristis</i>	BRY 127	Terceira, Azores	152	38.802, -27.071	M150-BIOD 526	* OP086183	* OP070984	
	BRY 128	Terceira, Azores	152	38.802, -27.071	M150-BIOD 528	* OP086184	* OP070983	* OP070838
	BRY 129	Terceira, Azores	152	38.802, -27.071	M150-BIOD 530	* OP086185	* OP070982	* OP070839
	BRY 143	Terceira, Azores	153	38.802, -27.071	M150-BIOD 549	* OP086186	* OP070974	* OP070837
	BRY 195	Santa Maria, Azores	151	37.055, -25.154	M150-BIOD 941	* OP086191		* OP070840

Species	Sample ID	Locality	Depth (m)	Coordinates DD	Collection voucher	COI Acc.	16S rRNA Acc.	28S rRNA Acc.
	BRY196	Santa Maria, Azores	151	37.055, -25.154	M150-BIOD 943	* OP086192	* OP070981	* OP070841
	BRY 198	Santa Maria, Azores	152	37.055, -25.154	M150-BIOD 946	* OP086193	* OP070980	* OP070842
	BRY 204	Santa Maria, Azores	152	37.055, -25.154	M150-BIOD 952	* OP086194	* OP070979	* OP070843
	BRY 216	Santa Maria, Azores	152	37.055, -25.154	M150-BIOD 964	* OP086195		* OP070844
	BRY 220	Santa Maria, Azores	153	37.055, -25.154	M150-BIOD 971	* OP086196	* OP070978	* OP070845
	BRY 222	Santa Maria, Azores	153	37.055, -25.154	M150-BIOD 974	* OP086197	* OP070977	* OP070846
	BRY 224	Santa Maria, Azores	152	37.055, -25.154	M150-BIOD 978	* OP086198	* OP070976	* OP070847
	BRY 225	Santa Maria, Azores	152	37.055, -25.154	M150-BIOD 980	* OP086189		* OP070848
	BRY 226	Santa Maria, Azores	152	37.055, -25.154	M150-BIOD 982	* OP086190		* OP070851
	DR10.B7	Sabrina, Azores	150	37.882, -25.907	DBUA:SAB2011	* OP086188		* OP070849
	M151-50341	São Miguel, Azores	200	37.675, -25.717	SaM-ID-50341	* OP086187	* OP070975	* OP070850
<i>R. oceanica</i>	9869A	Condor Bank, Azores	454.5	38.555, -29.044	DOP-9869	* OP086154		* OP070918
	9869B	Condor Bank, Azores	454.5	38.555, -29.044	DOP-9869	* OP086155		* OP070919
<i>Reteporella</i> sp. 1	BRY 116	Terceira, Azores	277	38.803, -27.05	M150-BIOD 514	* OP086182	* OP070973	* OP070836
<i>Reteporella</i> sp. 5	M151-100 359	São Miguel, Azores	311	37.675, -25.717	SaM-ID-100 359	* OP086153	* OP071029	* OP070921
<i>Reteporella</i> sp. 6	BRY 103	Terceira, Azores	160	38.811, -27.335	M150-BIOD 437	* OP086159	* OP071026	* OP070897
	BRY 117	Terceira, Azores	277	38.8021, -27.059	M150-BIOD 515	* OP086162	* OP071025	* OP070898
	BRY 136	Terceira, Azores	153	38.803, -27.071	M150-BIOD 539	* OP086158	* OP071022	* OP070899
	BRY 141	Terceira, Azores	152	38.802, -27.071	M150-BIOD 546	* OP086161	* OP071023	* OP070900
	BRY 146	Terceira, Azores	153	38.802, -27.071	M150-BIOD 553	* OP086160	* OP071024	* OP070901
	10302E	Gigante Bank, Azores	493	38.975, -29.852	DOP-10302	* OP086156	* OP071028	* OP070902
	10303	Gigante Bank, Azores	402	39.008, -29.929	DOP-10303	* OP086157		* OP070903
<i>Reteporella</i> sp. 7	BRY 159	Santa Maria, Azores	152	38.802, -27.071	M150-BIOD 720	* OP086175	* OP071038	* OP070906
	BRY166	Santa Maria, Azores	152	37.047, -25.057	M150-BIOD 721			* OP070904
	BRY 170	Santa Maria, Azores	144	37.047, -25.057	M150-BIOD 719	* OP086176	* OP071031	* OP070907
	BRY 217	Santa Maria, Azores	152	37.047, -25.057	M150-BIOD 966	* OP086177	* OP071033	* OP070908
	BRY 218	Santa Maria, Azores	153	37.055, -25.154	M150-BIOD 96	* OP086178	* OP071037	* OP070909
	BRY 223	Santa Maria, Azores	153	37.055, -25.154	M150-BIOD 976	* OP086181	* OP071034	* OP070910
	BRY-211-4	Santa Maria, Azores	153	37.055, -25.154	M150-BIOD 959	* OP086174	* OP071032	
	BRY-221-2	Santa Maria, Azores	152	37.055, -25.154	M150-BIOD 972	* OP086173		* OP070916
	M151-50332	São Miguel, Azores	153	37.055, -25.154	SaM-ID-50332	* OP086167	* OP071036	* OP070917
	M151-100 113	São Miguel, Azores	544	37.670, -25.713	SaM-ID-100 113	* OP086166		
	DR2.B2 A	Sabrina, Azores	309	37.675, -25.717	DBUA:SAB2011	* OP086170		* OP070911
	DR2.B2 B	Sabrina, Azores	50-100	37.850, -25.667	DBUA:SAB2011	* OP086171		* OP070912

Species	Sample ID	Locality	Depth (m)	Coordinates DD	Collection voucher	COI Acc.	16S rRNA Acc.	28S rRNA Acc.
	DR2.B2 C	Sabrina, Azores	50-100	37.850, -25.667	DBUA:SAB2011	* OP086172		
	DR3.B13 A	Sabrina, Azores	50-100	37.850, -25.667	DBUA:SAB2011	* OP086168		
	DR3.B13 B	Sabrina, Azores	200	37.855, -25.683	DBUA:SAB2011	* OP086169		
	DR4.B13	Sabrina, Azores	200	37.855, -25.683	DBUA:SAB2011	* OP086179	* OP071035	* OP070913
	A07	Santa Maria, Azores	140	37.873, -25.900	DOP-5282	* OP086180		* OP070905
	10302A	Gigante Bank, Azores	138	36.911, -25.022	DOP-10302	* OP086164	* OP071039	* OP070914
	10302C	Gigante Bank, Azores	493	38.975, -29.852	DOP-10302	* OP086165	* OP071030	* OP070915
	BRY 257	Formigas, Azores	150	37.274, -24.755	M150-BIOD 1140	* OP086163	* OP071027	* OP070920
<i>Reteporella</i> sp. 4	AW006	Marseille	-	-	-	JN680991		
<i>Reteporella</i> sp. 3	CR-PU1	Stoja, Croatia	5.1	44.855, 13.817	DBUA-BRY 061	* OP086204	* OP070988	* OP070922
	CR-PU2	Monte Kope, Croatia	7.1	44.806, 13.879	DBUA-BRY 062	* OP086205	* OP070990	* OP070923
	CR-PU3	Soline, Croatia	18.3	44.849, 13.831	DBUA-BRY 063	* OP086206		
	CR-PU4	Stoja, Croatia	5.2	44.855, 13.817	DBUA-BRY 064	* OP086207	* OP070989	
<i>Reteporella</i> sp. 2	Cors1	Elpa Nera, Corsica	65	42.405, 8.599	DBUA-BRY 065	* OP086200		
	Cors2	Gulf of Galéria, Corsica	80	42.429, 8.609	DBUA-BRY 066	* OP086201	* OP070985	
	Cors3	Gulf of Galéria, Corsica	80	42.429, 8.609	DBUA-BRY 067	* OP086202	* OP070986	
	Cors4	Gulf of Galéria, Corsica	80	42.429, 8.609	DBUA-BRY 068	* OP086203	* OP070987	* OP070924
<i>R. beaniana</i>	AW2966; BiolCE sample 3552	Iceland, Biolce cruise B-4-03, Station no. 384	300- 304	64.279, -25.689	NHMUK 2022.5.17.1	* OP086199		
	-	Kattegat, Sweden	-	-	-	-	-	FJ196142
<i>R. cf. grimaldii</i>	BLEED1851	Spain	31	42.284, 3.3		OK244991	OK244991	MZ677711
<i>R. cf. graeffei</i>	BLEED1059	Australia	36.2	-15.449, 124.154		OK244990	OK244990	MZ677710
<i>R. tuberosa</i>	BLEED420B	Saudi Arabia	-	-		OK244992	OK244992	-
<i>Iodictyum violaceum</i>	BLEED542	New Caledonia	17	-22.318, 166.431		OK244876	OK244876	MZ677606
<i>Iodictyum yaldwyni</i>	BLEED1387	New Zealand	82.9	-34.160, 172.09		MW160083	MW160083	-
<i>Hippellozoon novaezelandiae</i>	BLEED1639	New Zealand	82.9	-34.160, 172.09		MW160102	MW160102	MW173763
<i>Phidolopora avicularis</i>	BLEED1381	New Zealand	82.9	-34.160, 172.09		MW160081	MW160081	MW173808
<i>Triphyllozoon arcuatum</i>	BLEED1936	Thailand	2	12.559, 101.57		OK245068	OK245068	MZ677799
<i>Celleporaria cf. fusca</i>	BLEED1814	New Caledonia	17	-22.318, 166.431		OK244791	OK244791	MZ677543

Table A5.2. Estimates of evolutionary divergence (mean raw p-distances) between *Reteporella* species and additional phidoloporids. a) mitochondrial COI marker (552 bp); **b)** mitochondrial 16S rRNA marker (332 bp); and **c)** nuclear 28S rRNA marker (427 bp). The analysis, using MEGA v11 (Tamura et al., 2021), was performed after exclusion of positions containing gaps and/or missing data for each sequence pair analysed. Intraspecific mean p-distances were calculated among haplotypes for each species. Hyphen (-) indicates species represented by a single haplotype. Samples *R. atlantica* (BRY16; Flores) and *Reteporella* sp. 5 (BRY257; Formigas) are distinguished in a) and b) due to their differentiation when compared to conspecifics. Pairwise comparisons of other phidoloporid genera highlighted in yellow.

