High Altitude Phylogeography of Selected Moroccan Herpetofauna

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não vão sós,
não nos deixam sós.
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levam um pouco de nós."

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RESUMO

Quantificar a biodiversidade é muito importante para entender a história evolutiva do nosso planeta mas especialmente para tentar diminuir ou mesmo inverter a perda de diversidade que enfrentamos. A delimitação de espécies, além de ser um tema controverso, tem elevada importância, uma vez que a espécie é a unidade base em áreas como ecologia, biogeografia e evolução, e tem sérias implicações na conservação.
Os sistemas montanhosos, ricos em endemismos e com reduzido fluxo génico entre habitats isolados são, geralmente, pouco conhecidos e as suas espécies são particularmente sensíveis a flutuações climáticas. Isto deve-se a uma baixa tolerância a variações de temperatura e altitude, que podem restringir a sua capacidade de persistência.

Os objectivos específicos desta tese visaram contribuir para o aumento de conhecimento dos répteis de altitude de Marrocos. O que incluiu estudar a sua distribuição, níveis de diversidade genética, morfológica e ecológica e a relação evolutiva entre os diferentes grupos estudados. Com este trabalho pretendemos contribuir para o conhecimento da história evolutiva e diversidade dos répteis endémicos de altitude, não só de Marrocos mas também investigar em que medida é que os padrões observados neste estudo refletem o que se tem observado noutras regiões.

Numa primeira fase, as distribuições conhecidas para as espécies de *Quedenfeldtia* e *Atlantolacerta* foram ampliadas e algumas questões sobre a distribuição e requerimentos ecológicos das duas espécies de *Quedenfeldtia* foram clarificadas.
Numa segunda fase, identificou-se elevados níveis de diversidade genética nas duas espécies especialistas de altitude, *Quedenfeldtia trachyblepharus* e *Atlantolacerta andreanskyi* acompanhados de baixos níveis de variação morfológica. Esta diversidade críptica é concordante com resultados obtidos em outros répteis do Norte de África e em répteis Europeus de altitude. No entanto, os padrões de elevada diversidade encontrados identificam-se mais com os dos "paleoendémicos" observados nas montanhas de África do Sul. Especificamente no caso do género *Atlantolacerta*, quase todas as populações amostradas demonstraram ser linhagens isoladas, tendo sido proposta a classificação de seis diferentes espécies neste trabalho.
A análise de três espécies de *Chalcides* resultou num padrão complexo que põe em causa a atual taxonomia. Este resultado põe igualmente em causa a taxonomia de varias outras espécies de *Chalcides,* baseada principalmente em padrões de coloração.
Finalmente, este estudo reforça a importância do conhecimento da diversidade críptica, que aumenta o conhecimento da diversidade, dos processos de especiação e merece especial

atenção no planeamento da conservação. Além disso, o uso de uma taxonomia integrativa é avidamente aconselhada, tendo em conta contudo, que os processos de especiação são complexos e que diferentes tipos de variação não são adquiridos necessariamente ao mesmo tempo nem na mesma ordem. Os resultados inesperados obtidos na filogenia dos *Chalcides* realçam as limitações e potenciais problemas do uso de um único caractere em taxonomia.

Estes resultados salientam a importância do uso de múltiplas ferramentas no estudo da biodiversidade e dos processos evolucionários que lhe dão origem. Além disto, o estudo de taxa com um habitat limitado, isolado e de difícil acesso é importante para o conhecimento da biodiversidade real. A surpreendente biodiversidade observada nos répteis de Marrocos é, provavelmente, um exemplo da realidade em muitos outros habitats semelhantes, alertando para a necessidade do estudo dos mesmos.

ABSTRACT

Quantifying biodiversity is very important to understand the evolutionary history of life on Earth but specially to try to slow down or even reverse the loss of diversity that we are facing. Delimiting species, despite being a controversial issue, is of major importance since species are the basic units in areas such as ecology, biogeography and evolution and has serious implications for conservation biology.

Montane systems, with high levels of endemisms and reduced gene flow between isolated habitats are, generally, poorly known and their species are particularly sensitive to climatic flutuations. This sensitivity issue is due to the fact that their small window of tolerances to temperature and elevation ranges can restrict their ability to persist. The specific goals of this thesis aimed to increase the knowledge about high altitude reptiles from Morocco. This included to study their distribution, levels of genetic, morphological and ecological diversity and the evolutionary relations between related taxa. With all this we intended to contribute to the knowledge about evolution history and diversity of high altitude endemic reptiles, not just from Morocco but also to see how patterns observed here reflect the patterns observed in other geographic regions.

First, the known distributions of *Quedenfeldtia* and *Atlantolacerta* species were extended and some questions about the distributions and ecological requirements of the two *Quedenfeldtia* species were clarified.

Secondly, high levels of genetic diversity were identified in the two high altitude specialists' species, *Q. trachyblepharus* and *Atlantolacerta andreanskyi* accompanied by low levels of morphological variation. This cryptic diversity is concordant with previous results obtained for other North African reptiles, and high altitude reptiles from Europe. However the high diversity patterns found in these species are more likely to the "paleoendemics" found in southern African Mountains. Specifically in *Atlatolacerta* genus almost all sampled populations were demonstrated to be different isolated lineages, being proposed to be classified as six different species.

The analyses of the three *Chalcides* species recovered a complex pattern of relationship that questions the current taxonomy. Furthermore, the present results question the taxonomy of various *Chalcides* species that is based mostly on colour patterns.

Finally, this study reinforces the importance of assessing cryptic diversity as this provides information on diversity and speciation processes deserving special consideration in conservation planning. Additionally, the use of an integrative taxonomy is highly recommended, however, it is always necessary to bear in mind that, speciation is a complex process and different kinds of variation are not achieved necessarily at the same time or order.

The unexpected results obtained in *Chalcides* species highlight the limitation and potential problems of using only one character in taxonomy.

These results highlighted the importance of the use of multiple tools in the study of biodiversity and the evolutionary processes that gives rise. Additionally, the study of taxa with a limited and isolated habitat of difficult access is important to be aware of the real biodiversity. The amazing biodiversity observed in Moroccan reptiles, probably, is an example of what happen in several other similar habitats.

RESUMÉ

La quantification de la diversité est très importante pour comprendre l'histoire évolutive de notre planète mais surtout pour ralentir, voire inverser, l'actuelle perte de la diversité. La délimitation des espèces, en dépit d'être une question controversée, est d'une importance majeure car les espèces sont les unités de base dans des domaines tels que l'écologie, biogéographie et l'évolution et à de graves conséquences pour la biologie de la conservation.
Les systèmes de montagne, avec des niveaux élevés d' endémismes et le flux génétique réduit entre habitats isolés, sont généralement mal connus; de plus les espèces sont particulièrement sensibles aux fluctuations climatiques à cause de leur étroite fenêtre de tolérances aux fluctuations de température (comme une élevation) qui peut limiter leur capacité à persister.

Les objectifs spécifiques de cette thèse étaient d'augmenter notre connaissance sur les reptiles de haute altitude du Maroc. Cela comprenait la distribution, la diversité génétique, morphologique et écologique et les relations entre taxons apparentés. Nous avions l'intention par ce travail de contribuer à la connaissance de l'histoire de l'évolution et de la diversité de reptiles endémiques de haute altitude, non seulement du Maroc mais aussi voir comment les tendances observées ici sont reflétées dans d'autres régions géographiques.

Tout d'abord, les distributions connues des espèces *Quedenfeldtia* et *Atlantolacerta* ont été élargies et les quelques doutes sur les distributions et les exigences écologiques des deux espèces de *Quedenfeldtia* ont été clarifiées.
Deuxièmement, la diversité génétique élevée a été estimée sur les espèces spécialisées de haute altitude *Q. trachyblepharus* et *Atlantolacerta andreanskyi* accompagnée d'un faible niveau de variation morphologique. Cette diversité cryptique est concordante avec les résultats antérieurs obtenus pour les reptiles d'Afrique du Nord et de haute altitude en Europe. Cependant, ces patrons de diversité élevée sont plus susceptibles d'être "paleoendemics", comme celui (ceux) trouvés dans les montagnes de l'Afrique du Sud. Plus précisément, dans les populations d' *Atlatolacerta*, la quasi-totalité de l'échantillon appartenaient à différentes lignées isolées, qui ont été classés comme six espèces différentes.
Les analyses des trois espèces de *Chalcides* montrent une relation complexe et floue qui remettent en question la taxonomie actuelle des *Chalcides* qui est basée la plupart du temps sur les modèles de couleur.
Finalement, les résultats de cette étude renforcent l'importance d'évaluer la diversité cryptique car ils fournissent des informations sur la diversité et les processus de spéciation et méritent une attention particulière pour la planification de la conservation. En outre, l'utilisation d'une taxonomie integrée est extrêmement recommandé, cependant, il est toujours

nécessaire de rappeler que la spéciation est un processus complexe et différents types de variation ne sont pas atteints en même temps ou ordre. Les résultats inattendus obtenus chez les espèces *Chalcides* mettent en évidence le problème de la seule utilisation de seul caractère en matière de taxonomie.

Ces résultats mettent en évidence l'importance de l'utilisation de plusieurs outils dans l'étude de la biodiversité et les processus évolutifs qui lui donne lieu. En outre, l'étude des taxons dans un habitat limité et isolé, à l'accès difficile, est important pour connaître la biodiversité réelle. L'incroyable biodiversité observée chez les reptiles du Maroc, sans doute, est un exemple de ce qui se passe dans plusieurs autres habitats similaires.

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CHAPTER 1

GENERAL INTRODUCTION

Mafalda Barata, High Atlas, Morocco, 2009

"No one definition has as yet satisfied all naturalists; yet every naturalist knows vaguely what he means when he speaks of a species."

Darwin (1809/1882)

1.1. Quantifying Biodiversity - Species Definition and Delimitation

1.1.1. Quantifying Biodiversity

Human beings always felt the need of understanding the processes that rules everything around them, and this is the main reason for the evolution of any kind of Science.

The classification of biodiversity dates back at least to the 1700s when Linnaeus (1707-1778) developed a system of naming, ranking and classifying organisms, which is still in use today, his *Systema Naturae*. After Aristoteles classification of all known organisms in two groups, Kingdoms Plantae and Animalia, Linnaeus created the basis of the modern scientific systematics and his ideas on classification still influence all biologists even the ones that disagree with the roots of his ideas. On the other hand, the establishment of the fundamental ideas of evolutionary biology took place in 1859, with the publication of Darwin's book "On the Origin of Species", even though some of the ideas were older.

Nowadays, the loss of biodiversity, most of it driven by human activities (habitat destruction, pollution and introduction of exotic species), is increasing (Begon *et al.* 2006), and this fact weighs more than the simple curiosity in the need of knowledge. The attempt of slowing down the loss of biodiversity and reverse the processes that lead to extinctions, highlight the necessity of quantifying diversity accurately. According to Myers (2000), biodiversity hotspots are defined as areas containing exceptional concentrations of endemic species and facing exceptional loss of habitats. Following this definition, the identification of those areas is the first and most important step to prevent biodiversity loss (Myers 2003). Recently, Mittermeier *et al.* (2004) updated to 34 (Fig. 1), the initial hotspot list of Conservation International (CI) that identified 25 terrestrial areas of the world for priority conservation.

Nowadays, the knowledge of biodiversity remains unsatisfactory due to Linnean and Wallacean shortfalls or, in other words, there are many species that have not been formally described, and geographical distributions of most species are still poorly understood and usually contain many gaps (Whittaker *et al.* 2005).

1.1.2. The Problem of Species Concept

Species are the fundamental basic units for studies of ecology, evolution, systematic and conservation biology (Wiens 1999). However the seed of the discussion about their definition was growing long before Darwin (Britton 1908; Wilkins 2009), and even now it has not reached a general consensus (reviewed by Mayden 1997; de Queiroz 1998; Harrison 1998). Darwin's book "On the origin of species" (1859) further heated the discussion about the species concept, bringing uncertainty for some biologists due to Darwin's explanation of the

evolutionary process as a gradual process, but without giving details about how one species gives rise to two (Bailey 1896).

Figure 1. The 34 hotspots identified by Mittermeier *et al.* (2004) (Conservation International, 2005).

Therefore all species definitions are, in some way incomplete, since they are static concepts that attempt to define a continuous process that occurs in very different organisms in completely diverse habitats. Some of the most used are:

Biological species concept (Dobzhansky 1935; Mayr 1942; 1963): has historically been the most widely used and accepted definition. Ernest Mayr defined species as "groups of actually or potentially, interbreeding natural populations that are reproductively isolated from other such groups." This definition allows the existence of subspecies.

Ecological species concept (Vanvalen 1976; Ridley 1993): Ridley defined species as a “group of organisms that is exploiting or adapted to a set of resources - niche”. This group evolves separately from all other groups outside its range (Andersson 1990).

Evolutionary species concept (Simpson 1961; Wiley 1978; Templeton 1989): Wiley defined species as a “single lineage of ancestor-descendant populations, which maintain its identity from other such lineages and which has its own evolutionary tendencies and historical fate”

Cohesion species concept (Templeton 1989): “A species is the most inclusive group of organisms having the potential for genetic and/or demographic exchangeability”.

Phylogenetic species concept (Cracraft 1983; 1989): "species is an irreducible (basal) cluster of organisms, diagnosable distinct from other such clusters, and within which there is a parental pattern of ancestry and descent". It implies monophyly (commonly inferred from possession of shared derived character states), exclusive coalescence of alleles (all alleles of a given gene descend from a common ancestral allele not shared with those of other species) and diagnosability of qualitative, fixed differences according to different authors (de Queiroz 1998).

Cracraft (2002) argue that the question "what is a species?" remains the most important of the "seven great questions of systematic biology", which is true especially because after clarifying this question, biologists can more easily focus on the problem of species delimitation. According to de Queiroz (2007), one of the main problems is that the issue of species delimitation has long been confused with the definition of species itself, leading to disagreement in the methods to define boundaries and numbers of species. The same author (de Queiroz 2007) defends that this problem is not as "serious as it appears", because despite the differences between the several species concepts, they have an essential conceptual agreement which provides the basis for a "unified species concept". This common element associates species to separately evolving metapopulation lineages, or more specifically, an ancestor-descendent series (Simpson 1961; Hull 1980). de Queiroz (2007) finalizes this *unified species concept* saying that a species is not an entire metapopulation lineage but only a segment of that lineage in a way that species derive from other species. The only property needed to delimit species would be detecting a segment of a metapopulation lineage evolving separately (de Queiroz 1998). Other evidences mentioned in the several different species concepts, like reproductive isolation, reciprocal monophyly, phenetic distinguishability or occupation of a distinct niche or adaptive zone will be useful lines of evidence important to determine species delimitation but fail to be determinants of a species existence (de Queiroz 2007).

1.1.3. Species Delimitation

Defining the species boundaries and describing new ones is important to various biological fields including biogeography, ecology, evolutionary biology, and conservation (Sites and Marshall 2003; Agapow 2005). Assuming the general metapopulation lineage species concept explained above (section 1.1.2), speciation involves lineage separation and divergence that can lead to reproductive isolation, ecological divergence, morphological distinctness and reciprocal monophyly (de Queiroz 1998; Harrison 1998). These criteria were and still are the ones used by systematists as evidence to delimit species, and these criteria can arise at different times and in different order during lineage formation processes (de Queiroz 2007).

Species delimitation becomes extremely difficult, especially when any of the criteria are not achieved, but this is expected in recent or adaptive radiations, as speciation is a continuous process (Wake 2006).

The principal advantage of the separation of species concept from species delimitations criteria is to release the species delimitation studies from the controversy that is surrounding the species concept. Consequently, such studies can concentrate on investigating all evidence relevant to the recognition of evolutionary independent metapopulations lineages (de Queiroz 2007).

According to Knowles and Carstens (2007) species lineages can be delimited long before reciprocal monophyly is achieved as several recent works have shown that gene genealogies give information about the history of species split (gene trees), despite the presence of incomplete lineage sorting (Degnan and Salter 2005; Maddison and Knowles 2006; Carstens and Knowles 2007). Various authors agree that a substantial amount of time is needed for observing reciprocal monophyly after the initial divergence of species (Hudson and Coyne 2002; Hudson and Turelli 2003).

The use of one or two mitochondrial genes to reconstruct the "species tree" has been largely criticized due to the differences found between gene trees and species trees (Maddison 1997; Degnan and Rosenberg 2009; Edwards 2009). The absence of recombination that makes this molecule so attractive to perform phylogenetic research is also one of its most important limitations, differing from the species tree not only due to stochastic processes of lineage sorting but also due to events of hybridization and introgression. Furthermore, this molecule only reflects the evolution of female lineage, that can differ from the species history (Rosenberg and Nordborg 2002 see examples in Shaw 2002). Back in 1988, Pamilo and Nei suggested that it was better to combine information from different loci than to add more samples, but the Bayesian methods based on the coalescent theory makes possible the use of the gene trees to construct a species tree instead of simply concatenating all the information (Heled and Drummond 2010). New emerging methods to delimit species based on the coalescent theory are changing the species delimitation field. These methods use recent theoretical models that combine gene trees and species phylogenies from multilocus sequence data. The methodology starts from a sample of genes and trace backwards in time to infer the demographic history from the population since the most recent common ancestor of the sampled genes. Several recent works used this approach to determine species boundaries and identify new lineages (Heled and Drummond 2010; Leaché and Fujita 2010; Yang and Rannala 2010).

The recent conceptual advances in integrative taxonomy (Padial *et al.* 2010) support that the approach to deal with the problem of delimiting species is the use of multiple and complementary disciplines for a consistent identification of species. The use of multiple lines

of evidence, using different data types and diagnostic methods to get the most relevant information is achieving a consensus between biologists (Sites and Marshall 2004; de Queiroz 2007; Knowles and Carstens 2007; Leache *et al.* 2009). However, some uncertainty in the species boundaries of recent lineages may remain due to incomplete separation, secondary introgression, sampling deficiencies, or disagreement of criteria. Furthermore, the degree of congruence that different characters must show to consider a population or a group of populations as a separate species, splits integrative taxonomists (Padial *et al.* 2010).

Following these points of reasoning, to better understand the processes involved in species delimitation requires a model with particular characteristics. Firstly, distinct sources of data (for example, morphological and molecular data) must be obtainable. Reptiles are ideal for this kind of studies, since the alpha taxonomy, although complex is not particularly challenging, they can be found in large numbers, and genetic markers are widely available (see the review in Camargo *et al.* 2010). Additionally, preferably, the geographic locality should be discreet so that variation can be assessed across the range of the model organisms. Islands are often employed for this purpose, as "natural laboratories" to study evolution. Analogously, in this thesis, selected taxa of reptiles from Mountains have been used as model organisms to study the evolution history and diversity of the reptiles living on those conditions. Mountains share many of the advantages that make islands attractive settings for evolutionary studies – as Mayr (1967) states, "islands may demonstrate certain biological phenomena almost with the clarity of test-tube experiments; indeed every island is an experiment on its own" and mountains can be considered, for their lizard fauna, as islands.

1.2. Mountains as Centres of Speciation

Mountains, like most other isolated areas, are generally species deprived but rich in endemisms, as is typical of islands (Darwin 1859). Isolation is the key feature that makes these habitats so attractive models for evolutionary and ecological studies (Emerson 2002).

A variety of mechanisms could have produced montane restrictions in non-volant species such as reptiles. Mountains in northern latitudes, which were especially affected by the climatic fluctuations of the Pleistocene, are likely to have relatively recent fauna, possibly having been colonized by cold-tolerant fauna from lower regions that moved into the mountains during warmer periods. Moreover, the formation of temporary corridors of suitable habitat may have allowed pre-adapted mountain forms to invade new habitats. In other areas, lineages may have simply adapted into montane forms during the orogenesis of the mountain system. In this case, the geological age of the mountains will be correlated with the age of the species. Alternatively, mountain taxa may have been restricted or even displaced by other taxa in nearby lowlands and due to this developed into mountain specialists (Carranza *et al.* 2004). These different mechanisms will imprint identifiable characteristics in the genetic

make-up of the populations. However, it is possible that during colder climates, such as those that existed during glacial periods of the Pleistocene, these populations extended to lower elevations and more continuous ranges permitted gene flow between populations that were otherwise isolated by regions of unsuitable habitat (Wiens 2004). Nevertheless, high mountain populations may have persisted in separate refugia, resulting in high levels of genetic, and perhaps phenotypic, divergence (Garcia-Paris *et al.* 2000; Bowie *et al.* 2006; Cadena *et al.* 2007).

High altitude specialist species are particularly sensitive to climatic flutuations due to the fact that their small window of tolerances to temperature and elevation ranges can restrict their ability to persist in, or disperse across, different habitats (Janzen 1967; Ghalambor *et al.* 2006; Deutsch *et al.* 2008; McCain 2009). As many of the species that inhabit mountain biodiversity hotspots are endemic to a single mountain, or limited number of adjacent mountains they face higher levels of extinction risk (Gifford and Kozak 2011).

The mountains around the Mediterranean basin (Fig. 2), such as the Pyrenees, the Alps, the Balkans and Rhodope Mountains and the Atlas Mountains, have been glaciated on several occasions through the Quaternary (Hughes *et al.* 2006). Due to this fact and to its location at the interface between the North Atlantic Ocean, the western Mediterranean Sea and the Sahara Desert (Hughes 2008), the Atlas Mountains are an especially interesting setting for evolutionary studies.

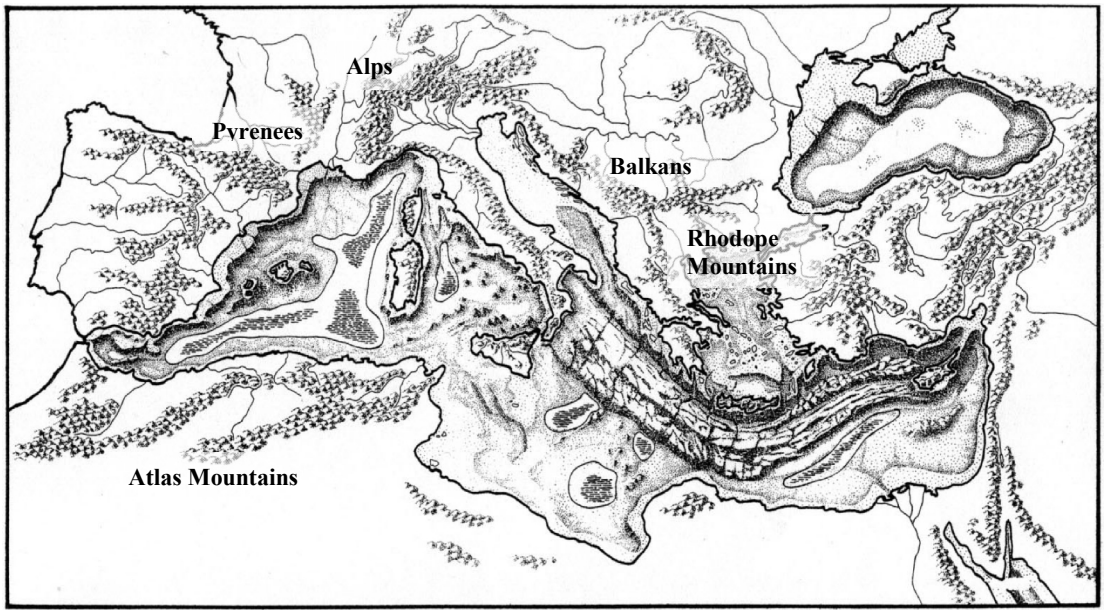

Figure 2. Mediterranean Basin desiccation representation (6 Ma) and mountain chains from Hsu *et al.* (1973).

1.3. Study Area: The Atlas Mountains, Morocco

1.3.1. Morocco

North Africa is a model region for phylogeographic studies of Montane fauna. Morocco (Fig. 3) is in the northwest extreme of the African continent and includes an area of 710 850 Km^2, with the Sahara Desert occupying half of it. Morocco together with Algeria and Tunisia form an area known as the Maghreb. The geology, weather, relief, fauna and flora make this area very different from the remaining continent and, in various aspects, more similar to Southern Europe. Morocco suffers the influence of the Mediterranean Sea, Atlantic Ocean and the Sahara climate. All these characteristics and diversity are responsible for the enormous richness and high level of endemism found in the herpetofauna of Morocco. Of the 104 species that constitute the herpetofauna, 22 are endemic. This makes Morocco the richest country in the Occidental Paleartic area in terms of herpetofauna (Bons and Geniez 1996). These combine to mean that the area contains considerable diversity but has definable boundaries and includes various features that can be used as calibration points to try to date phylogeographic breaks.

During the mid-Tertiary, the collision between the Eurasia and Africa plates resulted in a diverse, complex and unusual geographic and topographical scenario. A great diversity of confined climates arose and during the mid-Pliocene to Pleistocene this region was repeatedly affected by alternated humid and arid phases (Le Houerou 1997). The principal vegetation in Morocco are the forests, distributed in the Tingitana Peninsula, Rif, Middle Atlas, Beni Snassen, the plateaux of Debdou and Jerada, some parts of the North face of the High Atlas and Jebel Sirwa, the Souss plain and the occidental extreme of the Anti-Atlas. The principal tree species are *Quercus ilex, Tretraclinis articulata, Argania spinosa, Quercus suber, Juniperus* sp*., Cedrus libanotica atlântica, Pinus pinaster* and *Abies maroccana.* Other areas are, generally, covered by shrubs (Bons and Geniez 1996).

1.3.2. The Atlas Mountains

The Atlas Mountains are a mountain range that reaches 2500 Km through Morocco, Algeria and Tunisia. In Morocco, the Atlas Mountains are divided in three main mountains belts, Middle Atlas (max. 3340 m a.s.l.), High Atlas (max. 4167 m a.s.l.) and Anti-Atlas (max. 2531 m a.s.l.), the last extending in a NE/SW direction. The highest peak is the Toubkal, in the High Atlas, with 4167 m; this and other higher mountains are covered with snow during winter and spring and these mountains constitute a very important reservoir of water, where the most important rivers come from, including the Moulouya River (520 Km), Sebou river (458 Km), Oum-er-Rbia river (555 Km) and Tennsitt river (270 Km) (Schleich *et al.* 1996).

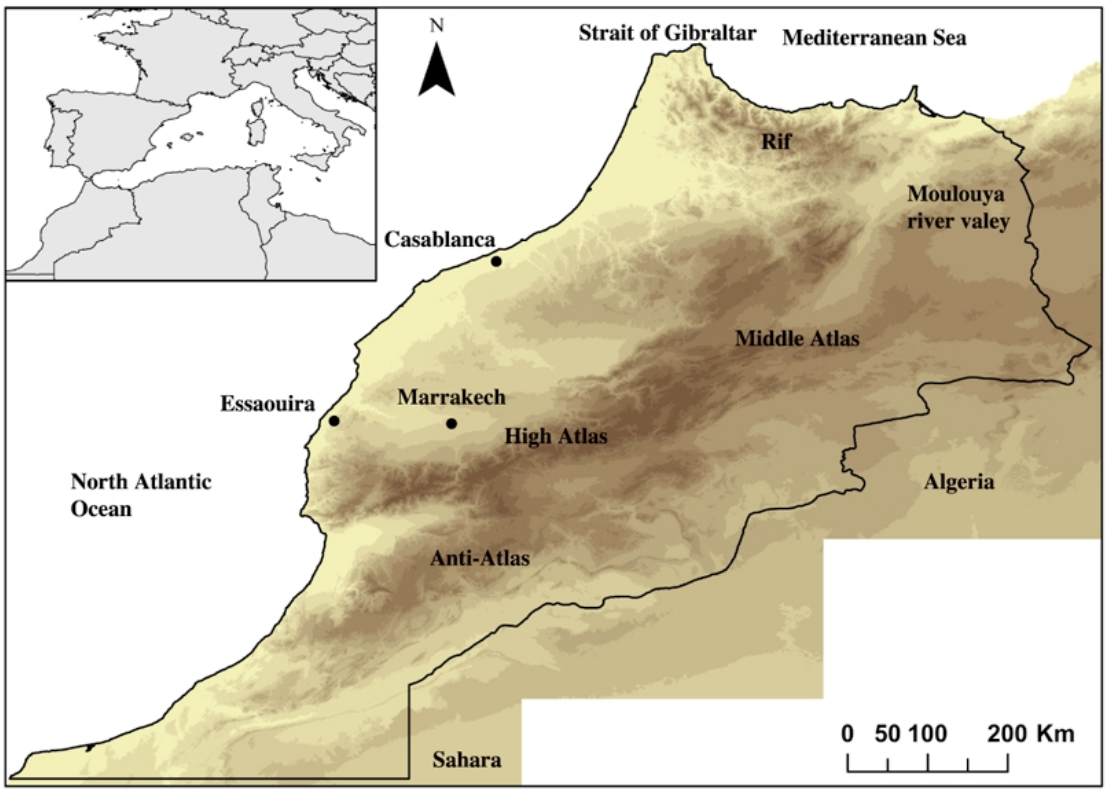

Figure 3. Map of Morocco with the identification of some important features, to frame the study area.

This Mountain range separates Morocco into two bioclimatic regions, acting as a barrier to the dispersal of several Mediterranean species coming from the north, and also to species coming from the Sahara (Bons and Geniez 1996).

The Atlas Mountains were formed in three subsequent events of Earth's history. The first tectonic deformation was in the Paleozoic (around 300 Mya) and involved only the Anti-Atlas formation. The Anti-Atlas chain is believed to have initially been formed as part of Alleghanian orogeny (Hatcher 2008) and as a result of the collision between America and Africa (Fig. 4). At that time, North America was part of the super-continent Euroamerica, while Africa was part of Gondwana. The collision between the two super-continents formed the super-continent Pangaea, which comprised all major continental landmasses.

In the Mesozoic Era (before 65 Mya) a second event took place. This event consisted in an extension of the Earth's crust that rifted and separated the American and African continents. Most of the rocks that form the High Atlas today were deposited under the ocean during this period.

In the Tertiary (between 68 to 1.8 Mya), approximately 35 Mya the landmasses of Europe and Africa collided in the area where is currently the Strait of Gibraltar in the Mesinian, and the mountain chains that today comprises the Atlas uplifted. This tectonic convergence was the responsible for the closure of the Strait of Gibraltar with the consequent elimination of much of the original

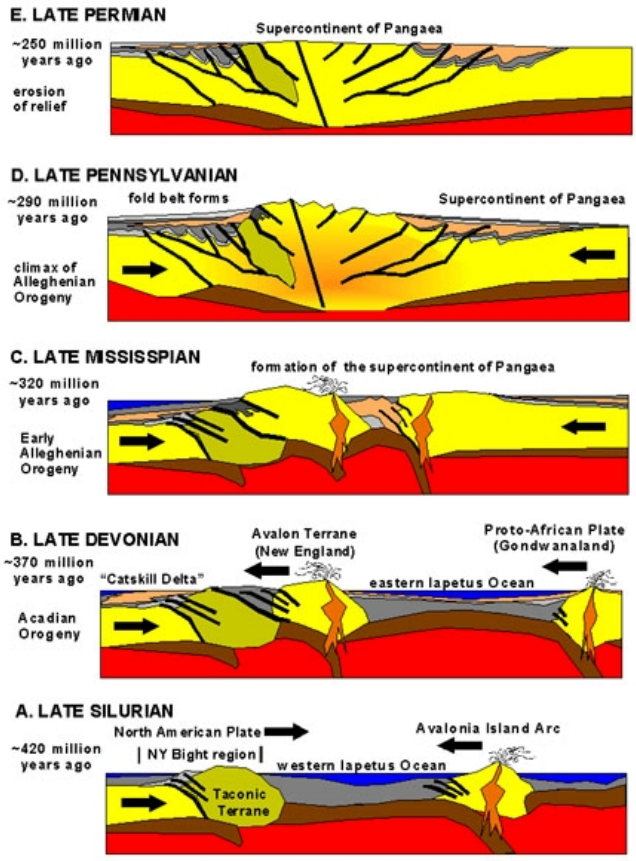

Figure 4. Origin of the Appalachian Mountains, a result of three separate continental collisions involving the North American continent and the collision of the African and North American continents during the Alleghenian Orogeny at the end of the Paleozoic (USGS –United States Geological Survey.)

Tethys and the formation of some of the more than twenty mountains ranges that today encircles the Mediterranean Basin (Fig. 5A), as the High Atlas, Alps and Pyrenees. From roughly 15 Mya ago (Fig. 5B), Africa continued moving northwards but, for the first time in many millions of years, it also started to move westwards clockwise, producing the opening of the Red Sea, and the formation of more mountains in Turkey, Southern Europe and North Africa and slowly the connection between the Mediterranean and the Atlantic Ocean at its western end was closed again (Teixell *et al.* 2005; Missenard *et al.* 2006).

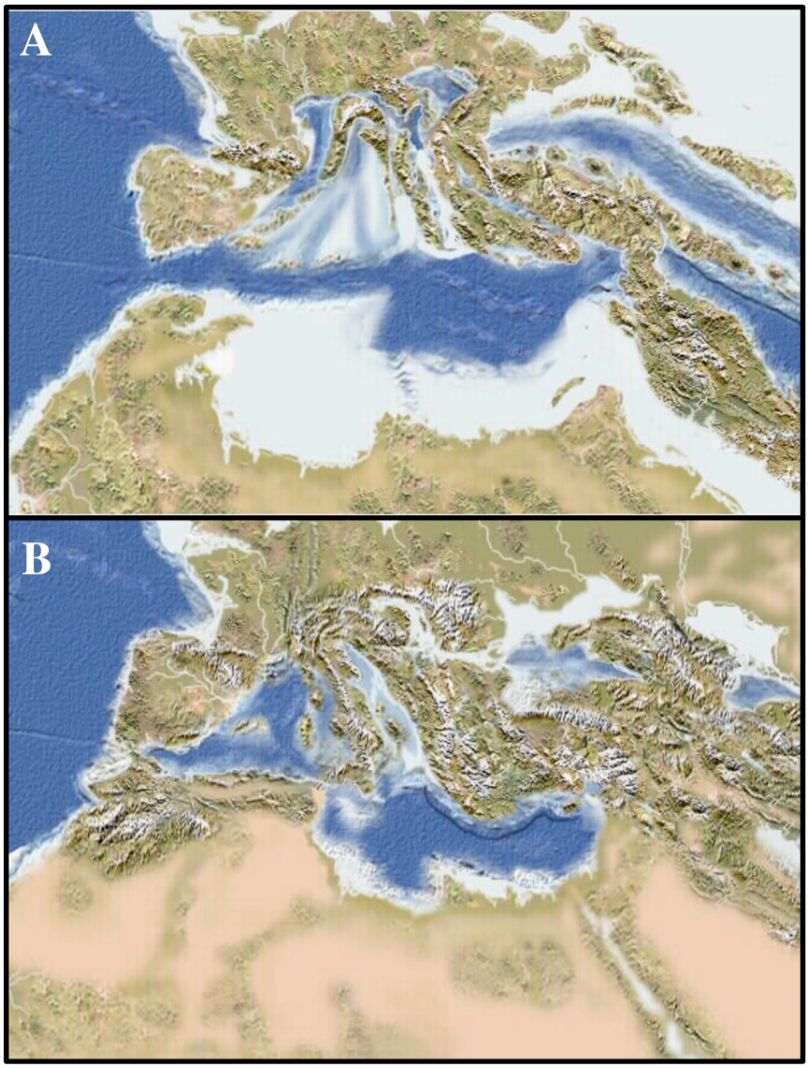

Figure 5. Representation of the movements of European and African continents, around 35 (A) and 15 (B) Mya.

Hsu (1978) estimated that the Atlas orogenesis occurred in the late Miocene, around 9 Mya. However, there is still a lack of evidence about the source of subduction in the Atlas. Ayarza *et al.* (2005) suggested that the uplift of the Middle and High Atlas could be due to processes in the Earth's mantle. Alternatively, Babault *et al*. (2008), based on scattered direct surface evidence, proposed a more recent uplift of the Middle and High Atlas, occurring in the post-Miocene around 7.1 to 5.3 Mya.

The Atlas Mountains, like the other mountains surrounding the Mediterranean Basin have been glaciated several times during the Quaternary. This period of glaciations were very important to the present biodiversity, since the Atlas Mountains like the Southern European Mountains, are thought to have hosted biotic refugia during Pleitocene cold stages (Hughes *et al.* 2006). Unlike Europe, in North Africa the climate change during Quaternary and its impact in species geographical distribution is poorly known, and the few existing studies are

based on pollen records and only show back to the Last Glacial Maximum (LGM), 20 ka, and are further limited to a specific area in the Middle Atlas (Rhoujjati *et al.* 2010). Cheddadi *et al*. (1998) recovered three apparently main climatic phases, the first in the early Holocene (10 to 6.5 ka) warm and dry (22°C July, 4.8°C January and 870 mm precipitation), an intermediate period and a cooler and moist period (20.5°C July, 2.8°C January and 940 mm precipitation). In the Last Glacial Maximum, temperatures were 15°C lower than now, with an average annual precipitation of 300 mm and an arid state condition prevailed (Cheddadi and Bar-Hen 2009). These condition, have probably affected the biodiversity and restricted many species to isolated refugia. After the Last Glacial period, the temperatures increased abruptly, and many data confirmed that the temperatures in the earlier Holocene were warmer than today by 2-3°C (Cheddadi and Bar-Hen 2009). In this same study, they concluded that the cedar (*Cedrus atlantica*) can migrate to high altitudes and become extinct at low altitudes as a response to climate change, and they suggest that the next global warming could be too rapid for them to migrate, which can also be true for other species, even for animals.

In Europe, several species survived the glacial periods in southern refugia and then expanded to the northern areas (Huntley and Birks 1983). This was also a response to the abundance and multiplicity of habitats in the Iberian Peninsula (Hewitt 1999; Hewitt 2000; Hewitt 2001) and is supported by the high level of endemism found in some Iberian organisms (Garcia-Barros *et al.* 2002). European montane herpetofauna tended to survive the last glacial maxima through limited altitudinal range shifts, unlike the classic larger contraction and recolonization patterns observed in lowland species (Mouret *et al.* 2011). On the other hand, places where there was greater climatic buffering through the Pleistocene like the tropical forests of East Africa, present an enormous diversity due to the ancient lineages that stayed isolated since the early Miocene/Oligocene. An example of this are the ancient lineages of East African forest chameleons (Tolley *et al.* 2011).

1.4. Study Group: High Altitude Reptiles from Morocco

In Morocco there are four high altitude reptile species that are not found at lower altitudes; *Quedenfeldtia trachyblepharus*, *Atlantolacerta andreanskyi*, *Chalcides montanus* and *Vipera monticola* (Bons and Geniez 1996), which are all endemic. We also included *Quedenfeldtia moerens* in this study since during our fieldwork it was found until 3000 m (Jebel Awlime), and its distribution and history can be related with *Q. trachyblepharus.* Two of them, *Q. trachyblepharus* and *A. andreanskyi* are restricted to very high altitudes; found only above 2000 m. *Quedenfeldtia moerens*, *C. montanus* and *V. monticola* are also found at slightly lower altitudes in the Middle Atlas. These high altitude species are found exclusively in humid bioclimatic habitats with cold winters from high mountains (Bons and Geniez 1996).

The present work was focused on the study of *Quedenfeldtia* spp., *A. andreanskyi* and three species of *Chalcides*, *C. montanus*, *C. polylepis* and *C. manueli*. Although *C. polylepis* and *C. manueli* are not from high altitude, species are not isolated units and the possibility of introgression between *C. montanus* and other *Chalcides* has previously been raised (Carranza *et al.* 2008). Thus it was necessary to widen the focus for this group. *Vipera monticola* was excluded from this thesis simply due to insufficient number of samples caught during the fieldwork for this study. It is a highly elusive species, and very few specimens have been reported (Fahd *et al.* 2007).

1.4.1. *Quedenfeldtia* spp. (Boettger, 1883)

The genus *Quedenfeldtia* Boettger, 1883 comprises diurnal Geckos, endemic from Morocco (Fig. 6), North Africa. Presently there are two species described for this genus, *Q. trachyblepharus* (Bons 1967) and *Q. moerens* (Arnold 1990), although the genus was for a long time considered monotypic prior to Arnold (1990).

Quedenfeldtia trachyblepharus (Fig. 7) has a restricted range in the High Atlas, and has been found only in mountain areas from 1400 up to above 4000 meters, where there are no other reptiles (Bons and Geniez 1996). It occurs on rocky faces, both near water and in dry places (Schleich *et al.* 1996). Jebel Hadid near Essaouira (on the coast) was described as the type locality for *Q. trachyblepharus,* but Arnold (1990) suggested a labelling error of the holotype collected by C. Von Fritsch and J. J. Rein, because, although it has been actively sought in that area (J. Bons; Hoogmoed 1974), it has never been found there again. Arnold (1990) considers this species more primitive, taking into consideration their anatomy. They have an accentuated sexual dichromatism, males are pale with dark or red spots that are more abundant in flanks and sides of the neck, and females are dark or grey, with darker ocelli with yellow borders forming longitudinal series. Dominant males normally have a reddish or yellowish head more evident during the mating period (Schleich *et al.* 1996).

Quedenfeldtia moerens (Fig. 7) is more widespread, usually found from lowland habitats to 3000 m, and presents little sexual dichromatism. Specimens are frequently quite uniform above with small pale and dark spots, and commonly have one to three dark spot (sometimes more) with yellow borders forming an ocellus, in the shoulder region. These characters imply differences in their behaviour; the pronounced dichromatism in *Q. trachyblepharus* may suggest a different social behaviour, and some differences in anatomy indicate that *Q. moerens* may be a sit-and-wait hunter (Arnold 1990) contrary to *Q. trachyblepharus* that seems to have a more predatory behaviour (Carretero *et al.* 2006). These authors also affirm that *Q. trachyblepharus* is an atypical member of its family, as a result of its high activity and specialized diet. Their primary predator is *Vipera monticola,* that often lives near the rocks populated by *Quedenfeldtia* (Schleich *et al.* 1996).

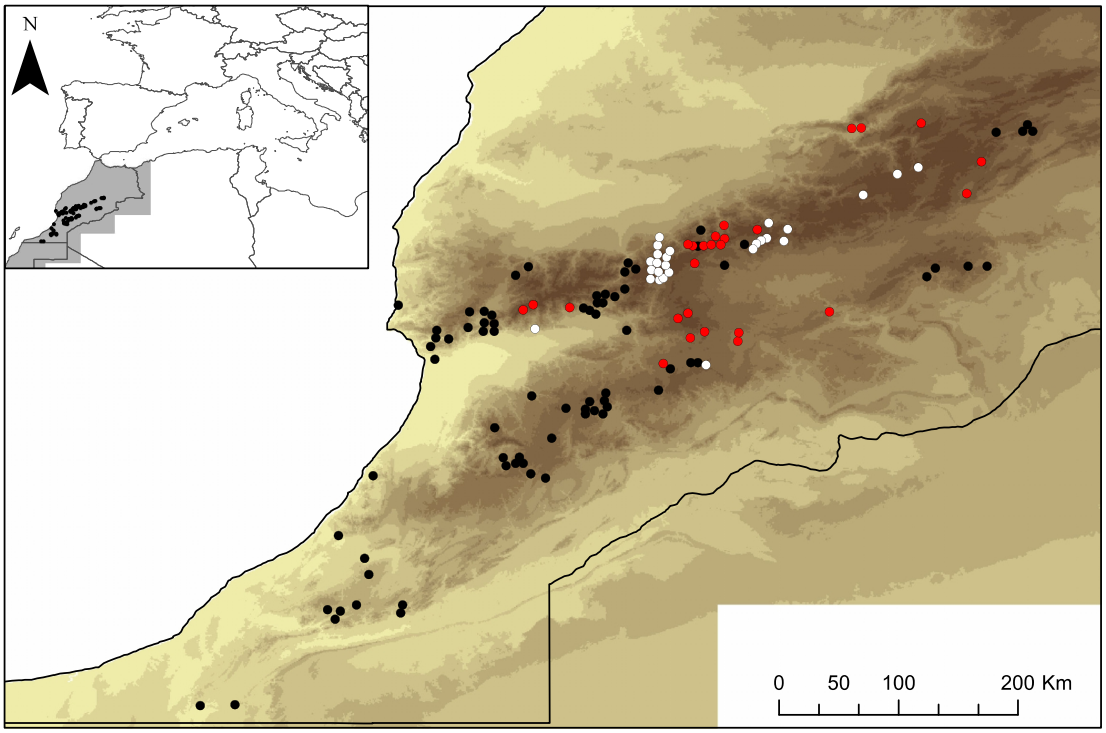

Figure 6. Distribution map of *Quedenfeldtia* species based on Bons and Geniez (1996). Black dots represent *Q. moerens,* white dots represent *Q. trachyblepharus* and the red dots are *Quedenfeldtia* spp.

In 1954, Underwood presented the first detailed study regarding the evolution, systematic and biogeography of geckos (Figure 8), and this work remains the backbone of the current taxonomy. (Gamble *et al.* 2008) presented a new organization to the Gekkota order. Where Eublepharidae, Gekkonidae, Sphaerodactylidae, Diplodactylidae, Carphodactylidae and Pygopodidae are families of the order Gekkota. Sphaerodactylidae comprises the gekos *Coleodacylus*, *Gonatodes, Lepidoblepharis, Pseudogonatodes, Sphaerodactylus, Saurodactylus, Teratoscincus, Quedenfeldtia, Aristellinger, Euleptes* and *Pristurus*. The same authors (Gamble *et al.* 2008) recently published a work where they review the phylogeny of the gecko lizards. This work confirmed some of the previous knowledge about this clade, such as the well-supported monophyly of the Gekkota relatively to outgroups, the Eublepharidae and Gekkonidae as sisters groups, and the basal location of the Diplodactylidae / Carphodactylidae / Pygopodidae (Donnellan *et al.* 1999; Han *et al.* 2004; Townsend *et al.* 2004). In that work the time frame proposed to the divergence between the two species could be as long ago as 15-17 Myr (Fig.9).

Figure 7. Photographs of males from the *Quedenfeldtia* species. *Q. trachyblepharus* (left) and *Q. moerens* (right).

Underwood (1954)
Gekkonoidea
Eublepharidae
Sphaerodactylidae: *Coleodactylus, Gonatodes, Lepidoblepharis, Pseudogonatodes, Sphaerodactylus*
Gekkonidae
Diplodactylinae: *Aristelliger, Saurodactylus, Teratoscincus*
Gekkoninae: *Euleptes*
incertae sedis: Pristurus, Quedenfeldtia

Kluge (1967, 1976)
Gekkonidae
Eublepharinae
Gekkoninae: *Aristelliger, Euleptes, Pristurus, Quedenfeldtia, Saurodactylus, Teratoscincus*
Spaherodactylinae: *Coleodactylus, Gonatodes, Lepidoblepharis, Pseudogonatodes, Sphaerodactylus*
Diplodactylinae
Diplodactylini
Carphodactylini
Pygopodidae
Pygopodinae
Lialisinae

Kluge (1987)
Gekkota
Eublepharoidea
Eublepharidae
Gekkonoidea
Gekkonidae
Gekkoninae
'Ptyodactylini': *Euleptes, Quedenfeldtia, Saurodactylus*
Gekkonini: *Aristelliger*
Sphaerodactylini: *Pristurus, Coleodactylus, Gonatodes, Lepidoblepharis, Pseudogonatodes, Sphaerodactylus*
Teratoscincinae: Teratoscincus
Pygopodidae
Diplodactylinae
Carphodactylini
Diplodactylini
Pygopodinae

Han *et al* (2004)
Gekkota
Eublepharidae
Gekkonidae
Gekkoninae: Aristelliger, *Euleptes, Pristurus, Quedenfeldtia, Saurodactylus, Teratoscincus*
Sphaerodactylinae: *Coleodactylus, Gonatodes, Lepidoblepharis, Pseudogonatodes, Sphaerodactylus*
Diplodactylidae
Carphodactylidae
Pygopodidae

Gamble *et al.* 2008
Gekkota
Eublepharidae
Gekkonidae
Sphaerodactylidae: *Coleodactylus, Gonatodes, Lepidoblepharis, Pseudogonatodes, Euleptes, Sphaerodactylus, Aristelliger, Pristurus, Quedenfeldtia, Saurodactylus, Teratoscincus*
Diplodactylidae
Carphodactylidae
Pygopodidae

Figure 8. Previous and current higher order classification of extant Gekkota (Gamble *et al.* 2008).

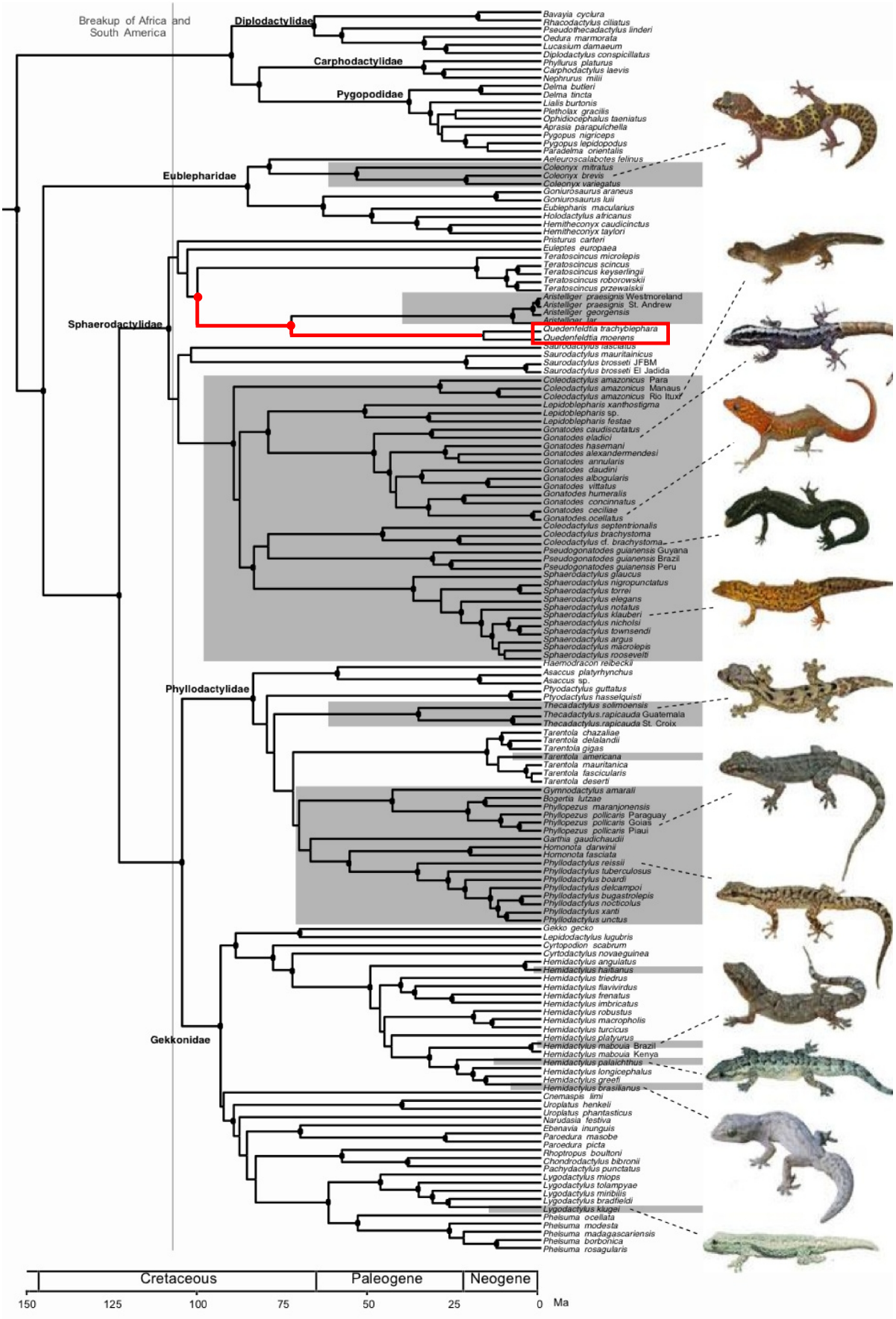

Figure 9. Gecko phylogeny with time-calibrated using a Bayesian uncorrelated relaxed clock from Gamble *et al*. (2010). *Quedenfeldtia* branch are marked in red.

The analyses of Rato and Harris (2008) indicated the paraphyly of *Saurodactylus* with *S. mauritanicus,* and *S. brosseti* closer to *Teratoscincus przewalskii* than to *S. fasciatus*. Based on this fact, they proposed to divide the *Saurodactylus* in two genera, with *mauritanicus* and *brosseti* remaining in *Saurodactylus,* and creating a new monotypic genus to *fasciatus*. Such a finding shows the importance of extensive sampling within other groups, such as *Quedenfeldtia,* to fully assess their phylogenetic relationships.

In general, geckos present high genetic diversity with very conservative morphology (Gamble *et al.* 2008; Rato and Harris 2008; Perera and Harris 2010), recent studies show that two North African genera (*Quedenfeldtia* and *Saurodactylus,* both endemic to Morocco) are basal to the American Sphaerodactylidae family that diverged by vicariance and dispersal events after fragmentation of Gondwana (Gamble *et al.* 2008; Gamble *et al.* 2010). In consequence, such North African endemics as *Quedenfeldtia* are expected to retain high levels of genetic diversity because of their old evolutionary histories (Busack 1986; Rato and Harris 2008).

1.4.2. *Atlantolacerta andreanskyi* (Werner, 1929)

The Atlas Dwarf Lizard, *Atlantolacerta andreanskyi*, is a lacertid lizard endemic from the highest peaks (2400 to 3800 m) of western and central parts of the High Atlas Mountains (Fig. 10) (Bons and Geniez 1996; Schleich *et al.* 1996). They can be found in alpine meadows, scree, amongst boulders, and in areas of thorn cushion vegetation and thickets near small watercourses or plateaux in the top of the mountains that retain some water from rain or snowmelt (Geniez 2005 ; authors personal observation). It is a small lacertid lizard similar to a half-grown *Zootoca vivipara* in size and pattern, with a light brown middorsal region and dark brown flanks. This species does not have accentuated sexual dimorphism: the male' head is relatively larger and the body shorter, its extended foreleg reaches the anterior border of the eye while in females only its posterior border is reached (Schleich *et al.* 1996).

The systematics of *Atlantolacerta andreanskyi* has been historically complex. Initially it was placed in several different genera and subgenera within the Lacertini subtribe, including *Zootoca* (Pasteur and Bons 1960), *Lacerta* part II (Arnold 1973), *Lacerta incertae sedis* (Guillaume 1987), and *Lacerta sensu lato* (Arnold 1989). Later, (Volobouev *et al.* 1990), based on cytochemical methods, suggested that *A. andreanskyi* and *L. vivipera* (within the subgenus *Zootoca*), considered sister species, presented similar patterns. The taxonomic situation was revised when Arnold *et al*. (2007) assigned *andreanskyi* as a basal member of Eremiadini. A reanalysis of the mtDNA dataset of Fu (2000) obtained the same results (Fig 11).

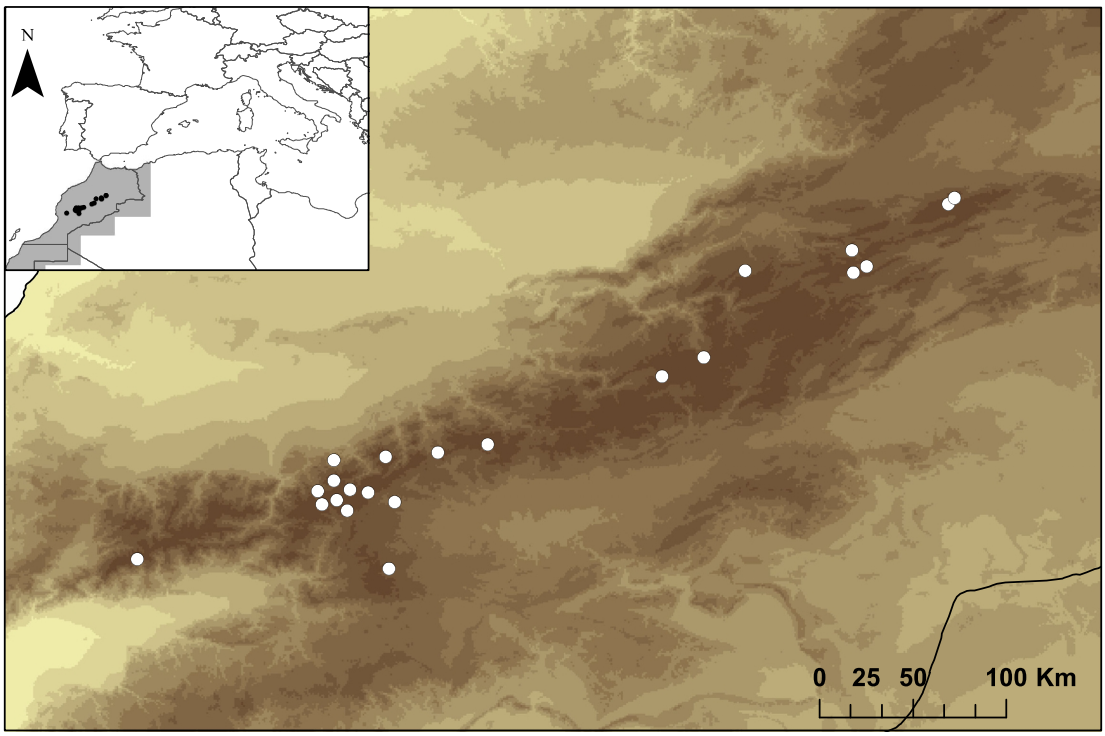

Figure 10. Distribution map of *Atlantolacerta andreanskyi* based on Bons and Geniez (1996).

This position was unexpected given that this species lacks the synapomorphies that characterize most other Eremiadini, namely a derived condition of the ulnar nerve and the presence of a fully developed armature in the hemipenis, which has folded lobes when retracted. Because of its probably basal position, without close relationship to any other genus of Eremiadini and its distinctive morphology, the High Atlas endemic was described as a new monotypic genus: *Atlantolacerta* by (Arnold *et al.* 2007). Besides the basal position, they suggest that *A. andreanskyi* is between 16 and 12 Mya (Fig. 12), an old origin inside the Lacetidae. Pavlicev and Mayer (2009) latter confirmed the phylogenetic relationships and generic status of *A. andreanskyi,* using a combined analysis of mitochondrial and nuclear sequences (C-MOS and RAG1).

The different populations of *A. andreanskyi* present an apparently disjunct distribution (Bons and Geniez 1996; Schleich *et al.* 1996), and this situation is similar to an archipelago, with the different "islands" being represented by mountaintops unconnected due to areas of unsuitable habitat below 2400 m. As a result of this scenario, minimal gene flow is currently expected between the different populations; even though it is not known how the different climatic events occurred during the Miocene and Pleistocene have affected this species. Despite this, some aspects of the biology of *A. andreanskyi* are already well known (Busack 1987; Carretero *et al.* 2006), although all available information comes from only one population, at Oukaimeden. The genetic structure of the different populations, as well as the relationships between them have never been assessed before.

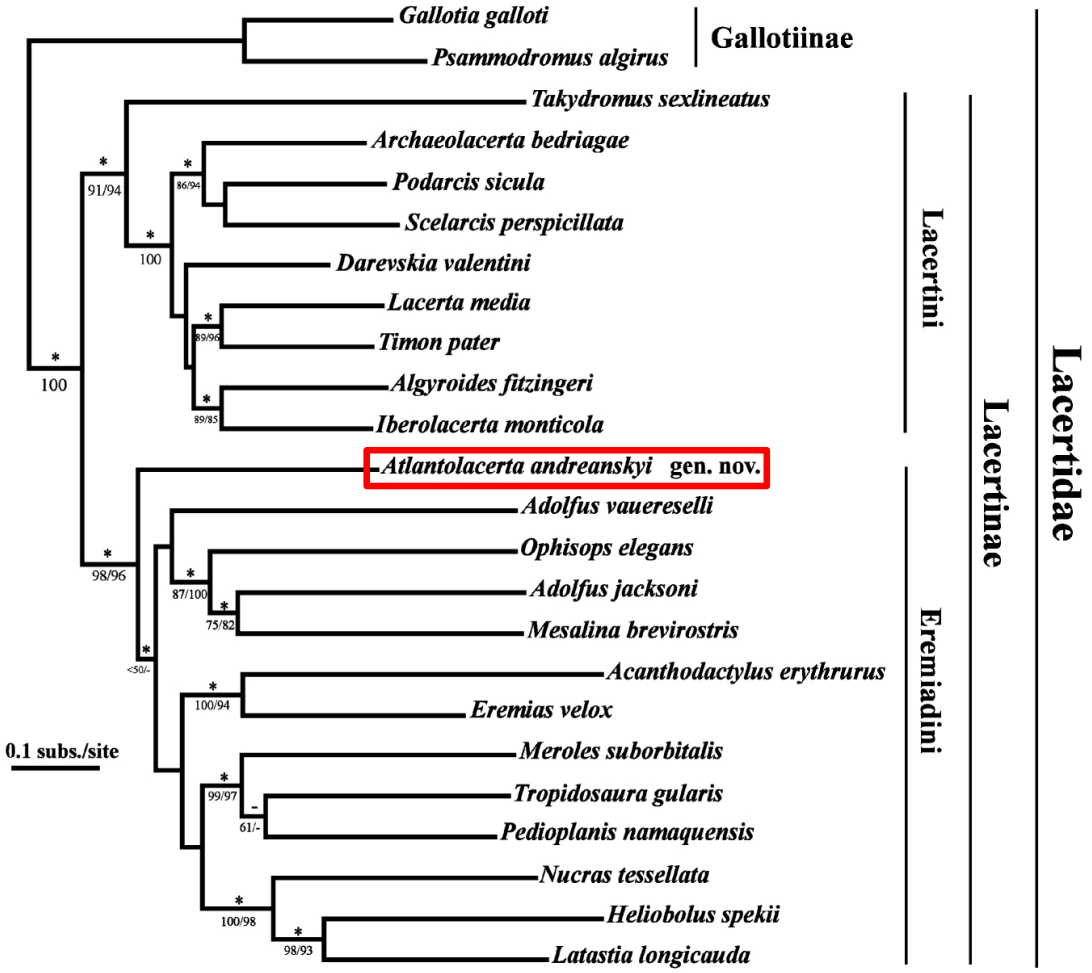

Figure 11. ML tree of a reanalysis of the mtDNA data set of Fu (2000), based on 4522 bp (1026 bp of cytochrome *b*, 1048 bp of cytochrome oxidase I and 2448 bp of the ribosomal genes 12S rRNA + 16S rRNA) (adapted from Arnold *et al.* 2007).

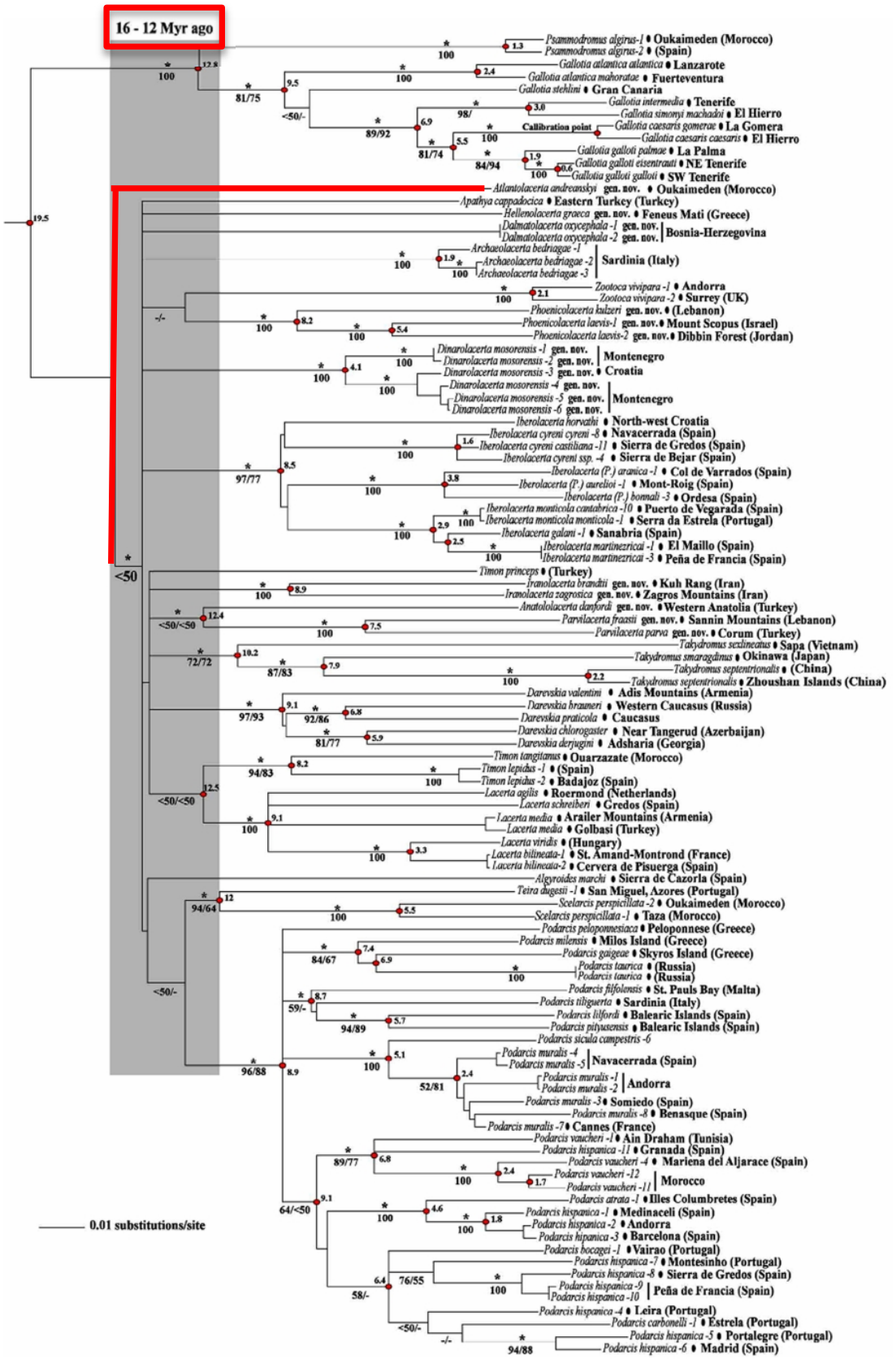

Figure 12. Bayesian phylogenetic tree of the Lacertini based on mitochondrial DNA sequence (cyt*b* and 12S rRNA) adapted from Arnold *et al*. (2007). These analyses support the origin of the Lacertini in the Mid-Miocene (between 12 and 16 Mya). *Atlantolacerta andreanskyi*, a member of the Eremiadini, is sister to the Lacertini.

1.4.3. *Chalcides* spp. (Laurenti 1768)

Chalcides is a genus of the Scincidae family, comprising approximately 24 species with a wide distribution, from Southern Europe, North Africa to Somalia and Kenya, Turkey, Iraq, Arabia, coastal Iran and Pakistan (Carranza *et al.* 2008). Pasteur (1981) suggested that Morocco was an important evolutionary centre for this genus, probably because most of the species occur there and in surrounding areas, including several endemisms.

This genus has a typical elongated body, round in cross sections and limbs short or reduced (Schleich *et al.* 1996). Many of the species are morphologically similar and difficult to identify.

The taxonomy of *Chalcides* has been revised, all or in part, by several authors (Boulenger 1887; 1890; 1896; Boulenger 1920; Lanza 1957; Pasteur 1981; Caputo 1993; Mateo *et al.* 1995; Greenbaum 2005; Greenbaum *et al.* 2006) However it was difficult to estimate a phylogeny based on morphological features and there are still uncertainty in species boundaries and in the relationships between them (Fig. 13; Carranza *et al.* 2008).

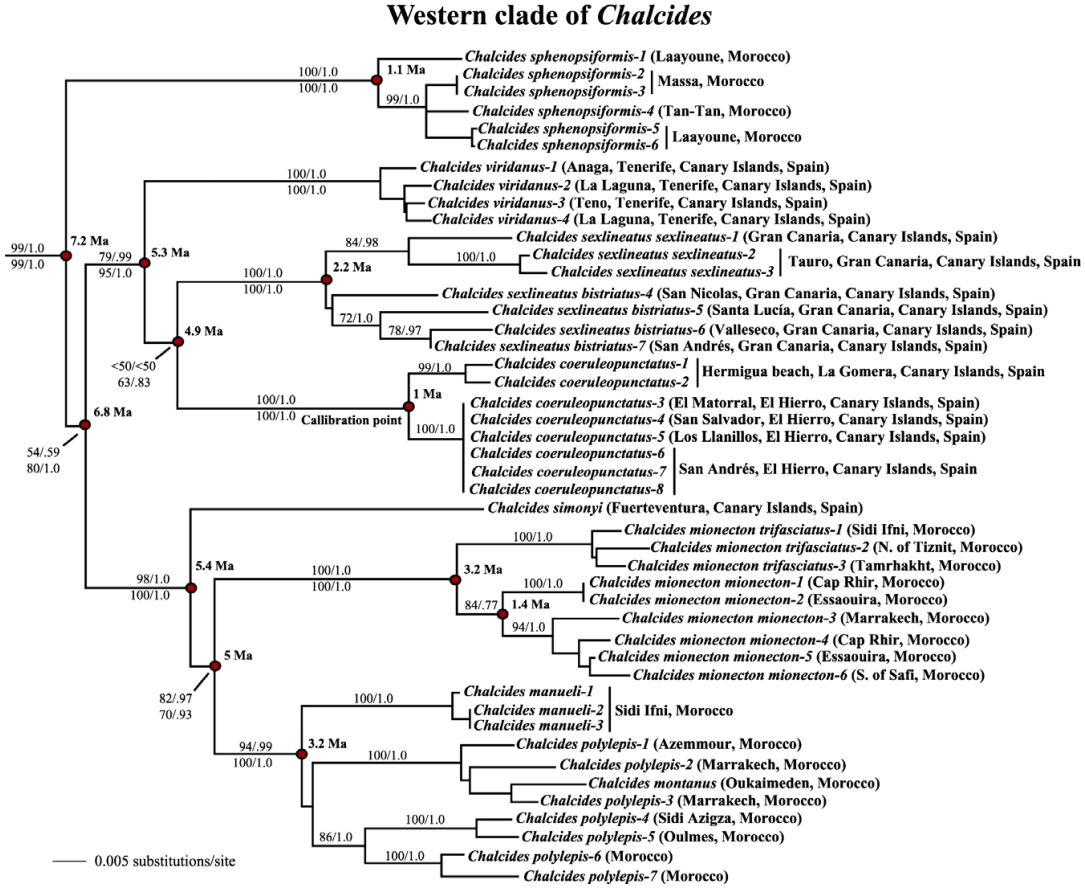

Figure 13. Adapted tree from Carranza *et al*. (2008) showing the detailed phylogenetic relationships in the Western clade. Note that *C. montanus* is nested with a paraphyletic *C. polylepis.*

In this study we focused on the relationships between three endemic *Chalcides* species from Morocco, *Chalcides montanus*, *Chalcides polylepis* and *Chalcides manueli* (distribution shown in Fig. 14).

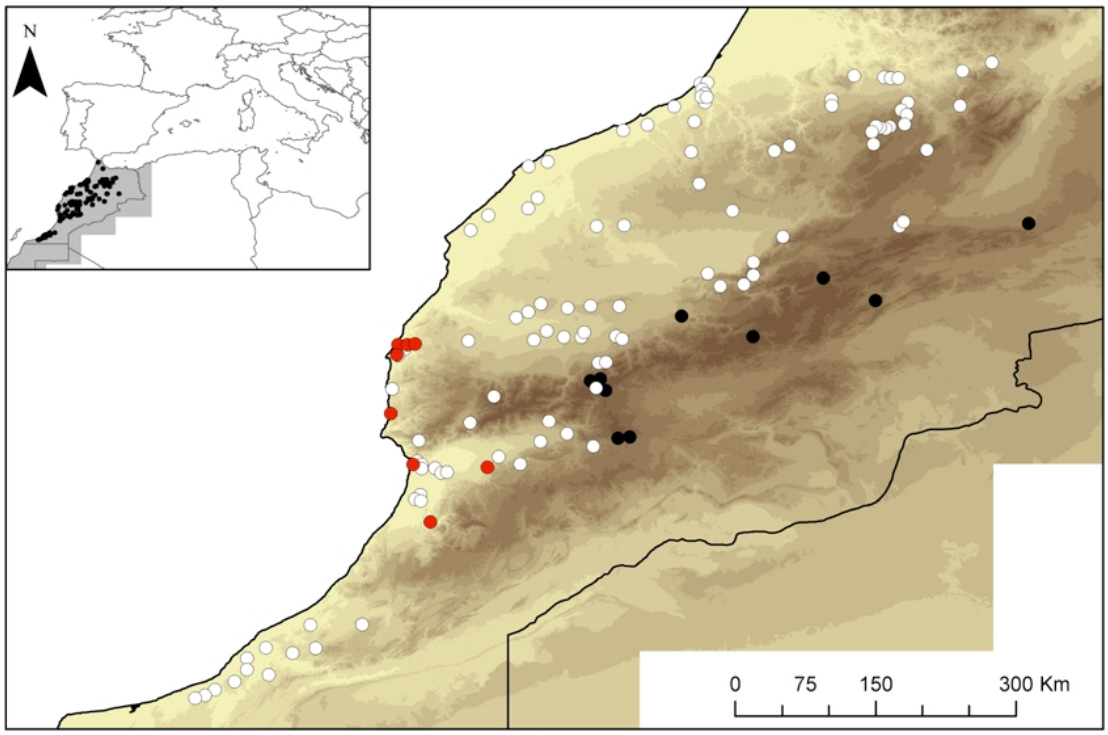

Figure 14. Distribution map of three *Chalcides* species based on Bons and Geniez (1996). Black dots represent *C. montanus*, white dots represent *C. polylepis* and red dots *C. manueli*.

Chalcides montanus Werner, 1931 is the *Chalcides* found at higher altitude (until 2500 m) in North Africa, normally found in cold and humid mountain regions, with low bushes. It is a small *Chalcides* (Fig. 15) with white parallel lines on the neck and a yellow venter. Morphologically it is similar to *C. polylepis* (but smaller), with which it is sympatric (Schleich *et al.* 1996). Before Caputo and Mellado (1992) attributed specie rank to this form, it was considered a subspecies of *Chalcides ocellatus*. *Chalcides lanzai* was long considered a subspecies of *C. montanus* (Schleich *et al.* 1996), but mtDNA confirmed that they are unrelated (Carranza *et al.* 2008).