a)	<i>R. atlantica</i>	<i>R. atlantica</i> (BRY16)	<i>R. oceanica</i>	<i>R. sp. 5</i>	<i>R. sp. 6</i>	<i>R. sp. 7</i>	<i>R. sp. 7</i> (BRY257)	<i>R. sp. 1</i>	<i>R. tristis</i>	<i>R. beaniana</i>	<i>R. sp. 2</i>	<i>R. sp. 3</i>	<i>R. cf. grimaldii</i>	<i>R. sp. 4</i>	<i>R. cf. graeffei</i>	<i>R. tuberosa</i>	<i>P. avicularis</i>	<i>Schizoretopora</i> sp.	<i>Schizotheca</i> sp.	<i>H. novaezelandiae</i>	<i>T. arcuatum</i>	<i>I. yaldwyni</i>	<i>I. violaceum</i>
<i>R. atlantica</i>	0.021																						
<i>R. atlantica</i> (BRY16)	0.033	-																					
<i>R. oceanica</i>	0.067	0.082	-																				
<i>Reteporella</i> sp. 5	0.083	0.098	0.038	-																			
<i>Reteporella</i> sp. 6	0.087	0.097	0.066	0.071	0.012																		
<i>Reteporella</i> sp. 7	0.070	0.078	0.083	0.083	0.057	0.017																	
<i>Reteporella</i> sp. 7 (BRY257)	0.089	0.101	0.089	0.091	0.063	0.034	-																
<i>Reteporella</i> sp. 1	0.179	0.183	0.178	0.183	0.185	0.187	0.194	-															
<i>R. tristis</i>	0.201	0.205	0.206	0.201	0.193	0.196	0.202	0.097	0.012														
<i>R. beaniana</i>	0.178	0.183	0.183	0.183	0.191	0.194	0.201	0.172	0.162	-													
<i>Reteporella</i> sp. 2	0.170	0.171	0.174	0.176	0.166	0.171	0.172	0.152	0.168	0.132	-												
<i>Reteporella</i> sp. 3	0.175	0.174	0.190	0.181	0.164	0.180	0.181	0.156	0.176	0.147	0.062	0.005											
<i>R. cf. grimaldii</i>	0.172	0.172	0.176	0.170	0.159	0.180	0.179	0.165	0.180	0.134	0.042	0.061	-										
<i>Reteporella</i> sp. 4	0.138	0.147	0.132	0.130	0.134	0.143	0.138	0.179	0.195	0.190	0.182	0.181	0.175										
<i>R. cf. graeffei</i>	0.135	0.143	0.129	0.132	0.136	0.142	0.138	0.176	0.196	0.192	0.185	0.187	0.181	0.002	-								
<i>R. tuberosa</i>	0.222	0.228	0.232	0.234	0.226	0.216	0.225	0.210	0.224	0.236	0.205	0.216	0.199	0.222	0.221	-							
<i>Phidolopora avicularis</i>	0.209	0.203	0.228	0.225	0.218	0.235	0.241	0.219	0.232	0.219	0.210	0.210	0.201	0.238	0.236	0.197	-						
<i>Schizoretopora</i> sp.	0.240	0.232	0.252	0.25	0.243	0.238	0.246	0.241	0.235	0.232	0.234	0.239	0.234	0.263	0.259	0.216	0.170	-					
<i>Schizotheca</i> sp.	0.203	0.205	0.219	0.208	0.209	0.218	0.23	0.210	0.214	0.208	0.223	0.231	0.221	0.233	0.230	0.205	0.194	0.201	-				
<i>Hippellozoon novaezelandiae</i>	0.215	0.216	0.241	0.23	0.230	0.218	0.23	0.205	0.231	0.212	0.212	0.226	0.210	0.242	0.245	0.188	0.181	0.174	0.194	-			
<i>Triphyllozoon arcuatum</i>	0.255	0.257	0.259	0.255	0.241	0.246	0.252	0.236	0.244	0.243	0.223	0.231	0.226	0.263	0.259	0.207	0.228	0.25	0.23	0.228	-		
<i>Iodictyum yaldwyni</i>	0.252	0.261	0.255	0.257	0.242	0.242	0.255	0.221	0.224	0.23	0.228	0.240	0.230	0.261	0.248	0.194	0.236	0.246	0.234	0.208	0.217	-	
<i>Iodictyum violaceum</i>	0.241	0.241	0.252	0.261	0.242	0.252	0.257	0.234	0.253	0.228	0.210	0.216	0.219	0.250	0.261	0.217	0.219	0.219	0.221	0.187	0.225	0.178	-

b)	<i>R. atlantica</i>	<i>R. atlantica</i> (BRY16)	<i>R. sp. 5</i>	<i>R. sp. 6</i>	<i>R. sp. 7</i>	<i>R. sp. 7</i> (BRY257)	<i>R. sp. 1</i>	<i>R. tristis</i>	<i>R. sp. 2</i>	<i>R. sp. 3</i>	<i>R. cf. grimaldii</i>	<i>R. cf. graeffei</i>	<i>R. tuberosa</i>	<i>I. yaldwyni</i>	<i>H. novaezelandiae</i>	<i>T. arcuatum</i>	<i>P. avicularis</i>
<i>R. atlantica</i>	0.002																
<i>R. atlantica</i> (BRY16)	0.003	-															
<i>Reteporella</i> sp. 5	0.022	0.020	-														
<i>Reteporella</i> sp. 6	0.013	0.011	0.010	0.005													
<i>Reteporella</i> sp. 7	0.016	0.010	0.008	0.007	0.002												
<i>Reteporella</i> sp. 7 (BRY257)	0.012	0.013	0.009	0.005	0.003	-											
<i>Reteporella</i> sp. 1	0.085	0.083	0.085	0.081	0.081	0.080	-										
<i>R. tristis</i>	0.085	0.083	0.080	0.080	0.077	0.080	0.026	0									
<i>Reteporella</i> sp. 2	0.043	0.036	0.036	0.040	0.039	0.039	0.067	0.063	-								
<i>Reteporella</i> sp. 3	0.044	0.038	0.037	0.041	0.040	0.040	0.069	0.061	0.004	0.002							
<i>R. cf. grimaldii</i>	0.045	0.039	0.031	0.035	0.034	0.033	0.070	0.065	0.007	0.008	-						
<i>R. cf. graeffei</i>	0.036	0.035	0.023	0.027	0.027	0.028	0.096	0.096	0.056	0.057	0.050	-					
<i>R. tuberosa</i>	0.198	0.198	0.198	0.197	0.192	0.196	0.208	0.213	0.197	0.198	0.193	0.196	-				
<i>Iodictyum yaldwyni</i>	0.177	0.177	0.172	0.171	0.166	0.169	0.184	0.184	0.175	0.173	0.169	0.169	0.111	-			
<i>Hippellozoon novaezelandiae</i>	0.181	0.179	0.175	0.176	0.169	0.173	0.178	0.183	0.174	0.178	0.172	0.180	0.094	0.087	-		
<i>Triphyllozoon arcuatum</i>	0.200	0.197	0.195	0.195	0.189	0.192	0.207	0.200	0.205	0.204	0.201	0.195	0.121	0.132	0.123	-	
<i>Phidolopora avicularis</i>	0.187	0.181	0.182	0.181	0.176	0.180	0.190	0.190	0.193	0.192	0.189	0.180	0.109	0.082	0.055	0.127	-

c)	<i>R. atlantica</i>	<i>R. oceanica</i>	<i>R. sp. 3</i>	<i>R. sp. 4</i>	<i>R. sp. 5</i>	<i>R. sp. 1</i>	<i>R. tristis</i>	<i>R. beaniana</i>	<i>R. sp. 2</i>	<i>R. sp. 3</i>	<i>R. cf. grimaldii</i>	<i>R. cf. graeffei</i>	<i>P. avicularis</i>	<i>H. novaezelandiae</i>	<i>I. violaceum</i>	<i>T. arcuatum</i>
<i>R. atlantica</i>	0															
<i>R. oceanica</i>	0.003	-														
<i>Reteporella</i> sp. 5	0.003	0	-													
<i>Reteporella</i> sp. 6	0.003	0	0	0												
<i>Reteporella</i> sp. 7	0.003	0	0	0	0											
<i>Reteporella</i> sp. 1	0.006	0.003	0.003	0.003	0.003	-										
<i>R. tristis</i>	0.006	0.003	0.003	0.003	0.003	0.003	0									
<i>R. beaniana</i>	0.006	0.003	0.003	0.003	0.003	0.006	0.006	-								
<i>Reteporella</i> sp. 2	0.012	0.015	0.015	0.015	0.015	0.015	0.012	0.012	-							
<i>Reteporella</i> sp. 3	0.012	0.015	0.015	0.015	0.015	0.015	0.012	0.012	0	-						
<i>R. cf. grimaldii</i>	0.012	0.015	0.015	0.015	0.015	0.015	0.012	0.012	0	0	-					
<i>R. cf. graeffei</i>	0.003	0	0	0	0	0.003	0.003	0.003	0.015	0.015	0.015	-				
<i>Phidolopora avicularis</i>	0.066	0.063	0.063	0.063	0.063	0.060	0.063	0.030	0.060	0.060	0.060	0.063	-			
<i>Hippellozoon novaezelandiae</i>	0.030	0.033	0.033	0.033	0.033	0.030	0.033	0.036	0.033	0.033	0.033	0.033	0.069	-		
<i>Iodictyum violaceum</i>	0.027	0.030	0.030	0.030	0.030	0.033	0.033	0.063	0.027	0.027	0.027	0.030	0.073	0.048	-	
<i>Triphyllozoon arcuatum</i>	0.018	0.021	0.021	0.021	0.021	0.021	0.018	0.024	0.012	0.012	0.012	0.021	0.054	0.039	0.036	-

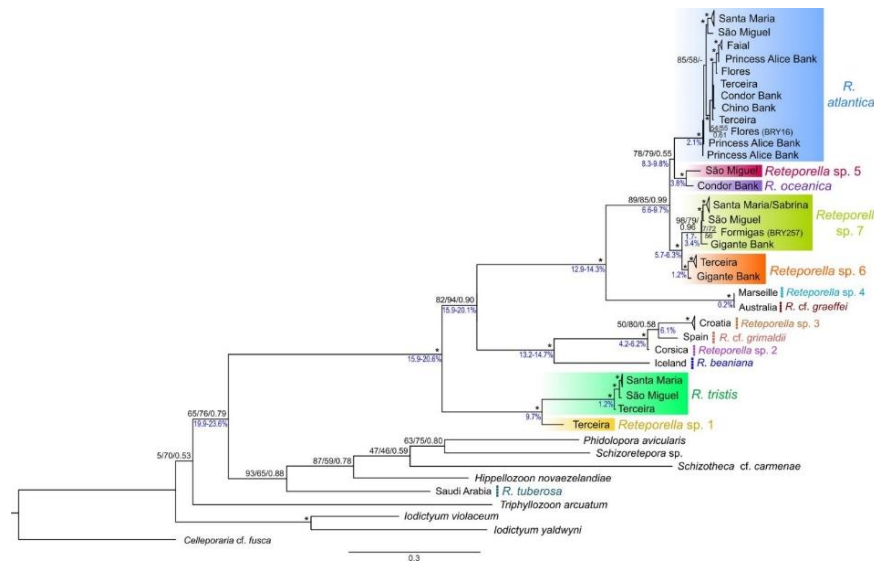


Figure A5.1. Maximum likelihood analysis of the COI data. Constructed using the W-IQ-Tree web server (Trifinopoulos et al., 2016) under the GTR+I (1st codon position), F81+I (2nd codon position) and GTR+G (3rd codon position) models of nucleotide evolution. Values at the nodes correspond to branch test (SH-aLTR) values, ultrafast bootstrap support (UFboot) values, and posterior probabilities (PP), inferred with W-IQ-Tree and MrBayes v3.2.7 software (Ronquist et al., 2012), respectively. Hyphen (-) indicates nodes absent in one of the phylogenetic reconstructions; asterisk (*) indicates nodes supported by both ML and BI analyses (SH-aLTR >= 80%, UFboot >= 95%, PP >= 95%). Mean divergence levels among *Reteporella* terminals, as estimated with MEGA11 (Tamura et al., 2021), are depicted at the nodes in blue. Known and putative new Azorean *Reteporella* species are indicated with different coloured shading. Non-Azorean species are indicated by dashed coloured lines. Other phidoloporids included in the analyses are given in normal black font. Geographic origins of *Reteporella* samples are given as terminal labels. The scale bar represents substitutions per site.