Figure 15. Photo from a *Chalcides montanus* specimen (photo from Gabriel Martínez).

Chalcides polylepis Boulenger 1990, also similar to *C. ocellatus* is one of the largest of the genus (Fig. 16) with a larger body and head, and ocelli forming parallel lines on the upper side. Unlike *C. montanus*, this species is usually found in hillsides or flat country up to 2000 m (Schleich *et al.* 1996).

In a recent phylogeny, three samples of *C. montanus* from one locality appear within the *C. polylepis* clade, making it paraphyletic (Carranza *et al.* 2008). The authors suggested two possible explanations: *C. montanus* might be just a highland form of *C. polylepis*, with a different altitudinal habitat, or the second possibility is that *C. montanus* mitochondrial DNA was received from *C. polylepis* through introgression. However this issue remained unresolved as Carranza *et al* (2008) used only mtDNA evidence.

Figure 16. Photo from a *Chalcides polylepis* specimen from near Guelmin (photo from Gabriel Martínez).

Chalcides manueli Hediger 1935, is a small *Chalcides* also similar to *C. ocellatus* but without any conspicuous dorsal pattern, see Fig. 17 (Schleich *et al.* 1996). It was known from localities in the coast between Essaouira and Sidi Ifni (Bons and Geniez 1996; Carranza *et al.* 2008). Recently, specimens morphologically identified as *C. montanus* from Jebel Sirwa and

Tizin Tichka where genetically (mtDNA, 12S rRNA) closer to *C. manueli* (Harris *et al.* 2010; Barata *et al.* 2011) from Sidi Ifni and sister taxa to *C. manueli* from Essaouira, confirming that taxonomy and relationships within Moroccan *Chalcides* are complex and in need of revision. This species is listed as vulnerable in the IUCN red list, due to its small range distribution being, in fact, only known for 11 localities (Joger *et al.* 2006).

Figure 17. Photo from *Chalcides manueli* specimen, from Essaouira (photo from Philippe Geniez).

Although this thesis was initially focussed only on high altitude reptile species from Morocco, given the clearly conflicting evidence between morphological characters and mtDNA for these three species of *Chalcides*, sampling was extended so that greater numbers of all three could be included in an improved estimate of phylogenetic relationships, and to assess fully if introgression was indeed taking place.

1.5. Tools and methods

Before molecular techniques where developed, morphological characters were the primary source of information used in the study of taxonomy. Different species were, for a long time, classified based only on different morphological characters (e.g. see Schleich *et al.* 1996). However, use of morphological characters can underestimate species diversity due to cryptic species (Baker and Bradley 2006), species can look very similar or even be identical but are reproductively isolated. Furthermore, unrelated taxa can acquire similar appearance as a consequence of convergent evolution or mimicry. On the other hand, sampling a few genetic markers does not reveal patterns that are identifiable using morphological, behavioural or ecological information, that could be crucial to separate two species (Knowles and Carstens

2007). A classical example is the case of the cichlid fish species from some African Lakes that show large functional diversity but have limited genetic variation. The hybrids are viable and fertile (Seehausen *et al.* 2003). In such situations, morphology is still a powerful tool to study ecology and behaviour and can be used to support the characterization of new species. Recently, another method that is earning the confidence of biologists, especially in ecology but even in species delimitation area, is ecological niche modelling.

1.5.1. Molecular Methods

The methods for delimiting species changed dramatically in the seventies, when molecular techniques become more widespread (Avise *et al.* 1979). Currently, the amount of DNA sequences published is still increasing exponentially, and the software available for analysing this data is also improving (Knowles and Carstens 2007; Kubatko and Degnan 2007). DNA has become the most popular source of data for reconstructing phylogenies (Harris 1999), and even for understanding the mechanisms acting in populations or lineages. However, despite the initial idea that molecular data would readily resolve all phylogenies (Diaz 2007), these new methods, besides keeping old methodological problems, brought new ones, maintaining a constant search for increasing objectivity (Suárez-Díaz and Anaya-Muñoz 2008).

Most early studies relied only mtDNA and although this genome is a very useful tool, it has particular problems. The non-recombinant characteristic can have important limitations that are now widely recognized. The first one is that the analyses of mtDNA correspond to the study of a single locus that reflects the history of that molecule rather that the species history (Zhang and Hewitt 2003). This can be due to the effects of natural selection and introgression. Furthermore, mtDNA only reflects the evolutionary history of the female lineage, which in some situations can be completely different from the population or species history (see Magri *et al.* 2006).

Currently, despite the relative ease of obtaining sequence data from multiple loci, the most appropriate methods used to analyse this data are still debatable. Although some authors argue that standard methods that concatenate multigene data are enough for accurate phylogenetic estimates (Chen and Li 2001; Rokas *et al.* 2003), many studies revealed that gene histories could be different from the species histories (Kolaczkowski and Thornton 2004; Mossel and Vigoda 2005; Kubatko and Degnan 2007). This lack of concordance between gene trees and species trees can result from diverse processes such as coalescence, gene flow, selection, hybridisation, and gene duplication (Maddison 1997; Kubatko and Degnan 2007; Edwards 2009). As a result of this, information from different unlinked genetic markers (mitochondrial and nuclear) is thus necessary for delimiting evolutionary lineages, as well as for establishing phylogenetic relationships.

Apart from that, a widespread problem in the use of mtDNA alone is introgression between different taxa. This event has being observed in several different groups as reptiles (Pinho *et al.* 2008), insects (Zakharov *et al.* 2009), and mammals (Alves *et al.* 2006; Boratynski *et al.* 2011) and can be confounded with ancestral polymorphism, showing how evolutionary inferences can be misrepresented when they are based on single locus.

Bayesian analysis was successively introduced to calculate trees and to test hypothesis (Huelsenbeck and Ronquist 2001; Drummond and Rambaut 2007). Recently, several new methods were developed in order to calculate species tree from multiple loci in a coalescent framework (see Blair and Murphy 2011). The well-known benefits of considering the stochasticity of genetic processes promoted the development of coalescent based approaches (Kubatko and Degnan 2007; Heled and Drummond 2008). Coalescent methods (mentioned before in section 1.1.3) do not uses gene genealogies directly to infer demographic history. Instead, this methods use those genealogies as a nuisance parameter to get estimations of biogeographically informative parameters (Hey and Machado 2003). Instead of focusing on gene trees, this method uses data from multiple loci and multiple specimens from population to estimate the "species tree", an estimate of the history of divergence (Belfiore *et al.* 2008; Brumfield *et al.* 2008). On the other hand, these coalescence-based methods are still inadequately applied in non-model species phylogenies, especially at the interface between populations and species, where the threat of incomplete lineage sorting is greatest (Degnan and Rosenberg 2009), and where lack of prior information about the organisms and surrounding environment is often lacking.

1.5.2. Morphology

Morphology is a branch of science dealing with the form and structure of the organisms and their specific structural descriptions as shape, colour, pattern and structure of internal parts as bones and organs. Most taxa differ morphologically from other taxa but there are exceptions like cryptic species and convergent evolution as mentioned before (section 1.5). For a long time and before molecular techniques were developed, taxonomy was based only on morphological information. There are several fields of morphology, including the study of the patterns of the locus of structures within the body plan of an organism (comparative morphology), the relationship between form and the function (functional morphology), effects of external factors upon the morphology of organisms under experimental condition (experimental morphology), the study of the internal structures of an organism (anatomy), and the external appearance of the organisms (eidonomy).

There are several studies regarding the morphology of reptiles, such as indicators in ecotoxicology (Amaral *et al.* 2012), detection of cryptic species (Kaliontzopoulou *et al.* 2012), parasitology (Carretero *et al.* 2011; Maia *et al.* 2011), geometric morphometrics to

study habitat related patterns (Kaliontzopoulou *et al.* 2010a), sexual dimorphism (Kaliontzopoulou *et al.* 2010b), study of contact zones (Martinez-Freiria *et al.* 2009), and evolution and species delimitation (Arnold 2009).

Focusing on the study of the external structures of the organisms, there are different variables that can be studied and different techniques as well as different statistical analysis to process the data. The measurements of several body parts (Fig. 18), scale counting (Fig. 18) and coloration patterns (Fig. 19) are examples of the most widely used.

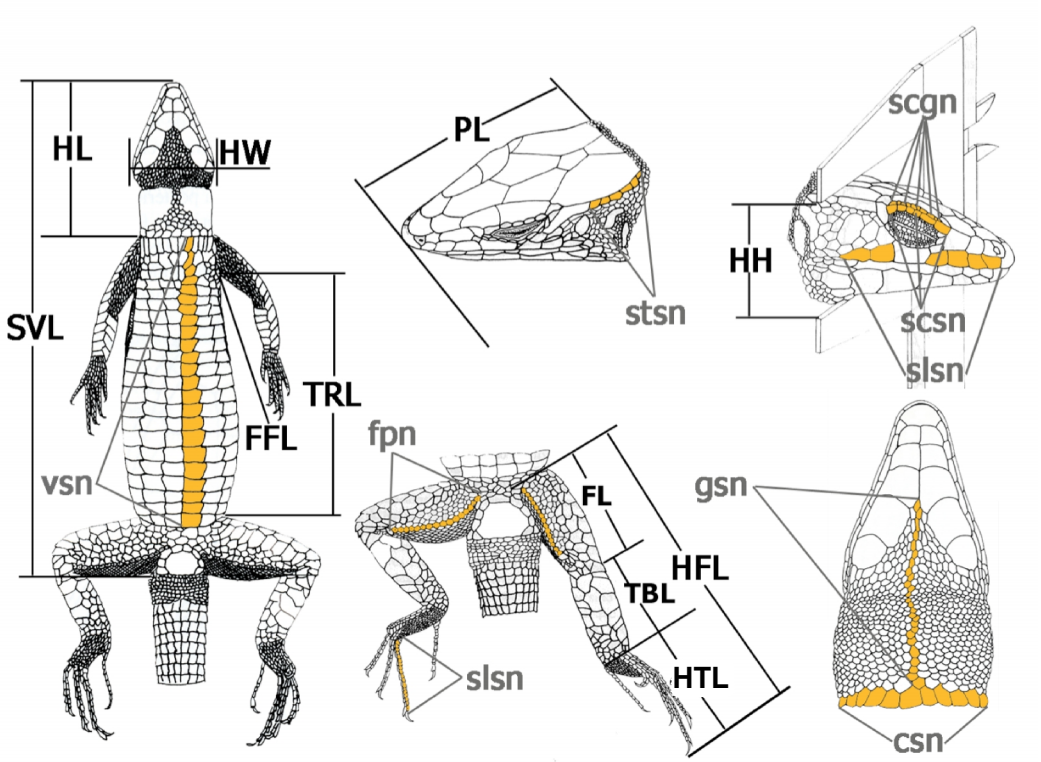

Figure 18. Representation of standard measurements (black) and scales counting (grey) used in lizards (Kaliontzopoulou *et al.* 2007).

Figure 19. Some examples of differentiation in colour patterns in *Quedenfeldtia* species.

1.5.3. Ecological Niche Modelling

Another relatively recent tool that is increasing in popularity among biologists is Ecological niche modelling. This is used for assessing the spatial context of the species and their relations with environmental variables, and has been widely used to describe patterns and make predictions of species distributions (Elith and Leathwick 2009; Sillero 2011). This spatial context methodology offers another approach to analyse the evolution history of the organisms, providing a different point of view from the molecular methods (Guisan and Zimmermann 2000). The first modelling study dates from 1924, when a cactus species from Australia was modelled (Johnston 1924). However it is only in recent years that computer capabilities have improved enough so that ecological niche modelling turned into a versatile tool that can investigate distinct questions in ecology, evolution and conservation (see Sillero 2011).

Due to the complexity of the organization and relations in evolutionary history of most organisms, several tools need to be used to address different questions. If all methods can raise some controversy, and each one has its weakness, the best way to delimit species or even to understand their evolutionary history, is to accumulate as much information as possible regarding the concerned species or populations of interest. This not only increases the possibility to detect recently separated lineages, but also provides stronger support to results (Sites and Marshall 2003; Wake 2006; de Queiroz 2007). Besides this, and focusing on the species delimitation issue, the failure of one method in a particular case does not contradict the existence of a species in nature (de Queiroz 1998) since speciation is a continuous process (Wake 2006), resulting from multiple evolutionary processes operating between and within populations across varying spatio-temporal scales (Harrison 1998; Lee 2004).

1.6. Objectives and organization of the thesis

1.6.1. Objectives

As the title of this work reveals, this thesis aims to investigate the evolutionary patterns, diversity and phylogenetic relations within endemic reptiles species from high altitude in the Atlas Mountains, Morocco (North Africa).

Reptiles were chosen as model organisms due to their non-volant nature, because they are relatively common in these habitats, their well-understood alpha-taxonomy, and the ease of sampling using non-invasive procedures (tail-tips). In recent years several phylogeographic studies have been made of the herpetofauna of the region (e.g. Carranza *et al.* 2002; Perera *et al.* 2007; Fonseca *et al.* 2008; Rato and Harris 2008; Fonseca *et al.* 2009; Perera and Harris 2010). However there are some that are still poorly studied, probably due to sampling

difficulties, such as typically low densities (*Chalcides*) or inhabiting inaccessible areas (*Quedenfeldtia* and *Atlantolacerta andreanskyi*).
Molecular methods are the main tools used in this work, since we intend to assess the genetic variation within and between species in each group (*Chalcides*, *Quedenfeldtia* and *Atlantolacerta*) using both mitochondrial and nuclear DNA sequences. However, morphology and landscape genetic approaches are also used to add information and support to the genetic results, contributing to the knowledge of the species evolutionary history, and present diversity patterns.

The specific aims of this work are:

1. Sample the high altitude reptiles from Morocco, covering all their distribution.

2. Identify levels of genetic diversity within endemic high altitude species, especially those with fragmented ranges, to detect the presence of cryptic variation.

3. Use a landscape genetic approach and morphological analysis to look for possible differences between genetic lineages.

4. Clarify the relation between *Chalcides montanus* and *Chalcides polylepis* assessing the hypothesis of possible introgression, or that *C. montanus* is only a recent morphotype of *C. polylepis*. Furthermore, the samples from J. Sirwa were morphologically identify as *C. montanus*, but a preliminary genetic study (mtDNA, 12S) placed them closer to *C. manueli* from Sidi Ifni that to *C. montanus.* So, we also intend to clarify the relation between *C. montanus* and *C. manueli.*

With all this we intend to contribute to the knowledge about evolution history and diversity of high altitude endemic reptiles not just from Morocco but also to see how patterns observed here are reflected in other geographic regions.

1.6.2. Organization and thematic of the thesis

The present thesis was organized in six chapters and includes five articles incorporated in four chapters. After providing a GENERAL INTRODUCTION to this work in the present Chapter, in CHAPTER 2 the biogeographic history of the Moroccan endemic genus *Quedenfeldtia* spp. was investigated, using tools including genetic, morphological variability and ecological niche modelling. These results are resumed in a scientific article publish in a peer-reviewed international journal:

ARTICLE 1.

Barata M. Perera A. Martínez-Freiria F. and Harris D.J. 2012. Cryptic diversity within the Moroccan endemic day geckos *Quedenfeldtia* (Squamata: Gekkonidae): a multidisciplinary approach using genetic, morphological and ecological data. *Biological Journal of Linnean Society*, 106(4): 828-850.

In CHAPTER 3, genetic and morphological structure of the different populations of *Atlantolacerta andreanskyi* was assessed, a lacetid lizard endemic to the Atlas Mountains, Morocco, whose populations are isolated in the peaks of high mountains, in most of the cases, with difficult access. Results are presented in two main scientific articles, one published in an international peer-reviewed international journal and the second was recently submitted to a peer-reviewed international journal:

ARTICLE 2.

Barata M. Carranza S. and Harris D.J. 2012. Extreme genetic diversity in *Atlantolacerta andreanskyi* (Werner, 1929): A mountain cryptic species complex. *BMC Evolutionary Biology*, 12: 167.

ARTICLE 3.

Barata M. Perera A. and Harris D.J. (submitted). Cryptic diversity in the Moroccan high altitude lacertid *Atlantolacerta andreanskyi* (Werner, 1929): a taxonomical assessment.

In ARTICLE 2, two mitochondrial and five nuclear loci and several different analytical approaches were used to assess the genetic structure of the *A. andreanskyi* populations. All the analyses classified most of the populations as different lineages and with high genetic differentiation between almost all of them. In the ARTICLE 3, we analysed the morphology of six populations of *A. andreanskyi*. The results revealed limited variation in morphological characters, that results are concordant with high levels of "cryptic" diversity inside *Atlantolacerta*. The classification of six different species is proposed.

In CHAPTER 4 we attempt to unveil the phylogeographic pattern within *Chalcides polylepis* and *Chalcides montanus,* and test the possibility of *C. montanus* receiving mitochondrial DNA from *C. polylepis* through introgression, as proposed by Carranza *et al.* (2008). We also pretend to clarify the relation between *C. montanus* and *C. manueli*, following the preliminary results that shows genetic similarities (mtDNA, 12S) between *C. montanus* from J. Sirwa and

C. manueli from Sidi Ifni. The results of this work were resumed in a scientific article in preparation to be published in an international peer-reviewed journal.

ARTICLE 4.

Barata M. Geniez P. Carranza S. and Harris D.J. (in preparation) Complex estimates of phylogenetic relationships between three species of *Chalcides* skinks from Morocco.

In CHAPTER 5, is given an explanation on the fieldwork, a very important part of this work. One of the scientific articles resulting from the time spent in the field is presented here. This work extends the distribution of some reptile and amphibian species from Morocco, and was published in an international scientific journal, ARTICLE 5.

ARTICLE 5.

Barata M. Perera A. Harris D.J. Van Der Meijden A. Carranza S. Ceacero F. García-Muñoz E. Gonçalves D. Henriques S. Jorge F. Marshall J.C. Pedrajas L. and Sousa P. 2011. New observations of amphibians and reptiles in Morocco, with a special emphasis on the Eastern Region. *Herpetological Bulletin*, 116: 4-14.

Finally in CHAPTER 6, a GENERAL DISCUSSION about the main results obtained during the four years of this work is presented. The major conclusions and future perspectives are also expanded.

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CHAPTER 2.

CRYPTIC DIVERSITY IN *QUEDENFELDTIA SPP.* (BOETTGER, 1883)

Mafalda Barata, Morocco, 2008

ARTICLE 1.

Barata M. Perera A. Martínez-Freiría F. and Harris D.J. 2012. **Cryptic diversity within the Moroccan endemic day geckos *Quedenfeldtia* (Squamata: Gekkonidae): a multidisciplinary approach using genetic, morphological and ecological data**. *Biological Journal of Linnean Society*. 106(4): 828-850.

Cryptic diversity within the Moroccan endemic day geckos *Quedenfeldtia* (Squamata: Gekkonidae): a multidisciplinary approach using genetic, morphological and ecological data

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Abstract

Quedenfeldtia (Boettger, 1883) is a genus of diurnal geckos, endemic to the Atlas Mountains in Morocco, with two species being recognized: *Quedenfeldtia moerens* and *Quedenfeldtia trachyblepharus*. *Quedenfeldtia moerens* is found across a wide variety of habitats, from sea level to 3000 m a.s.l., whereas *Q. trachyblepharus* occupies exclusively high mountain regions reaching up to 4000 m a.s.l. This differentiation, offers an interesting model for study biogeographical patterns and evolutionary scenarios in a North African endemic. Analysis of two mitochondrial (12S rRNA and ND4) and four nuclear (ACM4, MC1R, PDC, and Rag1) DNA markers revealed high genetic variation, consistent with other recent phylogeographical studies, and with the two currently described species. However, within each species, a subdivision into two groups with geographical consistence was found. Multivariate morphological analyses confirmed the existence of two main phenotypes, whereas ecological niche modelling identified various environmental variables associated with the distribution of each species, and helped to predict occurrences outside the confirmed ranges. The results obtained in the present study indicate the possible existence of additional 'cryptic' species within this genus, a condition found in many North African reptiles, and particularly common in geckos. In general, North African montane fauna appears to reflect the occurrence of diverse palaeoendemics, as seen in Central Africa Mountain systems, rather than the pattern of recent postglacial recolonization observed in Europe. © 2012 The Linnean Society of London, Biological Journal of the Linnean Society, 2012, 106(4), 828–850.

Keywords: Atlas Mountains – cryptic species – ecological modelling – mitochondrial DNA – morphology – North Africa – nuclear DNA – *Quedenfeldtia moerens* – *Quedenfeldtia trachyblepharus*.

Introduction

Understanding and quantifying biological diversity is one of the priority issues that need to be addressed in order to successfully apply conservation policies (Agapow 2005; Sattler *et al.* 2007). The Mediterranean Basin is considered one of the world hotspots of biodiversity (Myers *et al.* 2000), with much of its richness concentrated in the Southern European Peninsulas and the Western Maghreb (Medail and Quezel 1999). Past events, such as Pleistocene climatic oscillations, a high heterogeneity of habitats and climates, and a complex geological history have promoted this diversity. However, while biodiversity and the possible events that promoted it have been extensively studied in southern European Peninsulas (reviewed in Weiss and Ferrand 2007), underlying mechanisms promoting biodiversity in the Western Maghreb region of North Africa are still poorly understood.

Several recent studies have reported high levels of genetic diversity within reptiles of the Western Maghreb (Brown *et al.* 2002; Harris *et al.* 2004a; Harris *et al.* 2004b; Barata *et al.* 2008; Rato and Harris 2008; Gamble *et al.* 2010; Perera and Harris 2010; Rato *et al.* 2010), highlighting the relevance of high-mountain systems, particularly the High Atlas mountains, as a source of endemism (Medail and Quezel 1999). In this respect, geckos are an especially interesting group to study, since they have an ancient origin (Kluge 1987), and this region has been identified as one of the source areas for the currently observed worldwide diversity in this group (Gamble *et al.* 2008a). In particular, recent studies show that two North African genera (*Quedenfeldtia* and *Saurodactylus,* both endemic to Morocco) are basal to the American Sphaerodactylidae family that diverged by vicariance and dispersal events after fragmentation of Gondwana (Gamble *et al.* 2008a; Gamble *et al.* 2010). In consequence, such North African endemics are expected to retain high levels of genetic diversity because of their old evolutionary histories (Busack 1986; Rato and Harris 2008).

European montane herpetofauna tended to survive the last glacial maxima through limited altitudinal range shifts, unlike the classic larger contraction and recolonization patterns observed in lowland species (Mouret *et al.* 2011). Thus montane lizards such as *Iberolacerta bonnali* have minimal mtDNA diversity, and phylogeographic patterns reflect colonization history rather than current habitat (Mouret *et al.* 2011). On the other hand, in the African tropics, where there was greater climatic buffering through the Pleistocene, this may have allowed speciation through ecological diversification to predominate (Fjeldsa and Lovett 1997). The continuation of "palaeoendemics" in these stable refugia may also have retained biodiversity at greater levels than at higher latitudes. This seems to be the case for example in East African forest chameleons (Tolley *et al.* 2011). In this scenario, further complexities are likely; within networks of refugia species may still undergo some cycles of fragmentation and admixture, leading to refugia being "melting pots" as well as hotspots of diversity (Canestrelli *et al.* 2010; Canestrelli *et al.* 2012). An alternative hypothesis however is that much of the

diversity is more recent, as species adapted to exploit novel niches, resulting in shallower radiations (eg Blackburn and Measey 2009). How these alternative hypotheses relate to North African montane species remains essentially unknown. To fully determine between such competing, and not exclusive hypotheses, a complex approach involving both distribution modelling and phylogeographic assessments is needed. Furthermore, since "melting pot" scenarios involve particular complexities, multiple independent molecular markers are needed to identify possible examples of introgression and gene flow between refugia.

The genus *Quedenfeldtia* (Boettger 1883) was historically considered monotypic (Loveridge 1947) although several authors highlighted the presence of two different phenotypes (e.g. Bons 1959). Later, Arnold (1990) recognized two species, *Q. moerens* and *Q. trachyblepharus*, with distinctive external features including pholidosis (the arrangement or pattern of the scales) and colour pattern. Different ecological requirements should be expected for these species because *Q. moerens* inhabits a wide range of habitats from the sea level to 3000 m while *Q. trachyblepharus* is found in mountainous areas from 1400 m to 4000 m (Bons and Geniez 1996; Schleich *et al.* 1996). However, any study has quantified the exact distribution range of both species and possible differences in ecological requirements remain speculative.

The aim of this work is to understand the diversity of the endemic genus *Quedenfeldtia* using a multi-perspective approach. Molecular (two mitochondrial and four nuclear markers) and morphological (body measurements, pholidotic characters and colour pattern variation) data are used to assess the genotypic and phenotypic variability within the genus. Distribution/ecological data retrieved both from literature and from this study are used to identify ecological requirements distributional ranges, probable areas of sympatry and niche overlap for the members of the genus. We compare our results with other North African reptiles and assess possible evolutionary hypotheses that might explain the patterns observed. In particular we aim to 1) Determine if multiple distinct lineages occur within each species, using different tools to assess this; morphological characters, ecological modelling and molecular data 2) To assess if nuclear markers reflects the patterns obtained using mtDNA, or if evidence of gene flow or introgression occurs between lineages, 3) to determine if the lineages appear to be palaeoendemics, or more recent radiations, and therefore to assess if the pattern observed in North African montane fauna more closely resembles patterns recovered in Central African or European montane fauna.

2. Material and Methods

2.1. Field Sampling

The study area comprised South and Central Morocco, including the Anti Atlas and High Atlas Mountains, the distribution area of *Quedenfeldtia*. Initially, 42 individuals from 19 localities from throughout the range of the genus were sampled (Fig. 1 and Table 1). Specimens were noosed, photographed, identified on the basis of external features described by Arnold (1990) and tail tips collected and stored in 96% ethanol.

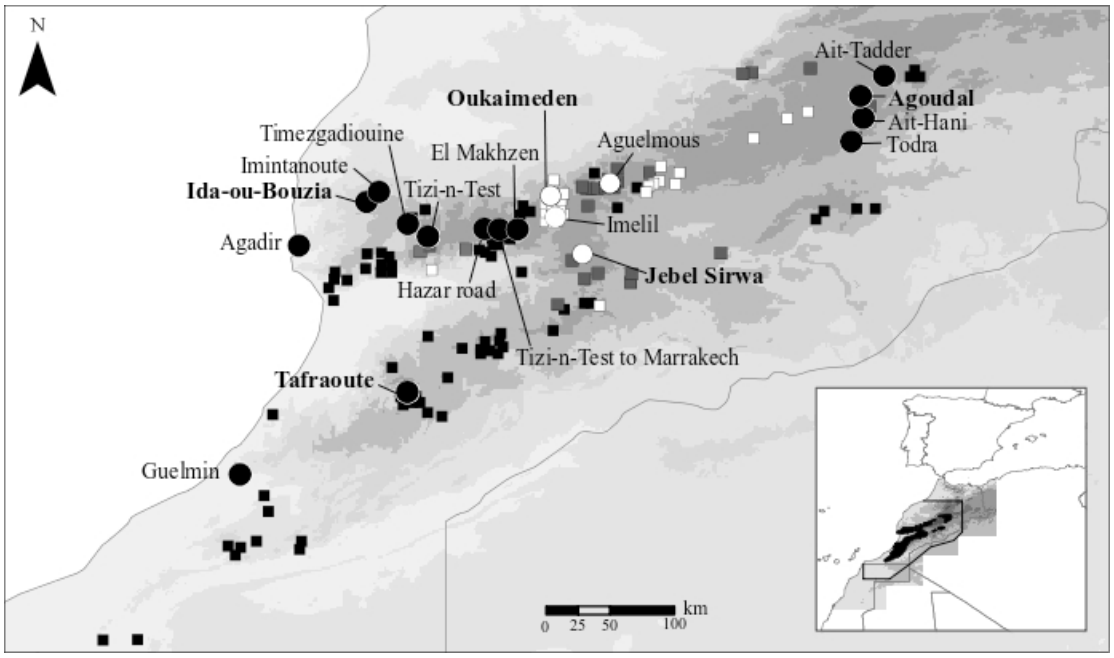

Figure 1. Study area location, toponomies used in text, and distribution of the *Quedenfeldtia* species, *Q. trachyblepharus* (white), *Q. moerens* (dark) and indeterminate species (grey). The map shows the distributions of the species by Bons and Geniez (1996) (squares) and the samples used for genetic analyses in this study (circles). The populations localities used for morphological analysis are identified in the map in bold (Tafraoute, Ida-ou-Bouzia, Oukaimeden. J. Sirwa and Agoudal). A dark line identifies the buffer area.

From these localities five populations, three from *Q. moerens* (Agoudal, Ida-ou-Bouzia and Tafraoute) and two from *Q. trachyblepharus* (Oukaimeden and Jebel Sirwa) were chosen to include in the morphological analysis (Fig. 1). In total, 111 adult males and 95 adult females were sampled. Populations were selected with regard to species distribution and to encompass genetic lineages recovered from preliminary phylogenetic analysis based on the mtDNA sequences. Specimens were sexed using colour pattern (Schleich *et al.* 1996) and the presence or absence of developed cloacal tubercles (Arnold 1990; Schleich *et al.* 1996). Seven linear measurements were also collected (Appendix 1). Pictures from ventral, lateral and dorsal areas were taken for posterior analysis of pholidosis and colouration characters. Only adult

individuals were included in the study. Individuals were released in the same place where they were caught after recording the exact location using a GPS.

2.2. Genetic analysis

2.2.1. Extraction, amplification and sequence alignment

Genomic DNA was extracted from tissue samples using standard high-salt protocols (Sambrook *et al.* 1989). Polymerase chain reaction (PCR) was used to amplify two portions of mitochondrial DNA: one including the 12S rRNA region and the other a fragment comprising the ND4 and flanking tRNAs (tRNA-His, tRNA-Ser and tRNA-Leu). Additionally, four partial nuclear protein-coding genes were amplified: recombination-activating gene 1 (Rag1), acetylcholinergic receptor M4 (ACM4), melanocortin receptor 1 (MC1R) and phosducin (PDC). The primers used for amplification and sequencing were 12Sa and 12Sb (Kocher *et al.* 1989) for 12S rRNA, ND4 and Leu (Arévalo *et al.* 1994) for ND4, L2408 and H2920 (Vidal and Hedges 2004) for Rag1, tg-F and tg-R (Gamble *et al.* 2008b) for ACM4, MC1RF and MC1RR (Pinho *et al.* 2010) for MC1R and PHOF2 and PHOF1 (Bauer *et al.* 2007) for PDC. PCR conditions included in bibliography (following primers information). PCR products were sequenced on an Applied Biosystem DNA Sequencing Apparatus. The genus *Saurodactylus* was used as an outgroup, with one sample representing each species, *S. mauritanicus*, *S. fasciatus* and *S. brosseti*. The sequences were aligned for each gene independently using the online version of MAFFT v.6 (Katoh *et al.* 2002) with default parameters (gap opening penalty = 1.53, gap extension = 0.0). We used the FFT-NS-1 algorithm since the sequences where not complicated to align and this is the simplest progressive option in MAFFT and one of the fastest methods currently available. New sequences have been submitted to GenBank (accession numbers in Table 1).

Table 1. Samples used in this study, specimen code, species, clade, localities, coordinates given in WGS84 coordinate system and GenBank accession numbers.

						GenBank accession numbers
Code	**Species**	**clade**	**Locality**	**Latitude**	**Longitude**	**12S rRNA / ND4 / ACM4 / MC1R / PDC / Rag1**
DB989	*Q. moerens*	Qm_North	Âit Tadder	32.13	-5.30	JQ686321 / JQ686385
DB1000	*Q. moerens*	Qm_North	Todra	31.62	-5.56	JQ686320 / JQ686384 / JQ686277 / JQ686264 / JQ686244 / JQ686342
DB1003	*Q. moerens*	Qm_North	Âit Hani	31.80	-5.47	JQ686322 / JQ686386 / JQ686278 / JQ686263 / JQ686246 / JQ686340
DB1009	*Q. moerens*	Qm_North	Âit Hani	31.80	-5.47	JQ686319 / JQ686387 / JQ686281 / JQ686265 / JQ686248 / JQ686341
DB1019	*Q. moerens*	Qm_North	Agoudal	32.04	-5.47	JQ686324 / JQ686388 / JQ686280 / JQ686267 / JQ686245 / JQ686343
DB1020	*Q. moerens*	Qm_North	Agoudal	32.04	-5.47	JQ686323 / JQ686389 / JQ686279 / JQ686266 / JQ686247 / JQ686344
DB1265	*Q. moerens*	Qm_North	Agoudal	32.04	-5.47	JQ686325 / JQ686390
DB1267	*Q. moerens*	Qm_North	Agoudal	32.04	-5.47	JQ686326 / JQ686391
DB1272	*Q. moerens*	Qm_North	Agoudal	32.04	-5.47	JQ686327 / JQ686392
DB809	*Q. moerens*	Qm_South	Tafraoute	29.70	-8.97	JQ686310 / JQ686381 / JQ686284 / JQ686262 / JQ686239 / JQ686349
DB810	*Q. moerens*	Qm_South	Tafraoute	29.70	-8.97	JQ686312 / JQ686380 / JQ686285 / JQ686261 / JQ686240 / JQ686345
DB811	*Q. moerens*	Qm_South	Tafraoute	29.70	-8.97	JQ686311 / JQ686379
DB1312	*Q. moerens*	Qm_South	Tizi-n-Test	30.89	-8.81	JQ686315 / JQ686376
DB1323	*Q. moerens*	Qm_South	Tizi-n-Test	30.89	-8.81	JQ686316 / JQ686377 / JQ686282 / JQ686257 / JQ686241 / JQ686346
DB1409	*Q. moerens*	Qm_South	Tizi-n-Test	30.89	-8.81	JQ686317 / JQ686378
DB1475	*Q. moerens*	Qm_South	Guelmin	29.07	-10.25	JQ686318 / JQ686375
DB1643	*Q. moerens*	Qm_South	Guelmin	29.07	-10.25	JQ686308 / JQ686373 / JQ686283 / JQ686260 / JQ686238 / JQ686348
DB1916	*Q. moerens*	Qm_South	Timezgadiouine	30.99	-8.96	JQ686302 / JQ686367
DB1924	*Q. moerens*	Qm_South	Hazar road	30.90	-8.33	JQ686303 / JQ686368
DB1925	*Q. moerens*	Qm_South	Hazar road	30.90	-8.33	JQ686304 / JQ686369
DB1935	*Q. moerens*	Qm_South	Tizi-n-Test to Marrakech	30.95	-8.26	JQ686305 / JQ686370
DB1997	*Q. moerens*	Qm_South	Tafraoute	29.70	-8.97	JQ686307 / JQ686372
DB2003	*Q. moerens*	Qm_South	Ida-ou-Buzia	31.16	-9.28	JQ686309 / JQ686374
DB3630	*Q. moerens*	Qm_South	El Makhzen	30.95	-8.12	JQ686306 / JQ686371
DB7994	*Q. moerens*	Qm_South	Agadir	30.82	-9.80	JQ686313 / JQ686382 / JQ686286 / JQ686259 / JQ686243 / JQ686347
DB7995	*Q. moerens*	Qm_South	Imintanoute	31.24	-9.18	JQ686314 / JQ686383 / JQ686287 / JQ686258 / JQ686242 / JQ686350
DB1050	*Q. trachyblepharus*	Qt_JSirwa	Jebel Sirwa	30.78	-7.65	JQ686298 / JQ686363 / JQ686276 / JQ686251 / JQ686235 / JQ686338
DB1107	*Q. trachyblepharus*	Qt_JSirwa	Jebel Sirwa	30.78	-7.65	JQ686300 / JQ686365 / JQ686274 / JQ686253 / JQ686237 / JQ686335
DB1123	*Q. trachyblepharus*	Qt_JSirwa	Jebel Sirwa	30.78	-7.65	JQ686301 / JQ686366 / JQ686271 / JQ686252 / JQ686236 / JQ686339
DB1126	*Q. trachyblepharus*	Qt_JSirwa	Aguelmous	31.30	-7.41	JQ686299 / JQ686364 / JQ686275 / JQ686250 / JQ686234 / JQ686337
DB1128	*Q. trachyblepharus*	Qt_JSirwa	Aguelmous	31.30	-7.41	JQ686297 / JQ686362
DB1932	*Q. trachyblepharus*	Qt_JSirwa	Jebel Sirwa	30.78	-7.64	JQ686294 / JQ686359 / JQ686272 / JQ686249 / JQ686232 / JQ686336
DB1933	*Q. trachyblepharus*	Qt_JSirwa	Jebel Sirwa	30.78	-7.64	JQ686295 / JQ686360 / JQ686273 / JQ686254 / JQ686233 / JQ686334
DB1934	*Q. trachyblepharus*	Qt_JSirwa	Jebel Sirwa	30.78	-7.65	JQ686296 / JQ686361
DB1155	*Q. trachyblepharus*	Qt_Oukaimeden	Oukaimeden	31.20	-7.86	JQ686292 / JQ686357
DB1159	*Q. trachyblepharus*	Qt_Oukaimeden	Oukaimeden	31.20	-7.86	JQ686293 / JQ686358
DB1914	*Q. trachyblepharus*	Qt_Oukaimeden	Toubkal	31.10	-7.91	JQ686328 / JQ686351 /. /. /. / JQ686330
DB1915	*Q. trachyblepharus*	Qt_Oukaimeden	Toubkal	31.10	-7.91	JQ686329 / JQ686352 / JQ686270 /. /. / JQ686331
DB1920	*Q. trachyblepharus*	Qt_Oukaimeden	Oukaimeden	31.20	-7.86	JQ686290 / JQ686353
DB1921	*Q. trachyblepharus*	Qt_Oukaimeden	Oukaimeden	31.20	-7.86	JQ686291 / JQ686354
DB1927	*Q. trachyblepharus*	Qt_Oukaimeden	Oukaimeden	31.20	-7.86	JQ686288 / JQ686355 / JQ686268 / JQ686256 / JQ686231 / JQ686332
DB1931	*Q. trachyblepharus*	Qt_Oukaimeden	Oukaimeden	31.20	-7.86	JQ686289 / JQ686356 / JQ686269 / JQ686255 / JQ686230 / JQ686333

2.2.2. Phylogenetic analysis

Mitochondrial genes (12S rRNA and ND4 with flanking tRNAs) and nuclear gene fragments (Rag1, ACM4, MC1R and PDC) were concatenated in two independent datasets and the most appropriate evolutionary model for each gene was calculated using jModeltest (Posada 2008) under the Akaike information criteria.

Two different phylogenetic approaches, Bayesian inference (BI) and Maximum Likelihood (ML) were implemented to analyse the phylogenetic relationships within the genus.

The BI analyses for the mitochondrial and nuclear datasets were performed using MrBayes v.3.1.2 (Huelsenbeck and Ronquist 2001) with partitions defined by gene, codon position for ND4, and without partitions. Partitioned Bayesian analysis was performed following Brandley *et al.* (2005), and Bayesian Factors (BF) were used to identify the best partitioning strategy for the concatenated dataset. All analyses started with randomly generated trees and ran for 2 million generations, saving one tree every 1000 generations. Two independent replicates were conducted and inspected for consistency to check for local optima (Huelsenbeck and Bollback 2001). Tracer v.1.4 (Drummond and Rambaut 2007) was used to verify if stationarity had been reached, both in terms of likelihood scores and parameters (ESS – Effective sample size - higher than 200 were considered acceptable), and the first 500 trees were discarded. A majority-rule consensus tree was generated from the remaining 19500 trees.

ML analysis was executed using RaxML version 7.0.4 (Stamatakis 2006) for concatenated data under the GTR+G+I model. Bootstrapping (1000 pseudo-replicates) was used to evaluate the stability of nodes of the phylogenetic trees (Felsenstein 1985) for ML analysis. Uncorrected distances (*p*-distance), between and within clades were calculated in Mega v.3.0 (Kumar *et al.* 2004). Any heterozygotes present in the nuclear data were treated as ambiguous (N) in all analyses, since we sequenced only a few samples from each lineage and the analysis of nuclear genes was based on concatenated sequences.

2.2.3. Species delimitation

Bayesian species delimitation was conducted using the program Bayesian Phylogenetics and Phylogeography, BPP v.2.0b (Rannala and Yang 2003; Yang and Rannala 2010) using the four nuclear loci. This method accommodates both species phylogeny and lineage sorting due to ancestral polymorphism. A gamma prior *G* (1, 10) was used on the population size parameters (qs). The age of the root in the species tree (t0) was assigned the gamma prior *G* (1, 10), while the other divergence time parameters were assigned the Dirichlet prior (Yang and Rannala 2010 : equation 2). We used algorithm 0 with the finetuning parameter $\varepsilon = 15.0$ in order to ensure adequate rjMCMC mixing. This involves specifying a reversible jump algorithm to achieve dimension matching between species delimitation models with different

number of parameters. Each species delimitation model was assigned equal prior probability and each analysis was run at least twice to confirm consistency between runs. The guide tree plays an important role in the result of the species delimitation model (Leaché and Fujita 2010); therefore we used the guide tree: ((Qt_Oukaimeden; Qt_JSirwa); (Qm_North; Qm_South)) based on the estimate of relationships from both mtDNA and nDNA trees (see results).

2.3. Morphological analysis

2.3.1. Variables quantification

In total, 111 adult males (M) and 95 adult females (F) from five different localities were analyzed: Oukaimeden (23M, 23F), Jebel Sirwa (22M, 21F), Agoudal (17M, 12F), Ida-ou-Buzia (28M, 21F) and Tafraoute (21M, 18F).

Seven linear measurements and eight pholidotic characters including the ones identified by Arnold (1990) for their taxonomic value, and five colour pattern variables were taken from each individual. Details of the variables collected can be found in Appendix 1. All linear measurements were recorded in the field by the same person (AP), to the nearest 0.01 mm, in order to minimize measurement error. Pholidotic and colour pattern variables were retrieved from digital pictures by the same person (MB) at least twice and the mean value was recorded. Pholidotic variables were treated as continuous when they had more than five categories (UPLAB, SUBLAB, SNEY, PRECL and LAM, Appendix 1), while variables with a lower number (DS, SLC_1 and SLC_2) were considered as categorical. All bilateral variables were taken from the right side of the animal when possible. In cases where data could not be collected due to member amputations or poor quality of pictures (3.4%, n = 85) they were replaced by the group mean.

2.3.2. Statistical analysis

Body measurements were log-transformed and checked for homoscedasticity (Levene's test) and normality (Shapiro-Wilks test) assumptions. Since linear measurements were highly correlated to the body size measured as snout-vent length (SVL, Pearson correlation in all cases $p < 0.01$), we used an isometric correction (Somers 1986) to separate the relative contribution of size and shape to the total variation analysed. Thus, the isometric body size (mSIZE) was used as a multivariate representation of size, whereas the combination of the remaining isometric-size corrected variables were used as representation of shape (Kaliontzopoulou *et al.* 2010).

In order to obtain a general approximation regarding the level of differentiation between sexes, species and populations, we performed multivariate analysis of the variance

(MANOVA) on shape (represented by the iso-corrected linear measurements) and pholidosis (only continuous variables) using SPECIES, SEX, POPULATION, nested in SPECIES, and their interaction, as factors. Since normality and homoscedasticity assumptions were not always met, non-parametric permutational MANOVAs were used (Anderson 2001) using the function adonis, implemented in the R package Vegan (Oksanen *et al.* 2012), and based on 1,000 permutations of the Euclidean distance matrix between group means. The same non-parametric procedure was used in univariate analysis of the variance (ANOVA) to estimate differences in SIZE and all continuous variables using the same factor design. Regarding categorical variables (colour pattern and non-continuous pholidotic variables), we plotted them in order to visualize their relative frequencies, and differences between SPECIES and POPULATION were compared using chi-squared tests.

In order to investigate the source of morphological variation in *Quedenfeldtia* at a multivariate level, the morphological relationships among populations and the relative contribution of each dataset to their variability, we performed a Principal Component Analysis (PCA) on linear measurements (iso-corrected variables) and pholidosis (only continuous ones). For colour pattern, we used a Multiple Correspondence Analysis (MCA) to assess, which characters contributed most to the differentiation between populations.

Finally, since we were interested in investigating the existence of further morphological differences between the two current *Quedenfeldtia* species besides the general colour pattern and pholidotic characteristics described by (Arnold 1990), we performed Canonical Discriminant Function Analysis (CDFA) on linear measurements (using both SIZE and SHAPE datasets) and continuous pholidotic variables. This allowed assessment at a multivariate level of which variables were the major contributors to the differentiation between species, and to create canonical discriminant functions to calculate the probability of classifying correctly the individuals based on them. We used the leave-one-out option to cross-validate the classification results. Since this procedure (Jacknife prediction) generates individual classifications using discriminate functions based on all observations except the given case, it provides a more accurate estimate of the classification values.

All statistical analyses were performed using SPSS v.17.0 and R (R Development Core Team, 2009). Significance level was considered at $p < 0.05$.

2.4. Ecological niche modelling analyses

2.4.1. Data sampling

A total of 92 and 35 localities for *Q. moerens* and *Q. trachyblepharus*, respectively, were gathered from published (Bons and Geniez 1996; Harris *et al.* 2010; Barata *et al.* 2011) and unpublished data (Table 1) and were initially considered for ecological modelling purposes.

However, in order to avoid spatial bias, and thus decrease the level of spatial autocorrelation in species presence, a randomly process of removing localities from clusters of species occurrence was performed (for details see Brito *et al.* 2011). The Nearest Neighbour Index was used to assess the degree of data clustering, along of this process, finally obtaining values of 0.97 and 0.89 for *Q. moerens* and *Q. trachyblepharus*, respectively, which indicate a random distribution for both species. Spatial analyses were done with the "Spatial Analyst" extension of ArcGIS (ESRI 2006). Following this, a total of 44 and 20 no clustered localities for *Q. moerens* and *Q. trachyblepharus*, respectively, were used for running ecological models (model samples), whereas 48 and 15 localities for *Q. moerens* and *Q. trachyblepharus*, respectively, were used for a secondary test of ecological models (validation samples).

2.4.2. Statistical analysis

Choosing a correct extension for the study area is a complex but necessary requirement for ecological niche modelling (VanDerWal *et al.* 2009; Anderson and Raza 2010). In presence-background-modelling techniques, such as MaxEnt (Phillips *et al.* 2006), different extensions may vary the nature of pseudoabsences randomly chosen by the algorithm, affecting the model output (VanDerWal *et al.* 2009). Therefore, calibration exercises with different sizes of the study area were performed (VanDerWal *et al.* 2009; Anderson and Raza 2010), and finally an area of 150 km of buffer from all localities was chosen to develop ecological models (Fig. 1). Subsequently, predictions were projected to a larger area of 837,034 km^2 covering all of Morocco and adjacent areas of Algeria, Western Sahara and Mauritania (Fig. 1).

Three sets of environmental and ecogeographical variables (hereafter EGV) were selected for the ecological models according to their meaning to the ecology and distribution of other reptiles (Brito *et al.* 2008; Kaliontzopoulou *et al.* 2008; Martinez-Freiria *et al.* 2008; Brito *et al.* 2011). These sets included one topographical grid (USGS, 2006) that was used to derive the variable Slope, with the "Slope" function of ArcGIS; 19 climate grids (Hijmans *et al.* 2005); and a land cover grid from the years 2004-2006 (Bicheron *et al.* 2008). In order to convert the categorical land cover EGV into a continuous variable, one binary grid was created for each habitat type that covered more than 2% of the study area. The Euclidean distance of each grid cell to the closest habitat-type was calculated for each individual habitat grid (13 habitat types) using the "Euclidean Distance" tool of ArcGIS (Brito *et al.* 2011). Finally, spatial correlation among all EGVs was tested using the "Band Collection Statistics" tool of ArcGIS and only 11 EGVs ($r < 0.750$) were chosen for modelling purposes (Appendix 2).

Ecological niche models were developed with the MaxEnt v.3.3 software (Phillips *et al.* 2006). The Maximum Entropy modelling approach requires only presence data as input, performs well in comparison to other methods, and has been used successfully in ecological niche-based modelling of many species distributions (Elith *et al.* 2006; Elith *et al.* 2011). Model samples and EGVs were imported into MaxEnt and a total of 40 and 20 model replicates for *Q. moerens* and *Q. trachyblepharus*, respectively, were run in the buffer area with random seed and 80% training / 20% testing data partition in each run. Samples for each replicate were chosen by bootstrap allowing sampling with replacement. Models were run with auto-features (Phillips *et al.* 2006), and the Area under the Curve (AUC) of the receiver-operating characteristics (ROC) plots was taken as a measure of individual model fit (Fielding and Bell 1997). Finally, each model replicate was projected to the projection area and the individual model replicates in the projection area were added to generate a mean forecast of probability of species occurrence (Marmion *et al.* 2009). Standard deviation between individual model probabilities of presence was used as an indication of prediction uncertainty (Buisson *et al.* 2010; Carvalho *et al.* 2010).

Mean models were reclassified according to the minimum training presence thresholds given by MaxEnt, which includes all presence data in suitable cells, to display areas of probable absence and presence for each species. To evaluate model quality, the model and validation samples were intersected with the threshold models to calculate the percentage of correct classification of presences in each probability category for each species. This technique was also used to assign a classification to the "indeterminate" *Quedenfeldtia* samples derived from Bons and Geniez (1996) (n = 26). Identification of areas of probable sympatry between both species was determined by the overlap of threshold models using the "Raster Calculator" function of ArcGIS.

The importance of each EGV for explaining the distribution of the species was determined by its average percent contribution to the models (Brito *et al.* 2008; Martinez-Freiria *et al.* 2008; Brito *et al.* 2011). The relation between occurrence of the species and EGVs was determined by the visual examination of response curves profiles from univariate models (Phillips *et al.* 2006). Similar profiles for a given EGV were taken as an indication of parallel relationships between the occurrence of these species and the range of variation of the EGV (Brito *et al.* 2008; Martinez-Freiria *et al.* 2008; Brito *et al.* 2011). This indicates also the possible occurrence of sympatry and eventual competition within the range of values of the EGV equally selected by both species. Conversely, a distinct profile among species was taken as an indication of divergent relationships and possible allopatry.

Finally, niche overlap and similarity between both species was tested using ENMtools v.1.3 (http://enmtools.blogspot.com). First, niche overlap was quantified from models generated for the two species using Schoener's D (Schoener 1968), I statistic (Warren *et al.* 2008) and

relative rank metrics (RR, Warren and Seifert 2011), which range from 0 (no overlap) to 1 (total overlap). The overlaps for I and D are calculated by taking the difference between species in suitability scores at each grid cell, after suitabilities are standardized so that they sum to 1 over the geographic space being measured (Warren *et al.* 2008; 2010; Warren and Seifert 2011). The relative rank statistic is an estimate of the probability that the relative ranking of any two patches of habitat is the same for the two models, irrespective of the quantitative difference in suitability estimates (Warren and Seifert 2011). Then, an identity test, using 50 randomized pseudoreplicates, was carried out to test the hypothesis of whether models generated from the two species are more different than expected if they were drawn from the same underlying distribution (Warren *et al.* 2008; 2010). By comparing the observed values of D, I and RR to the null distribution obtained using the identity test; it is possible to ask whether models produced by the two species are statistically significantly different (Warren *et al.* 2010).

3. Results

3.1. Genetic analysis

3.1.1. Mitochondrial DNA

The mitochondrial data consisted of 12S rRNA (407 bp), ND4 and tRNAs (692 bp) fragments totalling 1099 bp. A total of 42 haplotypes were retrieved from the 42 individuals of *Quedenfeldtia*, 16 from *Q. trachyblepharus* and 26 from *Q. moerens*.

Best-fit models of nucleotide substitution for each gene and the concatenated fragment were: 12S rRNA - GTR+G; ND4 1st position - HKY+G; ND4 2nd position - HKY; ND4 3rd position - HKY+G, tRNAs - K80+G, and concatenated data - GTR+I+G. The division of the data into five partitions had an improvement effect in the -lnLk (BF = 10.596; BF > 10 in favour of the hypothesis being tested (Kass and Raftery 1995; Brandley *et al.* 2005) and thus the partitioned analysis was preferred to estimate the phylogeny.

ML and BI analyses were mostly congruent and most estimates of relationships were well supported (Fig. 2A). The analyses divided the samples in two main groups concordant with the two current species, *Q. moerens* and *Q. trachyblepharus*. *Quedenfeldtia moerens* was further divided in two geographically congruent groups. One well-supported lineage (BI – 1 and ML - 92) grouped the samples from the South, and the other included the populations from the North, although the latter was paraphyletic with respect to *Q. moerens* from the South. The lineage that included the samples of *Q. moerens* from the South was further divided into two sub-lineages corresponding to the central and southern populations sampled. *Quedenfeldtia trachyblepharus* was also divided in two lineages, one that included all

samples from Oukaimeden and Toubkal and a second that included the remaining samples, all from the Jebel Sirwa area. All lineages exhibited high differentiation between them, with 13% (*p*-distance) between species and 8% and 9.5% between sub-lineages of *Q. moerens* and *Q. trachyblepharus*, respectively. Interestingly, there was low diversity within groups, up to a maximum of circa 3%, although sample size was limited (8 to 16 individuals)

3.1.2. Nuclear DNA

For the combined four nuclear genes from 23 individuals, 13 from *Q. moerens* and 10 from *Q. trachyblepharus* were analysed (Fig. 2B). The four partial genes Rag1 (471 bp), ACM4 (399 bp), MC1R (690 bp) and PDC (372 bp) were concatenated, giving a total length of 1932 base pairs. Best-fit models of nucleotide substitution for each gene fragment were: Rag1 - HKY+G; ACM4 - HKY+I; MC1R - HKI+I+G and PDC - K80+I, and for the concatenated data - GTR+I+G.

ML and BI analysis using nuclear markers were mostly congruent (Fig. 2B). The analyses of individual nuclear fragments (not shown) were not discordant but some did not resolve the two groups of *Q. moerens* (ACM4) or *Q. trachyblepharus* (Rag1) and one of the genes (PDC) showed the same polyphyly in the *Q. moerens* groups, as the estimate of relationships based on mtDNA tree. *Quedenfeldtia moerens* samples were divided in two lineages that coincide with the mtDNA results but the two groups were monophyletic. However, for the nDNA there was no differentiation within the Southern populations. Further, there was no support for the monophyly of *Q. trachyblepharus*. Moreover, results differ from the mitochondrial analysis, since individuals from Toubkal were well differentiated from Oukaimeden and almost identical to the remaining *Q. trachyblepharus* localities.

The nuclear divergence (*p*-distance) between the two species where 1.5%, and between the lineages, 0.5% between *Quedenfeldtia trachyblepharus* from Oukaimeden and J. Sirwa and 0.8% between *Quedenfeldtia moerens* from North and South.

3.1.3. Bayesian species delimitation

When assuming four lineages, Bayesian species delimitation analysis (BPP) strongly supports the guide tree, as found in previous analyses (four clades), with speciation probability ≥ 0.99 on all nodes (Guide tree with posterior probability for presence of nodes:
((Qt_Oukaimeden; Qt_JSirwa)#0.991; (Qm_North; Qm_South)#1.0)#1.0)

We used only one guide tree, the one obtained in both mtDNA and nDNA phylogenetic analysis, since this resulting tree was very simple with no ambiguous relationships. Following Leaché and Fujita (2010) the use of random trees, with an artificial increase of sister species, has a negative impact on the result.

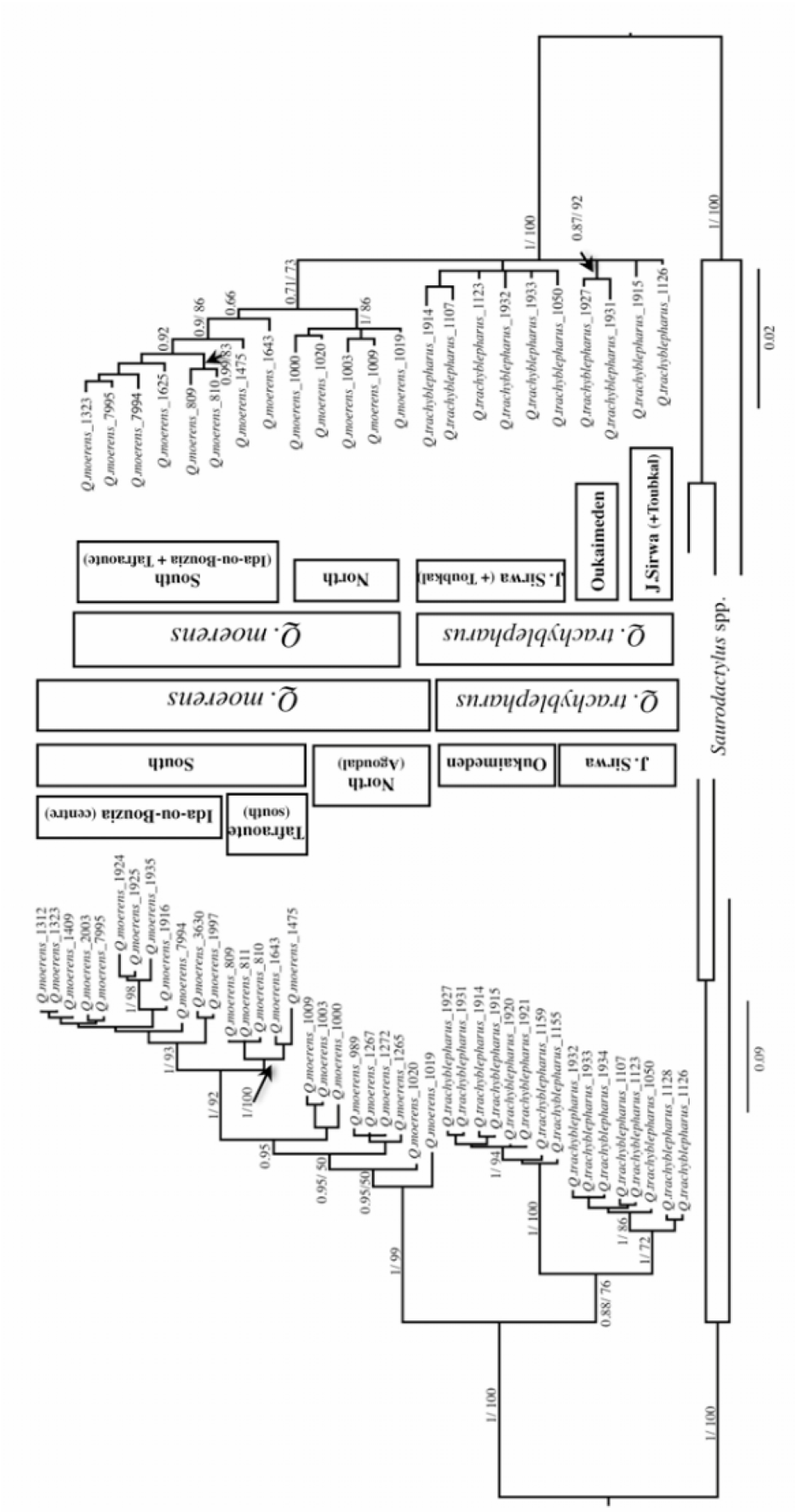

Figure 2. Trees derived from a Bayesian partitioned analysis for the mitochondrial DNA (12S rRNA, ND4 and tRNA's) on the left (A) and nuclear sequences (Rag1, ACM4, MC1R and PDC) on the right (B), using the models described in the text. Bayesian posterior probabilities (0-1) and bootstrap values (> 50%) for ML (1-100) are indicated near the branches. Between the trees are grouped the two species (*Q. trachyblepharus* and *Q. moerens*) and the clades found inside each species (Oukaimeden, J. Sirwa, South, North and in mtDNA the South clade is divided in two smaller groups, represented by the sampled population, Tafraoute and Ida-ou-Bouzia).

3.2. Morphological analysis

Details on the basic statistics of the two species by locality and sex can be found in Appendix 2.

3.2.1. Continuous variables

Our results from the MANOVAs showed general differences between SPECIES, SEX and POPULATION in size and shape, although interaction between factors was not always significant (Table 2). SEX was the main factor explaining the variation in size (R^2 = 0.27, Table 2), while SPECIES and POPULATION were the most explicative factors on shape (Table 2).

Regarding differentiation among body measurements (using size-corrected variables), males had wider heads (HW), while females exhibited a larger trunk length (ILL) (Table 2). The degree of sexual dimorphism (interaction SEX*SPECIES) was, in general, similar in the two species (except for HW and FLL, Table 2) although at the intraspecific level (interaction SEX*POPULATION, the latter nested to SPECIES) only differences in multivariate size were observed (Table 2). Regarding the morphological differentiation between the two species, *Q. moerens* showed significantly larger multivariate size and longer limbs (HLL and FLL) and head (HL), while *Q. trachyblepharus* exhibited wider heads (HW) and longer trunks (ILL; Table 2). Moreover, there were also differences between populations at the intraspecific level in all the characters analysed (Table 2 and Appendix 1).

For pholidosis, the results indicated differences between SPECIES, SEX and POPULATION, as well as their interactions (Table 2), although the most contributing factor to the variation in pholidosis was POPULATION (R^2 = 0.18, Table 2). In fact, populations differed in all the pholidotic characters analyzed, except for the number of supralabial scales (UPLAB; Table 2). Such differences were also evident at the species level (Table 2).

Differences in morphological and pholidotic traits were reflected in a relative grouping of the individuals in populations when analysing variation at the multivariate level using a PCA (Table 3 and Fig. 3A). Individuals from the two species appeared differentiated across the first two axes, although a high overlap was observed, especially between females of *Q. trachyblepharus* and *Q. moerens* from the South-Central locality of Ida-ou-Bouzia (Fig. 3A). Individuals from the other two *Q. moerens* populations from North (Agoudal) and South (Tafraoute) were better differentiated (Fig. 3A). Variation across the first axes was mostly explained by shape-related variables in males and females, including limbs length (both FLL and HLL), trunk length (ILL) and head width (HW, only in females) the most contributing ones (Table 3). However, number of lamellae scales (LAM) was also an important factor for the variation observed across PC1 in both sexes. Regarding PC2 and PC3, size contributed more across PC2 in males and PC3 in females, while SNEY was also important to explain the

Table 2. Results of the non-parametric (M) ANOVAs on the effect of species, sex and population (as factor nested in species) and their interactions on size (isometric size), shape (remaining iso-corrected linear measurements) and pholidosis (only continuous). For each factor and dataset analysed, degrees of freedom (df) and results of the R^2, F-value and level of significance (*p*) are provided. Analyses were based on 999 permutations. See materials and methods for more details. Numbers in bold indicates statistically significant values.

			species	sex	pop	sex*species	sex*pop	Residuals	Total
		df	1	1	3	1	3	197	206
MANOVA		R^2	0.16	0.27	0.17	0.00	0.03	0.37	1.00
	size	**F**	88.06	143.26	30.28	2.61	4.81		
		p	**0.001**	**0.001**	**0.001**	0.114	**0.006**		
		R^2	0.18	0.02	0.21	0.01	0.01	0.58	1.00
	shape	**F**	62.30	5.14	23.68	1.74	1.30		
		p	**0.001**	**0.001**	**0.001**	0.121	0.198		
		R^2	0.09	0.02	0.18	0.03	0.03	0.66	1.00
	pholidosis	**F**	25.46	4.77	18.12	7.57	2.87		
		p	**0.001**	**0.001**	**0.001**	**0.001**	**0.002**		
ANOVA		R^2	0.38	0.01	0.09	0.00	0.01	0.50	1.00
Body	**SVL**	**F**	152.30	3.96	12.38	0.54	1.95		
measurements		**p**	**0.001**	0.054	**0.001**	0.464	0.113		
		R^2	0.32	0.02	0.10	0.01	0.00	0.55	1.00
	HW	**F**	112.34	6.96	11.65	4.58	0.18		
		p	**0.001**	**0.022**	**0.001**	**0.033**	0.914		
		R^2	0.02	0.01	0.07	0.00	0.03	0.87	1.00
	HL	**F**	4.61	2.74	4.92	0.64	2.08		
		p	**0.034**	0.092	**0.001**	0.423	0.094		
		R^2	0.01	0.00	0.32	0.00	0.01	0.66	1.00
	HH	**F**	2.87	0.37	32.25	0.83	0.75		
		p	0.080	0.526	**0.001**	0.336	0.508		
		R^2	0.12	0.06	0.19	0.01	0.01	0.61	1.00
	ILL	**F**	39.72	18.89	20.83	1.74	1.36		
		p	**0.001**	**0.001**	**0.001**	0.195	0.244		
		R^2	0.22	0.00	0.29	0.01	0.01	0.47	1.00
	FLL	**F**	90.53	0.07	40.93	4.39	1.50		
		p	**0.001**	0.790	**0.001**	**0.036**	0.235		
		R^2	0.21	0.00	0.39	0.00	0.01	0.39	1.00
	HLL	**F**	107.35	2.14	66.62	0.01	0.93		
		p	**0.001**	0.153	**0.001**	0.928	0.426		
ANOVA	**UPLAB**	R^2	0.02	0.03	0.01	0.03	0.04	0.88	1.00
Pholidosis		**F**	4.06	6.22	0.50	6.23	2.61		
		p	**0.041**	**0.013**	0.683	**0.020**	0.058		
	SNEY	R^2	0.01	0.01	0.16	0.06	0.00	0.76	1.00
		F	3.69	2.68	13.45	15.41	0.06		
		p	0.062	0.098	**0.001**	**0.002**	0.978		
	PRECL	R^2	0.10	0.04	0.14	0.05	0.09	0.57	1.00
		F	35.06	14.51	16.69	18.87	10.18		
		p	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**		
	SUBLAB	R^2	0.02	0.00	0.23	0.01	0.01	0.73	1.00
		F	5.05	0.38	20.49	3.11	0.61		
		p	**0.03**	0.56	**0.00**	0.07	0.60		
	LAM	R^2	0.27	0.01	0.28	0.00	0.01	0.43	1.00
		F	123.46	3.66	42.15	0.16	1.77		
		p	**0.00**	0.06	**0.00**	0.70	0.15		

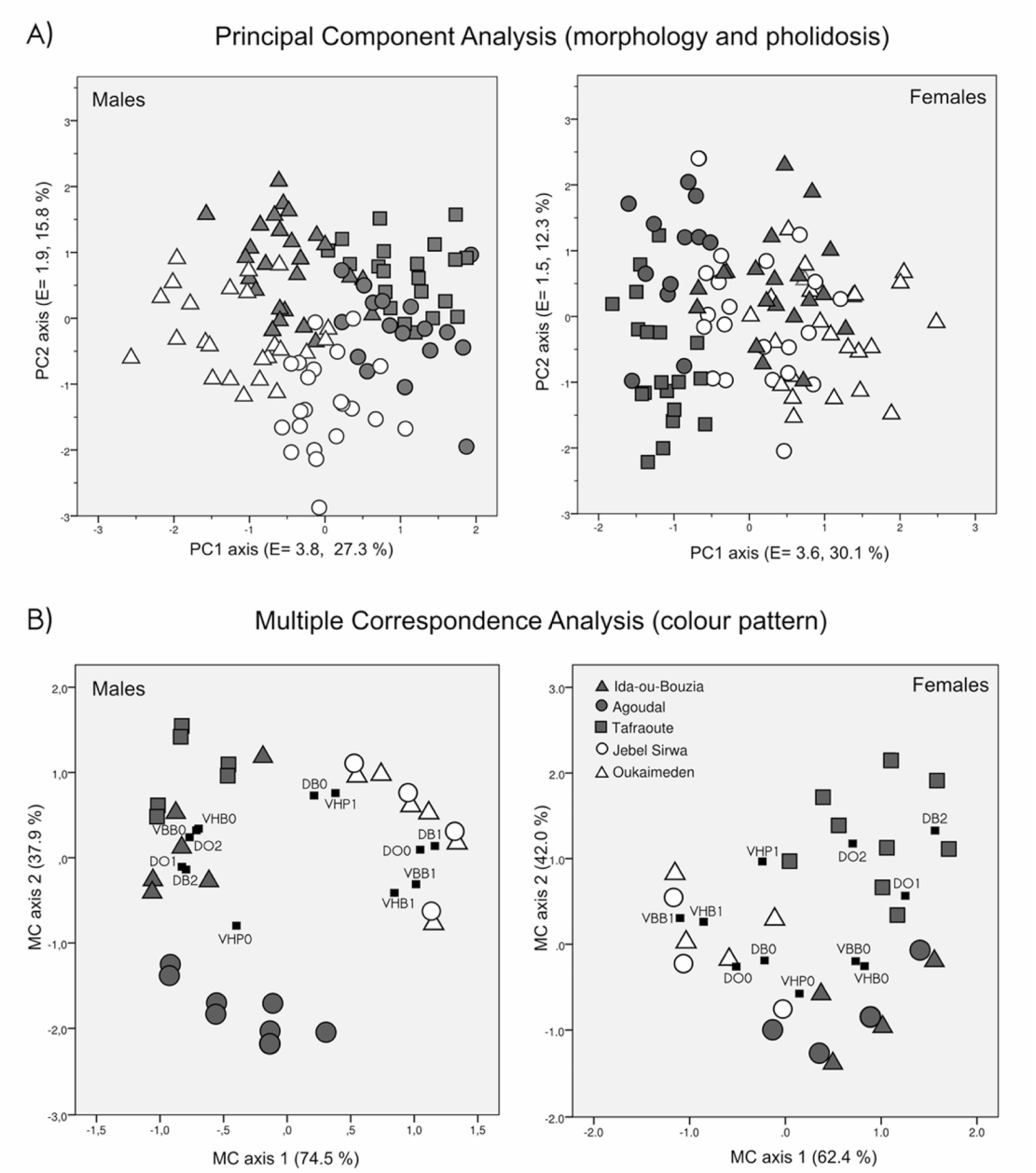

Figure 3. Scatterplots of the first two axes of the multivariate Principal Component Analysis (PCA) and Multiple Correspondence Analysis (MCA). (A) Plot of the PC1 against PC2 individual scores of the five populations of *Quedenfeldtia* included in the PCA using size, shape (size-corrected measurements) and pholidosis (continuous) variables. (B) Multivariate representation of the colour pattern variables (VHB0, VHB1, VBB0, VBB1, VHP0, VHP1, DO0, DO1, DO2, DB0, DB1, DB2 - Appendix 1) and individuals against the first two axes of the MCA. Left graphs: males, right graphs: females. See text for more details. The five populations are identified by: filled triangle (Ida-ou-Bouzia), open triangle (Oukaimeden), opens circle (J. Sirwa), closed circle (Agoudal) and closed square (Tafraoute). *Quedenfeldtia trachyblepharus* (open) and *Q. moerens* (closed).

variation across males PC2 (Table 3). The remaining variables showed a lower contribution (Table 3).

The Discriminant analysis (CDFA) using linear measurements and pholidotic characters allowed a good discrimination of the two species in both sexes. In males 95.5% of the *Q. moerens* individuals (n = 63) were correctly classified, while 91.1% of *Q. trachyblepharus* were properly discriminated (n = 41). In females, similar percentages were observed, with correct classifications ranging from 92.3% for *Q. moerens* (n = 48) to 97.7% for *Q. trachyblepharus* (n = 43). The most important discriminating body measurements were SIZE, limb length (FLL and HLL), head width (HW) and trunk length (ILL), the latter for females only (Table 3). The most important discriminating pholidotic characters were the number of lamellae, and the number of precloacal scales (the latter in males only, Table 3).

Table 3. Resume of the multivariate PCA and CDFA. PCA: Correlations between the first three principal components (PC1, PC2 and PC3) and linear measurements (size-corrected variables) and pholidotic characters. Group's correlations between the discriminating variables and the retrieved standardized canonical discriminant function (CDF1). Values in bold represent the most contributing variables. For each axis, eigenvalues (Eigenv), and total (% total) and cumulative (% cum) percentage of their contribution to the total variation are provided. See Material and Methods for more details.

	PCA						CDFA	
	Males			Females			Males	Females
	PC1	PC2	PC3	PC1	PC2	PC3	CDF1	CDF1
SIZE	0.046	**0.696**	-0.410	0.116	-0.231	**0.770**	**0.286**	**0.229**
HW	**-0.540**	-0.245	-0.285	**0.648**	-0.190	-0.451	**-0.321**	**-0.406**
HL	0.153	-0.295	0.482	-0.031	**0.553**	0.109	0.049	0.096
HH	**-0.540**	0.184	**0.531**	0.376	0.244	0.354	0.035	0.069
ILL	**-0.663**	0.169	-0.394	**0.721**	-0.214	0.191	-0.157	**-0.231**
FLL	**0.846**	0.074	-0.056	**-0.900**	-0.028	0.038	**0.221**	**0.327**
HLL	**0.843**	0.102	0.050	**-0.861**	-0.076	-0.209	**0.281**	**0.253**
UPLAB	0.183	0.470	-0.199	-0.058	0.486	-0.267	0.146	-0.028
SNEY	-0.198	**0.739**	0.110	0.460	-0.040	0.094	0.184	-0.082
PRECL	-0.013	**-0.528**	-0.249	-0.138	**-0.591**	-0.353	**-0.286**	-0.051
SUBLAB	**-0.502**	0.301	**0.538**	0.461	**0.577**	-0.281	-0.013	-0.143
LAM	**0.693**	0.214	0.213	**-0.724**	0.224	0.260	**0.302**	**0.334**
Eigenv	3.28	1.89	1.37	3.62	1.48	1.37	3.62	3.85
% total	27.33	15.76	11.44	30.13	12.31	11.38	100.00	100.00
% cum	27.33	43.10	54.54	30.13	42.44	53.82	100.00	100.00

3.2.2. Colouration patterns

Regarding the colour pattern, the Multiple Correspondence analysis allowed a relatively good separation of the two species across the first axis (figure 3B). The presence of dorsal colouration (DB), dorsal ocelli (DO) and the existence of ventral colouration, both in gular and trunk (VHB and VBB) were the characters that contributed most to the variation of the two species across the first axis (Fig. 3B). The two populations of *Q. trachyblepharus* showed

yellow ventral colouration, two tonalities of dorsal colouration and lack of ocelli in the dorsum, while *Q. moerens* populations were grouped based on white ventral colours and homogeneous dorsal colouration. The second axis (MC axis 2) separated *Q. moerens* populations with different patterns in males and females. Thus, in males, the Southern (Tafraoute) and Central (Ida-ou-Bouzia) populations grouped together while in females the northern population (Agoudal) was closer to the central one. *Quedenfeldtia moerens* males from the North differed from the others in the lack of ventral head pigmentation (VHP) while *Q. moerens* females from the South had a more uniform dorsal colouration (DB), more visible ocelli (VHP), and a yellowish gular (VHB).