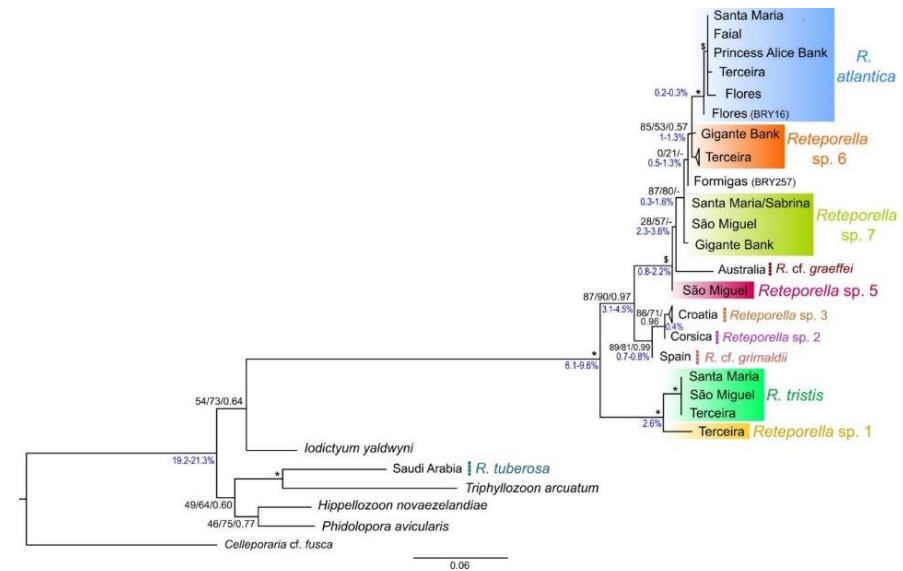


Figure A5.2. Maximum likelihood analysis of the 16S rRNA data. Constructed using the W-IQ-Tree web server (Trifinopoulos et al., 2016) under the GTR+I+G model of nucleotide evolution. Values at the nodes correspond to branch test (SH-aLTR) values, ultrafast bootstrap support (UFboot) values, and posterior probabilities (PP), inferred with W-IQ-Tree and MrBayes v3.2.7 software (Ronquist et al., 2012), respectively. Hyphen (-) indicates nodes absent in one of the phylogenetic reconstructions; asterisk (*) indicates nodes supported by both ML and BI analyses (SH-aLTR >= 80%, UFboot >= 95%, PP >= 95%). Dollar sign (\$) indicates nodes supported only by ML analyses (SH-aLTR >= 80%, UFboot >= 95%). Mean divergence levels among *Reteporella* terminals, as estimated with MEGA11 (Tamura et al., 2021), are depicted at the nodes in blue. Known and putative new Azorean *Reteporella* species are indicated with different coloured shading. Non-Azorean *Reteporella* species are indicated by dashed coloured lines. Geographic origins of the *Reteporella* samples are given as terminal labels. Other phidoloporids included in the analyses are given in normal black font. Geographic origins of the *Reteporella* samples are given as terminal labels. The scale bar represents substitutions per site.

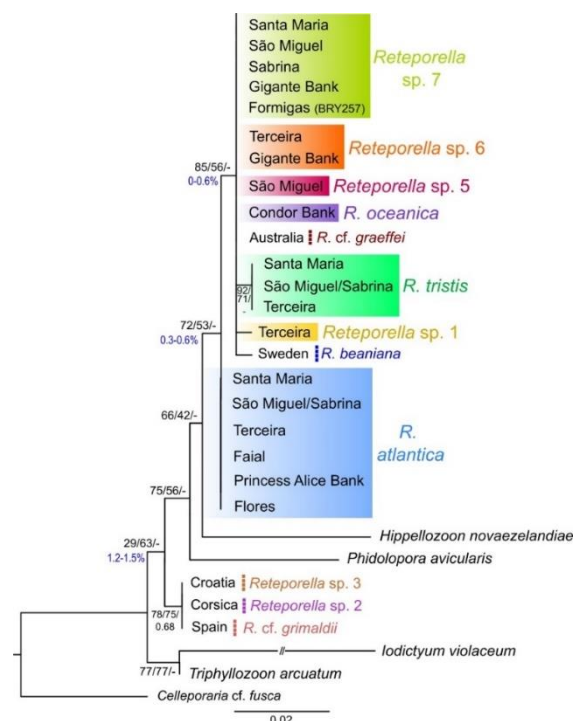


Figure A5.3. Maximum likelihood analysis of the 28S rRNA data. Constructed using the W-IQ-Tree web server (Trifinopoulos et al., 2016) under the GTR+I+G model of nucleotide evolution. Values at the nodes correspond to branch test (SH-aLTR) values, ultrafast bootstrap support (UFboot) values, and posterior probabilities (PP), inferred with W-IQ-Tree and MrBayes v3.2.7 software (Ronquist et al., 2012), respectively. Hyphen (-) indicates nodes absent in one of the phylogenetic reconstructions; asterisk (*) indicates nodes supported by both ML and BI analyses (SH-aLTR $\geq$ 80%, UFboot $\geq$ 95%, PP $\geq$ 95%). Mean divergence levels among *Reteporella* terminals, as estimated with MEGA11 (Tamura et al., 2021), are depicted at the nodes in blue. Known and putative new Azorean *Reteporella* species are indicated with different coloured shading. Non-Azorean *Reteporella* species are indicated by dashed coloured lines. Geographical origins of the *Reteporella* samples are given as terminal labels. Other phidoloporids included in the analyses are given in normal black font. Geographic origins of *Reteporella* samples are given as terminal labels. The scale bar represents substitutions per site.

References

- Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61, 539–42. <https://doi.org/10.1093/sysbio/sys029>
- Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, 38, 3022–7. <https://doi.org/10.1093/molbev/msab120>
- Trifinopoulos, J., Nguyen, L. T., & von Haeseler, A. (2016). Minh BQ. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research*, 44, W232–5. <https://doi.org/10.1093/nar/gkw256>
- Wisshak, M., Berning, B., Jakobsen, J., & Freiwald, A. (2015). Temperate carbonate production: biodiversity of calcareous epiliths from intertidal to bathyal depths (Azores). *Marine Biodiversity*, 45(1), 87–112. <https://doi.org/10.1007/s12526-014-0231-6>

Supplementary Information for Section 5.2.

Ecological differentiation in the bryozoan genus *Reteporella* suggested by microsatellite data

List of Supplementary Tables and Figures

Table A6.1. Detailed information of *Reteporella* samples used in this work.

Table A6.2. Complete list of all SSR primers designed in this study, listing their sequences, reference library, repetition motifs, and number of repeats in the original sequence from which the primers were designed.

Table A6.3. Estimates of genetic diversity per species.

Table A6.4. Hardy–Weinberg Equilibrium tests and frequency of null allele estimates per species.

Table A6.5. Hierarchical analysis of molecular variance, based on the SSR-GBAS dataset.

Table A6.6. Hierarchical analysis of molecular variance, based on the COI dataset.

Table A6.7. Hierarchical analysis of molecular variance among populations of *R. atlantica* in the Azores Archipelago.

Figure A6.1. Genetic variation among groups on individual SSR loci, explained by partition in species and geographical localities.

Table A6.1. Detailed information of *Reteporella* samples used in this work. Localities with coordinates in decimal degrees (when available), depth (in meters), collection voucher (if applicable), and COI accession numbers are provided. Column “SSR data” indicates samples for which short-sequence repeats were successfully amplified and analysed (x) or that failed amplification (f; less than 60 % loci successfully genotyped); n.a. indicates samples not included in the Illumina MiSeq run.

Species	Sample ID	Locality	Depth (m)	Coordinates DD	Collection voucher	COI Acc.	SSR data
<i>R. atlantica</i>	P8	Santa Maria, Azores	24	36.935, -25.092	DBUA:BRY 009	OP086115	x
	P10	Santa Maria, Azores	24	36.935, -25.092	DBUA:BRY 011	OP086116	x
	P15	Santa Maria, Azores	24	36.935, -25.092	DBUA:BRY 016	OP086117	x
	P16	Santa Maria, Azores	28	36.935, -25.092	DBUA:BRY 017	OP086106	x
	P17	Santa Maria, Azores	28	36.935, -25.092	DBUA:BRY 018	OP086118	x
	MB4	Santa Maria, Azores	27	36.995, -25.187	DBUA:BRY 023	OP086107	x
	MB5	Santa Maria, Azores	27	36.995, -25.187	DBUA:BRY 024	OP086104	x
	MB6	Santa Maria, Azores	27	36.995, -25.187	DBUA:BRY 025	OP086105	x
	MB10	Santa Maria, Azores	27	36.995, -25.187	DBUA:BRY 029	OP086108	x
	MB12	Santa Maria, Azores	27	36.995, -25.187	DBUA:BRY 031	OP086109	x
	GA8	Santa Maria, Azores	11-13	36.982, -25.039	DBUA:BRY 031	OP086110	x
	GA16	Santa Maria, Azores	11-13	36.982, -25.039	DBUA:BRY 055	OP086111	f
	GA17	Santa Maria, Azores	11-13	36.982, -25.039	DBUA:BRY 057	OP086112	f
	GA20	Santa Maria, Azores	11-13	36.982, -25.039	DBUA:BRY 059	OP086113	x
	GA21	Santa Maria, Azores	11-13	36.982, -25.039	DBUA:BRY 060	OP086114	x
	SM15-1-1	Santa Maria, Azores	10	-	Personal:BBerning	OP086119	n.a.
	SM15-6-1	Santa Maria, Azores	10	-	Personal:BBerning	OP086120	n.a.
	SM15-6-2	Santa Maria, Azores	10	-	Personal:BBerning	OP086121	n.a.
	BRY15	Flores, Azores	151	39.570, -31.217	M150-BIOD 072	OP086135	f
	BRY16	Flores, Azores	151	39.570, -31.217	M150-BIOD 073	OP086099	f
	BRY18	Flores, Azores	151	39.570, -31.217	M150-BIOD 076	OP086136	f
	BRY19	Flores, Azores	151	39.570, -31.217	M150-BIOD 077	OP086137	f
	BRY20	Flores, Azores	151	39.570, -31.217	M150-BIOD 078	OP086138	f
	BRY21	Flores, Azores	151	39.570, -31.217	M150-BIOD 079	OP086139	f
	BRY22	Flores, Azores	151	39.570, -31.217	M150-BIOD 080	OP086140	f
	BRY23	Flores, Azores	151	39.570, -31.217	M150-BIOD 081	OP086141	f
	BRY24	Flores, Azores	151	39.570, -31.217	M150-BIOD 082	OP086142	f
	BRY25	Flores, Azores	151	39.570, -31.217	M150-BIOD 083	OP086143	x
	BRY 100	Terceira, Azores	160	38.811, -27.335	M150-BIOD 434	OP086126	f
	BRY 102	Terceira, Azores	160	38.811, -27.335	M150-BIOD 436	OP086127	x
	BRY 104	Terceira, Azores	160	38.811, -27.335	M150-BIOD 438	OP086128	f
	BRY 105	Terceira, Azores	160	38.811, -27.335	M150-BIOD 439	OP086129	x
	BRY 130	Terceira, Azores	152	38.802, -27.071	M150-BIOD 532	OP086130	x
	BRY 134	Terceira, Azores	152	38.802, -27.071	M150-BIOD 536	OP086131	x