3.2.3. Categorical variables

Quedenfeldtia trachyblepharus in contrast with *Q. moerens,* typically showed contact between the nostril and the first sublabial scale (SLC_1, around 75%), and with the supralabial scale fused to the postnasal scale (SLC_2, around 75%). The *Q. moerens* females from the North were an exception since 60% present the rostral scale fused with the postnasal scale, as were *Q. trachyblepharus* females from Oukaimeden, with more than 65% of individuals with nostril and first sublabial scales not in contact. Interestingly, more than 80% of the specimens from Oukaimeden (80% males and 90% females), but only a small percentage from Jebel Sirwa (15% females and 10% males) and a single female from *Q. moerens* from the South had double lamellae in the tip of the fourth toe (DS).

3.3. Ecological niche modelling analyses

The ROC plots exhibited high average AUCs with low standard deviations for both training and test datasets in the two species models (Table 4). Threshold models identified suitable cells for both species, with reasonable extensions in the projection area (Table 4). The average number and percentage of model and validation samples identified in suitable cells was very high (Table 4). A total of eleven and nine samples from *Quedenfeldtia* spp. were located in suitable cells for *Q. moerens* and *Q. trachyblepharus*, respectively (Table 4), whereas one and five were located in and out of suitable cells for both species, respectively.

Ecological models identified suitable cells for the occurrence of both species with relatively low prediction uncertainty (Fig. 4). Suitable cells for *Q. moerens* were found in almost all the High Atlas Mountains, and Atlantic coast of Morocco, and several areas where the species was not reported, including the mountains around the Moulouya River in north-eastern Morocco and western mountains of Tellien and Saharien Atlas of Algeria (Fig. 4). Suitable cells for the occurrence of *Q. trachyblepharus* were found only in the High Atlas Mountains

Table 4. Number (n) of replicates, number (n) of total, model (training and test) and validation samples, average (Av) and standard deviation (SD) training and test AUC and minimum training presence logistic threshold value (MTP) for the models of *Q. moerens* and *Q. trachyblepharus*. Area (Km^2) and percentage (%) of the projection area with presence of each species, number and percentage of correct classification (CC) model and validation samples and number and percentage of classified *Quedenfeldtia* spp. according to the MTP.

	Q. moerens	***Q. trachyblepharus***
n replicates	40	20
n total samples	92	35
n model samples (training – test)	36 - 8	16 - 4
n validation samples	48	15
Av (SD) training AUC	0.938 (0.010)	0.987 (0.004)
Av (SD) test AUC	0.882 (0.044)	0.969 (0.021)
MTP	0.129	0.155
Projection area [km^2] (%)	123333.312 (14.735)	21905.308 (2.617)
n (%) CC model samples	44 (100)	23 (95.833)
n (%) CC validation samples	46 (95.833)	15 (100)
n (%) *Quedenfeldtia spp.* (n=26)	11 (42.308)	9 (34.615)

and Jebel Sirwa (Fig. 4). A large area of probable sympatry between both species was found in the central part of the High Atlas (Fig. 4).

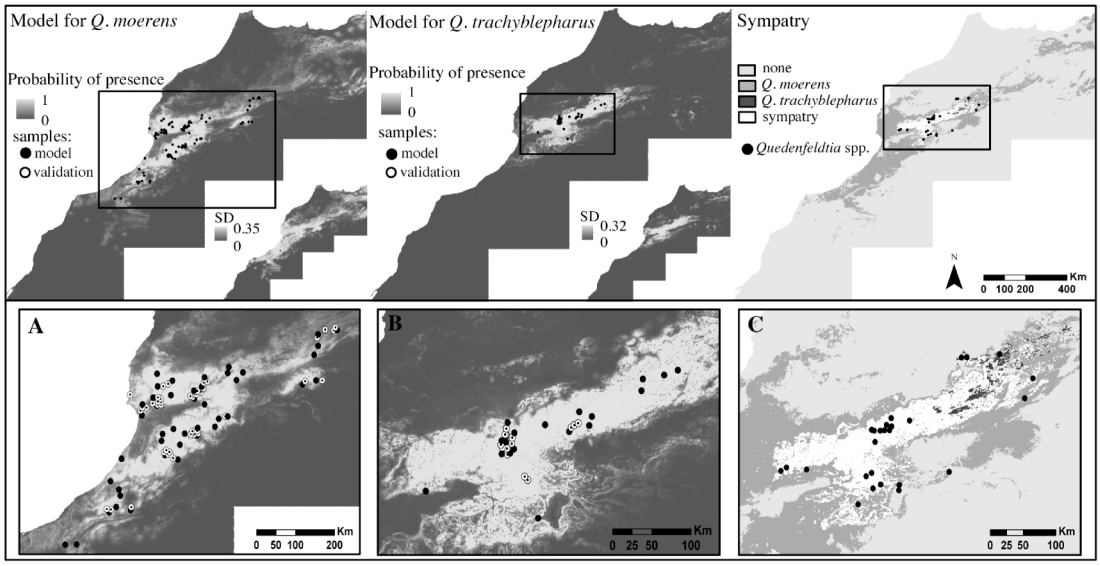

Figure 4. Average and standard deviation of probability of occurrence and areas of probable sympatry for *Quedenfeldtia moerens* and *Q. trachyblepharus* in North-West Africa. A) zoom to the area where *Q. moerens* was reported; B) zoom to the area where *Q. trachyblepharus* was reported; C) zoom to the area where *Quedenfeldtia* spp was reported.

A set of EGVs was identified as explaining the distribution of both species (Table 5, Fig. 5). The distribution of *Q. moerens* was mostly related to SLOPE, MTWQ and DFOR, whereas the distribution of *Q. trachyblepharus* was mostly related to SLOPE, PWQ, PCQ and DFOR.

The distribution of both species was influenced by common EGVs, such as SLOPE, DFOR and PWQ (Table 5, Fig. 5).

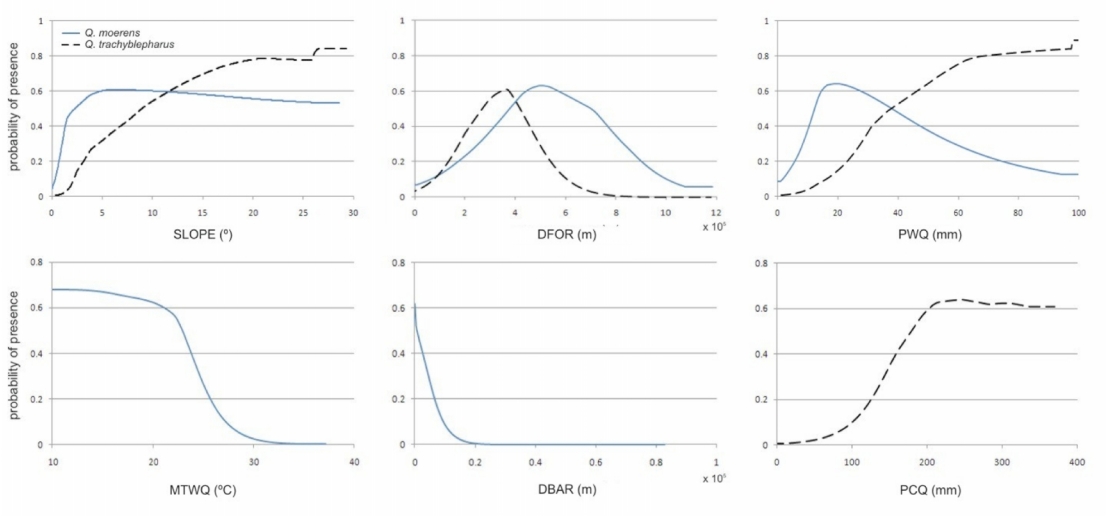

Figure 5. Response curves for the most related ecogeographical variables to the distribution of *Quedenfeldtia moerens* and *Q. trachyblepharus* in North-West Africa. Names for ecogeographical variables are given in appendix 3.

The profiles of the response curves for the common EGVs related to the distribution of the species revealed distinct patterns of occurrence (Fig. 5); 1) opposite occurrence, with *Q. moerens* occurring in flat and dry areas whereas *Q. trachyblepharus* occurring in sloping and humid areas; 2) similar occurrence, with both species occurring at high distance to open forests. Also, specific patterns were observed (Fig. 5): 1) *Q. moerens* occurred in areas with temperature below 25ºC during summer and close to sparse vegetation; *Q. trachyblepharus* occurred in areas with high levels of precipitation during winter.

Table 5. Average percent contribution and standard deviation (SD) of each variable for the models of *Quedenfeldtia. moerens* and *Q. trachyblepharus.*

	Q. moerens		*Q. trachyblepharus*	
variables	Average	SD	Average	SD
TAR	1.054	1.191	3.803	1.749
MTDQ	3.399	4.712	0.232	0.695
MTWQ	21.093	7.588	2.975	4.342
PS	0.239	0.433	0.001	0.004
PWQ	10.72	7.076	19.632	13.572
PCQ	2.976	3.555	12.69	5.693
DFOR	20.041	5.069	10.87	5.185
DVEG	11.841	7.156	3.382	4.33
DBAR	4.649	4.428	4.498	5.706
DWAT	1.046	1.148	2.998	2.652
SLOPE	22.943	10.245	38.921	16.213

4. Discussion

Even though the distribution of biodiversity in North Africa is not so well known as in other areas such as the Iberian Peninsula, in recent years several studies have shown similar histories, and in particular, high levels of genetic diversity (Harris *et al.* 2003; Pinho *et al.* 2007; Carranza *et al.* 2008; Fonseca *et al.* 2008; Kaliontzopoulou *et al.* 2008; Fonseca *et al.* 2009; Perera and Harris 2010). Geckos especially have often been shown to conceal cryptic species with very conservative morphology (Gamble *et al.* 2008a; Rato and Harris 2008; Perera and Harris 2010). The present study confirmed these trends in *Quedenfeldtia* as well as the differentiation of the genus into two well defined clades corresponding to *Q. moerens* and *Q. trachyblepharus*, corroborating the separation established by Arnold (1990). This differentiation was well supported by the genetic (mtDNA and nuclear markers) and morphological data, while ecological niche modelling also clearly identified different ecological preferences for the two species. However, considerable variation also occurs within each species.

Quedenfeldtia moerens comprises two groups supported by mtDNA and nuclear markers, geographically matching the Southern and Northern samples of this species, although the Northern group was not monophyletic in the analysis of mtDNA (Fig. 2). Divergence between the Northern and Southern group was considerable (8% ND4). Using the concatenated nuclear markers the same groups were recovered. Both lineages analysed were geographically separated by more than 250 km, and although Bons and Geniez (1996) report a few presences in the intermediate area, as far as we currently know, there is no geographic connection between these clades. This fact is corroborated by ENM: the only connection between both clades is in the High Atlas where *Q. trachyblepharus* occurs (Fig. 4). Moreover, the Southern clade showed a genetic distinction (at least at the mitochondrial level) between the southernmost and central-south populations with 5.3% divergence between them (ND4). Furthermore, although the morphology of the three populations corresponded to the definition of *Q. moerens* by Arnold (1990), the multivariate analysis allowed a partial distinction among populations, mostly on colour patterns (number of dorso-lateral ocelli, uniformity of the dorsal pattern, and colour of the gular area), but also on other characteristics such as head dimensions and trunk length. However, such patterns of differentiation between populations differed in both sexes, which might be related to differences in the degree of sexual dimorphism. These forms may therefore actually represent different species that are difficult to distinguish based on morphological characters in the field.

Quedenfeldtia trachyblepharus was also split into two well supported highly divergent clades based on mtDNA (9.5% ND4), which again is high enough to be indicative of a possible

species complex. One lineage (Oukaimeden) comprises the samples from only two localities, Oukaimeden and Toubkal, both in the High Atlas and separated by 10 km from each other. The other clade (J. Sirwa), grouped the remaining specimens of *Q. trachyblepharus* from the eastern High Atlas (Aguelmous), and an isolated mountain in the southern High Atlas (Jebel Sirwa and El Jazib n-Triri). However, nuclear markers only separated Oukaimeden (only two sequences available) from the remaining localities, with samples from Toubkal being very similar to the samples from the J. Sirwa clade. This discrepancy between the markers again highlights the limitations of using only mtDNA for phylogeographic studies (Godinho *et al.* 2008), and also seems to confirm that the complexity of these species may reflect their "melting pot" characteristics (Canestrelli *et al.* 2010; Canestrelli *et al.* 2012), although incomplete lineage sorting in the nuclear markers is also a possibility. Morphologically the two populations were considered *Q. trachyblepharus* (Arnold 1990), suggesting a conservative morphology. The analysis of colour patterns (MCA) did not reveal differences between them, although some biometric traits such as head dimensions or trunk length discriminate partially both populations, but only in males. Such discrimination, as also seen in *Q. moerens,* may be indicative of different selective pressures on sexual dimorphism. We also found in most of the individuals from Oukaimeden the presence of a double scale under the fourth hind limb toe, which was only found occasionally in J. Sirwa. It is necessary to assess this character in other populations to determine how well it corresponds with the two genetically distinct forms identified.

The main four lineages, two within each species, are genetically very different from each other which can be a result of a long-term isolation while the variation found between them suggests that they diverge approximately at the same time. Following the time frame proposed by Gamble *et al.* (2010), this could be as long ago as 15-17 Myr, and would therefore precede the formation of the Atlas mountains 9.0 Myr (Gomez *et al.* 2000; Babault *et al.* 2008). Thus, these species can reasonably be considered as "palaeoendemics", mirroring the situation in central African mountains much more closely than the patterns observed in Iberian montane species (Mouret *et al.* 2011; Tolley *et al.* 2011). The Bayesian species delimitation using the nuclear markers supports the existence of the four clades with high speciation probability (≥ 0.99%), indicating the possible presence of cryptic species. The magnitude of divergence between the subclades (8% in *Q. moerens* and 9.5% in *Q. trachyblepharus,* ND4), was similar to that found in other groups that were suggested to be cryptic species (Brown *et al.* 2002; Perera and Harris 2010).

Quedenfeldtia trachyblepharus showed high levels of divergence in a geographically restricted area, yet very low variation within each clade (1%). This seems to suggest that the current distribution is the result of a rapid expansion from very small populations or a

bottleneck effect. Assessment of additional populations would be needed to confirm this, but again it seems to indicate that rather than a pattern of expansion from a single primary refugium, as is often observed in European herpetofauna (eg Rowe *et al.* 2006), in these species many small but distinct refugia existed during the last glaciations leading to maintenance of high genetic diversity between lineages but with limited variation within them.

Previous studies (Bons and Geniez 1996; Schleich *et al.* 1996; Sindaco and Jeremcenko 2008) showed that while *Q. moerens* had a more widespread distribution from the south, almost from the desert to the mountains in central East Morocco, *Q. trachyblepharus* was restricted to high mountain altitudes of the High Atlas. However, many records for these species were considered undetermined, particularly in the High Atlas (Bons and Geniez 1996 ; Fig. 1). Results from ecological niche models identified areas for the occurrence of both species mostly in concordance with previous studies (Bons and Geniez 1996) and successfully assigned a probable classification for most of the indeterminate localities (classification > 76%). Moreover, ecological models showed suitable cells for the occurrence of *Q. moerens* in areas where the species was not reported. Historical reasons (e.g expansion from the south and/or barriers to dispersal) could have prevented *Q. moerens* from reaching these areas, but fieldwork should be conducted to investigate its possible presence. This is particularly important given the case of *Podarcis* lizards in North Africa, where models indicated possible presence in extra-range regions that were later confirmed after prospection (Kaliontzopoulou *et al.* 2008).

Probable areas for the occurrence of both species were almost allopatric and environmental variables related to the distribution of both species were different or similar but with mostly different profiles in the response curves, which also suggests allopatry. For instance, *Q. moerens* occur in flat and dry areas with temperatures below 25ºC during summer and close to sparse vegetation whereas *Q. trachyblepharus* occurs in sloping and humid areas with high levels of precipitation during winter. Nevertheless, there are specific environmental conditions where both species could coexist, with a large area of habitat in the High Atlas that could be suitable for both species. However, no regions of sympatry have been found in the field to date. Possibly *Q. trachyblepharus* is isolated as a result of competition with *Q. moerens,* since *Q. moerens* is limiting the south and north limits of *Q. trachyblepharus,* and according to Bons and Geniez (1996) is present between Oukaimeden and J. Sirwa, where there seems to be some kind of geographic barrier between these two clades. Probably these two species split and then adapted to different environmental conditions, temperate and drier climate for *Q. moerens* and high and humid mountains in *Q. trachyblepharus*. Following this hypothesis, when the temperature increased after the last glacial maxima *Q. moerens*

expanded its distribution upwards, forcing *Q. trachyblepharus* to move to higher altitudes, where long-term isolation coupled with a reduction in population sizes (bottlenecks) might have promoted the high inter-population and low intra-population differentiation observed today. The presence of the two species in the central area of the distribution, between *Q. moerens* from the South and *Q. trachyblepharus* from Oukaimeden, indicates this could be where the species originated.

5. Conclusions

The extreme genetic variability found within the genus *Quedenfeldtia*, including within the two species, is more than the level used to distinguish species in various other groups. However, geckos generally show high genetic variability (Harris *et al.* 2004a; Rato and Harris 2008), and species definition is still a controversial issue (de Queiroz 2007). Indications are that both currently accepted species might mask additional units that could deserve recognition as full species. Within *Q. moerens* subgroups show more phenotypic variation, probably related to the different selective pressures resulting from the wider range of habitats they occupy. On the other hand *Q. trachyblepharus*, despite the limited distribution, show higher genetic diversity but low morphological differentiation between populations. Our results indicate that, for this genus, phylogeographic patterns are much more similar to those recovered from montane species in Central Africa – highly divergent palaeoendemics - rather than the predominantly genetically uniform pattern observed in European montane reptiles. Some evidence of a "melting pot" scenario in which mtDNA patterns do not fully coincide with nuclear markers highlights the need for further assessments prior to any alterations to taxonomy. However, assessment of morphological data indicates some characters that might be useful to discriminate genetically divergent lineages in the field. In the Pyrenean rock lizard, *Iberolacerta bonnali*, phylogeographic patterns tended to reflect recolonization history rather than current habitat (Mouret *et al.* 2011). Our modelling approach indicates that, at least at a two-taxon level, patterns in *Quedenfeldtia* can be associated with habitat. It may be that within these two major lineages, genetic patterns better reflect refugial areas, but this can only be tested by sampling extensively across the region. It will be important in the future therefore to increase the sampling of *Q. trachyblepharus* to other high mountain localities, as well as investigate the possible existence of contact areas between the two species, in order to assess in more detail the evolutionary history of this group.

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APPENDIX

Table A1. Morphological, pholidotic and colour patterns variables included in the study.

1-Body measurements:
SVL- snout-vent length, from the tip of the snout until the cloaca.
HL- head length, from the tip of the snout to the posterior ear cavity.
HW- total head width at its widest part at the level of the temporal region.
HH- head height from occipital to jaws
ILL- inter-limb length from the posterior edge of forelimb insertion to the anterior edge of hindlimb insertion.
HLL- hind-limb length from the longest toe to the base of the hindlimb.
FLL- forelimb length from the longest toe to the base of the forelimb.

2-Scales:
Continuous variables
UPLAB- number of upper labial scales on the right side until the limit of the mouth opening counted on the right side of the head.
SUBLAB- number of sub-labial scales until the limit of the mouth opening counted on the right side of the head.
SNEY- number of linear scales between the eye and the nostril counted on the right side of the head.
PRECL- number of "pre-cloacal" scales, counted from leg axis to cloaca
LAM- number of non-divided enlarged side to side lamellae on the fourth right hind toe

Discrete variables
DS- presence of a divided subdigital scale on the fourth hind toe: no (0), yes (1)
SLC_1- contact between nostril and first supralabial scale: no (0), yes (1)
SLC_2- supralabial scale fused with postnasal or rostral scale: no (0), yes (1)

3-Colouration:
Ventral
VHB- Gular colour- white (0), yellow (1)
VHP-Head pigmentation- white (0), Black (1)
VBB-Trunk ventral pigmentation- white (0), yellow (1)

Dorsal
DB-Colouration- brown uniform (0), two colours (1), uniform with dots (2)
DO-Ocelli- without (0), 1 to 3 (1), more than three (2)

Table A2. Descriptive statistics for all the linear measurements and pholidotic variables of the different *Quedenfeldtia* localities analysed in this study. For each variable, mean ± standard deviation, minimum and maximum and sample size (N) per sex is shown.

	Q. moerens						*Q. trachyblepharus*			
	Ida-ou-Bouzia		Agoudal		Tafraoute		Jebel Sirwa		Oukaimeden	
	Males (N =28)	Females (N=21)	Males (N =17)	Females (N=12)	Males (N =21)	Females (N=19)	Males (N=22)	Females (N=21)	Males (N=23)	Females (N=23)
SVL	47.00 ± 1.71 43.00 – 50.00	44.45 ± 2.09 40.00 – 49.00	44.26 ± 1.06 42.00 – 46.00	43.46 ± 1.31 41.00 – 46.00	47.45 ± 2.58 42.00 – 52.50	43.13 ± 2.64 39.00 – 47.50	45.29 ± 2.14 41.00 – 48.50	43.07 ± 1.89 40.00 – 46.50	47.54 ± 1.61 44.00 – 50.0	45.8 ± 1.04 44.00 – 48.00
HL	9.38 ± 0.34 8.77 - 10.13	8.74 ± 0.35 7.95 - 9.28	8.51 ± 0.36 7.91 - 9.04	8.14 ± 0.34 7.83 - 8.99	9.33 ± 0.50 8.26 - 10.12	8.51 ± 0.31 7.96 - 9.25	8.97 ± 0.35 8.38 - 9.61	8.64 ± 0.62 7.62 - 9.56	9.26 ± 0.34 8.41 - 9.82	8.88 ± 0.31 8.27 - 9.46
HW	11.08 ± 0.59 9.92 - 12.05	10.32 ± 0.61 9.07 - 11.12	10.32 ± 0.35 9.65 - 11.23	9.90 ± 0.39 9.19 - 10.49	11.14 ± 0.59 9.86 - 12.06	10.06 ± 0.59 8.95 - 11.06	10.25 ± 0.47 9.30 - 10.94	9.40 ± 0.39 8.76 - 10.08	10.28 ± 0.40 9.33 - 10.85	9.82 ± 0.36 9.12 - 10.44
HH	5.97 ± 0.20 5.52 - 6.34	5.65 ± 0.28 5.08 - 6.15	5.30 ± 0.27 4.95 - 5.95	5.08 ± 0.20 4.79 - 5.44	5.58 ± 0.36 4.95 - 6.41	5.14 ± 0.35 4.24 - 5.53	5.20 ± 0.33 4.64 - 5.76	4.87 ± 0.39 4.17 - 5.56	5.47 ± 0.23 4.97 - 5.84	5.22 ± 0.26 4.87 – 5.90
ILL	21.06 ± 1.46 18.64 – 24.40	20.75 ± 1.88 16.77 - 23.22	18.84 ± 1.01 17.26 - 20.99	19.20 ± 0.75 18.03 - 20.42	21.05 ± 1.17 19.10 - 23.79	19.50 ± 1.44 17.01 – 22.10	19.34 ± 1.29 16.98 - 21.65	19.41 ± 0.89 18.23 – 21.50	21.43 ± 1.08 19.00 – 23.60	21.46 ± 1.40 19.11 - 24.62
FLL	19.10 ± 0.83 17.69 - 20.68	17.95 ± 1.02 16.00 - 19.86	19.09 ± 0.52 17.99 – 19.80	18.36 ± 0.45 17.62 - 18.99	21.20 ± 1.26 18.50 - 23.23	19.08 ± 1.05 17.20 - 20.97	17.64 ± 1.00 15.94 - 19.58	16.59 ± 0.71 14.96 - 18.05	18.14 ± 1.09 16.32 - 20.24	16.61 ± 0.92 14.90 - 18.23
HLL	25.87 ± 1.24 23.22 - 28.39	23.47 ± 1.37 20.48 - 26.41	25.29 ± 0.85 23.50 - 26.37	23.69 ± 1.17 22.26 - 26.37	29.28 ± 1.86 25.49 - 32.29	25.95 ± 0.69 24.67 - 27.04	23.91 ± 1.08 21.44 - 25.36	22.05 ± 1.20 19.52 - 24.45	23.38 ± 1.24 20.25 – 25.10	21.72 ± 1.34 18.98 - 24.27
UPLAB	7.11 ± 0.63 6 - 8	6.57 ± 0.68 6 - 8	6.73 ± 0.66 5 - 8	6.89 ± 0.67 6 - 8	7.24 ± 0.7 6 - 9	6.58 ± 0.61 6 - 8	6.62 ± 0.65 5 - 8	6.67 ± 0.55 6 - 8	6.74 ± 0.62 6 - 8	6.73 ± 0.45 6 - 7
LOLAB	5.04 ± 0.43 4 – 6	4.48 ± 0.6 4 - 6	4.4 ± 0.47 4 - 5	4.78 ± 0.38 4 - 5	4.71 ± 0.64 4 - 6	4.37 ± 0.5 4 - 5	5.1 ± 0.61 4 - 6	5.06 ± 0.38 4 - 6	5.09 ± 0.42 4 - 6	5.27 ± 0.54 4 - 6
SNEY	11.88 ± 0.96 10 – 14	11.25 ± 0.94 10 - 13	10.82 ± 0.69 9 - 12	10.33 ± 0.55 9 - 11	11.17 ± 0.57 10 - 12	10.67 ± 0.51 10 - 12	10.53 ± 0.70 9 - 12	10.86 ± 0.43 10 - 12	11.00 ± 0.85 10 - 13	11.27 ± 1.05 10 - 14
PRECL	11.14 ± 1.38 9 – 14	11.00 ± 0.95 9 - 13	11.25 ± 1.44 9 - 15	10.56 ± 0.75 9 - 12	11.53 ± 1.07 10 - 13	12.11 ± 1.29 10 - 15	14.38 ± 1.46 11 - 17	11.55 ± 0.74 10 - 13	11.6 ± 0.63 10 - 13	11.37 ± 0.62 10 - 13
SUBLAB	7.79 ± 0.92 6 – 9	7.57 ± 0.75 6 - 9	6.94 ± 0.66 5 - 8	6.80 ± 0.83 5 - 8	6.40 ± 1.11 5 - 9	6.00 ± 0.75 5 - 7	6.89 ± 0.87 5 - 9	7.35 ± 0.95 6 - 10	7.45 ± 1.16 5 - 9	7.43 ± 1.07 5 - 9
LAM	9.32 ± 0.65 8 – 11	9.06 ± 0.67 8 - 10	10.31 ± 1.04 9 - 13	10.11 ± 0.67 9 - 11	9.95 ± 0.59 9 - 11	9.74 ± 0.56 9 - 11	9.53 ± 0.65 8 - 11	9.06 ± 0.86 8 - 11	7.91 ± 0.79 7 - 10	8.05 ± 0.47 7 - 9
DS	1.00 ± 0.00 1 – 1	1.00 ± 0.00 1 - 1	1.00 ± 0.00 1 - 1	1.00 ± 0.00 1 - 1	1.00 ± 0.00 1 - 1	1.06 ± 0.23 1 - 2	1.11 ± 0.29 1 - 2	1.12 ± 0.3 1 - 2	1.82 ± 0.39 1 - 2	1.9 ± 0.29 1 - 2
SLC	3.54 ± 0.88 3 – 6	3.43 ± 0.75 3 – 5	4.19 ± 0.73 3 – 5	4.4 ± 0.63 3 – 5	3.25 ± 0.62 3 – 5	3.06 ± 0.23 '3 - 4	3.72 ± 0.81 3 - 5	4 ± 0.84 3 - 5	3.32 ± 0.55 3 - 5	3.24 ± 0.51 3 - 5

Table A3. Environmental factors used to model the distribution of *Q. moerens* and *Q. trachyblepharus* and their codes, units and range of variation.

Code	Name	Units	Range
TAR	Temperature Annual Range	ºC	10.9 – 42.7
MTDQ	Mean Temperature of Driest Quarter	ºC	8.6 – 34.0
MTWQ	Mean Temperature of Warmest Quarter	ºC	10.4 – 36.2
PS	Precipitation Seasonality (Coefficient of Variation)	adimensional	19 - 122
PWQ	Precipitation of Warmest Quarter	mm	0 - 110
PCQ	Precipitation of Coldest Quarter	mm	2 - 573
DFOR	Distance to closed to open (>15%) mixed broadleaved and needleleaved forest (>5m)	m	$0 – 15.561*10^5$
DVEG	Distance to sparse (<15%) vegetation	m	0 - 301024
DBAR	Distance to bare areas	m	0 - 69180
DWAT	Distance to water bodies	m	0 - 502573
SLOPE	Slope (derived from altitude)	degrees	0 – 31.42

FINAL NOTE

This article is formatted accordingly to the Journal where it was published, Biological Journal of Linnean Society.

CHAPTER 3.

CRYPTIC DIVERSITY IN *ATLANTOLACERTA ANDREANSKYI* (WERNER, 1929)

Salvador Carranza, Oukaimeden, 2009

ARTICLE 2.

Barata M. Carranza S. and Harris D.J 2012. **Extreme genetic diversity in the lizard *Atlantolacerta andreanskyi* (Werner, 1929): A montane cryptic species complex.** *BMC Evolutionary Biology*, 12:167.

ARTICLE 3.

Barata M. Perera A. and Harris D.J. (submited). **Cryptic diversity in the Moroccan high altitude lacertid *Atlantolacerta andreanskyi* (Werner, 1929): a taxonomical revision**.

ARTICLE 2.

EXTREME GENETIC DIVERSITY IN *ATLANTOLACERTA ANDREANSKYI* (WERNER, 1929)

Mafalda Barata, Jebel Awlime, 2010

Barata M. Carranza S. and Harris D.J. 2012. **Extreme genetic diversity in *Atlantolacerta andreanskyi* (Werner, 1929): A mountain cryptic species complex**. *BMC Evolutionary Biology*, 12:167.

Extreme genetic diversity in the lizard *Atlantolacerta andreanskyi* (Werner, 1929): A montane cryptic species complex

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Abstract

Background: *Atlantolacerta andreanskyi* is an enigmatic lacertid lizard that, according to the most recent molecular analyses, belongs to the tribe Eremiadini, family Lacertidae. It is a mountain specialist, restricted to areas above 2400 m of the High Atlas Mountains of Morocco with apparently no connection between the different populations. In order to investigate its phylogeography, 92 specimens of *A. andreanskyi* were analysed from eight different populations across the distribution range of the species for up to 1108 base pairs of mitochondrial DNA (*12S, ND4* and flanking *tRNA-His*) and 2585 base pairs of nuclear DNA including five loci (*PDC, ACM4, C-MOS, RAG1, MC1R*).

Results: The results obtained with both concatenated and coalescent approaches and clustering methods, clearly show that all the populations analysed present a very high level of genetic differentiation for the mitochondrial markers used and are also generally differentiated at the nuclear level.

Conclusions: These results indicate that *A. andreanskyi* is an additional example of a montane species complex.

Keywords: *Atlantolacerta andreanskyi*, Lacertidae, Mountain specialist, High Atlas Mountains, Phylogeography, Morocco

Background

An emerging pattern among European biotas is that the accentuated environmental instability that occurred during the Pleistocene did not lead to increased speciation rates, with many species and populations originating during the Miocene and proceeding through the Quaternary [1-2]. In many species, population fragmentation was triggered by the beginning of the Messinian Salinity Crisis, a short (600 000 years) but crucial period that occurred between 5.9 and 5.3 Mya during which the Mediterranean Sea desiccated almost completely, producing a general and drastic increase in aridity around the Mediterranean Basin [3-4]. As a result of this increased aridity, forests continued to be replaced by more open and arid landscapes forcing the mesic species to retreat to the moister Atlantic-influenced areas and to the mountainous regions, leading to high speciation in some groups [5-6].

Various studies have attempted to unravel the different roles that the global aridification at the end of the Miocene and the Pleistocene glacial cycles have played in the diversity and distribution of European faunas [7]. However, little is known about the effects that these climatic changes had on species living further South, in the African continent. Recent assessments of central African chameleons have uncovered evidence of long-isolated evolutionary histories, with the survival of palaeoendemics leading to considerable diversity [8]. In general, reptiles are excellent model organisms to assess the relative role that the Pre-Quaternary and Quaternary major climatic events have played in the origin, evolution and distribution of species [9]. Available data from some herpetofauna indicate that a similar pattern to the neighboring Iberian Peninsula exists in North Africa, with deep lineages originating at the end of the Miocene (*Chalcides* [10], *Acanthodactylus* [11-13], *Podarcis* [2, 14-15], *Saurodactylus* [16], *Ptyodactylus* [17], *Salamandra* [18], *Pleurodeles* [19]). However, the lack of informative nuclear markers in most of these studies may prevent the recovery of the true evolutionary history of the group [20-21], and makes it difficult to ascertain if these lineages correspond to species complexes or not. Since there is a strong likelihood of discordance between gene trees and species trees [22-24], information from different genetic markers (mitochondrial and nuclear) is thus necessary for delimiting evolutionary lineages, as well as for establishing phylogenetic relationships.

Despite being key concepts in the fields of systematic and evolutionary biology, recognizing and delimiting species are highly controversial issues [e.g. 25-26]. Recognizing species is not only a taxonomic challenge, but is also essential for other biological disciplines such as biogeography, ecology and evolutionary biology [27], and has serious consequences for conservation biology and the design of effective conservation plans [28-29]. Delimiting species is also the first step towards discussing broader questions on evolution, biogeography, ecology or conservation. Recently, thanks to intellectual progress made in the field with the

aim of identifying a common element among all the different species concepts, a single, more general, concept of species known as General Lineage Species Concept has been suggested [30]. This unified species concept emphasizes the common element found in many species concepts, which is that species are separately evolving lineages. Therefore, properties like reciprocal monophyly at one or multiple loci, phenotypic diagnosability, ecological distinctiveness, etc. are not part of the species concept but are used to assess the separation of lineages and to species delimitation [31]. This separation between species conceptualization and species delimitation and the proposal of a unified species concept has concentrated efforts in the development of new approaches for species delimitation, as for example with "integrative taxonomy" [32-33, among others]. Under this new approach, species delineation is regarded as an objective scientific process that results in a taxonomic hypothesis. Therefore, the level of confidence in the taxonomic hypothesis supported by several independent character sets is much higher than for species supported by only one character [34]. Such an integrative view is especially useful in the case of taxonomic groups that are morphologically conservative, where cryptic species have probably been overlooked [17, 35-36].

Normally, high altitude species carry signatures of the expansion and contraction cycles occurred during glacial and interglacial periods [37-39]. Because of this, they are of particular interest to study historical responses to climate change, since they are adapted to a small window of environmental changes, and usually present low tolerance to high temperatures [40]. In Europe, high altitude species often seem to have persisted through glacial periods by short movements to lower altitudes rather than to the classic "southern refugia" of lowland species. In this way current ranges may primarily reflect postglacial expansions [41]. However, it is not clear if the same phenomenon occurs in African montane taxa.

Atlantolacerta andreanskyi (Werner, 1929) is a lacertid lizard endemic to the western and central parts of the High Atlas Mountains of Morocco. It is restricted to areas above 2400 m [42-43], where it is frequently found in the vicinity of small watercourses or plateaus in the top of the mountains that retain some water from rain or snowmelt. Habitat is normally screes and areas with boulders, meadows and, in particular, the base of cushion-like thorny plants in these places [42; personal observation]. Although *A. andreanskyi* had initially been placed in several different genera within the subtribe Lacertini [44-48], recent phylogenetic analyses based on mitochondrial DNA and a combination of mitochondrial and nuclear markers [49-50] suggest that *A. andreanskyi* is a member of the subtribe Eremiadini, and apparently sister to the remaining Eremiadini. This position would conform to this species lacking the synapomorphies that characterize most other Eremiadini, namely a derived condition of the ulnar nerve and the presence of a fully developed armature in the hemipenis, which has folded

lobes when retracted. It is also distinctive within the Eremiadini regarding the presence of enlarged masseteric scale [49]. Because of its phylogenetic position, without close relationship to any other genus of Eremiadini and its distinctive morphology it was recently placed in a new monotypic genus, *Atlantolacerta* [49]. *Atlantolacerta andreanskyi* is distributed across 440 Km (straight line) of mountainous terrain, with the different populations presenting an apparently disjunct distribution [42-43; see Fig. 1]. As with many montane species, the situation observed in *A. andreanskyi* is similar to an archipelago, with the different "islands" being represented by mountaintops disconnected due to areas of unsuitable habitat below 2400 m. As a result of this scenario, minimal gene flow is currently expected between the different populations; however, it is not known how the different climatic events occurred during the Miocene and Pleistocene have affected this species. Even though some aspects of the biology of *A. andreanskyi* are already well known [e.g. 51-52], the genetic structure of the different populations, as well as the relationships between the different populations have never been assessed before.

Therefore, in order to shed some light on the previous questions and attempt to assess the evolutionary history of the species and identify the number of lineages, we sampled the distribution area of the species and performed several combined phylogenetic reconstructions and clustering analyses, using both mtDNA and nuclear markers.