Species	Sample ID	Locality	Depth (m)	Coordinates DD	Collection voucher	COI Acc.	SSR data
	BRY 139	Terceira, Azores	152	38.802, -27.07	M150-BIOD 543	OP086132	x
	BRY-95-1	Terceira, Azores	151	38.811, -27.335	M150-BIOD 504	OP086122	n.a.
	BRY-95-2	Terceira, Azores	151	38.811, -27.335	M150-BIOD 504	OP086125	n.a.
	SP-60m-2	Faial, Azores	60	38.520, -28.605	Personal:BBerning-SP	-	n.a.
	SP-60m-3	Faial, Azores	60	38.520, -28.605	Personal:BBerning-SP	OP086145	n.a.
	SP-60m-5	Faial, Azores	60	38.520, -28.605	Personal:BBerning-SP	OP086150	n.a.
	SP-60m-6	Faial, Azores	60	38.520, -28.605	Personal:BBerning-SP	OP086146	n.a.
	SP-60m-7	Faial, Azores	60	38.520, -28.605	Personal:BBerning-SP	OP086147	n.a.
	SP-60m-8	Faial, Azores	60	38.520, -28.605	Personal:BBerning-SP	OP086144	n.a.
	SP-150m-1	Faial, Azores	150	38.509, -28.610	Personal:BBerning-SP	OP086151	n.a.
	SP-150m-3	Faial, Azores	150	38.509, -28.610	Personal:BBerning-SP	-	n.a.
	SP-150m-6	Faial, Azores	150	38.509, -28.610	SP-150m	OP086152	n.a.
	DR5.B9	Sabrina, Azores	150	37.869, -25.902	DBUA:SAB2011	OP086101	x
	A02	Princess Alice Bank, Azores	265	37.753, -28.991	DOP-4193	OP086102	f
	A08	São Miguel, Azores	117	37.77, -25.116	DOP-5300	OP086100	x
	A10	Chino Bank, Azores	371	37.987, -29.53	DOP-5892	OP086123	x
	A11	Princess Alice Bank, Azores	256	37.694, -28.935	DOP-6132	OP086103	x
	A12	Princess Alice Bank, Azores	393	37.990, -29.565	DOP-6622	OP086149	x
	A13	Princess Alice Bank, Azores	368	37.975, -29.531	DOP-6627	-	f
	A19	Terceira, Azores	368	37.975, -29.531	DOP-5325	OP086124	f
	A20	Terceira, Azores	86	38.815, -27.302	DOP-5325	OP086134	f
	9860B	Condor Bank, Azores	86	38.815, -27.302	DOP-9860	OP086133	f
<i>R. tristis</i>	BRY 127	Terceira, Azores	152	38.802, -27.071	M150-BIOD 526	OP086183	f
	BRY 128	Terceira, Azores	152	38.802, -27.071	M150-BIOD 528	OP086184	x
	BRY 129	Terceira, Azores	152	38.802, -27.071	M150-BIOD 530	OP086185	x
	BRY 143	Terceira, Azores	153	38.802, -27.071	M150-BIOD 549	OP086186	f
	BRY 195	Santa Maria, Azores	151	37.055, -25.154	M150-BIOD 941	OP086191	f
	BRY 196	Santa Maria, Azores	151	37.055, -25.154	M150-BIOD 943	OP086192	f
	BRY 198	Santa Maria, Azores	152	37.055, -25.154	M150-BIOD 946	OP086193	f
	BRY 204	Santa Maria, Azores	152	37.055, -25.154	M150-BIOD 952	OP086194	f
	BRY 216	Santa Maria, Azores	152	37.055, -25.154	M150-BIOD 964	OP086195	
	BRY 220	Santa Maria, Azores	153	37.055, -25.154	M150-BIOD 971	OP086196	f
	BRY 222	Santa Maria, Azores	153	37.055, -25.154	M150-BIOD 974	OP086197	f
	BRY 224	Santa Maria, Azores	152	37.055, -25.154	M150-BIOD 978	OP086198	f
	BRY 225	Santa Maria, Azores	152	37.055, -25.154	M150-BIOD 980	OP086189	x
	BRY 226	Santa Maria, Azores	152	37.055, -25.154	M150-BIOD 982	OP086190	x
	DR10.B7	Sabrina, Azores	150	37.882, -25.907	DBUA:SAB2011	OP086188	f
	M151-50341	São Miguel, Azores	200	37.675, -25.717	SaM-ID-50341	OP086187	n.a.

Species	Sample ID	Locality	Depth (m)	Coordinates DD	Collection voucher	COI Acc.	SSR data
<i>R. oceanica</i>	9869 ^a	Condor Bank	455	38.555, -29.044	DOP-9869A	OP086154	
	9869B	Condor Bank	455	38.555, -29.044	DOP-9869B	OP086155	
<i>Reteporella</i> sp. 6	BRY 103	Terceira, Azores	160	38.811, -27.335	M150-BIOD 437	OP086159	x
	BRY 117	Terceira, Azores	277	38.802, -27.059	M150-BIOD 515	OP086162	x
	BRY 136	Terceira, Azores	153	38.803, -27.071	M150-BIOD 539	OP086158	x
	BRY 141	Terceira, Azores	152	38.802, -27.071	M150-BIOD 546	OP086161	x
	BRY 146	Terceira, Azores	153	38.802, -27.071	M150-BIOD 553	OP086160	x
	10302E	Gigante Bank, Azores	493	38.975, -29.852	DOP-10302	OP086156	x
	10303	Gigante Bank, Azores	402	39.008, -29.929	DOP-10303	OP086157	x
	BRY 159	Santa Maria, Azores	152	38.802, -27.071	M150-BIOD 720	OP086175	f
<i>Reteporella</i> sp. 7	BRY 170	Santa Maria, Azores	144	37.047, -25.057	M150-BIOD 719	OP086176	x
	BRY 217	Santa Maria, Azores	152	37.047, -25.057	M150-BIOD 966	OP086177	x
	BRY 218	Santa Maria, Azores	153	37.055, -25.154	M150-BIOD 968	OP086178	x
	BRY 223	Santa Maria, Azores	153	37.055, -25.154	M150-BIOD 976	OP086181	x
	BRY-211-4	Santa Maria, Azores	153	37.055, -25.154	M150-BIOD 959	OP086174	n.a.
	BRY-221-2	Santa Maria, Azores	152	37.055, -25.154	M150-BIOD 972	OP086173	n.a.
	M151-50332	São Miguel, Azores	153	37.055, -25.154	SaM-ID-50332	OP086167	n.a.
	M151-100 113	São Miguel, Azores	544	37.670, -25.713	SaM-ID-100 113	OP086166	n.a.
	DR2.B2 A	Sabrina, Azores	309	37.675, -25.717	DBUA:SAB2011	OP086170	f
	DR2.B2 B	Sabrina, Azores	50-100	37.850, -25.667	DBUA:SAB2011	OP086171	f
	DR2.B2 C	Sabrina, Azores	50-100	37.850, -25.667	DBUA:SAB2011	OP086172	x
	DR3.B13 A	Sabrina, Azores	50-100	37.850, -25.667	DBUA:SAB2011	OP086168	f
	DR3.B13 B	Sabrina, Azores	200	37.855, -25.683	DBUA:SAB2011	OP086169	f
	DR4.B13	Sabrina, Azores	200	37.855, -25.683	DBUA:SAB2011	OP086179	x
	A07	Santa Maria, Azores	140	37.873, -25.900	DOP-5282	OP086180	x
	10302A	Gigante Bank, Azores	138	36.911, -25.022	DOP-10302	OP086164	f
	10302C	Gigante Bank, Azores	493	38.975, -29.852	DOP-10302	OP086165	x
	BRY 257	Formigas, Azores	150	37.274, -24.755	M150-BIOD 1140	OP086163	x
unknown	A22	Azores Bank	548	38.117, - 29.18	DOP-1085	-	x

Table A6.2. Complete list of all SSR primers designed in this study, listing their sequences, reference library, , repetition motifs and number of repeats in the original sequence from which the primers were designed. The mixes in which primers were included for the multiplex PCRs are also indicated. The last six primers failed during single PCR tests and were omitted from any SSR genotyping. It is also indicated if the primer was informative for population genetic analyses or if it was excluded from the dataset after applying criteria for missing data (> 60%). The number of alleles of the loci analysed is indicated in the last column.

Primer/ Loci	Forward (5'-3')	Reverse (5'-3')	Reference library	Repetition motif	Mix	Population genetic analyses?	# alleles
Bry1_CATA	GGTTGGTTGATTGTGACCAG	G TAGGGAGCTAGCCAATTGA	BRY16 (<i>R. atlantica</i>)	(CATA)6	1	Yes	25
Bry2_TATG	TACACCCAAATTCCTGCCA	G TGCCTCCCACAAATTCATG	BRY16 (<i>R. atlantica</i>)	(TATG)5	3	Yes	15
Bry3_TATC	GTTTGGAGCTCAACATTCGT	A CCGCATCATCACTTACTGT	BRY141 (<i>Reteporella</i> sp. 6)	(TATC)23	3	Yes	31
Bry4_GGTA	TCTGCAGAGCCGAATCATT	A CCGGGACTATGTGAAGCTAT	BRY141 (<i>Reteporella</i> sp. 6)	(GGTA)5	4	Yes	35
Bry6_TACC	TATGACAGTTCGATGGACG	A CATCTAAAGGCACAACAGC	BRY141 (<i>Reteporella</i> sp. 6)	(TACC)6	4	Yes	39
Bry7_GATA	GCCAAGGAGTCTACATGTC	A ATTCTGGAACGAAGTGGGA	BRY141 (<i>Reteporella</i> sp. 6)	(GATA)6	4	Yes	40
Bry8_TGTC	CCCTACCTTGTCATACACT	A TGGTAAGGCTATTGTTGACCA	BRY141 (<i>Reteporella</i> sp. 6)	(TGTC)11	4	Yes	66
Bry9_AATA	ACCATGTTCTCACTTTGGT	A TAGTCCGCAAATGACCAGA	BRY141 (<i>Reteporella</i> sp. 6)	(AATA)5	2	Yes	29
Bry10_CACTA	GTCCAAAGTTGATCACTGCG	A TCGCCACTGCTCTATTTGAT	BRY141 (<i>Reteporella</i> sp. 6)	(CACTA)5	1	Yes	46
Bry11_CCTA	GGATAGCTTCTTGGCTTGGA	A GTCCACAAAGCTCACATTTG	BRY141 (<i>Reteporella</i> sp. 6)	(CCTA)5	2	Yes	42
Bry12_CTGT	TCAACCGCTATAGCCATTGT	A TGTGTGTTCTTGCAGACTCT	BRY141 (<i>Reteporella</i> sp. 6)	(CTGT)6	1	Yes	16
Bry14_ATAC	TGTCTCGAAGCTGTCTTGAA	A AACATGGCTAACTACCCTC	BRY141 (<i>Reteporella</i> sp. 6)	(ATAC)6	3	Yes	39
Bry15_CATA	TCCATCCATACGTGTATGCA	A AGTGCAAACCTCCACAATTG	BRY141 (<i>Reteporella</i> sp. 6)	(CATA)5	2	Yes	28
Bry16_CCTA	TTAAGTCACTGCAAGTTGGC	A CACAACGTGAGTGGTGTGTA	BRY141 (<i>Reteporella</i> sp. 6)	(CCTA)8	3	Yes	38
Bry18_ATAC	ACATCGAATTTACAGTTGCT	A CGTGTACAACAAAGCACAGA	BRY141 (<i>Reteporella</i> sp. 6)	(ATAC)5	2	No; > 60% missing	-
Bry19_TGTC	CTGATGTAGCGTTGTAGAGC	A CCACAACAAATTGAGCCCAT	BRY141 (<i>Reteporella</i> sp. 6)	(TGTC)6	2	Yes	52
Bry21_GTTA	TGCTTCTTAGTGCTCATGT	A TGCGGAACCTCCACATGTTAT	BRY141 (<i>Reteporella</i> sp. 6)	(GTTA)8	1	Yes	32
Bry22_TGTC	GCGGTTATTCACCCAGACT	A AAGGCCACAAAAGCTGAAAG	BRY141 (<i>Reteporella</i> sp. 6)	(TGTC)5	3	Yes	31
Bry23_CATA	ACAACCTCTCATTCCAGACC	A AACTCCAGAAGTGGCTGAA	BRY141 (<i>Reteporella</i> sp. 6)	(CATA)6	4	Yes	21
Bry24_CAA	TCAGGCAATACGAGCAGAAT	A ACACAGGAGCTGGTACCTAG	BRY141 (<i>Reteporella</i> sp. 6)	(CAA)10	2	Yes	39
Bry25_TGT	CAGAAAAGGTTGATGCGGTC	A GGCTTCCTTTGAGGGATTTT	BRY141 (<i>Reteporella</i> sp. 6)	(TGT)9	4	No; > 60% missing	-
Bry26_ATA	GTTGTGACAGATCGTGACAG	A GTGAGTGTGACAGCCATAGA	BRY141 (<i>Reteporella</i> sp. 6)	(ATA)9	2	Yes	15
Bry27_ATA	GGTTTACAACCGGCTTTACC	A TCCATAGTGGTGCTTGTCA	BRY141 (<i>Reteporella</i> sp. 6)	(ATA)9	4	No; > 60% missing	-
Bry28_TTA	ACCTACACTGACAGCAACAA	A ATTACTTGATGACGGGTCCC	BRY141 (<i>Reteporella</i> sp. 6)	(TTA)8	1	Yes	28
Bry30_TTA	CTCTCGTGTGCTGGAATT	A GCTATTGTACACTTGCTAGCC	BRY141 (<i>Reteporella</i> sp. 6)	(TTA)8	2	Yes	42
Bry31_TAA	ATCCTCGACTCCGTCATTTT	A GCAAGTTGCATTACAGAGT	BRY141 (<i>Reteporella</i> sp. 6)	(TAA)9	3	Yes	25
Bry32_TTG	TCTGTTGTTGAGGGGTTTGT	A CAATGGCTTCATTACCGCAT	BRY141 (<i>Reteporella</i> sp. 6)	(TTG)13	3	Yes	46
Bry33_ACT	CATACACTCACTGCTTCCCT	A GACTGCATTGGTCAGGACTA	BRY141 (<i>Reteporella</i> sp. 6)	(ACT)8	1	Yes	38
Bry34_AAT	GACTTCAACACAGGGTAAGAA	A GTACGGCTACTTTGTGCAAG	BRY141 (<i>Reteporella</i> sp. 6)	(AAT)8	4	No; > 60% missing	-