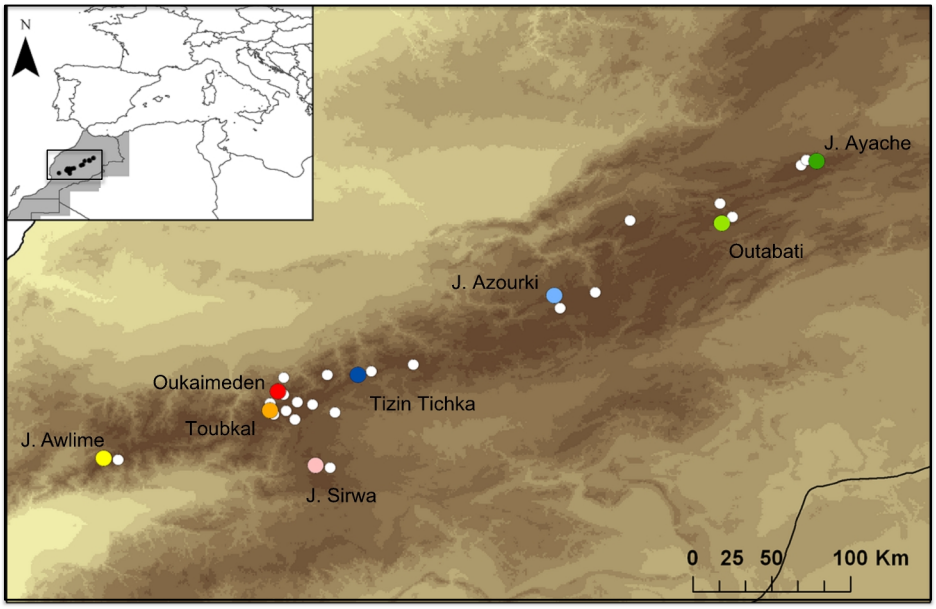

Figure 1. *Atlantolacerta andreanskyi* distribution map. The colour dots represent the localities of the populations sampled for this work. The white dots represent the distributions of the species by Bons and Geniez (1996). J. Awlime (1 yellow), Oukaimeden (2 red), Toupkal (3 orange), J. Sirwa (4 pink), Tizin Tichka (5 dark blue), Outabati (6 light blue), J. Azourki (7 light green) and J. Ayache (8 dark green).

Results

Mitochondrial genealogies

A total of 1108 base pairs (bp) of concatenated mtDNA (*12S rRNA* 330 bp, *ND4* 592 bp and *tRNA-His* 186 bp) were obtained for 89 *A. andreanskyi*. The concatenated alignment of the ingroup sequences revealed 30 haplotypes (3 from Tizin Tichka, 7 from J. Ayache, 5 from J. Sirwa, 2 from Oukaimeden, 7 from J. Azourki, 2 from Outabati, 2 from Toubkal and 2 from J. Awlime) and contained 241 variable sites, of which 232 were parsimony informative.

Analyses of the concatenated mtDNA data were mostly congruent (Fig. 2A). Seven well-supported lineages were recovered from these analyses (pp > 0.95 and BP > 70%), corresponding to the populations from J. Awlime, J. Sirwa, Tizin Tichka, J. Azourki, Outabati, J. Ayache, and Oukaimeden and nearby Toubkal that were grouped together. Regarding the relationships among these clades, we could distinguish three main groups, Oukaimeden and Toubkal with J. Sirwa from the southern end of the distribution range; J. Ayache with Outabati from the northern distribution, and Tizin Tichka with J. Azourki from the central distribution range. The population from J. Awlime, from the extreme South of the range, is a genetically distinct lineage related to the northern group, although, both ML and BI analysis weakly support this topology (see Fig. 2A).

All the populations present a low level of diversity in the mitochondrial DNA (uncorrected genetic distances 0 - 0.5% for the *ND4+tRNA-His* and 0 – 0.2% for the *12S*; see Table 1), and a very high level of genetic divergence between populations (5.5 – 16.5% in the *ND4+tRNA-His* and 2.5 – 6.6% in the *12S;* see Table 1).

Nuclear genealogies

A total of 77 specimens of *A. andreanskyi* were sequenced for five nuclear genes. The *ACM4* was 447 bp long, presenting 47 haplotypes and 34 polymorphic sites, 33 of them parsimony informative; *C-MOS* was 534 bp long, with 32 haplotypes and 21 polymorphic sites, all of them parsimony informative; *MC1R* was 635 bp long, with 57 haplotypes and 36 variable sites, 35 of them parsimony informative; *PDC* was 441 bp long, with 60 haplotypes and 29 variable sites, 26 of them parsimony informative; *RAG1* was 528 bp long, with 38 haplotypes and 19 variable sites, 18 of them parsimony informative.

The differences in the genetic distances between the lineages are congruent with the geographic distance between them, supporting the grouping of the lineages in three main groups as seen in the analysis of mitochondrial sequences.

The concatenated analyses of the 5 unphased nuclear markers are congruent with the results obtained in the mitochondrial DNA tree, although with some differences (Fig. 2B). Despite recovering the three main groups observed in the mtDNA analysis, according to the nuclear

markers the J. Awlime population is not sister to the northernmost populations but branches off inside a polytomy with the westernmost lineages at the base of the tree. It is possible to distinguish some of the lineages, although in some cases they are not monophyletic. The J. Ayache population is monophyletic but makes Outabati paraphyletic. The same happens with Tizin Tichka, which makes the population from J. Azourki paraphyletic. The population from Oukaimeden is polyphyletic.

Concatenated analysis (mtDNA and nDNA)

The results of the ML and BI analyses of the mtDNA and nDNA (Fig. 2C) support the same seven lineages as recovered in the mitochondrial analysis, although in this case J. Awlime is sister to the central and northern lineages (Tizin Tichka, J. Azourki, Outabati, and J. Ayache) instead of being sister to only the northernmost lineages (Fig. 2A). As in the mtDNA analysis (Fig. 2A), the relationship of J. Awlime with the central and northern lineages is very poorly supported. This result was expected, given the higher resolving power of the mtDNA that contributed with 241 variable sites versus the 150 from the nDNA.

Nuclear networks

As show in Fig. 3 and Table 2, there is a moderate degree of haplotype sharing between populations, with most f them lacking private alleles for the nuclear genes analysed.

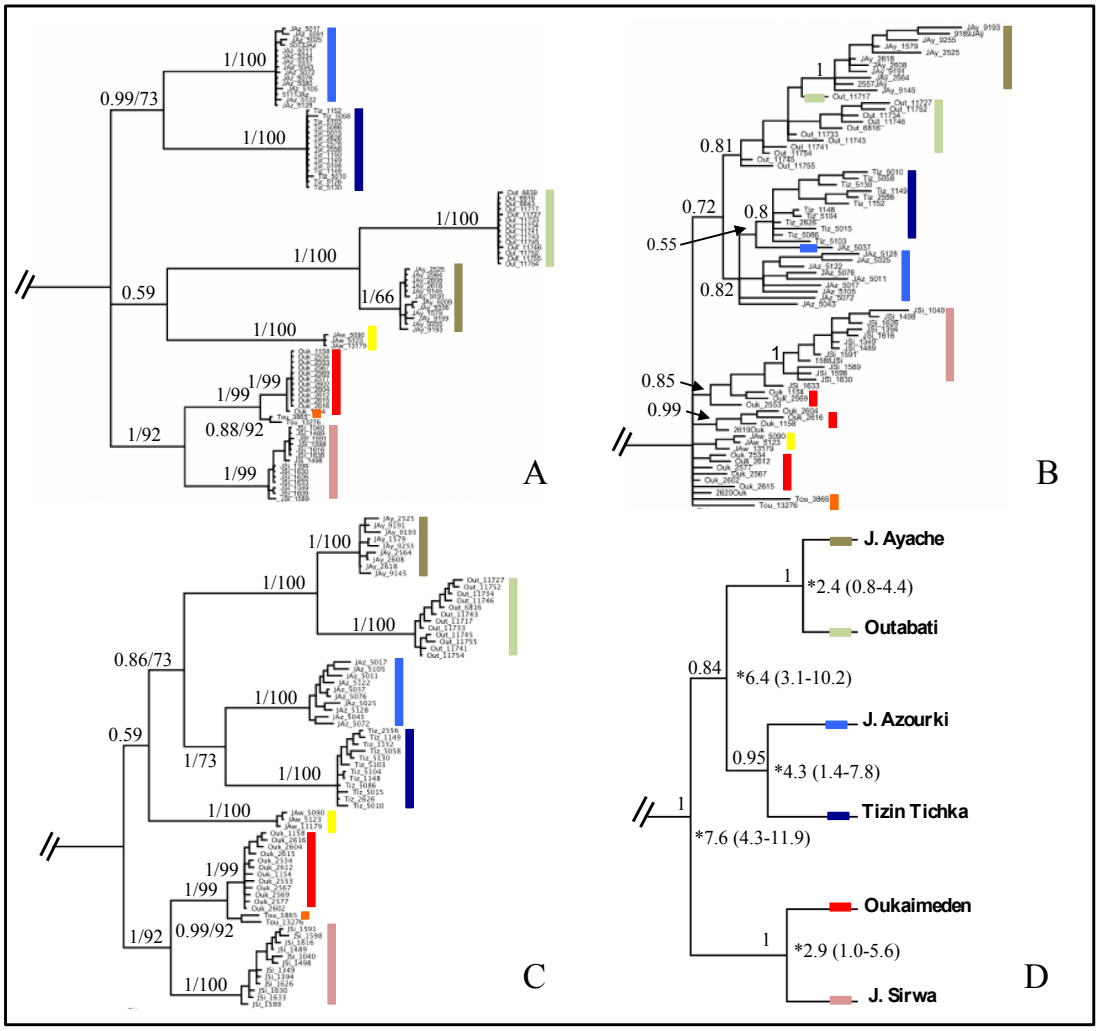

Figure 2. Trees resulting from partitioned Bayesian analysis. (A) Mitochondrial DNA tree (*12S, ND4* and flanking *tRNA-His*), (B) nuclear concatenated tree (*RAG1, ACM4, MC1R, PDC* and *C-MOS*), (C) Concatenated tree from the combined mitochondrial and nuclear DNA data. The partitions used the models described in the text. Bayesian posterior probabilities (0–1) and bootstrap values (> 50%) for ML (1–100) are indicated near the branches, (D) Species tree from mitochondrial and nuclear DNA data from the Bayesian Inference of Species Trees (STARBEAST). Clade posterior probabilities are shown to the left of the nodes, and divergence times and 95% intervals (calculated in BEAST using only *ND4* + *tRNA-His*), to the right of the nodes. The trees were rooted using *Podarcis bocagei*, *P. hispanica* and *P. carbonelli*. The colours represent the different populations.

Clustering analysis and individual assignment

In our study, the obtained K differs with the combination between the ancestry model and the allele frequency model. When combined the No Admixture Model (ancestry model) with the Allele Frequencies Independent Model (allele frequency model) the best resulting K values were for K = 3: South (Oukaimeden, J. Sirwa, Toubkal and J. Awlime), center (Tizin Tichka and J. Azourki) and North (Outabati and J. Ayache) groups. With the other three combinations between the models the best result were for K = 6: J. Sirwa, Tizin Tichka, J. Azourki, Outabati, J. Ayache, and a group formed by Oukaimeden, Toubkal and J. Awlime (Fig. 4).

Table 1. Genetic distances and divergence time estimate between populations. (A) Genetic distance (*12S* and *ND4* + *tRNA-His*) between all the populations and (B) between main groups; and (C) divergence time estimates, calculated using BEAST with *ND4* and *tRNA-His*. The diversity of each population is below the population's names.

A

Pop	**Tizin Tichka**	**Oukaimeden**	**J.Sirwa**	**J.Ayache**	**J.Azourki**	**Outabati**	**Toubkal**	**J.Awlime**
p-distance (%) *12S/ ND4*	0.1	0.4	0.3	0.5	0.2	0	0.4	0.1
Tizin Tichka 0		13.1	12.7	14.5	10.5	15.3	12.9	13.6
Oukaimeden 0	4		**7.7**	15	13.2	**16.1**	**1.7**	13.2
J.Sirwa 0.2	4.2	**2.8**		**16.1**	12.7	**16.5**	7.5	11.6
J.Ayache 0.1	5.4	**5.7**	4.8		12.7	5.5	14.4	14.1
J.Azourki 0.1	4.3	4.3	3.8	**6.6**		14.2	13.2	13.1
Outabati 0	5.4	**5.7**	4.2	1.6	6		**16**	14
Toubkal 0	3.7	**0.3**	2.5	5.4	4	5.4		12.6
J.Awlime 0	4	4.7	4.5	5.1	**6.4**	5	4.3	

B

Pop	**JAy+Out**	**Tiz+JAz**	**J.Awlime**	**Ouk+JSi+Tou**
p-distance (%) *12S/ ND4*	0.9	2.3	0	1.5
JAy+Out 2.9		13.7	13.4	14.6
Tiz+JAz 5.2	5.9		12.9	12.4
J.Awlime 0.1	5	5.2		12.2
Ouk+JSi+Tou 0.4	5.1	4.1	4.6	

C

Beast Ma (95% HPD)	***ND4***
North-South	7.6 (4.3-11.9)
JAw - Ouk	5.6 (2.5-9.7)
JAy+Out - Tiz+JAz	6.4 (3.1-10.2)
Ouk - JSi	2.9 (1.0-5.6)
Tiz - JAz	4.3 (1.4-7.8)
JAy - Out	2.4 (0.8-4.4)
Ouk - Tou	0.5 (0.1-1.2)

Species tree and divergence time estimates

The results of the clustering analysis with K = 6 were used to define the species for the species tree analysis in STARBEAST. The tree inferred with information from mitochondrial and nuclear markers (phased) (figure 2D) recovered the same topology as in Fig. 2C, with all the relationships between the lineages supported by previous analyses.

The divergence time estimates were calculated for the six populations (Table 2). High effective sample sizes were observed for all parameters in all BEAST analysis (posterior ESS

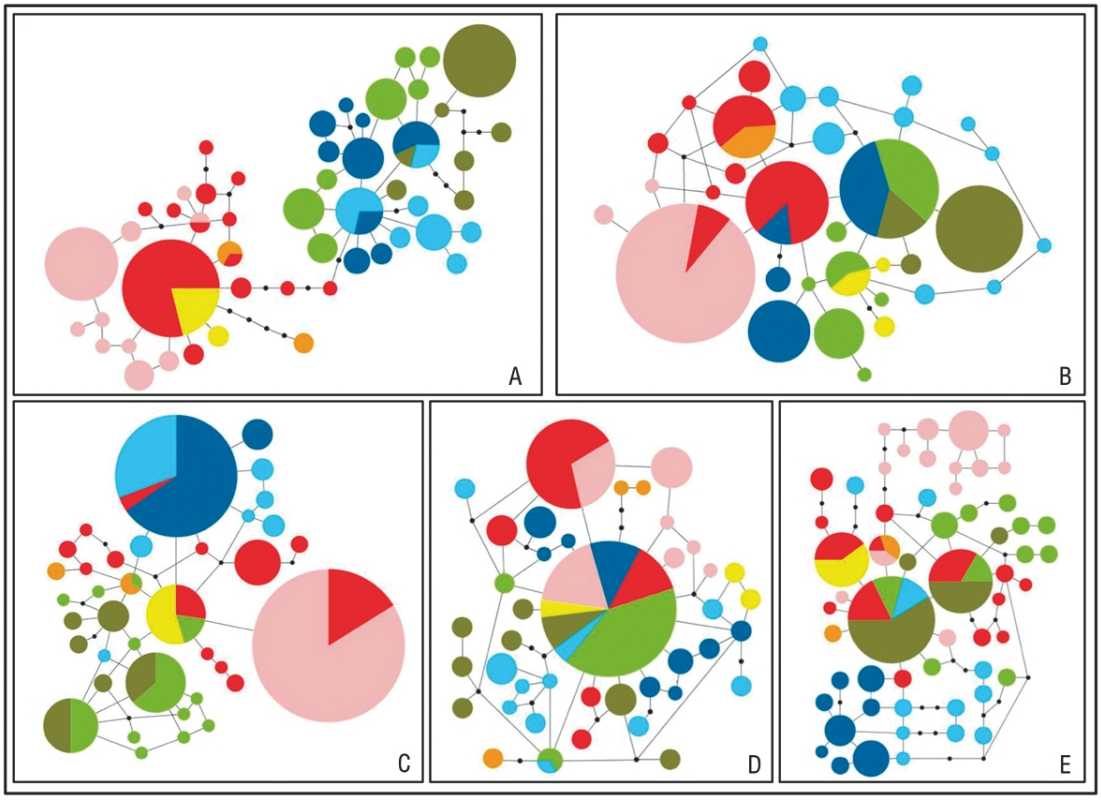

Figure 3. Parsimony networks corresponding to *MC1R* (A), *RAG1* (B), *C-MOS* (C), *ACM4* (D) and *PDC* (E) nDNA sequence variation from all the populations. The colours used were the same as the used in the map (Figure 1) and trees (Figure 2), J. Awlime (yellow), Toubkal (orange), Oukaimeden (red), J. Sirwa (pink), Tizin Tichka (dark blue), J. Azourki (light blue), Outabati (light green), and J. Ayache (dark green). Lines represent a mutation step, circles represent haplotypes and dots missing haplotypes. The size of the circles is proportional to the number of alleles.

values > 1000 for all four analyses) and assessment of convergence statistics in Tracer indicated that all analyses had converged. Maximum clade credibility tree for *ND4+tRNA-His* was identical in topology to those produced by Bayesian and ML analyses. According to the inferred dates resulted from BEAST (Fig. 2D), the two main mitochondrial lineages of *A. andreanskyi* (South versus central and North) split approximately 7.6 Ma (95% high posterior density (HPD) interval 4.3-11.9 Ma). The populations that are grouped in the three main clades (South, central and North) split approximately at the same time, being Tizin Tichka and J. Azourki the first to split at about 4.3 Ma (1.4-7.8), followed by Oukaimeden and J. Sirwa 2.9 Ma (1-5.6), and Outabati and J. Ayache 2.4 Ma (0.8-4.4). Tizin Tichka and J. Azourki diverged from Outabati and J. Ayache approximately 6.4 Ma (3.1-10.2).

Table 2. Percentage of private alleles in all the populations and for each nuclear locus.

Private Alleles (%)	*MC1R*	*RAG1*	*C-MOS*	*ACM4*	*PDC*
J. Awlime	33	50	0	67	0
J. Sirwa	96	12	0	42	92
Toubkal	50	0	50	100	50
Oukaimeden	41	33	70	29	57
Tizin Tichka	75	59	23	71	100
J. Azourki	60	100	60	84	90
Outabati	100	54	43	9	83
J. Ayache	92	85	57	80	20

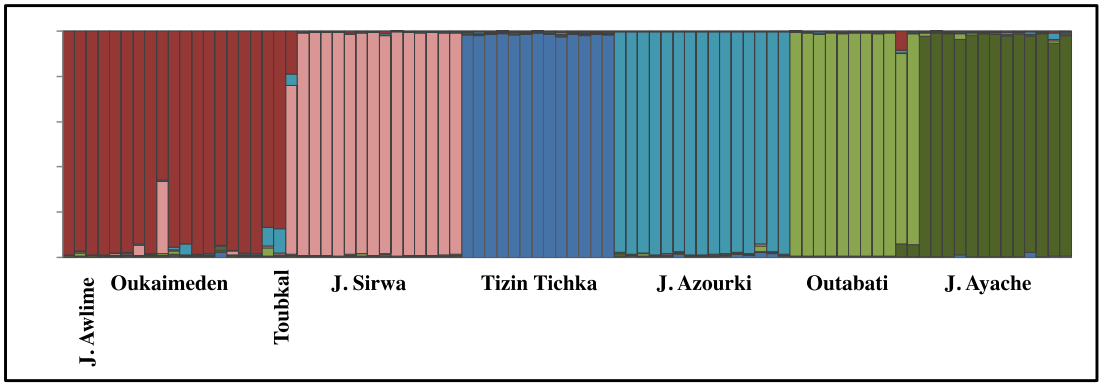

Figure 4. Population structure estimation. Each individual is represented by a thin vertical line, which is partitioned into K coloured segments that represent the individual's estimated membership fractions in K clusters. The bigger vertical divisions separate individuals from different populations. Populations are labeled below the figure. The colours used are the same used in Figure 1 and Figure 2.

Discussion

Extreme mtDNA diversity in *A. andreanskyi*

Several recently published analyses of North African herpetofauna have revealed high levels of endemism and cryptic species [12, 14-15, 17]. In this analysis, the surprising result was the extreme diversity of mitochondrial DNA found between almost all the populations analysed. The genetic differentiation observed between populations (2.8% - 6.6% in *12S* and 5.5% - 16.5% in *ND4+tRNA-His*) is similar and, in some cases, higher than the divergence found between *Iberolacerta* species (between 7.4% and 8.2% in the *cytochrome b* gene, [53]), a lacertid genus with most of its species occurring in the mountains of the Iberian Peninsula [41, 54]. Initially considered one species, there are now seven recognized species of *Iberolacerta* in the Iberian Peninsula. Genetic differentiation between these species is lower than between the different populations of *A. andreanskyi*.

Although the mitochondrial phylogeny supports the existence of seven distinct groups, the clustering analysis only supports the existence of six lineages (J. Sirwa, Tizin Tichka, J. Azourki, Outabati, J. Ayache and a lineage formed by Oukaimeden, Toubkal and J. Awlime).

Toubkal samples were always part of the same lineage as Oukaimeden, although, they show some divergence at least at the mitochondrial DNA level (1.7% in *ND4+tRNA-His* and 0.3% in *12S*). This is not unexpected, as these populations are geographically very close and are part of the High Atlas Mountains, where interconnectivity between populations could occur. The mitochondrial phylogenetic analyses supported the existence of a seventh isolated lineage, J. Awlime, however clustering analysis and the nuclear phylogeny did not support the distinctiveness of this population, possibly because of the small sampling size. Unfortunately, despite multiple attempts to sample in this remote region, only three individuals were captured. The analyses also could not recover the genetic relationship between J. Awlime and the other populations, because its position in the trees fluctuated between the two main groups (North and South), without support in any of the trees.

Non-reciprocal monophyly in nuclear markers and species delimitation

In the phylogenetic analyses of the concatenated nuclear loci, some of the lineages supported by mtDNA data were not monophyletic. This was observed only between the geographically closest lineages, as in the case of Oukaimeden and J. Sirwa; Tizin Tichka and J. Azourki; and Outabati and J. Ayache, that presumably were in contact more recently than the others. This may be due to the larger effective population size of the nuclear DNA compared to the mitochondrial DNA and the consequent stronger effect of the incomplete lineage sorting at each single nuclear loci [55]. Additionally, the slow evolutionary rate of some of these markers may be a factor. The conjugation of these two effects probably explains the absence of concordance in the single nuclear gene networks (figure 3), although the same general topology was recovered in the concatenated nuclear phylogeny. Reciprocal monophyly is one of the primary criteria to delimit species [31, 56]. Although it is possible to delimit species without observing monophyly in gene trees, since a considerable amount of time must pass after the beginning of divergence of species until they show reciprocal monophyly at a sample of multiple loci [57-58]. Pinho *et al.* [59] have shown that *Podarcis* from the Iberian Peninsula and North Africa have a similar pattern (between mtDNA and nuclear) but in a smaller time window and using faster evolving nuclear loci and, in contrast to our case, some populations are in contact.

Although we are aware that the determination of K, in STRUCTURE, is only an *ad hoc* guide to describe consistence between models and the data [60], the program has been commonly used for this end [61]. Several methods based on Bayesian clustering have been developed [62-64], however, STRUCTURE is the most widely used, and various studies show its efficiency in assigning individuals to their population of origin [65-68] and its ability to construct an appropriate clustering hypothesis [61]. However, in the present example the

analysis was limited because it was based only in haplotype information. The obtained K differ with the combination model used, but in most of the combinations the analysis supports a K = 6 corresponding to the geographical populations and to the results recovered by the other analyses. This analysis also placed the samples from the J. Awlime population together with the Oukaimeden lineage, possibly due to the limited haplotype sampling. Similarly, the concatenated phylogenetic tree, based on all the genes, supports the existence of 7 lineages giving once more a low support to the relationship between J. Awlime and the other lineages. The networks of the individual nuclear loci show high percentage of private alleles in some of the lineages, which fluctuate depending on the gene (figure 3).

Dating the trees

All the lineages are grouped in two main clusters, the northern group composed by J. Ayache, Outabati, J. Azourki and Tizin Tichka; and the southern group that includes Oukaimeden and J. Sirwa. The divergence obtained for these two lineages was around 7.6 Mya, (4.3-11.9), which coincides approximately with the time of the final closing of the Rifian Strait (7.2 Mya; [3]). During the Miocene, tectonic activity in the region was intense and included the uplift of the Atlas Mountains that occurred around 9.0 Mya [69-70]. It was more or less at the same time that *Podarcis* invaded North Africa (7.5 ± 1.2 Mya, [2]) and the Iberian clade of *Iberolacerta* started to fragment (6.1 Mya, [1]). The split of the six lineages must have occurred later, probably during the Quaternary Glaciations (4.3 ± 3; 2.4 ± 2; 2.9 ± 2 Mya). However, the confidence intervals obtained were very large, increasing the time window for the events and the associated error. Determination of the time of the speciation events is important to understand the evolutionary biogeography of species [71]. However, it is difficult to estimate ages in phylogenies without several sources of error. Clearly the lineages of *A. andreanskyi* are pre-Pleistocenic and, as found in Central African chameleons [8] can be considered paleoendemics. However, without better calibration points it is difficult to date the split of the lineages more precisely than this.

Conclusions

Phylogeographic assessments of several taxa in northwest Africa have indicated the presence of cryptic diversity in organisms ranging from scorpions [72] to mammals [73], and reptiles are not an exception [e.g. 11, 17, 74]. What is exceptional in the case of *A. andreanskyi* are the high levels of mitochondrial divergence between almost every sampled populations, ranging from 5.5 up to 16.5% (*ND4+tRNA-His*) between populations separated by low geographic distances (for example just 60 Km between Oukaimeden and J. Sirwa and 45 Km

between Oukaimeden and Tizin Tichka). Six of the eight analysed populations are highly distinct based on both mtDNA and multiple nuclear markers. This raises the issue not of whether *A. andreanskyi* is a species complex, but just how many species may occur within the group. Presumably, far more than the six possible species identified in this study, since, probably, many populations remain unsampled. However, preliminary morphological analyses suggest that all the different populations included in the present study are very homogeneous (unpublished data). This may imply the presence of cryptic diversity, but definitive conclusions should wait until a complete morphological study is carried out (work in progress).

Current models of reptiles species accessed for the region indicate low levels of diversity across much of the High Atlas Mountains [75]. Indeed only a few species are recorded at altitudes above 2000 m; typically *A. andreanskyi*, *Quedenfeldtia* species (*Q. trachyblepharus* and *Q. moerens*), *Chalcides montanus* and *Vipera monticola* [e.g. 42, 76]. However, the finding of high genetic diversity in *A. andreanskyi* indicates that unidentified lineages occur, and that the other high mountain species should also be assessed as possible cryptic species candidates. Our results are also essential from a conservation point of view, as many forms may actually have smaller ranges than currently thought, and small isolated populations on high mountains have been identified as those of high concern under typical global warming scenarios [77]. Given these results it is necessary to increase the sampling in order to understand the relationship of J. Awlime with the other populations and try to find new populations. Furthermore it is very important to conduct a through morphological study to determine if there is phenotypic variation, and then to revise the taxonomy of the genus *Atlantolacerta*.

Methods

Species concept and integrative approach

Although the present study does not include a taxonomic revision of the genus *Atlantolacerta*, like many other works in which some of the authors of the present manuscript have participated [35, 78-79], we advocate for the use of the General Lineage Species Concept proposed by de Queiroz [30]. Two lines of evidence have been defined on the basis of alleged independence of their respective datasets: mitochondrial DNA and nuclear DNA. In the present study, we have decided to retain as "putative species" only these lineages that were recovered as monophyletic in the phylogenetic analysis of the mtDNA data and that were supported by the analysis of the nDNA using STRUCTURE v.2.3.2 [60]. Within the framework of an integrative approach, and pending the inclusion of morphological data, this

would correspond to Integration by total congruence (ITC). However, it is important to take into account that in the absence of a thorough morphological analysis we do not consider the molecular data presented here enough to revise the taxonomy of the genus *Atlantolacerta.*

DNA extraction, amplification, and sequencing

A total of 92 individuals from eight different populations distributed across the entire range of *Atlantolacerta andreanskyi* were sampled for this study: 14 from Oukaimeden, 15 from Tizin Tichka, 14 from Jebel Ayache, 15 from Jebel Azourki, 14 from Outabati, 15 from Jebel Sirwa and 2 from Toubkal and 3 from J. Awlime (Fig. 1 and Table 3). Specimens were caught by hand, identified on the basis of external features, measured and photographed for later morphological studies. Tail tips where collected and stored in 96% ethanol, after which individuals were released in the same place where they were caught.

Genomic DNA was extracted from ethanol-preserved tissue samples using standard high-salt protocols [80]. A total of 89 specimens (from the 92 sampled) of *Atlantolacerta andreanskyi* plus three outgroups (*Podarcis hispanica*, *Podarcis carbonelli* and *Podarcis bocagei*) were sequenced for two mitochondrial regions: partial *12S rRNA* (*12S*) and partial *NADH dehydrogenase 4* (*ND4*) and flanking *tRNA (tRNA-His*) and 77 specimens (plus the outgroups, same as for mtDNA) for five nuclear gene fragments, *recombination-activating gene 1* (*RAG1*), *acetylcholinergic receptor M4* (*ACM4*), *melanocortin receptor 1* (*MC1R*), *oocyte maturation factor Mos* (*C-MOS*) and *phosducin* (*PDC*). Primers used for both amplification and sequencing were: 12Sa and 12Sb [81] for the *12S* following the PCR conditions described in Harris and Arnold [82], ND4 and Leu for *ND4+tRNA-His*, PCR conditions described in Arévalo *et al.* [83]; L2408 and H2920 for *RAG1* following the PCR conditions from Vidal and Hedges [84]; tg-F and tg-R [85] for *ACM4* with PCR conditions following Gamble *et al.* [86]; MC1RF and MC1RR for *MC1R* following PCR conditions described in Pinho *et al.* [87]; Lsc1 and Lsc2 for *C-MOS* following the PCR conditions from Godinho *et al.* [88]; and PHOF2 and PHOF1 for *PDC*, following PCR conditions described in Bauer *et al.* [89]. PCRs were carried out in 25 μl volumes, containing 5.0 μl of 10 reaction Buffer, 2.0 mM of MgCl2, 0.5 mM each dNTP, 0.2 μM each primer, 1 U of Taq DNA polymerase (Invitrogen), and approximately 100 ng of template DNA. Finally, PCR products were purified using exosap IT and the resulting amplified fragments were sequenced on an Applied Biosystem DNA Sequencing Apparatus. Chromatographs were checked manually, assembled and edited using Bioedit 7.0.1 [90].

Table 3. Samples used in the work with localities (PS coordinates; WGS84 coordinate system) and GenBank accession numbers for all the sequenced genes.

Specimen code	Alleles	Population	Latitude	Longitude	Altitude	GenBank accession codes *12S / ND4+tRNA-His / PDC / ACM4 / C-MOS / MC1R / RAG1*
1152	1152a	Tizin Tichka (Tiz)	31.30077	-7.40984	2800	JX462053 / JX462189 / JX461527 / JX461879 / JX485185 / JX461693 / JX461351
	1152b					JX461528 / JX461880 / JX485186 / JX461694 / JX461352
1149	1149a	Tizin Tichka (Tiz)	31.30077	-7.40984	2800	JX462062 / JX462194 / JX461523 / JX461875 / JX485189 / JX461689 / JX461349
	1149b					JX461524 / JX461876 / JX485190 / JX461690 / JX461350
1148	1148a	Tizin Tichka (Tiz)	31.30077	-7.40984	2800	JX462064 / JX462195 / JX461521 / JX461873 / JX485191 / JX461687 / JX461347
	1148b					JX461522 / JX461874 / JX485192 / JX461688 / JX461348
1150	1150	Tizin Tichka (Tiz)	31.30077	-7.40984	2800	JX462061 / JX462191 / ... / ... / ... / ... / ...
2556	2556a	Tizin Tichka (Tiz)	31.30077	-7.40984	2800	JX462060 / JX462192 / JX461593 / JX461947 / JX485195 / JX461947 / JX461417
	2556b					JX461594 / JX461948 / JX485196 / JX461948 / JX461418
2578	2578	Tizin Tichka (Tiz)	31.30077	-7.40984	2800	JX462059 / JX462193 / ... / ... / ... / ... / ...
2626	2626a	Tizin Tichka (Tiz)	31.30077	-7.40984	2800	JX462058 / JX462190 / JX461625 / JX461979 / JX485193 / JX461793 / JX461447
	2626b					JX461626 / JX461980 / JX485194 / JX461794 / JX461448
5058	5058a	Tizin Tichka (Tiz)	31.30077	-7.40984	2800	JX462054 / JX462196 / JX461643 / JX461999 / JX485205 / JX461815 / JX461469
	5058b					JX461644 / JX462000 / JX485206 / JX461816 / JX461470
5010	5010a	Tizin Tichka (Tiz)	31.30077	-7.40984	2800	JX462065 / JX462203 / JX461629 / JX461983 / JX485199 / JX461799 / JX461453
	5010b					JX461630 / JX461984 / JX485200 / JX461800 / JX461454
5126	5126	Tizin Tichka (Tiz)	31.30077	-7.40984	2800	JX462066 / JX462197 / ... / ... / ... / ... / ...
5086	5086a	Tizin Tichka (Tiz)	31.30077	-7.40984	2800	JX462056 / JX462198 / JX461649 / JX462009 / JX485197 / JX461825 / JX461479
	5086b					JX461650 / JX462010 / JX485198 / JX461826 / JX461480
5103	5103a	Tizin Tichka (Tiz)	31.30077	-7.40984	2800	JX462055 / JX462202 / JX461655 / JX462015 / JX485207 / JX461831 / JX461483
	5103b					JX461656 / JX462016 / JX485208 / JX461832 / JX461484
5104	5104a	Tizin Tichka (Tiz)	31.30077	-7.40984	2800	JX462063 / JX462199 / JX461657 / JX462017 / JX485209 / JX461833 / JX461485
	5104b					JX461658 / JX462018 / JX485210 / JX461834 / JX461486
5015	5015a	Tizin Tichka (Tiz)	31.30077	-7.40984	2800	JX462057 / JX462200 / JX461633 / JX461987 / JX485203 / JX461803 / JX461457
	5015b					JX461634 / JX461988 / JX485204 / JX461804 / JX461458
5130	5130a	Tizin Tichka (Tiz)	31.30077	-7.40984	2800	JX462067 / JX462201 / JX461667 / JX462031 / JX485201 / JX461847 / JX461497
	5130b					JX461668 / JX462032 / JX485202 / JX461848 / JX461498
1040	1040a	Jebel Sirwa (JSi)	30.77671	-7.65299	2561	JX462083 / JX462153 / JX461519 / JX461871 / JX485240 / JX461685 / JX461345
	1040b					JX461520 / JX461872 / JX485241 / JX461686 / JX461346
1349	1349a	Jebel Sirwa (JSi)	30.77671	-7.65299	2561	JX462084 / JX462150 / JX461557 / JX461911 / JX485242 / JX461725 / JX461383
	1349b					JX461558 / JX461912 / JX485243 / JX461726 / JX461384
1394	1394a	Jebel Sirwa (JSi)	30.77671	-7.65299	2561	JX462085 / JX462147 / JX461559 / JX461913 / JX485244 / JX461727 / JX461385
	1394b					JX461560 / JX461914 / JX485245 / JX461728 / JX461386
1489	1489a	Jebel Sirwa (JSi)	30.77671	-7.65299	2561	JX462086 / JX462152 / JX461561 / JX461915 / JX485246 / JX461729 / JX461387
	1489b					JX461562 / JX461916 / JX485247 / JX461730 / JX461388
1498	1498a	Jebel Sirwa (JSi)	30.77671	-7.65299	2561	JX462087 / JX462151 / JX461563 / JX461917 / JX485248 / JX461731 / JX461389
	1498b					JX461564 / JX461918 / JX485249 / JX461732 / JX461390