Bry36_TAA	TCACGCATTAGCCTACACAT	CTAGACAGCACTGGCATGTA	BRY141 (<i>Reteporella</i> sp. 6)	(TAA)8	2	Yes	41
Bry37_TAT	TGTCCAACACTTACACCTTCA	TTTTCAGACACTGCTCAAGC	BRY141 (<i>Reteporella</i> sp. 6)	(TAT)8	1	Yes	37
Bry38_TTA	GCTGGGTGTCTTGCAATTATG	CTAGTCCCTAATTGTGCAACG	BRY141 (<i>Reteporella</i> sp. 6)	(TTA)17	3	Yes	35
Bry39_TAC	ATAATCTCCTCTACTCACCCC	ATACCATACGTACTGGTGGC	BRY141 (<i>Reteporella</i> sp. 6)	(TAC)15	1	Yes	39
Bry40_ACT	CGCAATGGCTAAGGCAAATT	GAGCCACTACTACAGCAGAT	BRY141 (<i>Reteporella</i> sp. 6)	(ACT)9	3	No; > 60% missing	
Bry41_ACA	AGGCGAGTACACAAATGTAAC	ACGTGGAGATAGAGGATTTGG	BRY141 (<i>Reteporella</i> sp. 6)	(ACA)8	1	Yes	58
Bry42_AATA	ATAGCACTCTGTACTCGCAT	TTGCGCAACTTCCAGATTTC	BRY141 (<i>Reteporella</i> sp. 6)	(AATA)5	4	Yes	38
Failed during single PCR test							
Bry5_ATAC	CAGGAGATGCAGAGTAAGCA	TCTCACCCACAAATGCACTA	BRY141 (<i>Reteporella</i> sp. 6)	(ATAC)5	-	No	
Bry13_CACCA	TCTGGTACCGGTACAATCAA	TAGGTGGCAATGCATCTCTT	BRY141 (<i>Reteporella</i> sp. 6)	(CACCA)5	-	No	
Bry17_TATG	GCATGTATGTGCATGATGATG	AATTGCTGCCACAAGAATGG	BRY141 (<i>Reteporella</i> sp. 6)	(TATG)15	-	No	
Bry20_ATGT	TGCACATAGAAAAGGGTCGA	GGTCGCCTGTCAACAAATAC	BRY141 (<i>Reteporella</i> sp. 6)	(ATGT)6	-	No	
Bry29_AAC	GGTGATGATTGGGTAGTGGA	TCAGCTACTGTAGTGAAGTGT	BRY141 (<i>Reteporella</i> sp. 6)	(AAC)20	-	No	
Bry35_AAT	GACTTCAACACAGGGTAAGAA	GTACGGCTACTTTGTGCAAG	BRY141 (<i>Reteporella</i> sp. 6)	(AAT)8	-	No	

Table A6.3. Estimates of genetic diversity per species based on: **a)** the mitochondrial marker COI; **b)** the SSR-GBAS dataset. Estimates of the number of haplotypes (h), haplotype ($Hd \pm$ standard deviation) and nucleotide (π) diversity in the COI dataset, conducted in DnaSP v6. Estimates of the mean number of alleles (Na), observed (Ho) and expected (He) heterozygosity in the SSR-GBAS dataset conducted on GenAlEx v6.51; cases in which Ho is higher than He are identified with an asterisk (*). Number of individuals (n) assigned to each group is depicted within brackets; the number of localities sampled for each species (per dataset) is shown in the first column (# locs). Genetic diversity indices in *R. atlantica* are estimated for all representatives, as well as separately for deep and shallow lineages.

a)	# locs	h	Hd	π
<i>R. atlantica</i> – all (n=54)	8	16	0.857 ± 0.028	0.01791
<i>R. atlantica</i> – deep (n=13)	7	13	0.843 ± 0.041	0.01392
<i>R. atlantica</i> – shallow (n=18)	1	3	0.397 ± 0.132	0.00058
<i>Reteporella</i> sp. 6 (n=4)	2	4	0.810 ± 0.130	0.01104
<i>Reteporella</i> sp. 7 (n=7)	3	7	0.824 ± 0.061	0.00848
<i>R. tristis</i> (n=4)	3	4	0.700 ± 0.090	0.00900
All (n=93)	9	31	0.939 ± 0.011	0.08799

b)	# locs	Na	Ho	He
<i>R. atlantica</i> (n=22)	7	15.452	0.463	0.841
<i>R. atlantica</i> – deep (n=9)	4	8.645	0.524	0.823
<i>R. atlantica</i> – shallow (n=13)	3	7.161	0.419	0.665
<i>Reteporella</i> sp. 6 (n=7)	2	6.935	0.576	0.794
<i>Reteporella</i> sp. 7 (n=10)	3	9.065	0.583	0.826
<i>R. tristis</i> (n=4) *	2	3.161	0.737	0.632

Table A6.4. Hardy–Weinberg Equilibrium tests and frequency of null allele estimates per species, conducted on the SSR-GBAS dataset using GenAIEx v6.5 and FreeNA, respectively. Significant (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) deviations from HWE are highlighted in bold. Monomorphic loci (*mono*), loci which completely failed genotyping in the group (*All null*), and impossibility to estimate the parameter (-) are also depicted. For *R. atlantica*, these parameters were estimated for all representatives, as well as separately for deep and shallow lineages.

	<i>R. tristis</i>		<i>R. oceanica</i>		<i>Reteporella</i> sp. 6		<i>Reteporella</i> sp. 7		<i>R. atlantica</i> – all		<i>R. atlantica</i> – deep		<i>R. atlantica</i> – sh	
	HWE	f(null)	HWE	f(null)	HWE	f(null)	HWE	f(null)	HWE	f(null)	HWE	f(null)	HWE	f(null)
Bry1 CATA		<i>All null</i>	<i>mono</i>	0.00100	0.464	0.00000	0.423	0.000	0.000 ***	0.09389	0.600	0.00038	0.993	0.00001
Bry2 TATG	0.637	0.00000	-	<i>All null</i>	0.244	0.08587	0.403	0.09314	0.004 **	0.30987	0.116	0.28634	0.106	0.24535
Bry3 TATC	-	<i>All null</i>	0.317	0.00000	0.285	0.00058	0.423	0.00000	0.000 ***	0.33614	0.001 **	0.46667	0.004 **	0.22377
Bry4 GGTA	0.172	0.00000	0.637	0.00000	0.317	0.08739	0.423	0.00000	0.008 **	0.15951	0.349	0.10294	0.427	0.06859
Bry6 TACC	0.172	0.00000	-	<i>All null</i>	0.299	0.00041	0.121 ***	0.13241	0.000 ***	0.10819	0.023 *	0.07629	0.721	0.00000
Bry7 GATA	0.112	0.00058	0.317	0.00000	0.073	0.24490	0.001 *	0.22222	0.000 ***	0.33159	0.003 **	0.28173	0.000 ***	0.29109
Bry8 TGTC	0.526	0.00000	0.157	0.33333	0.297	0.00045	0.047	0.08333	0.002 **	0.07270	0.108	0.13333	0.084	0.00000
Bry9 AATA	0.386	0.00000	-	<i>All null</i>	0.006 **	0.40322	0.206	0.40322	0.000 ***	0.07500	0.081	0.15000	0.001 **	0.00000
Bry10 CACTA	0.285	0.00000	0.157	0.00000	0.039 *	0.24490	0.160	0.05176	0.000 ***	0.20162	0.087	0.19992	0.021 *	0.16095
Bry11 CCTA	0.083	0.31500	0.261	0.00071	0.400	0.00000	0.063	0.23579	0.001 **	0.17557	0.477	0.00000	0.004 **	0.27992
Bry12 CTGT	<i>mono</i>	0.00100	0.637	0.00000	0.112	0.00082	0.009 **	0.39936	0.057	0.04445	0.343	0.17564	0.540	0.00000
Bry14 ATAC	0.931	0.00000	0.637	0.00000	0.008 **	0.38961	0.002 **	0.21439	0.000 ***	0.07774	0.514	0.00035	0.002 **	0.00001
Bry15 CATA	0.062	0.23625	<i>mono</i>	0.00100	0.012 *	0.19353	0.000 ***	0.39864	0.039 *	0.23660	0.073	0.24490	0.261	0.00071
Bry16 CCTA	0.046	0.38687	0.157	0.33333	0.026 *	0.16870	0.097	0.20104	0.000 ***	0.34864	0.001 ***	0.34974	0.001 **	0.30597
Bry19 TGTC	0.174 *	0.00000	0.423	0.00000	0.143	0.20000	0.166	0.13333	0.000 ***	0.22843	0.112	0.15904	0.050	0.21679
Bry21	0.083	0.00000	-	<i>All null</i>	0.092	0.24000	0.030 *	0.31196	0.001 ***	0.24003	0.097	0.30420	0.115	0.15000

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GTTA Bry22 TGTC	0.174	0.00000	mono	0.00100	0.058	0.18204	0.321	0.04770	0.000 ***	0.15735	0.227	0.06133	0.885	0.00002
Bry23 CATA	0.931	0.00000	mono	0.00100	0.284	0.00000	0.001 ***	0.10230	0.000 ***	0.05798	0.469	0.00000	mono	0.00100
Bry24 CAA	0.392	0.00000	0.572	0.00000	0.516	0.00000	0.102	0.04167	0.010 **	0.09668	0.513	0.00000	0.034 *	0.16114
Bry26 ATA	0.317	0.00000	-	All null	0.062	0.42857	0.017 *	0.28301	0.006 **	0.33794	0.245	0.19665	0.057	0.30824
Bry28 TTA	mono	0.00100	0.637	0.00000	0.100	0.30000	0.001 ***	0.25924	0.000 ***	0.32943	0.023	0.29154	0.001 **	0.29359
Bry30 TTA	0.386	0.00000	0.637	0.00000	0.451	0.00000	0.295	0.03996	0.007 **	0.07500	0.108	0.05137	0.293	0.03578
Bry31 TAA	0.174	0.00100	mono	0.00000	0.023 *	0.29154	0.423	0.00000	0.000 ***	0.21468	0.153	0.14144	0.045 *	0.14358
Bry32 TTG	0.317	0.00058	0.637	0.00000	0.261	0.00071	0.204	0.10458	0.227	0.04445	0.301	0.00038	0.966	0.00035
Bry33 ACT	mono	0.00100	0.317	0.00000	0.126	0.18555	0.018 *	0.31746	0.280	0.17308	0.339	0.14250	0.704	0.12004
Bry36 TAA	0.217	0.20190	0.637	0.00000	0.012 *	0.34958	0.357	0.09828	0.000 ***	0.32828	0.037 *	0.31023	0.025 *	0.28861
Bry37 TAT	0.521	0.00000	0.157	0.00000	0.451	0.00000	0.179	0.13333	0.089	0.24490	0.440	0.00050	0.112	0.40000
Bry38 TTA	0.392	0.00000	mono	0.00100	0.464	0.07528	0.315	0.00038	0.000 ***	0.36174	0.006 **	0.40322	0.000 ***	0.25508
Bry39 TAC	0.217	0.20190	0.317	0.00000	0.214	0.09524	0.073	0.13500	0.000 ***	0.29738	0.005 **	0.35498	0.205	0.15226
Bry41 ACA	0.532	0.00000	0.423	0.00000	0.206	0.08782	0.266	0.07407	0.000 ***	0.19081	0.115	0.15000	0.004 **	0.18210
Bry42 AATA	0.100	0.00050	0.637	0.00000	0.087	0.19682	0.033 *	0.27778	0.000 ***	0.27262	0.017 *	0.14761	0.001 ***	0.29344