Specimen code	Alleles	Population	Latitude	Longitude	Altitude	GenBank acession codes *12S / ND4+tRNA-His / PDC / ACM4 / C-MOS / MC1R / RAG1*	2/5
1598	1598a	Jebel Sirwa (JSi)	30.77671	-7.65299	2561	JX462158 / JX462158 / JX461573 / JX461927 / JX485256 / JX461741 / JX461399	
	1598b					JX461574 / JX461928 / JX485257 / JX461742 / JX461400	
1633	1633a	Jebel Sirwa (JSi)	30.77671	-7.65299	2561	JX462096 / JX462148 / JX461583 / JX461937 / JX485264 / JX461751 / JX461409	
	1633b					JX461584 / JX461938 / JX485265 / JX461752 / JX461410	
1638	1638	Jebel Sirwa (JSi)	30.77671	-7.65299	2561	JX462097 / JX462159 / ... / ... / ... / ... / ...	
1588	1588a	Jebel Sirwa (JSi)	30.77671	-7.65299	2561	... / ... / JX461567 / JX461921 / JX485250 / JX461735 / JX461393	
	1588b					JX461568 / JX461922 / JX485251 / JX461736 / JX461394	
1626	1626a	Jebel Sirwa (JSi)	30.77671	-7.65299	2561	JX462160 / JX462160 / JX461579 / JX461933 / JX485260 / JX461747 / JX461405	
	1626b					JX461580 / JX461934 / JX485261 / JX461748 / JX461406	
1616	1616a	Jebel Sirwa (JSi)	30.77671	-7.65299	2561	JX462154 / JX462154 / JX461577 / JX461931 / JX485258 / JX461745 / JX461403	
	1616b					JX461578 / JX461932 / JX485259 / JX461746 / JX461404	
1609	1609	Jebel Sirwa (JSi)	30.77671	-7.65299	2561	JX462155 / JX462155 / ... / ... / ... / ... / ...	
1589	1589a	Jebel Sirwa (JSi)	30.77671	-7.65299	2561	JX462090 / JX462156 / JX461569 / JX461923 / JX485252 / JX461737 / JX461395	
	1589b					JX461570 / JX461924 / JX485253 / JX461738 / JX461396	
1630	1630a	Jebel Sirwa (JSi)	30.77671	-7.65299	2561	JX462088 / JX462149 / JX461581 / JX461935 / JX485262 / JX461749 / JX461407	
	1630b					JX461582 / JX461936 / JX485263 / JX461750 / JX461408	
1591	1591a	Jebel Sirwa (JSi)	30.77671	-7.65299	2561	JX462157 / JX462157 / JX461571 / JX461925 / JX485254 / JX461739 / JX461397	
	1591b					JX461572 / JX461926 / JX485255 / JX461740 / JX461398	
1158	1158a	Oukaimeden (Ouk)	31.20426	-7.86705	2600	JX462069 / JX462162 / JX461531 / JX461883 / JX485211 / JX461697 / JX461355	
	1158b					JX461532 / JX461884 / JX485212 / JX461698 / JX461356	
1154	1154a	Oukaimeden (Ouk)	31.20426	-7.86705	2600	JX462068 / JX462161 / JX461529 / JX461881 / JX485214 / JX461695 / JX461353	
	1154b					JX461530 / JX461882 / JX485215 / JX461696 / JX461354	
2534	2534a	Oukaimeden (Ouk)	31.20426	-7.86705	2600	JX462070 / JX462163 / JX461587 / JX461941 / JX485220 / JX461755 / JX461411	
	2534b					JX461588 / JX461942 / JX485221 / JX461756 / JX461412	
2553	2553a	Oukaimeden (Ouk)	31.20426	-7.86705	2600	JX462071 / JX462164 / JX461591 / JX461945 / JX485218 / JX461759 / JX461415	
	2553b					JX461592 / JX461946 / JX485219 / JX461760 / JX461416	
2619	2619a	Oukaimeden (Ouk)	31.20426	-7.86705	2600	... / ... / JX461621 / JX461975 / JX485238 / JX461789 / JX461443	
	2619b					JX461622 / JX461976 / JX485239 / JX461790 / JX461444	
2620	2620a	Oukaimeden (Ouk)	31.20426	-7.86705	2600	... / ... / JX461623 / JX461977 / JX485236 / JX461791 / JX461445	
	2620b					JX461624 / JX461978 / JX485237 / JX461792 / JX461446	
2577	2577a	Oukaimeden (Ouk)	31.20426	-7.86705	2600	JX462074 / JX462167 / JX461603 / JX461957 / JX485216 / JX461771 / JX461427	
	2577b					JX461604 / JX461958 / JX485217 / JX461772 / JX461428	
2567	2567a	Oukaimeden (Ouk)	31.20426	-7.86705	2600	JX462072 / JX462165 / JX461599 / JX461953 / JX485222 / JX461767 / JX461423	
	2567b					JX461600 / JX461954 / JX485223 / JX461768 / JX461424	
2569	2569a	Oukaimeden (Ouk)	31.20426	-7.86705	2600	JX462073 / JX462166 / JX461601 / JX461955 / JX485234 / JX461769 / JX461425	
	2569b					JX461602 / JX461956 / JX485235 / JX461770 / JX461426	
2602	2602a	Oukaimeden (Ouk)	31.20426	-7.86705	2600	JX462075 / JX462168 / JX461607 / JX461961 / JX485232 / JX461775 / JX461429	
	2602b					JX461608 / JX461962 / JX485233 / JX461776 / JX461430	
2604	2604a	Oukaimeden (Ouk)	31.20426	-7.86705	2600	JX462076 / JX462169 / JX461609 / JX461963 / JX485230 / JX461777 / JX461431	
	2604b					JX461610 / JX461964 / JX485231 / JX461778 / JX461432	

Specimen code	Alleles	Population	Latitude	Longitude	Altitude	GenBank acession codes *12S / ND4+tRNA-His / PDC / ACM4 / C-MOS / MC1R / RAG1*	3/5
2612	2612a	Oukaimeden (Ouk)	31.20426	-7.86705	2600	JX462077 / JX462170 / JX461613 / JX461967 / JX485224 / JX461781 / JX461435	
	2612b					JX461614 / JX461968 / JX485225 / JX461782 / JX461436	
2615	2615a	Oukaimeden (Ouk)	31.20426	-7.86705	2600	JX462078 / JX462171 / JX461615 / JX461969 / JX485228 / JX461783 / JX461437	
	2615b					JX461616 / JX461970 / JX485229 / JX461784 / JX461432	
2616	2616a	Oukaimeden (Ouk)	31.20426	-7.86705	2600	JX462079 / JX462172 / JX461617 / JX461971 / JX485226 / JX461785 / JX461439	
	2616b					JX461618 / JX461972 / JX485227 / JX461786 / JX461440	
1579	1579a	Jebel Ayache (Jay)	32.53671	-4.79110	3043	JX462098 / JX462178 / JX461565 / JX461919 / JX485276 / JX461733 / JX461391	
	1579b					JX461566 / JX461920 / JX485277 / JX461734 / JX461392	
2552	2552a	Jebel Ayache (Jay)	32.53671	-4.79110	3043	JX462099 / JX462177 / JX461589 / JX461943 / JX485278 / JX461757 / JX461413	
	2552b					JX461590 / JX461944 / JX485279 / JX461758 / JX461414	
2564	2564a	Jebel Ayache (Jay)	32.53671	-4.79110	3043	JX462101 / JX462176 / JX461597 / JX461951 / JX485272 / JX461765 / JX461421	
	2564b					JX461598 / JX461952 / JX485273 / JX461766 / JX461422	
2608	2608a	Jebel Ayache (Jay)	32.53671	-4.79110	3043	JX462102 / JX462175 / JX461611 / JX461965 / JX485270 / JX461779 / JX461433	
	2608b					JX461612 / JX461966 / JX485271 / JX461780 / JX461434	
2618	2618a	Jebel Ayache (Jay)	32.53671	-4.79110	3043	JX462103 / JX462174 / JX461619 / JX461973 / JX485268 / JX461787 / JX461441	
	2618b					JX461620 / JX461974 / JX485269 / JX461788 / JX461442	
9189	9189a	Jebel Ayache (Jay)	32.53671	-4.79110	3043	... / ... / JX461675 / JX462043 / JX485282 / JX461859 / JX461509	
	9189b					JX461676 / JX462044 / JX485283 / JX461860 / JX461510	
9199	9199	Jebel Ayache (Jay)	32.53671	-4.79110	3043	JX462108 / JX462183 / ... / ... / ... / ... / ...	
9255	9255a	Jebel Ayache (Jay)	32.53671	-4.79110	3043	JX462110 / JX462179 / JX461681 / JX462051 / JX485290 / JX461867 / JX461515	
	9255b					JX461682 / JX462052 / JX485291 / JX461868 / JX461516	
9209	9209	Jebel Ayache (Jay)	32.53671	-4.79110	3043	JX462109 / JX462184 / ... / ... / ... / ... / ...	
9191	9191a	Jebel Ayache (Jay)	32.53671	-4.79110	3043	JX462106 / JX462181 / JX461677 / JX462045 / JX485292 / JX461861 / JX461511	
	9191b					JX461678 / JX462046 / JX485293 / JX461862 / JX461512	
9336	9336	Jebel Ayache (Jay)	32.53671	-4.79110	3043	JX462111 / JX462182 / ... / ... / ... / ... / ...	
9193	9193a	Jebel Ayache (Jay)	32.53671	-4.79110	3043	JX462107 / JX462188 / JX461679 / JX462047 / JX485288 / JX461863 / JX461513	
	9193b					JX461680 / JX462048 / JX485289 / JX461864 / JX461514	
2557	2557a	Jebel Ayache (Jay)	32.53671	-4.79110	3043	... / ... / JX461595 / JX461949 / JX485274 / JX461763 / JX461419	
	2557b					JX461596 / JX461950 / JX485275 / JX461764 / JX461420	
9145	9145a	Jebel Ayache (Jay)	32.53671	-4.79110	3043	JX462104 / JX462185 / JX461673 / JX462041 / JX485286 / JX461857 / JX461507	
	9145b					JX461674 / JX462042 / JX485287 / JX461858 / JX461508	
5076	5076a	Jebel Azourki (Jaz)	31.75847	-6.28826	2789	JX462120 / JX462206 / JX461647 / JX462005 / JX485296 / JX461821 / JX461475	
	5076b					JX461648 / JX462006 / JX485297 / JX461822 / JX461476	
5128	5128a	Jebel Azourki (Jaz)	31.75847	-6.28826	2789	JX462126 / JX462207 / JX461665 / JX462029 / JX485298 / JX461845 / JX461495	
	5128b					JX461666 / JX462030 / JX485299 / JX461846 / JX461496	
5091	5091	Jebel Azourki (Jaz)	31.75847	-6.28826	2789	JX462122 / JX462208 / ... / ... / ... / ... / ...	
5017	5017a	Jebel Azourki (Jaz)	31.75847	-6.28826	2789	JX462113 / JX462209 / JX461635 / JX461989 / JX485308 / JX461805 / JX461459	
	5017b					JX461636 / JX461990 / JX485309 / JX461806 / JX461460	
5122	5122a	Jebel Azourki (Jaz)	31.75847	-6.28826	2789	JX462125 / JX462210 / JX461661 / JX462023 / JX485300 / JX461839 / JX461491	
	5122b					JX461662 / JX462024 / JX485301 / JX461840 / JX461492	

Specimen code	Alleles	Population	Latitude	Longitude	Altitude	GenBank acession codes *12S / ND4+tRNA-His / PDC / ACM4 / C-MOS / MC1R / RAG1*
5105	5105a	Jebel Azourki (Jaz)	31.75847	-6.28826	2789	JX462123 / JX462211 / JX461659 / JX462019 / JX485302 / JX461835 / JX461487
	5105b					JX461660 / JX462020 / JX485303 / JX461836 / JX461488
5072	5072a	Jebel Azourki (Jaz)	31.75847	-6.28826	2789	JX462118 / JX462212 / JX461645 / JX462001 / JX485304 / JX461817 / JX461471
	5072b					JX461646 / JX462002 / JX485305 / JX461818 / JX461472
5037	5037a	Jebel Azourki (Jaz)	31.75847	-6.28826	2789	JX462116 / JX462213 / JX461639 / JX461995 / JX485322 / JX461811 / JX461465
	5037b					JX461640 / JX461996 / JX485323 / JX461812 / JX461466
5011	5011a	Jebel Azourki (Jaz)	31.75847	-6.28826	2789	JX462112 / JX462204 / JX461631 / JX461985 / JX485312 / JX461801 / JX461455
	5011b					JX461632 / JX461986 / JX485313 / JX461802 / JX461456
5034	5034	Jebel Azourki (Jaz)	31.75847	-6.28826	2789	JX462115 / JX462205 / ... / ... / ... / ... / ...
5080	5080	Jebel Azourki (Jaz)	31.75847	-6.28826	2789	JX462121 / JX462216 / ... / ... / ... / ... / ...
5025	5025a	Jebel Azourki (Jaz)	31.75847	-6.28826	2789	JX462114 / JX462214 / JX461637 / JX461991 / JX485314 / JX461807 / JX461461
	5025b					JX461638 / JX461992 / JX485315 / JX461808 / JX461462
5043	5043a	Jebel Azourki (Jaz)	31.75847	-6.28826	2789	JX462117 / JX462218 / JX461641 / JX461997 / JX485324 / JX461813 / JX461467
	5043b					JX461642 / JX461998 / JX485325 / JX461814 / JX461468
5073	5073	Jebel Azourki (Jaz)	31.75847	-6.28826	2789	JX462119 / JX462215 / ... / ... / ... / ... / ...
5111	5111	Jebel Azourki (Jaz)	31.75847	-6.28826	2789	JX462124 / JX462217 / ... / ... / ... / ... / ...
6016	6816a	Outabati (Out)	32.17714	-5.33214	2441	JX462128 / JX462221 / JX461671 / JX462037 / JX485330 / JX461853 / JX461503
	6816b					JX461672 / JX462038 / JX485331 / JX461854 / JX461504
11754	11754a	Outabati (Out)	32.17714	-5.33214	2441	JX462140 / JX462230 / JX461551 / JX461903 / JX485350 / JX461717 / JX461375
	11754b					JX461552 / JX461904 / JX485351 / JX461718 / JX461376
11746	11746a	Outabati (Out)	32.17714	-5.33214	2441	JX462137 / JX462228 / JX461547 / JX461899 / JX485346 / JX461713 / JX461371
	11746b					JX461548 / JX461900 / JX485347 / JX461714 / JX461372
11743	11743a	Outabati (Out)	32.17714	-5.33214	2441	JX462135 / JX462226 / JX461543 / JX461895 / JX485342 / JX461709 / JX461367
	11743b					JX461544 / JX461896 / JX485343 / JX461710 / JX461368
11717	11717a	Outabati (Out)	32.17714	-5.33214	2441	JX462130 / JX462222 / JX461533 / JX461885 / JX485332 / JX461699 / JX461357
	11717b					JX461534 / JX461886 / JX485333 / JX461700 / JX461358
11755	11755a	Outabati (Out)	32.17714	-5.33214	2441	JX462139 / JX462231 / JX461553 / JX461905 / JX485352 / JX461719 / JX461377
	11755b					JX461554 / JX461906 / JX485353 / JX461720 / JX461378
11727	11727a	Outabati (Out)	32.17714	-5.33214	2441	JX462131 / JX462232 / JX461535 / JX461887 / JX485334 / JX461701 / JX461359
	11727b					JX461536 / JX461888 / JX485335 / JX461702 / JX461360
11752	11752a	Outabati (Out)	32.17714	-5.33214	2441	JX462138 / JX462229 / JX461549 / JX461901 / JX485348 / JX461715 / JX461373
	11752b					JX461550 / JX461902 / JX485349 / JX461716 / JX461374
6643	6643	Outabati (Out)	32.17714	-5.33214	2441	JX462129 / JX462220 / ... / ... / ... / ... / ...
11741	11741a	Outabati (Out)	32.17714	-5.33214	2441	JX462134 / JX462225 / JX461541 / JX461893 / JX485340 / JX461707 / JX461365
	11741b					JX461542 / JX461894 / JX485341 / JX461708 / JX461366
11734	11734a	Outabati (Out)	32.17714	-5.33214	2441	JX462133 / JX462224 / JX461539 / JX461891 / JX485338 / JX461705 / JX461363
	11734b					JX461540 / JX461892 / JX485339 / JX461706 / JX461364
11745	11745a	Outabati (Out)	32.17714	-5.33214	2441	JX462136 / JX462227 / JX461545 / JX461897 / JX485344 / JX461711 / JX461369
	11745b					JX461546 / JX461898 / JX485345 / JX461712 / JX461370
11733	11733a	Outabati (Out)	32.17714	-5.33214	2441	JX462132 / JX462223 / JX461537 / JX461889 / JX485336 / JX461703 / JX461361
	11733b					JX461538 / JX461890 / JX485337 / JX461704 / JX461362

Specimen code	Alleles	Population	Latitude	Longitude	Altitude	GenBank acession codes *12S / ND4+tRNA-His / PDC / ACM4 / C-MOS / MC1R / RAG1*	5/5
6639	6639	Outabati (Out)	32.17714	-5.33214	2441	JX462127 / JX462219 / ... / ... / ... / ... / ...	
3865	3865a	Toubkal (Tou)	31.09415	-7.91367	2600	JX462142 / JX462236 / JX461627 / JX461981 / JX485360 / JX461797 / JX461451	
	3865b					JX461628 / JX461982 / JX485361 / JX461798 / JX461452	
13276	13276a	Toubkal (Tou)	31.09415	-7.91367	2600	JX462143 / JX462237 / ... / JX461909 / JX485362 / JX461723 / JX461381	
	13276b					... / JX461910 / JX485363 / JX461724 / JX461382	
5090	5090a	Jebel Awlime (JAw)	30.81708	-8.86298	2967	JX462144 / JX462234 / JX461651 / JX462011 / JX485354 / JX461827 / JX461481	
	5090b					JX461652 / JX462012 / JX485355 / JX461828 / JX461482	
13179	13179a	Jebel Awlime (JAw)	30.81708	-8.86298	2967	JX462146 / JX462235 / JX461555 / JX461907 / JX485358 / JX461721 / JX461379	
	13179b					JX461556 / JX461908 / JX485359 / JX461722 / JX461380	
5123	5123a	Jebel Awlime (JAw)	30.81708	-8.86298	2967	JX462145 / JX462233 / JX461663 / JX462025 / JX485356 / JX461841 / JX461493	
	5123b					JX461664 / JX462026 / JX485357 / JX461842 / JX461494	

Sequences were aligned for each gene independently using the online version of MAFFT v.6 [91] with default parameters (gap opening penalty = 1.53, gap extension = 0.0) and FFT-NS-1 algorithm. Coding gene fragments (*ND4*, *C-MOS*, *ACM4*, *RAG1*, *PDC* and *MC1R*) were translated into amino acids and no stop codons were observed, suggesting that the sequences were all functional. Heterozygous individuals were identified based on the presence of two peaks of approximately equal height at a single nucleotide site. SEQPHASE [92] was used to convert the input files, and the software PHASE v2.1.1 to resolve phased haplotypes [93]. Default settings of PHASE were used except for phase probabilities that were set as ≥ 0.7 [94]. All polymorphic sites with a probability of < 0.7 were coded in both alleles with the appropriate IUPAC ambiguity code. Phased nuclear sequences were used for the structure analysis; networks and species tree analysis, and the unphased sequences for the phylogenetic analyses (see below). DnaSP [95] was used to calculate the number of haplotypes (h) and mutations (η). Mega v.3.0 [96] was used to estimate uncorrected *p*-distances and to obtain the number of variable and parsimony informative sites.

Phylogenetic analyses

Phylogenetic analyses were performed using maximum likelihood (ML) and Bayesian (BI) methods. JModelTest [97] was used to select the most appropriate model of sequence evolution under the Akaike Information Criterion [98]. ML analyses were performed with RAxML v.7.0.4 [99] with 100 random addition replicates. A GTR+I+G model was used and parameters were estimated independently for each partition (by gene). Reliability of the ML tree was assessed by bootstrap analysis [100] including 1000 replications. Bayesian analyses were performed with MrBayes v.3.1.2 [101] with best fitting models applied to each partition by gene and all parameters unlinked across partitions. The models selected for the different partitions were: *12S*, GTR+I+G; *ND4*, GTR+G; *tRNA-His*, GTR+I+G; *ACM4*, HKY+I; *C-MOS*, GTR+I+G; *MC1R*, HKY+I+G; *PDC,* GTR+I+G; and *RAG1*, GTR+I. Two independent runs of $5x10^6$ generations were carried out, sampling at intervals of 1000 generations producing 5000 trees. Convergence and appropriate sampling were confirmed examining the standard deviation of the split frequencies between the two simultaneous runs and the Potential Scale Reduction Factor (PSRF) diagnostic. Burn-in was performed discarding the first 1250 trees of each run (25%) and a majority-rule consensus tree was generated from the remaining trees. In both ML and BI alignment gaps were treated as missing data and the nuclear gene sequences were not phased.

Nuclear Networks

The genealogical relationships between the populations were assessed with haplotype networks for all the individual nuclear genes, constructed using statistical parsimony [102] implemented in the program TCS v 1.21 [103] with a connection limit of 95%. This analysis was made with the phased sequences. Haplotypes were coloured taking into account the population of origin.

Population structure – Clustering analyses

A model-based Bayesian clustering method was applied to all haplotypes using STRUCTURE v.2.3.2 [60, 104-105]. In this analysis, individuals are probabilistically assigned to either a single cluster (the population of origin), or more than one cluster (if there is admixture). STRUCTURE was run with haplotype information from the nuclear fragments independently. We ran our data with the all parameters combinations between the Ancestry Model and the Allele Frequency Model to compare the results. The genetic structure was forced to vary from K = 2 to K = 10 clusters, the latter corresponding to the number of geographic populations sampled plus two. STRUCTURE ran for 550 000 steps, of which the first 50 000 were discarded as burn-in. For each value of K ten independent replicates of the Markov Chain Monte Carlo (MCMC) were conducted. To detect the true number of clusters (K) we followed the graphical methods and algorithms outlined in Evanno *et al.* [61], with the comparison of the average posterior probability values for K (log likelihood; ln L) using the online version, STRUCTURE HARVESTER v0.6.5 (available at: http://taylor0.biology.ucla.edu/struct_ harvest/, April 2011).

Species tree, and divergence time estimates

Here we applied the coalescent-based species-tree approach implemented in STARBEAST [106] an extension of BEAST v1.6.1 [107] to test the origin and diversification patterns in *Atlantolacerta*, and to compare these results to those obtained from the ML and BI analyses of the concatenated dataset. This analysis needs *a priori* information regarding the species/populations delimitation and the species/populations assignation of the individuals in order to reconstruct the topology of the species tree. For this approach, we used the results obtained from previous clustering analyses to define the groups of individuals to be used as "species" (populations) in STARBEAST [106]. The clustering analysis supported the existence of six lineages, as Oukaimeden, Toubkal and J. Awlime were included in the same lineage.

All five nuclear gene fragments, *12S* and the fragment consistent of the *ND4* and flanking *tRNA-His* were included in the analyses as 7 independent partitions. The phased dataset was

used for the nuclear loci.

The input file was formatted with the BEAUti utility included in the software package. We performed two independent runs of 1.5 x 10^8 generations, sampling every 15 000 generations, from which 10% were discarded as burn-in. Models and prior specifications applied were as follows (otherwise by default): *12S* - GTR+G; *ND4* and *tRNA-His* - HKY+G; *MC1R* - HKY+I; *ACM4* - HKY+I; *C-MOS* - GTR+I+G; *RAG1* - HKY+I; *PDC* - GTR+I; Relaxed Uncorrelated Lognormal Clock (estimate); Yule process of speciation; random starting tree; alpha Uniform (0, 10).

For all analyses implemented in BEAST, convergence for all model parameters was assessed by examining trace plots and histograms in Tracer v1.5 [108] after obtaining an effective sample size (ESS) > 200. The initial 10% of samples were discarded as burn-in. Runs were combined using LogCombiner, and maximum credibility trees with divergence time means and 95% highest probability densities (HPDs) were produced using Tree Annotator (both part of the BEAST package). Trees were visualized using the software FigTree v1.3.1 [109].

Several studies have already calculated divergence rates for reptiles, and particularly for lacertids [2, 15, 49]. Pinho *et al*. [15] used well-known and dated independent geological events in the Aegean [110] to estimate a maximum and minimum mutation rate for the *ND4* mitochondrial fragment (and flanking *tRNA-His*) for the lacertid lizards of the genus *Podarcis* (0.0278 and 0.0174 mutation/site/million years, respectively). However, this was the only information available for our data, since we did not have any fossils or calibrations for nuclear markers. It is important to bear in mind that, in the absence of accurate calibration points in the phylogeny from external and independent data (fossil records, known biogeographic events, or paleoclimatic reconstructions) or as a result of the heterogeneity in the evolutionary rate between the calibrated and uncalibrated taxa, temporal estimates by means of molecular data could be a potential source of inference error, and, therefore, they should be treated with caution [111]. Despite the limitations of molecular clocks [111-112], divergence time estimates can still provide a proxy for the temporal window of evolutionary diversification in species groups of interest. Therefore and taking into account our data limitations and availability, we used BEAST v.1.6.1 [107] to estimate dates of the cladogenetic events using only *ND4* and flanking *tRNA-His*. We used a phylogeny pruned arbitrarily to include one representative from each of the major lineages uncovered with the concatenated analysis (6 specimens in total, we excluded J. Awlime population, because of the lack of support of the branch in previous analyses). This method excludes closely related terminal taxa because the Yule tree prior (see below) does not include a model of coalescence, which can complicate rate estimation for closely related sequences [113]. Analyses were run four times for 5x10^7 generations with a sampling frequency of 10 000.

Models and prior specifications applied were as follows (otherwise by default): GTR+G for *12S*; HKY+G for *ND4* and *tRNA-His*; HKY+I for *MC1R*; HKY+I for *ACM4;* GTR+G+I for *C-MOS*; HKY+I for *RAG1*; GTR+I for *PDC*; Relaxed Uncorrelated Lognormal Clock (estimate); Yule process of speciation; random starting tree; alpha Uniform (0, 10); ucld.mean of *ND4* Normal (initial value: 0.0226, mean: 0.0226, Stdev: 0.0031).

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Authors' contributions

MB carried out the molecular laboratory work, analysed the data and drafted a preliminary version of the manuscript. All authors participated in the conception and design of the study, collection of samples, writing and approval of the final manuscript.

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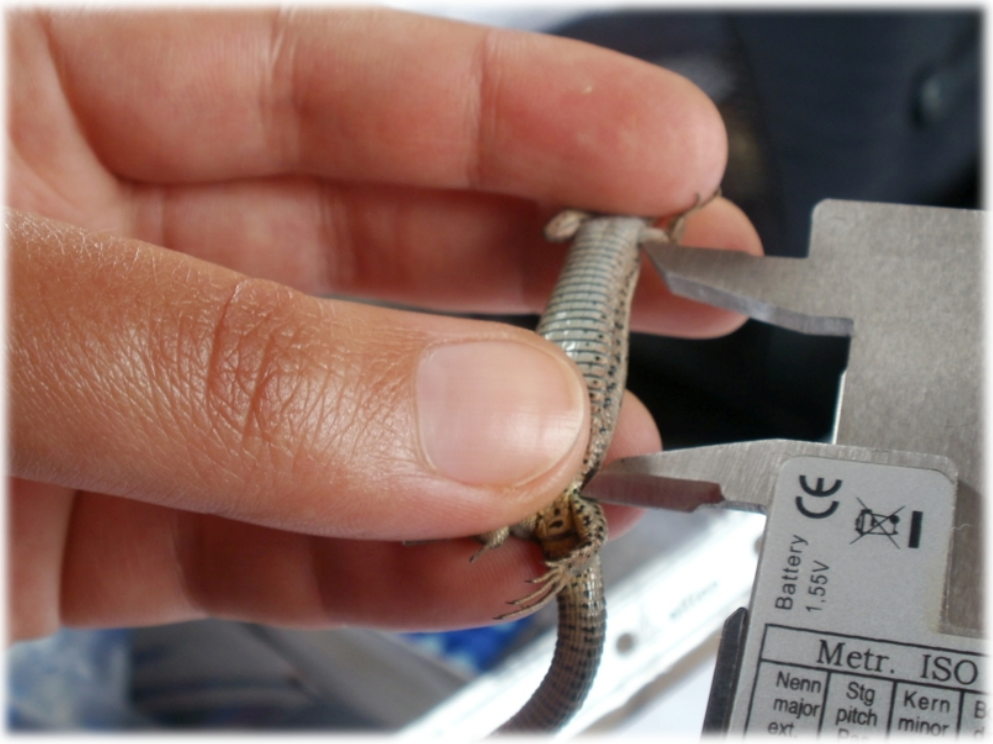
FINAL NOTE

This article is formatted accordingly to the Journal where it was published, BMC Evolutionary Biology.

ARTICLE 3.

MORPHOLOGICAL ANALYSES OF *ATLANTOLACERTA ANDREANSKYI* (WERNER, 1929)



Dianna Steiner, Tizin Tichka, 2010

Barata M., Perera A. and Harris D.J. (submitted) **Cryptic diversity in the Moroccan high altitude lacertid *Atlantolacerta andreanskyi* (Squamata: Lacertidae): a taxonomic assessment**.

Cryptic diversity in the Moroccan high altitude lizard *Atlantolacerta andreanskyi* (Squamata: Lacertidae): a taxonomic assessment

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Abstract

Atlantolacerta andreanskyi is a mountain specialist lacertid lizard, restricted to areas above 2400 m of the High Atlas Mountains of Morocco, with apparently no connection between different populations. In a recent molecular study, populations from *A. andreanskyi* collected across its distribution area were analysed, showing unprecendent levels of genetic differentiation for mitochondrial markers, that were also partially differentiated for nuclear markers using different approaches (concatenated, coalescent approaches and clustering methods). In the present study we aim to investigate for the first time the phenotypic variability of this species. For this, univariate and multivariate analyses were performed on linear measurements, pholidotic and colour characters in six populations of *A. andreanskyi,* previously analysed genetically and covering most of its distribution range. The results show that despite the high genetic divergence previously detected, morphological variation among populations was quite low, while variation within populations was generally high. Thus, although some genetic forms can be relatively well discriminated at a multivariate level, simple diagnostic traits could not be identified, and thus, they can be considered as essentially cryptic species. Due to the extreme genetic diversity observed and the results obtained using coalescent based approaches each of the six lineages analysed is recognized as a distinct species.

Keywords: Lacertids – Morocco – cryptic species – morphology – high altitude

Introduction

Delimiting species, despite being a controversial issue, is of major importance since species are the basic unit in areas such as ecology, biogeography and evolution, with serious implications for conservation biology (Myers *et al.*, 2000; Sites & Marshall, 2003). However, species concepts and their delimitation are still controversial, and recently several new approaches, such as the "unified species concept" (de Queiroz, 2007) and conceptual advances in integrative taxonomy (Dayrat, 2005; Padial *et al.*, 2010), have emerged to try to reconcile the different species concepts. The principal difficulty of determining if a population constitutes an independent evolving lineage occurs in recently separated species, which are less likely to achieve criteria such as morphological distinctiveness, reproductive isolation, ecological exclusivity and monophyly (de Queiroz, 2007). Moreover, speciation is not always accompanied with phenotypic changes, potentially leading to an underestimation of the actual levels of biodiversity. Cryptic species are an example that can be difficult to classify, particularly because morphology has been traditionally the main tool to identify and classify new species. Although in the past two decades the study of cryptic species has increased (Detwiler *et al.*, 2010; Florio *et al.*, 2012; Padial & de la Riva, 2009), see a review in (Bickford *et al.*, 2007) mainly due to the advances in molecular and analytical methods, cryptic diversity remains a challenge for taxonomists. Additionally, delimitation of allopatric forms is a further challenge, as it is difficult to measure objectively some of the criteria that, usually, determine reproductive isolation.

Cryptic species occur with regularity across all biogeographical regions and major metazoan taxa, and are more common than previously thought (Pfenninger & Schwenk, 2007). North Africa is a region where cryptic diversity has been described in several taxa such as plants (Abdelaziz *et al.*, 2011), spiders (Duncan *et al.*, 2010), mammals (Ben Faleh *et al.*, 2012) and reptiles (Perera & Harris, 2010; Rato *et al.*, 2012). The diverse geographical and geological features and the variety of climates exert different selective pressures that have promoted speciation processes in the region. The Atlas Mountains are especially interesting as a source of speciation. They formed at the Africa-Eurasia plate boundaries, and uplifted during the cenozoic (Gómez *et al.*, 2000) and have been identified as refugia during the Pleistocenic climatic fluctuations (Medail & Diadema, 2009), harbouring a diversity that is still underexplored. However, there are an increasing number of examples (Brown *et al.*, 2002; Fritz *et al.*, 2006; Rato *et al.*, 2010; Recuero *et al.*, 2007) demonstrating the role of the Atlas system in species diversification. The most recent study of the Moroccan day gecko (genus *Quedenfeldtia*) endemic from the Atlas region, confirms once more the interest of this region as source of cryptic speciation (Barata *et al.*, 2012b).

Atlantolacerta andreanskyi (Werner, 1929) is a small lacertid lizard endemic to the western and central High Atlas Mountains of Morocco. It is the lacertid found at higher altitudes in the region, being restricted to areas between 2400 m and 3800 m a.s.l. (Bons & Geniez, 1996; Schleich *et al.*, 1996). It is often found near watercourses and in the base of cushion-like thorny plants (Bons & Geniez, 1996) that offer a buffered microclimate with humidity, food, and protection against predators and wind (Schleich *et al.*, 1996). In a recent study, individuals from eight different geographic populations, covering the distribution range of *A. andreanskyi*, were compared using a multilocus approach (see Figure 1), which included two mitochondrial and five nuclear markers (Barata *et al.*, 2012a) results revealed an extreme genetic diversity among seven of the eight populations analysed in mtDNA, showing divergence levels ranging from 1.6% to 6.6% in 12S rRNA and 5.5% to 16.5% in ND4. The nuclear markers (ACM4, MC1R, C-MOS, PDC and RAG1) were concordant with the mtDNA even if monophyly was not achieved between some populations. In view of these results, the authors suggested the possibility that *A. andreanskyi* might be a complex of cryptic species. Unfortunately, due to the restricted, and in many cases inaccessible, distribution range, there is a profound lack of knowledge regarding this species, and the few existing studies (Busack, 1987; Klemmer, 1969; Pasteur & Bons, 1960; Saint Girons, 1953; Stemmler, 1972; Volobouev *et al.*, 1990; Werner, 1929, 1931, 1935) are mostly based on individuals from two geographically close populations from the High Atlas (Oukaimeden and Toubkal). The only available studies on morphology and sexual dimorphism are also based on these populations (Busack, 1987; Rykena & Bischoff, 1992; Schleich *et al.*, 1996), although Joger & Bischoff (1989) mention the population from Jebel Ayache and the possibility that this population is distinct from the others at a subspecific or specific level, but without any detailed explanation other than its geographical isolation.

In consequence, there is an urgent need to investigate the morphological variation across the distribution range of the species, in order to evaluate its real "cryptic nature". In this study we investigate the morphological variation within *A. andreanskyi* in different populations, in order to compare this with the genetic variation described by Barata *et al.* (2012a). To achieve this, a morphological analysis of body measurements, scalation and colour characters were performed. The main objective is to assess the cryptic nature of this species, and, as necessary, to describe the new taxa observed.

Material and Methods

Sampling and data collection

The study area comprises the western and central parts of the High Atlas Mountains of Morocco, covering the whole distribution range of *A. andreanskyi* (Bons & Geniez, 1996). A total of 139 specimens from 6 of the 8 populations genetically characterized in Barata *et al.* (2012a) covering most of the species distribution range were sampled (Figure 2): Jebel Sirwa (13 males (M) and 9 females (F)), Oukaimeden (14M, 17F), Tizin Tichka (12M, 12F), Jebel Azourki (10M, 21F), Outabati (6M, 5F) and Jebel Ayache (8M, 12F). Despite considerable effort to sample in the other two populations included in the genetic study (Toubkal and Jebel Awlime), only two and three specimens could be found, respectively, and thus, although they were examined for descriptive purposes, they were not included in the multivariate analysis. Specimens were caught by hand, identified and sexed on the basis of external features (e.g. developed femoral pores, see Schleich *et al.*, 1996).

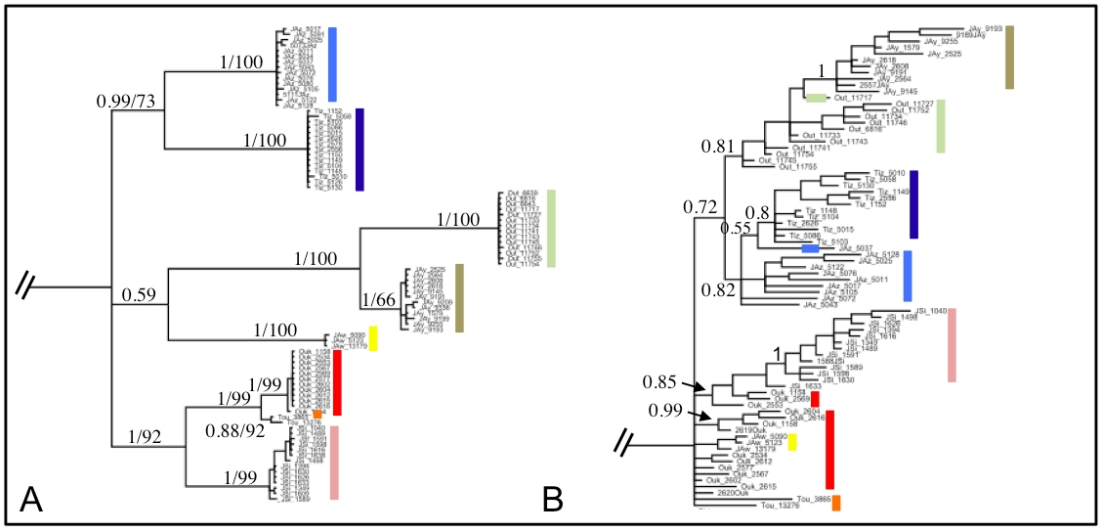

Figure 1. *Atlantolacerta andreanskyi* phylogenetic trees adapted from Barata *et al.* (2012a). A - mtDNA tree (12S and ND4+tRNA-His) and B - nuclear DNA concatenated tree (MC1R, PDC, ACM4, C-MOS and RAG1). Populations are represented with the same colours in all the figures; Jebel Sirwa (pink), Oukaimeden (red), Tizin Tichka (dark blue), Jebel Azourki (light blue), Outabati (light green) and Jebel Ayache (dark green), Jebel Awlime (orange) and Toubkal (yellow). The last two populations were not included in this study due to the small sample size.