Table A6.5. Hierarchical analysis of molecular variance based on the SSR-GBAS dataset among: **a)** species: *R. tristis*, *R. atlantica*, *R. oceanica*, *Reteporella* spp. 6 and 7; **b)** geographical localities: Gigante Bank, Condor Bank, Princess Alice Bank, Terceira, São Miguel, Santa Maria, and shallow waters around Santa Maria; **c)** geographical locations previously mentioned, excluding shallow waters. Analysis performed in GenAlEx v6.51, with 999 permutations.

a) Among species						
Source	df	SS	MS	Est. Var.	%	
Among populations	4	157.586	39.397	1.286	9%	
Among individuals	40	799.769	19.994	6.919	48%	
Within individuals	45	277.000	6.156	6.156	43%	
Total	89	1234.356		14.361	100%	

b) Among geographical localities						
Source	df	SS	MS	Est. Var.	%	
Among populations	6	263.227	43.871	2.193	15%	
Among individuals	37	670.285	18.116	5.956	41%	
Within individuals	44	273.000	6.205	6.205	43%	
Total	87	1206.511		14.337	100%	

c) Among geographical localities, except Santa Maria-shallow water						
Source	df	SS	MS	Est. Var.	%	
Among populations	5	170.137	34.027	1.579	11%	
Among individuals	25	471.669	18.867	6.079	42%	
Within individuals	31	208.000	6.710	6.710	47%	
Total	61	849.806		14.367	100%	

Table A6.6. Hierarchical analysis of molecular variance, based on the COI dataset among: **a)** species: *R. tristis*, *R. atlantica*, *R. oceanica*, *Reteporella* spp. 6 and 7; **b)** geographical localities: Condor Bank, Gigante Bank, Princess Alice Bank, Terceira, São Miguel, Santa Maria, and shallow waters around Santa Maria. Analysis performed in GenAlEx v6.51, with 999 permutations.

a) Among species						
Source	df	SS	MS	Est. Var.	%	
Among populations	4	1789.496	447.374	29.202	83%	
Within populations	92	568.164	6.176	6.176	17%	
Total	96	2357.660		35.378	100%	

b) Among geographical localities						
Source	df	SS	MS	Est. Var.	%	
Among populations	8	1049.615	131.202	11.355	43%	
Within populations	87	1296.156	14.898	14.898	57%	
Total	95	2345.771		26.254	100%	

Table A6.7. Hierarchical analysis of molecular variance among populations of *R. atlantica* in the Azores Archipelago, based on: **a)** the SSR-GBAS dataset: Gruta Azul, Mar da Barca, and Pedrinha in Santa Maria, Terceira, and Princess Alice Bank; **b)** the mitochondrial COI marker: Gruta Azul, Mar da Barca, and Pedrinha in Santa Maria, São Miguel, Terceira, Princess Alice Bank, Faial, and Flores. Analysis performed in GenAlEx v6.51, with 999 permutations.

a) WAI dataset

Source	df	SS	MS	Est. Var.	%
Among populations	4	1789.496	447.374	29.202	83%
Within populations	92	568.164	6.176	6.176	17%
Total	96	2357.660		35.378	100%

b) COI dataset

Source	df	SS	MS	Est. Var.	%
Among populations	8	1049.615	131.202	11.355	43%
Within populations	87	1296.156	14.898	14.898	57%
Total	95	2345.771		26.254	100%

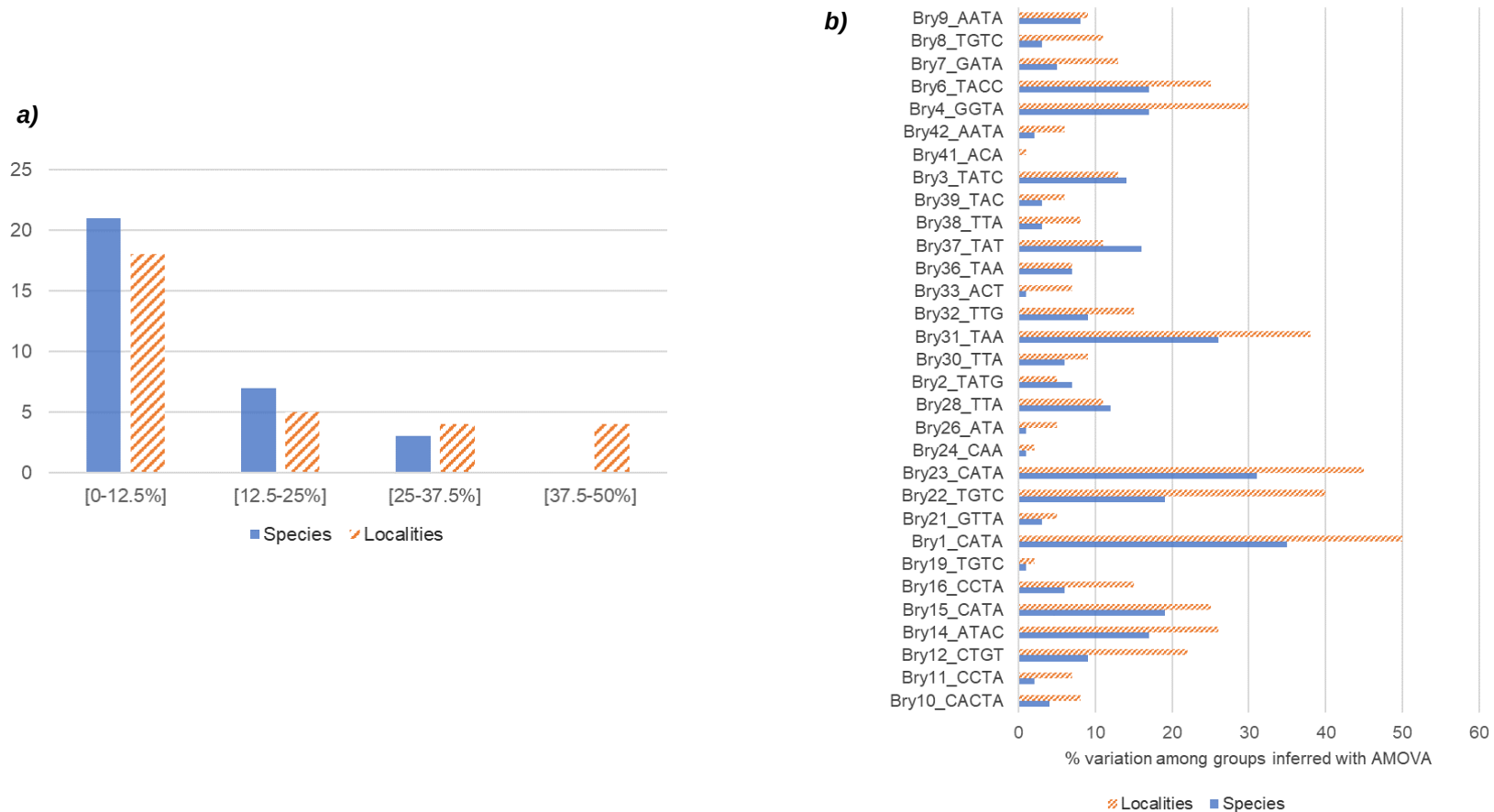


Figure A6.1. Genetic variation among groups on individual SSR loci, explained by partition in species and geographical localities. a) Number of SSR loci for which the genetic variation among groups was estimated into the following intervals: 0-12.5%, 12.5-25%, 25-37.5%, 37.5-50%. **b)** Estimates of the genetic variation among groups estimated in the AMOVA for individual SSR loci. Analysis performed in GenAlEx v6.51 on the WAI dataset, with 999 permutations.

Appendix B

Other publications during the Ph.D.

Evaluating the taxonomic status of the large sized *Tricolia* Risso, 1826 in the Northeast Atlantic and Mediterranean Sea.

(under review for *Molecular Phylogenetics and Evolution*, December 2022)

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Abstract

Despite a long history of taxonomic studies on the genus *Tricolia* Risso, 1826, there is a shortfall on thorough systematic molecular reviews of the taxa from the NE Atlantic and Mediterranean coasts. Aiming to assess the genetic distinctness among morphospecies and the taxonomic status of currently accepted large sized species in these areas, we conducted a molecular phylogenetic analysis of the genus based on one mitochondrial (COI) and two nuclear (28S and ITS2) markers. Seven *Tricolia* species were consistently retrieved in the analyses, including a new genetic lineage in the NE Atlantic designated as *Tricolia* sp. 1. Molecular analyses revealed that only one species, *T. azorica*, occurs in the NE Atlantic archipelagos. The sister taxa *T. pullus* (Mediterranean) and *T. picta* (NE Atlantic) should be classified as distinct species, instead of subspecies of the *T. pullus* group (*sensu* Gofas 1982). *Tricolia miniata* is also a complex of species in the Mediterranean and future studies across the distribution range are necessary to clarify its status.

Keywords: phylogeny, sympatric occurrence, new species, Atlantic archipelagos, oceanographic barriers, taxonomic revision

Marine Biology

(2019) 166:88

<https://doi.org/10.1007/s00227-019-3536-y>

ORIGINAL PAPER

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Intertidal or subtidal/circalittoral species: which appeared first? A phylogenetic approach to the evolution of non-planktotrophic larvae in Atlantic Archipelagos

Lara Baptista^{1,2,3} · António M. Santos^{2,4} · M. Pilar Cabezas⁴ · Ricardo Cordeiro^{1,2,5} · Carlos Melo^{1,2,6,7} · Sérgio P. Ávila^{1,2,5}

Received: 19 December 2018 / Accepted: 29 May 2019
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Abstract
Volcanic oceanic archipelagos are fascinating natural laboratories of evolutionary patterns and processes in remote, unique conditions. In the insular marine realm, deepwaters and sea-surface circulation hamper dispersal and, for marine invertebrates, this ability is linked to larval development: planktotrophic organisms disperse easily, whereas non-planktotrophic species usually have restricted ranges. Similarly, bathymetric zonation also influences dispersal: intertidal species are more prone to engage in the process than subtidal/circalittoral species. Therefore, the presence of endemic congeneric non-planktotrophic marine gastropods in two Atlantic archipelagos, hundreds of kilometers apart, inspired a biogeographical hypothesis. It predicts that when two congeneric non-planktotrophic gastropod species, with different bathymetric specific ranges, simultaneously occur and are restricted to two remote archipelagos, the subtidal/circalittoral species is expected to be evolutionarily older than the intertidal species. The present study aims to test this theoretical prediction from a multi-disciplinary perspective, with a molecular, Bayesian, fossil-calibrated, phylogenetic analysis of selected Rissoiidae species to test the theoretical predictions. We hereby corroborate the earlier speciation of the subtidal/circalittoral *Alvania steari*, compared to the congeneric intertidal *Alvania mediterranea*. Supported by ecological and palaeontological observations in the Azores and Madeira archipelagos, our study provides the first phylogenetic approach to this biogeographical hypothesis, unravelling the evolution of Rissoiidae in two insular Atlantic systems. In a broader perspective, combining molecular and palaeontological data contributes to better understand past processes that shaped current diversity in Atlantic Archipelagos. This approach can be further replicated in other related non-planktotrophic invertebrates in remote Archipelagos, to corroborate the biogeographical hypothesis in other marine taxa.

Responsible Editor: T. Reusch.

Reviewed by: Undiscovered experts.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00227-019-3536-y>) contains supplementary material, which is available to authorised users.

Keywords: Archipelagos · Biogeography · Evolution · Fossils · Marine invertebrates · Phylogenetics · Remote islands

Introduction
Remote volcanic oceanic archipelagos gather ideal conditions to study evolution in ecosystems formed de novo, as life appeared and thrived in unique conditions of isolation

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Published online: 04 June 2019

Springer

Marine Biology

(2021) 168:9

<https://doi.org/10.1007/s00227-020-03808-5>

ORIGINAL PAPER

Check for updates

Untangling the origin of the newcomer *Phorcus sauciatus* (Mollusca: Gastropoda) in a remote Atlantic archipelago

Lara Baptista^{1,2,3} · António M. Santos^{2,4} · Carlos S. Melo^{1,2,5,6} · Ana C. Rebelo^{1,2,7,8} · Patrícia Madeira^{1,2,9} · Ricardo Cordeiro^{1,2,9} · Andrea Z. Botelho^{1,2} · Ana Hipólito^{6,10} · Joana Pombo^{6,11} · Antje H. L. Voelker^{12,13} · Sérgio P. Ávila^{1,2,3,9}

Received: 21 June 2020 / Accepted: 15 November 2020
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Abstract
The marine topshell *Phorcus sauciatus* is currently found along the temperate-subtropical shores of the Northeast Atlantic Ocean. Although present in the Iberian Peninsula, Madeira, and Canaries for centuries, *P. sauciatus* has only recently reached another oceanic volcanic archipelago in the region. In 2013, a small population was recorded for the first time in Santa Maria Island (Azores), widening its distribution around the entire island and to the neighbouring island of São Miguel in a short period of time. The success of such colonization of the remote archipelago by *P. sauciatus* still awaits an explanation. To better understand the population dynamics of the species in the NE Atlantic Ocean, we used a molecular approach to evaluate the genetic structure of *P. sauciatus* aiming at the determination of a potential origin for the first individuals that reached the Azores. On the foundations of detailed oceanographic, palaeontological, and ecological data, we discuss the impact of climate change as a trigger for colonization of remote oceanic islands and suggest a mechanism that might explain the long-distance dispersal of the non-planktotrophic gastropod *P. sauciatus* across important biogeographical barriers in the NE Atlantic.

Responsible editor: A. Zhan.

Reviewed by: Y. Lin and J. Sun.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00227-020-03808-5>.

Keywords: Archipelagos · Biogeography · Evolution · Fossils · Marine invertebrates · Phylogenetics · Remote islands

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Published online: 04 January 2021

Springer

Baptista et al. BMC Ecology and Evolution

(2021) 21:28

<https://doi.org/10.1186/s12862-021-01862-1>

BMC Ecology and Evolution

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Dispersal ability, habitat characteristics, and sea-surface circulation shape population structure of *Cingula trifasciata* (Gastropoda: Rissoiidae) in the remote Azores Archipelago

L. Baptista^{1,2,3,4*}, S. P. Ávila^{2,3,4,5}, A. M. Santos^{4,6} and M. Curto^{1,7}

Abstract
Background: In the marine realm, dispersal ability is among the major factors shaping the distribution of species. In the Northeast Atlantic Ocean, the Azores Archipelago is home to a multitude of marine invertebrates which, despite their dispersal limitations, maintain gene flow among distant populations, with complex evolutionary and biogeographic implications. The mechanisms and factors underlying the population dynamics and genetic structure of non-planktotrophic gastropods within the Azores Archipelago and related mainland populations are still poorly understood. The Rissoid *Cingula trifasciata* is herewith studied to clarify its population structure in the Northeast Atlantic Ocean and factors shaping it, with a special focus in intra-archipelagic dynamics.
Results: Coupling microsatellite genotyping by amplicon sequencing (SSR-GBAS) and mitochondrial datasets, our results suggest the differentiation between insular and continental populations of *Cingula trifasciata*, supporting previously raised classification issues and detecting potential cryptic diversity. The finding of connectivity between widely separated populations was startling. In unique ways, dispersal ability, habitat type, and small-scale oceanographic currents appear to be the key drivers of *C. trifasciata* population structure in the remote Azores Archipelago. Dispersal as non-planktotrophic larvae is unlikely, but its small-size adults easily engage in rafting. Although the typical habitat of *C. trifasciata*, with low hydrodynamics, reduces the likelihood of rafting, individuals inhabiting algal mats are more prone to dispersal. Sea-surface circulation might create dispersal pathways for rafts, even between widely separated populations/islands.
Conclusions: Our results show that gene flow of a marine non-planktotrophic gastropod within a remote archipelago can reveal unanticipated patterns, such as the understanding of life in such areas is far from well-understood. We expect this work to be the starting of the application of SSR-GBAS in other non-model marine invertebrates, providing insights on their population dynamics at distinct geographical scales and on hidden diversity. How transversal is the role played by the complex interaction between functional traits, ecological features, and sea-surface circulation in the population structure of marine invertebrates can be further addressed by expanding this approach to more taxa.
Keywords: Rissoiidae, *Cingula trifasciata*, Population structure, SSR-GBAS, Speciation

Responsible Editor: T. Reusch.

Reviewed by: Undiscovered experts.

Electronic supplementary material The online version of this article (<https://doi.org/10.1186/s12862-021-01862-1>) contains supplementary material, which is available to authorised users.

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Published online: 04 June 2019

Springer

Baptista et al. BMC Ecology and Evolution

(2022) 22:128

<https://doi.org/10.1186/s12862-022-02080-2>

BMC Ecology and Evolution

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Morphospecies and molecular diversity of 'lace corals': the genus *Reteporella* (Bryozoa: Cheilostomatida) in the central North Atlantic Azores Archipelago

Lara Baptista^{1,2,3,4*}, Björn Berning^{1,3,6}, Manuel Curto^{1,7}, Andrea Waeschbach⁸, Harald Meimberg⁵, António M. Santos^{4,9} and Sérgio P. Ávila^{1,2,3,10}

Abstract
Background As in most bryozoans, taxonomy and systematics of species in the genus *Reteporella* Busk, 1884 (family Philidoporidae) has hitherto almost exclusively been based on morphological characters. From the central North Atlantic Azores Archipelago, nine *Reteporella* species have historically been reported, none of which have as yet been revised. Aiming to characterise the diversity and biogeographic distribution of Azorean *Reteporella* species, phylogenetic reconstructions were conducted on a dataset of 103 Azorean *Reteporella* specimens, based on the markers cytochrome C oxidase subunit 1, small and large ribosomal RNA subunits. Morphological identification was based on scanning electron microscopy and complemented the molecular inferences.
Results Our results reveal two genetically distinct Azorean *Reteporella* clades, paraphyletic to eastern Atlantic and Mediterranean taxa. Moreover, an overall concordance between morphological and molecular species can be shown, and the actual bryozoan diversity in the Azores is greater than previously acknowledged as the dataset comprises three historically reported species and four putative new taxa, all of which are likely to be endemic. The inclusion of Mediterranean *Reteporella* specimens also revealed new species in the Adriatic and Ligurian Seas, whilst the inclusion of additional philidoporida taxa hints at the non-monophyly of the genus *Reteporella*.
Conclusion Being the first detailed genetic study on the genus *Reteporella*, the high divergence levels inferred within the genus *Reteporella* and family Philidoporidae calls for the need of further revision. Nevertheless, the overall concordance between morphospecies and COI data suggest the potential adequacy of a 3% cut-off to distinguish *Reteporella* species. The discovery of new species in the remote Azores Archipelago as well as in the well-studied Mediterranean Sea indicates a general underestimation of bryozoan diversity. This study constitutes yet another example of the importance of integrative taxonomical approaches on understudied taxa, contributing to cataloguing genetic and morphological diversity.
Keywords: Biodiversity, Biogeography, Integrative taxonomy, Oceanic islands, Phylogeny

Responsible Editor: A. Zhan.

Reviewed by: Y. Lin and J. Sun.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1186/s12862-022-02080-2>.

Keywords: Archipelagos · Biogeography · Evolution · Fossils · Marine invertebrates · Phylogenetics · Remote islands

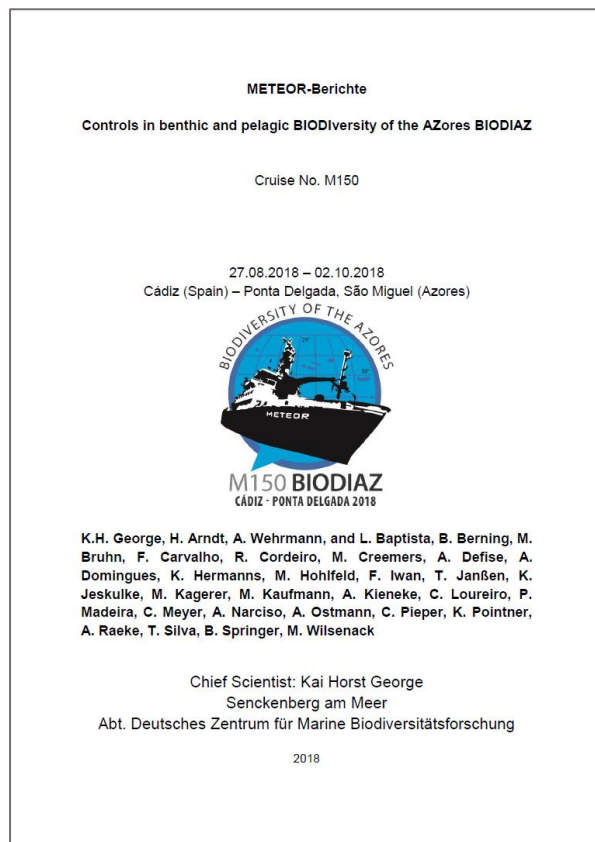
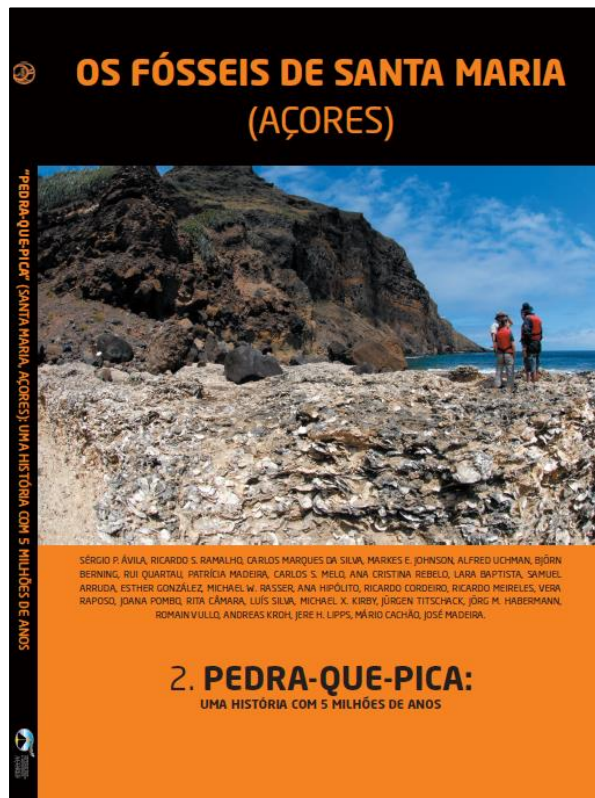
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Published online: 04 January 2021

Springer

Pliocene and late Pleistocene (MIS 5e) decapod crustaceans from Santa Maria Island (Azores Archipelago: Central Atlantic): systematics, palaeoecology and palaeobiogeography

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Revised 14 September 2020; Accepted 4 October 2020¹⁷Researcher in July 2020; Revised 14 September 2020; Accepted 4 October 2020¹⁸Researcher in July 2020; Revised 14 September 2020; Accepted 4 October 2020¹⁹Researcher in July 2020; Revised 14 September 2020; Accepted 4 October 2020²⁰Researcher in July 2020; Revised 14 September 2020; Accepted 4 October 2020²¹Researcher in July 2020; Revised 14 September 2020; Accepted 4 October 2020²²Researcher in July 2020; Revised 14 September 2020; Accepted 4 October 2020²³Researcher in July 2020; Revised 14 September 2020; Accepted 4 October 2020²⁴Researcher in July 2020; Revised 14 September 2020; Accepted 4 October 2020²⁵Researcher in July 2020; Revised 14 September 2020; Accepted 4 October 2020²⁶Researcher in July 2020; Revised 14 September 2020; Accepted 4 October 2020²⁷Researcher in July 2020; Revised 14 September 2020; Accepted 4 October 2020²⁸Researcher in July 2020; Revised 14 September 2020; Accepted 4 October 2020²⁹Researcher in July 2020; 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Appendix C

Other relevant activities during the Ph.D.

Ph.D. grant awarded by **Fundação para a Ciência e Tecnologia, I.P.** (ref. [SFRH/BD/135918/2018](#))

Conference and workshop presentations

2022

Baptista L, Berning B, Curto M, Waeschenbach A, Meimberg H, Santos AM, Ávila SP. *Morphospecies and molecular diversity of Reteporella in the Azores*. In Programme and Abstract Volume, p. 13. 19th International Bryozoology Association Conference (Dublin, Ireland).

Baptista L. *Factors shaping the biogeography of shallow water marine invertebrates*. 1st International Workshop Palaeontology in Atlantic Islands: Cabo Verde (Santiago, Cabo Verde)

Baptista L, Meimberg H, Ávila SP., Santos AM, Curto M. *Connectivity of the Azorean populations of Cingula trifasciata (Gastropoda: Rissoidae)*. 5th Spring Seminar – Biodiversity and Islands BIOISLE (Ponta Delgada, Azores, Portugal; online)

Baptista L, Berning B, Curto M, Waeschenbach A, Meimberg H, Santos AM, Ávila SP. *The bryozoan genus Reteporella: morphospecies and contrasting population structure inferred from mitochondrial and microsatellite data*. In Program and Abstracts, p. 2. 23rd Annual Meeting of the Society of Biological Systematics (online)

Baptista L, Berning B, Waeschenbach AW, Curto M, Meimberg H, Santos AM, Ávila SP. *Morphospecies and genetic diversity of Bryozoa collected during M150 BIODIAZ in the remote Azores Archipelago*. In: *Status Conference Research Vessels 2022*. Schriftenreihe Projektträger Jülich 15: 351–354. (poster)

George KH, Bruhn M, Iwan F, Janßen T, Jeskulke K, Kagerer M, Kieneke A, Ostmann A, Richter K, Springer B, Wehrmann A, Wilsenack M, Arndt H, Hermanns K, Hohlfeld M, Meyer C, Kaufmann M, Ávila SP, Loureiro C, Madeira P, Pieper C, **Baptista L**, Berning B, Carvalho F, Creemers M, Defise A, Silva T, Narciso A & Xavier JR. In: *Status Conference Research Vessels 2022*. Schriftenreihe Projektträger Jülich 15: 75–82. *Controls in benthic and pelagic BIODiversity of the AZores – BIODIAZ*. (poster)

2021

Baptista L. *Settlement and diversification of marine invertebrates in Macaronesia*. 1st Workshop of Marine Paleontology and Littoral Geology (Fuerteventura, Canaries, Spain).

Baptista L. *Studying the genetics of bryozoans and gastropods in the Azores*. 2^o Ciência em Zonas Costeiras: a plataforma costeira das Lajes do Pico e a Fajã da Caldeira do Santo Cristo (São Jorge and Pico Islands, Azores, Portugal)

Baptista L, Berning B, Curto M, Santos AM, Meimberg H, Ávila SP. *Estimating the diversity of Reteporella species in the Azores combining molecular and morphological data*. In: Programme and Abstracts, pp. 8. Australarwood X/17th Larwood Meeting “Bryozoans of the World” (online)

Madeira P, Melo C, Rebelo AC, **Baptista L**, Arruda S, Ávila SP. *Palaeobiodiversity of Santa Maria Island (Azores, North Atlantic): from nature to digital*. IUCN World Conservation Congress: Forum Digital tools for empowering citizen science-based conservation (online)

Baptista L, Santos AM, Melo C, Rebelo AC, Madeira P, Cordeiro R, Botelho AZ, Hipólito A, Pombo J, Voelker AHL, Ávila SP. *A viagem de Phorcus sauciatus (Mollusca: Gastropoda) para o remoto arquipélago dos Açores*. In: – Biodiversidade e Ilhas: Livro de Resumos, pp. 37. 4th Spring Seminar – Biodiversity and Islands; BIOISLE Webinar (online)

Baptista L, Santos AM, Melo C, Rebelo AC, Madeira P, Cordeiro R, Botelho AZ, Hipólito A, Pombo J, Voelker AHL, Ávila SP. *The journey of Phorcus sauciatus to the remote Azores Archipelago*. In: Book of Abstracts, pp. 20. BIODIV Annual Meeting (online)

2020

Baptista L. *The story of rissoids in the Azores Archipelago*. Ciência em Zonas Costeiras: a plataforma costeira das Lajes do Pico e a Fajã da Caldeira do Santo Cristo (São Jorge and Pico Islands, Azores, Portugal)

2019

Baptista L, Berning B, Santos AM, Curto M, Melo C, Ávila SP. *Bryozoa in isolated volcanic oceanic islands: evaluating their evolution and population dynamics in the Azores Archipelago*. In: Abstract Volume, pp. 9. 18th International Bryozoology Association Conference (Liberec, Czech Republic)

Baptista L, Santos AM, Curto M, Berning B, Ávila SP. *Understanding the biogeographical patterns of marine invertebrates in the remote Azores Archipelago and surrounding NE Atlantic Ocean*. In: Congress Programme, pp. 24. Congreso de Jóvenes Investigadores del Mar (Malaga, Spain)

Baptista L. *The genetic structure in populations of marine invertebrates in remote islands: A study of bryozoans and gastropods in the Azores (NE Atlantic)*. Student Seminar CIBIO-InBIO (Vairão, Portugal)

Baptista L. *Insights on biogeographical analyses: the role of genetic approaches*. 15th International Workshop Palaeontology in Atlantic Islands (Vila do Porto, Azores, Portugal)

Baptista L, Santos AM, Curto M, Berning B, Ávila SP. *"The genetic structure in populations of marine organisms in remote islands: a study of bryozoans and gastropods in the Azores (NE Atlantic Ocean)" – An overview*. In: Book of Abstracts, pp. 46. BIODIV Annual Meeting (Vairão, Portugal) ([poster](#))

Invited communications**2022**

A biodiversidade é mais do que os nossos olhos veem. "Cientista Regressa à Escola" (Vila do Porto, Azores, Portugal). Native Scientist program to promote scientific literacy with Elementary School Students (10 yo)

Reconstructing the phylogeny and biogeography of the bryozoan family Phidoloporidae. NHM Science Seminars, (Natural History Museum of London, online)

Studying the biogeographical patterns of marine invertebrates in the Azores. Quintas do MARE, (University of Lisbon, online)

2021

A viagem dos invertebrados marinhos dos Açores (Pitch). Noite Europeia dos Investigadores (Vila do Porto, Azores, Portugal)

Organization of scientific events

2022 Azores Summer School in Marine Island (Palaeo)Biogeography, 6-17 Jul (Santa Maria, Azores, Portugal; M3.3.B/ORG.R.C./008/2022/EDIÇÃO 1)

18th International Workshop "Palaeontology in Atlantic Islands", 7-15 Jul (Santa Maria, Azores, Portugal)

3rd "Ciência nas Zonas Costeiras", 18-21 Jul (São Jorge, Azores, Portugal)

"A Ciência deu à costa nas Lajes do Pico", 22-28 Aug (Pico, Azores Portugal).

2021 17th International Workshop "Palaeontology in Atlantic Islands", 15-19 Aug (Santa Maria, Azores, Portugal; M3.3.B/ 002/2021)

2nd "Ciência nas Zonas Costeiras: a plataforma costeira das Lajes do Pico e a Fajã da Caldeira de Santo Cristo (São Jorge)", 20-30 Aug (Pico and São Jorge, Azores, Portugal; M3.3.B/ORG.R.C./002/2021).

2020 16th International Workshop "Palaeontology in Atlantic Islands", 13-19 Sep (Santa Maria, Azores, Portugal; M3.3.B/ORG.R.C./016/2020)

"Ciência nas Zonas Costeiras: a plataforma costeira das Lajes do Pico e a Fajã da Caldeira de Santo Cristo (São Jorge)", 2-12 Sep (Pico and São Jorge, Azores, Portugal; M3.3.B/ORG.R.C./016/2020).

2019 15th International Workshop "Palaeontology in Atlantic Islands", 7-12 Jul (Santa Maria, Azores, Portugal; M3.3.B/ORG.R.C./034/2019).

Chair of the Evolution and Ecology Session, 18th International Bryozoology Association Conference (Liberec, Czech Republic).

Research Supervision

2022: Titouan Seimandi. *Morphological and genetic diversity of shallow-water bryozoans on algal substrata in the Azores (central North Atlantic)*. Report for CMI IEN, HLSE309 - validation of professional experience, Facult des Sciences de l'Université de Montpellier, France (co-supervision with Björn Berning)

Field expeditions

- 2022 1st International Workshop “Paleontology in Atlantic Islands” – Cabo Verde, 13-29 Jun (Maio, Santiago and Sal Islands, Cabo Verde)
18th International Workshop “Palaeontology in Atlantic Islands”, 7-15 Jul (Santa Maria, Azores, Portugal)
3rd “Ciência nas Zonas Costeiras”, 18-21 Jul (São Jorge, Azores, Portugal)
- 2021 17th International Workshop “Palaeontology in Atlantic Islands”, 15-19 Aug (Santa Maria, Azores, Portugal)
2nd “Ciência nas Zonas Costeiras: a plataforma costeira das Lajes do Pico e a Fajã da Caldeira de Santo Cristo (São Jorge)”, 20-30 Aug (Pico and São Jorge, Azores, Portugal).
1st Workshop of Marine Paleontology and Littoral Geology, 6-14 Nov (Fuerteventura, Canaries, Spain)
- 2020 16th International Workshop “Palaeontology in Atlantic Islands”, 13-19 Sep (Santa Maria, Azores, Portugal)
“Ciência nas Zonas Costeiras: a plataforma costeira das Lajes do Pico e a Fajã da Caldeira de Santo Cristo (São Jorge)”, 2-12 Sep (Pico and São Jorge, Azores, Portugal).
- 2019 15th International Workshop “Palaeontology in Atlantic Islands”, 7-12 Jul (Santa Maria, Azores, Portugal).

Participation in research projects

- 2022-2023 PO Azores **Portal da Biodiversidade dos Açores** – M1.1.A/INFRAEST CIENT/001/2022, Direcção Regional da Ciência e Tecnologia do Governo Regional dos Açores; Fundação Gaspar Frutuoso (PI: Paulo Borges) (<https://azoresbiportal.uac.pt/pt/equipa-actual/>)
- 2021-2024 Cursos Intensivos de Verão, 2021-2024, PRO-SCIENTIA (PI: Luís A. Silva)
- 2021-2023 **MPDb** - Macaronesian Palaeobiodiversity Infrastructure - M1.1.A/INFRAEST CIENT/A/001/2021 (PI: Patrícia Madeira)
- 2019-2023 **VRPROTO** – Virtual-Reality PROTOtype: the geological history of “Pedra-que-pica” (ACORES-01-0145-FEDER-000078), PO Azores 2020 (PI: Carlos Melo)
- 2019-2021 **PORBIOTA** – AZORESBIOPORTAL (ACORES-01-0145-FEDER-000072), PO Azores 2020 (PI: Paulo Borges)
- 2018 Research cruise RV METEOR 150 (Cruise application “MerMet 17-11 George”), Controls in benthic and pelagic BIODiversity of the Azores: **BIODIAZ** (PI: Kai Horst George)

AWARDS and DISTINCTIONS

- 2022 Best Oral Presentation (PhD Student) at the 19th IBA Conference (Dublin, Ireland)
Roger Bamber Award by the Porcupine Marine Natural History Society (995£)
- 2021 Synthesys+ project “Reconstructing the phylogeny and biogeographical history of phidolopod bryozoans”, Natural History Museum London (grant ref. GB-TAF-037; 5906.86 €)
- 2019 Full financial support to attend the OTGA Training course: Research Data Management, by the UNESCO/IOC Project Office for IODE (Oostende, Belgium) (~1200 €)