In total, twelve linear measurements, nine pholidotic characters (Table 1) and seven colour characters were taken. Snout vent length (SVL) was measured from the tip of the snout to the cloaca opening; trunk length (TRL) was measured from the posterior edge of the forelimb insertion to the anterior edge of the hindlimb insertion. Tail width (TW) was recorded at its widest point. Head length (HL) was measured from tip of the snout to the collar, head width

(HW) at its widest part, usually at the level of the temporal region, and head height (HH) from occiput to jaws. Pileus length (PL) was measured from the occipital to the limit of the rostral scale. The total lengths of front (FLL) and hind (HLL) limbs were measured from the longest toe to the base of the limb. Femur length (FL) was measured from the base of the hindlimb to the knee joint, tibia length (TBL) from the knee joint to the ankle joint and fourth toe length (HTL) from the insertion of the toe to its extremity, including the claw. Also, detailed pictures of dorsal, lateral and ventral body were taken in the field, and nine pholidotic variables recorded a posteriori from them: number of ventral scales (VSN) including all the large scales counted in a midline from the collar to the anterior insertion of hindlimbs; number of gular scales (GSN) in a midline from the collar to the chin shields scales; number of collar scales (CSN), number of femoral pores (FPN) counted in males only; number of supratemporal scales (STSN); number of supra labial scales (SLSN); number of supraciliary scales (SCSN); number of supraciliary granules (SCGN), and number of enlarged side to side lamellae under the fourth toe (Lam). Regarding colour pattern, the following variables were also recorded from pictures: presence of black pigmentation (spots) in the lateral head (HPL, 1 = absent, 2 = scarce, 3 = abundant), dorsal head (HPD, 1 = absent, 2 = scarce, 3 = abundant), ventral head (HPV, 1 = absent, 2 = scarce, 3 = abundant), ventral body (VBP, 1 = absent, 2 = scarce, 3 = abundant) and cloacal region (CD, 0 = absent, 1 = one single dot in the anal plate, 2 = two dots in the anal plate; 3 = three dots, in the anal plate); presence of a central dorsal line (CBL, 1 = absent, 2 = discontinuous, 3 = continuous) and presence of light dorsolateral lines (1 = absent, 2 = present).

Since morphological variation can be affected by inter-observer differences (Roitberg *et al.*, 2011) all linear measurements were recorded in the field by the same author (MB) to the nearest 0.01 mm, using a digital calliper. Pholidotic and colour variables were also retrieved from digital pictures by the same author (MB) at least twice and the mean value was recorded. All bilateral variables were taken from the right side of the animal. In cases where data could not be collected due to member amputations or poor picture quality they were replaced by the group mean.

Only adult individuals were included in this study. Individuals were released in the same place where they were caught after recording the coordinates of the location with a GPS. Tissue samples from the localities in this study were already characterized genetically in Barata *et al*. (2012a).

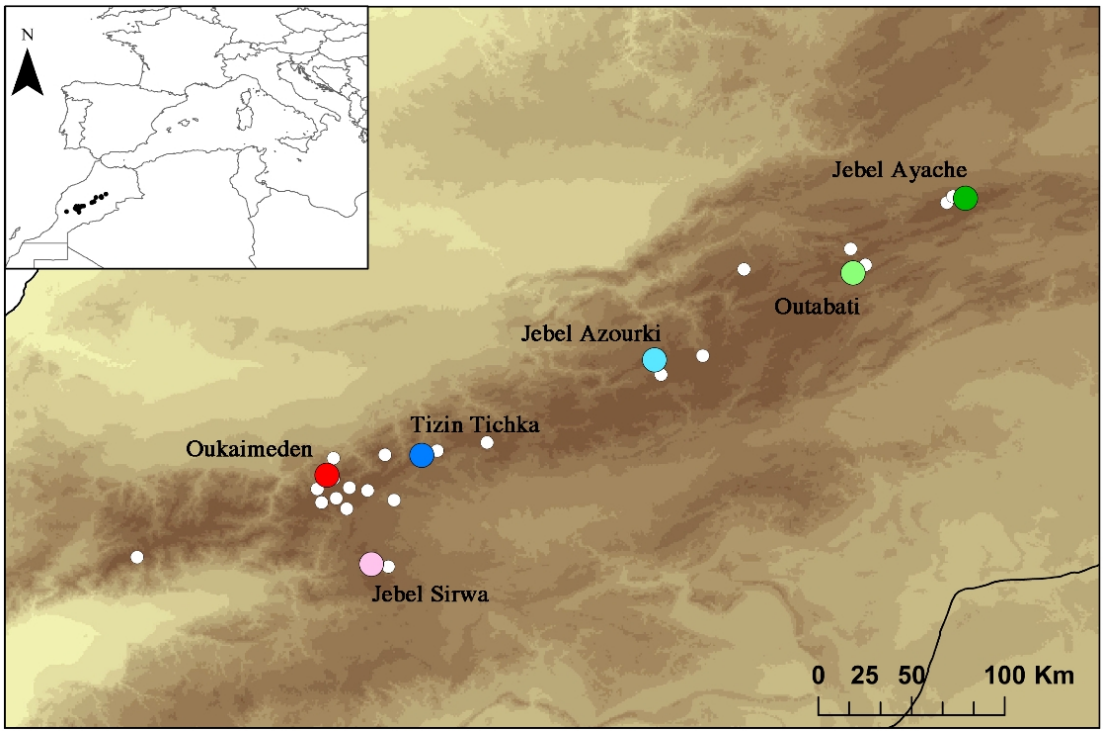

Figure 2. Distribution map of *Atlantolacerta andreanskyi.* Populations investigated in the current study: Jebel Sirwa (pink), Oukaimeden (red), Tizin Tichka (dark blue), Jebel Azourki (light blue), Outabati (light green) and Jebel Ayache (dark green). White dots represent the known distribution of the species as available in Bons & Geniez (1996).

Morphological analysis

Body measurements and pholidotic variables were log-transformed and checked for homoscedasticity (Levene's test) and normality (Shapiro-Wilks test) assumptions. Since linear measurements were highly correlated to body size, namely snout-vent length (SVL, Pearson correlation in all cases $p < 0.01$), we used an isometric correction (Somers, 1986) to estimate body-size-corrected variables that were then used to investigate the existence of possible differentiation patterns not related to body size. For this, all linear measurements (log transformed) were projected on an isometric vector, in order to obtain a multivariate representation of the isometric size of each individual (mSIZE). Each variable was then regressed on this isometric vector and the residuals were used as size-corrected variables. Thus, the multivariate representation of isometric size (mSIZE) was used as a size estimator, whereas the remaining isometric-size corrected variables were used as a representation of shape (Kaliontzopoulou *et al.*, 2010). Correction was done using the R package (R, 2011).

In order to investigate the morphological differences between sexes and populations, (multi)variate analysis of the variance (M)ANOVAs were performed on size (multivariate representation of the isometric size, mSIZE), shape (the remaining iso-corrected linear measurements) and pholidotic variables separately using POPULATION, SEX and its interaction (POPULATION*SEX), as factors. Analyses were done using the software

STATISTICA 7.1 (Statsoft, 2005). Since factor SEX was significant in several variables, further analyses were performed on males and females separately. Additionally, sexual dimorphism within each population was investigated using Analysis of Covariance (ANCOVAs) on linear measurements using SVL as covariate, and Student's t test on pholidotic variables.

To investigate the generalized morphological relationships among the different *A. andreanskyi* populations, and the contribution of each dataset to the differentiation among populations, Canonical Discriminant Function Analyses (CDFA) were performed on linear measurements (mSIZE and iso-corrected variables) and pholidotic variables separately.

CDFA allowed assessment at a multivariate level of which variables were the major contributors to the differentiation between populations, and to create canonical discriminant functions to calculate the probability of classifying correctly the individuals based on them. We used the leave-one-out option to cross-validate the classification results. Since this procedure (Jacknife prediction) generates individual classifications using discriminate functions based on all observations except the given case, it provides a more accurate estimate of the classification values. CDFA analyses were performed using SPSS v.20.0.0 (IBM, 2011).

Finally, variation in colour pattern between populations was investigated at a multivariate level using a Multiple Correspondence Analysis (MCA) using the software R (R, 2011). In all analysis, significance level was considered at $p < 0.05$.

Genetic diagnosis

In order to identify diagnosable nucleotide positions among different populations of *A. andreanskyi* for descriptive purposes, sequences from Barata *et al.* (2012a) were investigated. Diagnosable positions were detected with the help of the software Mega 5 (Tamura *et al.*, 2011) and confirmed by eye in BioEdit (Hall, 1999).

In order to locate the exact diagnosable positions, sequences from Barata *et al.* (2012a) were aligned to the complete genome of *Podarcis muralis* (GenBank accession number NC_011607.1, (Podnar *et al.*, 2009). The 1108 bp fragments from Barata *et al*. (2012a) correspond to the positions 484 to 812 (329 bp) of the *P. muralis* 12S rRNA and 10948 to 11539 (592 bp) of the *P. muralis* ND4.

Bayesian Species delimitation

Bayesian species delimitation was used in order to support the existence of the lineages observed previously in genetic analysis (Barata *et al.*, 2012a).

Bayesian species delimitation was conducted using the program Bayesian Phylogenetics and Phylogeography, BPP v.2.0b (Rannala & Yang, 2003; Yang & Rannala, 2010) using the five nuclear loci. This method accommodates both species phylogeny and lineage sorting due to ancestral polymorphism. A gamma prior *G* (1, 10) was used on the population size parameters (qs). The age of the root in the species tree (t0) was assigned the gamma prior *G* (1, 10), while the other divergence time parameters were assigned the Dirichlet prior (Yang & Rannala, 2010): equation 2). We used algorithm 0 with the finetuning parameter ε = 15.0 in order to ensure adequate rjMCMC mixing. This involves specifying a reversible jump algorithm to achieve dimension matching between species delimitation models with different number of parameters. Each species delimitation model was assigned equal prior probability and each analysis was run at least twice to confirm consistency between runs. The guide tree plays an important role in the result of the species delimitation model (Leaché & Fujita, 2010); therefore we used the guide tree: ((JSi, Ouk), ((Tiz, JAz), (Out, JAy))), based on the estimate of relationships from both mtDNA and nDNA trees (Bayesian and ML analysis, see results from(Barata *et al.*, 2012a).

Results

Detailed descriptive statistics for all the variables in all the lineages analysed are presented in Table 1, while Table 2 includes the detailed results regarding sexual dimorphism within each lineage.

Inter-lineage variation

Linear measurements

The (M)ANOVA analysis showed general differences between sexes, populations and its interaction in both size (mSIZE) and shape (Table 3). Regarding size, individuals from Jebel Azourki and Jebel Ayache were larger than the other ones, although males and females had a different pattern (Table 3; Figure 3). Regarding shape, there were differences among populations in all iso-corrected linear measurements (Table 3). On the other hand, males and females also showed differences in most of them, with the exception of tail width (TW), front limbs (FLL) and hind limbs (TBL, HTL and HLL, but not FL; Table 3, Figure 3). Finally, the degree of sexual dimorphism (POPULATION*SEX interaction) was similar among populations, with the exception of trunk length (TRL; Table 3; Figure 3).

Table 1. Descriptive statistics of linear measurements and pholidotic variables for males and females of all the populations included in the study. For each group, mean, standard deviation (Std Dev), minimum (Min) and maximum (Max) values and sample size (n) is detailed.

	Jebel Sirwa					Oukaimeden					Tizin Tichka					Outabati					Jebel Azourki					Jebel Ayache				
Males	**Mean**	**Std.Dev.**	**Min.**	**Max.**	**N**	**Mean**	**Std.Dev.**	**Min.**	**Max.**	**N**	**Mean**	**Std.Dev.**	**Min.**	**Max.**	**N**	**Mean**	**Std.Dev.**	**Min.**	**Max.**	**N**	**Mean**	**Std.Dev.**	**Min.**	**Max.**	**N**	**Mean**	**Std.Dev.**	**Min.**	**Max.**	**N**
SVL	42,24	2,96	36,24	45,50	13	42,02	3,48	35,68	47,50	14	42,53	2,84	36,92	45,97	12	41,49	1,41	40,36	44,14	6	**47,30**	3,68	42,86	52,44	10	**48,99**	2,07	45,28	51,67	13
TRL	21,64	1,84	18,08	23,82	13	22,96	1,63	19,68	25,92	14	22,47	2,12	18,38	26,65	12	22,35	1,26	21,01	23,92	6	24,62	2,86	20,19	29,48	10	26,27	0,66	25,37	27,23	13
PL	9,85	0,75	8,73	10,74	13	9,58	0,65	8,44	10,79	14	9,50	0,60	8,39	10,30	12	9,37	0,25	9,04	9,75	6	10,95	0,53	10,32	11,80	10	11,58	0,47	10,73	12,11	13
HL	15,56	1,09	12,95	16,58	13	14,54	1,53	12,00	16,89	14	15,23	1,14	12,88	17,20	12	15,18	0,48	14,40	15,70	6	17,02	1,30	14,51	19,29	10	17,37	1,31	15,43	18,84	13
HW	6,39	0,56	5,53	7,00	13	5,77	0,29	5,32	6,40	14	5,90	0,45	5,23	6,58	12	5,47	0,14	5,26	5,67	6	6,68	0,39	6,03	7,20	10	7,18	0,37	6,65	7,78	13
HH	4,14	0,37	3,56	4,74	13	4,18	0,58	3,27	5,31	14	4,45	0,46	3,77	5,15	12	3,90	0,35	3,36	4,25	6	4,79	0,45	4,18	5,35	10	5,11	0,36	4,68	5,75	13
TW	4,92	0,17	4,64	5,30	13	3,90	0,32	3,37	4,45	14	3,88	0,46	3,20	4,31	5	3,79	0,16	3,49	3,92	6	4,59	0,42	4,11	5,21	10	4,94	0,33	4,51	5,32	13
FFL	12,67	0,81	11,40	14,01	13	12,25	0,79	11,02	13,55	14	12,18	1,00	10,12	13,23	12	12,80	0,38	12,29	13,20	6	13,85	0,98	12,05	15,34	10	14,95	0,84	13,22	15,75	13
FL	8,22	0,53	7,11	9,11	13	8,11	0,30	7,54	8,56	14	7,90	0,53	7,03	8,58	12	7,74	0,69	6,55	8,31	5	9,21	0,90	7,45	10,82	10	9,77	0,52	9,29	10,65	13
TBL	5,80	0,48	4,96	6,43	13	5,24	0,51	4,27	5,90	14	5,45	0,18	5,03	5,68	12	5,68	0,13	5,50	5,88	6	6,16	0,39	5,46	6,79	10	6,42	0,31	5,86	6,78	13
4TL	8,77	0,86	7,34	10,06	13	8,27	0,82	6,60	9,48	14	9,07	0,31	8,46	9,59	12	9,13	0,61	8,39	9,89	6	10,27	0,51	9,43	10,96	10	10,53	0,98	8,63	11,55	13
HFL	21,06	1,81	18,16	24,25	13	17,61	1,50	15,56	20,50	13	19,77	2,02	16,04	22,87	12	20,14	1,16	18,38	21,83	6	22,41	0,33	21,87	22,87	10	23,05	1,43	20,00	24,90	13
VSN	26,63	1,30	25,00	29,00	8	27,00	2,65	24,00	29,00	3	28,57	1,27	27,00	30,00	7	26,00	0,89	25,00	27,00	6	28,30	1,64	26,00	31,00	10	26,75	1,49	24,00	29,00	13
FPN	16,50	0,97	15,00	18,00	10	16,43	0,85	15,00	18,00	14	17,17	1,12	16,00	19,00	12	18,33	1,63	17,00	21,00	6	18,20	2,10	16,00	23,00	10	19,13	1,13	18,00	21,00	13
Lam	22,29	1,25	21,00	25,00	7	18,54	1,56	16,00	21,00	13	19,83	2,04	17,00	24,00	12	19,83	1,72	17,00	22,00	6	21,22	0,83	20,00	22,00	9	21,13	0,99	20,00	23,00	13
STSN	4,63	0,74	4,00	6,00	8	3,50	0,65	3,00	5,00	14	4,00	0,95	2,00	5,00	12	4,33	0,52	4,00	5,00	6	4,60	1,08	3,00	6,00	10	4,13	1,13	3,00	6,00	13
GSN	21,00	1,94	17,00	24,00	9	20,83	1,40	19,00	24,00	12	20,50	2,02	18,00	25,00	12	21,33	1,86	19,00	24,00	6	22,10	1,45	20,00	25,00	10	23,75	1,58	21,00	26,00	13
CSN	7,90	0,57	7,00	9,00	10	7,36	1,08	7,00	11,00	14	7,58	0,90	6,00	9,00	12	7,67	0,82	7,00	9,00	6	8,00	0,47	7,00	9,00	10	8,38	0,52	8,00	9,00	13
SCGN	2,14	1,22	0,00	3,00	7	4,36	1,60	2,00	7,00	14	2,40	0,52	2,00	3,00	10	3,33	1,75	2,00	6,00	6	4,20	1,69	2,00	7,00	10	3,50	1,60	1,00	6,00	13
SCSN	5,00	0,87	3,00	6,00	9	4,64	0,63	3,00	5,00	14	4,92	0,79	4,00	6,00	12	5,50	0,55	5,00	6,00	6	4,50	0,71	3,00	5,00	10	4,88	0,35	4,00	5,00	13
SLSN	6,56	0,53	6,00	7,00	9	6,00	0,39	5,00	7,00	14	6,64	0,51	6,00	7,00	11	6,67	0,82	6,00	8,00	6	6,70	0,48	6,00	7,00	10	7,38	0,92	6,00	8,00	13
Females																														
SVL	45,51	1,12	43,88	47,70	9	**41,98**	2,65	37,43	46,61	17	45,03	2,48	40,80	48,30	12	44,53	1,22	43,28	46,26	5	**51,79**	3,47	46,50	58,91	21	44,89	5,90	33,76	52,35	12
TRL	25,74	2,92	21,27	29,35	9	24,38	2,27	20,95	28,35	17	26,75	1,86	23,30	28,70	12	26,92	2,47	24,10	29,72	5	30,87	2,28	27,52	34,80	21	26,15	3,93	18,19	33,16	12
PL	9,07	0,57	8,34	9,97	9	8,73	0,30	8,16	9,21	17	9,01	0,38	8,40	9,70	12	8,66	0,19	8,47	8,89	5	10,18	0,39	9,49	11,06	21	9,35	0,64	7,81	9,94	12
HL	14,15	1,17	12,70	15,90	9	13,81	0,87	11,98	15,45	17	13,89	0,68	12,90	15,20	12	13,73	0,68	12,89	14,76	5	15,99	0,83	14,53	17,32	21	14,11	1,32	11,50	16,31	12
HW	5,74	0,36	5,28	6,32	9	5,39	0,28	4,93	5,84	17	5,35	0,29	4,90	5,80	12	4,90	0,22	4,60	5,21	5	6,15	0,29	5,67	6,81	21	5,78	0,47	4,99	6,34	12
HH	3,96	0,51	3,31	4,64	9	3,76	0,30	3,36	4,45	17	3,96	0,39	3,20	4,50	12	3,23	0,32	2,86	3,65	5	4,39	0,33	3,69	4,95	21	4,13	0,40	3,32	4,60	12
TW	4,31	0,34	3,85	4,89	9	3,71	0,18	3,41	4,07	17	3,81	0,14	3,70	4,00	3	3,47	0,26	3,16	3,75	5	4,37	0,35	3,70	4,98	18	4,12	0,50	3,31	4,89	12
FFL	11,59	0,72	10,73	12,96	9	11,45	0,76	10,43	12,87	17	11,87	0,36	11,30	12,40	12	11,48	0,32	11,02	11,73	4	13,52	0,58	12,23	14,72	21	12,41	0,87	10,83	13,72	12
FL	7,19	0,43	6,52	7,90	9	7,10	0,71	5,71	8,14	17	7,17	0,65	5,80	8,40	12	5,66	0,59	5,06	6,40	5	8,30	0,56	7,29	9,13	21	7,29	0,59	6,16	8,17	12
TBL	5,23	0,30	4,78	5,73	9	4,95	0,29	4,34	5,30	17	5,19	0,21	4,70	5,50	12	5,30	0,29	4,88	5,55	4	5,86	0,29	5,26	6,50	21	5,42	0,28	4,95	5,92	12
4TL	8,79	0,33	8,07	9,22	9	7,76	0,70	6,30	9,14	17	8,75	0,51	7,80	9,50	12	8,34	1,10	7,03	9,36	4	10,08	0,72	8,69	11,56	21	9,05	0,64	7,67	9,93	12
HFL	19,76	1,80	16,50	21,72	9	16,69	1,01	14,94	18,56	17	18,47	1,18	16,40	19,90	12	17,55	0,91	16,80	19,01	5	21,54	1,25	19,59	25,07	21	19,23	1,44	16,47	21,17	12
VSN	30,00	2,12	27,00	32,00	5	29,50	1,00	29,00	31,00	4	30,50	1,07	29,00	32,00	8	29,20	1,92	27,00	32,00	5	31,05	1,86	27,00	35,00	21	29,67	1,83	26,00	32,00	12
Lam	20,60	1,14	19,00	22,00	5	19,00	2,34	13,00	22,00	16	20,00	0,95	18,00	21,00	12	20,20	1,79	18,00	22,00	5	20,65	1,76	18,00	24,00	20	20,58	1,62	18,00	23,00	12
STSN	5,20	1,30	4,00	7,00	5	3,88	0,99	2,00	6,00	17	4,00	0,60	3,00	5,00	12	4,80	0,45	4,00	5,00	5	3,76	0,77	2,00	5,00	21	4,83	1,12	3,00	6,00	12
GSN	21,40	2,61	17,00	23,00	5	20,93	2,02	18,00	25,00	14	19,75	2,05	17,00	24,00	12	19,80	1,64	18,00	21,00	5	21,33	1,74	19,00	24,00	21	22,08	1,78	20,00	27,00	12
CSN	7,71	0,76	7,00	9,00	7	7,13	0,99	5,00	9,00	15	6,92	0,90	6,00	8,00	12	6,80	1,10	5,00	8,00	5	8,24	0,70	7,00	9,00	21	8,50	0,67	8,00	10,00	12
SCGN	2,00	1,41	0,00	3,00	4	4,59	1,46	2,00	8,00	17	3,36	1,63	2,00	7,00	11	2,80	0,84	2,00	4,00	5	4,10	1,30	3,00	7,00	21	2,70	0,95	1,00	4,00	10
SCSN	4,00	1,00	3,00	5,00	7	4,82	0,53	4,00	6,00	17	5,17	0,84	4,00	6,00	12	5,00	1,00	4,00	6,00	5	4,86	0,48	4,00	6,00	21	4,83	0,39	4,00	5,00	12
SLSN	6,14	0,69	5,00	7,00	7	6,24	0,44	6,00	7,00	17	6,67	0,65	6,00	8,00	12	6,20	0,84	5,00	7,00	5	6,67	0,66	5,00	8,00	21	6,83	0,58	6,00	8,00	12

Table 2. Sexual dimorphism in linear measurements and pholidosis within lineages. For each population, mean value of the covariate used to estimate the adjusted means, and adjusted means for each sex are shown. Significant values ($p < 0.05$) are in bold.

Pop / Sex	LogTRL	LogPL	LogHL	LogHW	LogHH	LogTW	LogFFL	LogFL	LogTBL	LogHTL	LogHFL	VSN	FPN	Lam	STSN	GSN	CSN	SCGN	SCSN	SLSN
J.Sirwa (LogSVL 1.69)																				
Males	1.411	**1.050**	**1.241**	**0.835**	**0.692**	**0.673**	**1.148**	**0.977**	**0.799**	1.018	**1.355**	26.63	16.50	**22.29**	4.63	21.00	7.90	2.14	5.00	6.56
Females	**1.466**	0.997	1.193	0.781	0.630	0.625	1.123	0.907	0.759	0.994	1.332	**30.00**	-	20.60	5.20	21.40	7.71	2.00	4.00	6.14
Oukaimeden (LogSVL 1.62)																				
Males	1.357	**0.978**	1.159	0.758	0.616	0.589	1.085	0.908	0.715	0.915	1.245	27.00	16.43	18.54	3.50	20.83	7.36	4.36	4.64	6.00
Females	**1.383**	0.940	1.138	0.730	0.573	0.568	1.057	0.848	0.693	0.887	1.221	29.50	-	19.00	3.88	20.93	7.13	4.59	4.82	6.24
TizinTichka (LogSVL 1.62)																				
Males	1.344	**0.975**	1.172	**0.747**	**0.621**	0.584	1.080	0.896	**0.731**	0.947	1.250	28.57	17.17	19.83	4.00	20.50	7.58	2.40	4.92	6.64
Females	**1.381**	0.950	1.138	0.717	0.542	0.582	1.075	0.837	0.698	0.925	1.227	**30.50**	-	20.00	4.00	19.75	6.92	3.36	5.17	6.67
J.Azourki (LogSVL 1.64)																				
Males	1.353	**1.009**	**1.205**	**0.822**	**0.631**	**0.694**	**1.113**	**0.923**	**0.776**	0.956	**1.336**	28.30	18.20	21.22	**4.60**	22.10	8.00	4.20	4.50	6.70
Females	**1.389**	0.941	1.135	0.740	0.578	0.631	1.052	0.847	0.704	0.929	1.280	**31.05**	-	20.65	3.76	21.33	8.24	4.10	4.86	6.67
Outabati (LogSVL 1.62)																				
Males	1.356	**0.986**	**1.200**	**0.748**	**0.632**	0.574	1.102	**0.915**	0.746	0.998	**1.318**	26.00	18.33	19.83	4.33	21.33	7.67	3.33	5.50	6.67
Females	1.403	0.924	1.115	0.683	0.452	0.554	1.063	0.731	0.730	0.872	1.213	**29.20**	-	20.20	4.80	19.80	6.80	2.80	5.00	6.20
J.Ayache (LogSVL 1.66)																				
Males	1.403	**1.053**	**1.225**	**0.850**	**0.697**	**0.678**	**1.168**	**0.978**	**0.804**	**1.017**	**1.357**	26.75	19.13	21.13	4.13	**23.75**	8.38	3.50	4.88	7.38
Females	1.429	0.980	1.161	0.766	0.624	0.626	1.099	0.872	0.737	0.959	1.288	**29.67**	-	20.58	4.83	22.08	8.50	2.70	4.83	6.83

Considering only males, there were differences among populations in general size and shape (Table 3), including most of the iso-corrected measurements with the exception of head length, front limb length and tibia length (HL, FLL and TBL, respectively; Table 3; Figure 3). Regarding females, general differences in size and shape were also observed, and all the linear measurements analysed were significantly different among populations with the exception of front limb length (FLL; Table 3; Figure 3).

Table 3. Summary of the ANOVA/MANOVA results regarding the effect of sex, population and their interaction. Significant values are in bold.

	Total						Males		Females	
Variables	**Pop**		**Sex**		**Pop* Sex**		**Pop**		**Pop**	
mSize	50.10	**<0.01**	79.00	**<0.01**	6.80	**<0.01**	23.7	**<0.01**	37.60	**<0.01**
Shape	6.15	**<0.01**	28.75	**<0.01**	1.66	**<0.01**	3.63	**<0.01**	4.36	**<0.01**
Scales	4.14	**<0.01**	4.42	**<0.01**	0.94	0.58	2.72	**<0.01**	2.81	**<0.01**
Morphometric										
TrL	4.41	**<0.01**	237.91	**<0.01**	5.25	**<0.01**	5.69	**<0.01**	5.77	**<0.01**
PL	5.28	**<0.01**	19.38	**<0.01**	2.23	0.06	3.80	**<0.01**	3.68	**<0.01**
HL	3.09	**0.01**	7.23	**<0.01**	1.29	0.27	1.05	0.40	3.79	**<0.01**
HW	7.57	**<0.01**	17.19	**<0.01**	1.24	0.29	4.74	**<0.01**	5.02	**<0.01**
HH	7.49	**<0.01**	6.03	**0.02**	1.66	0.15	4.95	**<0.01**	4.70	**<0.01**
TW	19.09	**<0.01**	0.11	0.75	2.21	0.06	19.86	**<0.01**	4.39	**<0.01**
FFL	3.97	**<0.01**	1.73	0.19	0.78	0.56	2.18	0.07	2.38	0.05
FL	8.90	**<0.01**	46.24	**<0.01**	1.57	0.17	2.61	**0.03**	7.43	**<0.01**
TBL	5.63	**<0.01**	1.57	0.21	1.21	0.31	1.92	0.10	5.53	**<0.01**
HTL	5.49	**<0.01**	3.00	0.09	0.78	0.57	3.87	**<0.01**	2.98	**0.02**
HFL	9.52	**<0.01**	0.00	0.97	0.67	0.65	5.94	**<0.01**	3.84	**<0.01**
Scales										
VSN	4.20	**<0.01**	34.30	**<0.01**	0.40	0.82	3.23	**0.02**	1.41	0.24
FPN	---	---	---	---	---	---	3.30	**0.02**	---	---
Lam	7.18	**<0.01**	0.26	0.61	0.40	0.84	8.25	**<0.01**	2.25	0.07
STSN	2.64	**0.03**	0.59	0.45	2.33	0.05	1.38	0.26	4.74	**<0.01**
GSN	6.78	**<0.01**	0.13	0.72	1.15	0.34	4.66	**<0.01**	3.57	**<0.01**
CSN	6.34	**<0.01**	3.87	0.05	1.89	0.11	1.32	0.29	7.82	**<0.01**
SCGN	4.42	**<0.01**	0.32	0.58	0.30	0.91	1.56	0.21	3.77	**<0.01**
SCSN	1.44	0.22	2.69	0.11	1.66	0.16	1.96	0.12	0.61	0.69
SLSN	3.20	**0.01**	1.01	0.32	0.37	0.87	2.36	0.07	1.26	0.30

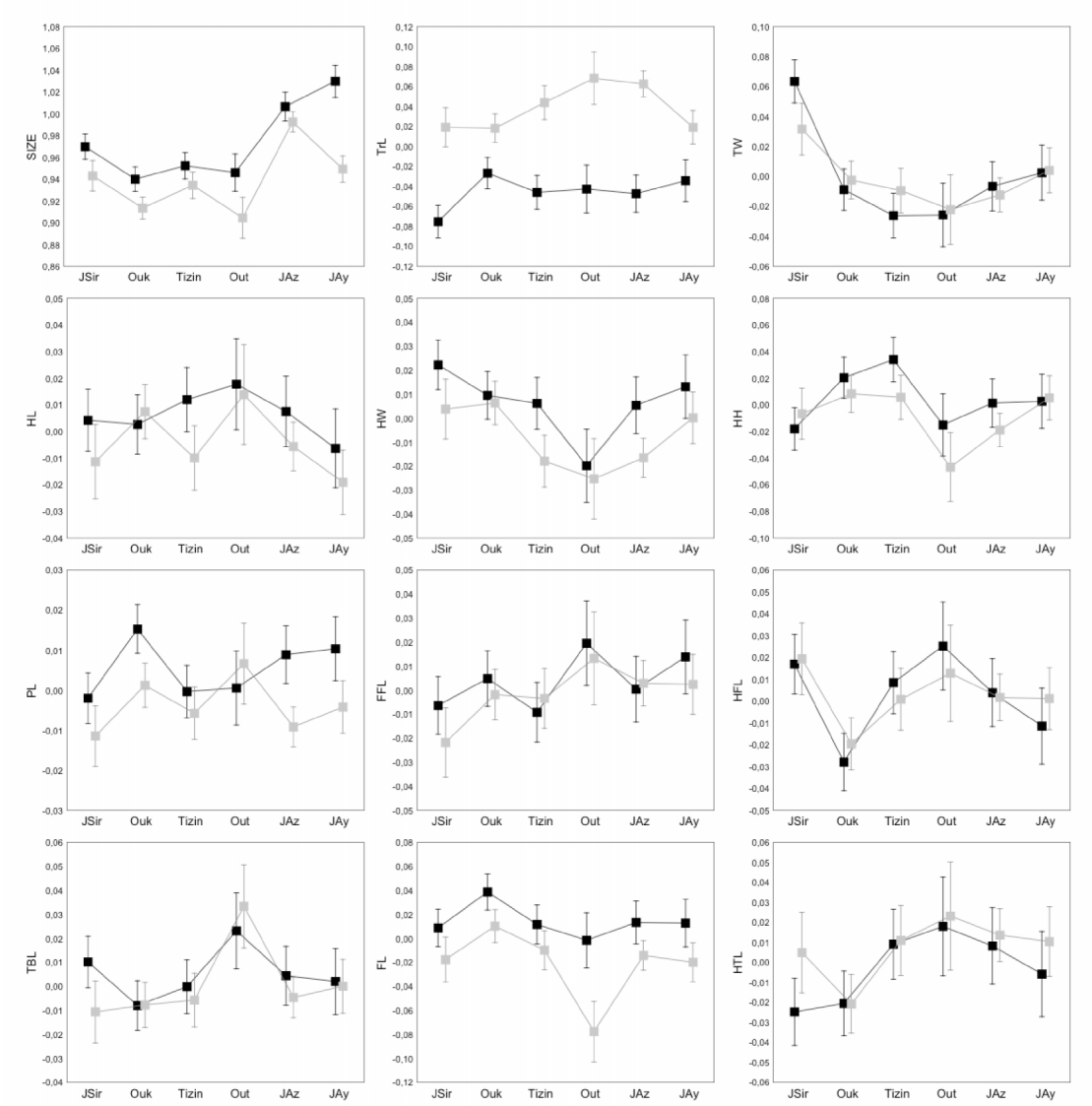

Figure 3. Variation in multivariate size (mSIZE) and iso-corrected linear measurements in males (in black) and females (in grey) of the *A. andreanskyi* lineages included in this study. For each population, mean values ± 95% confidence interval for the standard error is shown.

The Canonical Discriminant Function Analyses (CDFA, Figure 4) showed a partial discrimination of the lineages. However, in general, this separation did not have a consistent pattern neither with their genetic relationships nor with their geographic location. In males, populations were grouped in three entities across the multivariate space: 1) Jebel Sirwa, 2) Oukaimeden, Outabati and Tizin-Tichka, and 3) Jebel Ayache and Jebel Azourki.

The first discriminant function, which explains 71.3% of the total variation separates the group formed by Oukaimeden, Tizin-Tichka and Outabati from the other ones, mSIZE and TW being the most contributing variables, with this group having smaller size and narrower tails (Figure 4). The second function (15.80% of the total variation) discriminates the population from Jebel Sirwa from the others, with trunk length (TRL) and again mSIZE and

tail width (TW) being the most important variables (Table 4). According to this, specimens from Jebel Sirwa have smaller size, shorter trunks, and wider tails than individuals from Jebel Ayache and Jebel Azourki (Figure 4). Regarding the third discriminant function, that explains 9.8% of the variation, femur length (FL), pileus length (PL) and head width (HW) were the most contributing variables (Table 4). In this axis, individuals from Oukaimeden are discriminated due to shorter femur, wider heads and longer pileus.

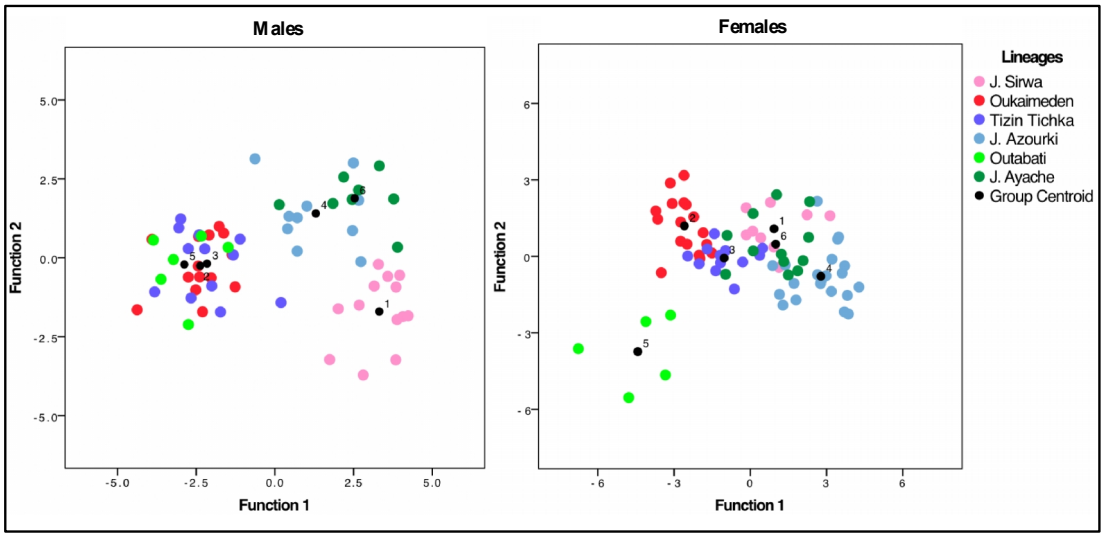

Figure 4. Canonical discriminate function analysis (CDFA) of linear measurements (mSIZE and corrected variables), for males (left) and females (right). Black circles represent the group centroids (1- J. Sirwa (pink), 2- Oukaimeden (red), 3- Tizin Tichka (dark blue), 4- J. Azourki (light blue), 5- Outabati (light green) and 6- J. Ayache (dark green)).

The classification percentage based on the discriminant functions showed relatively high scores, with males from Jebel Sirwa and females from Oukaimeden and Outabati being the better classified (>80%, Table 5), and the Jebel Ayache and Jebel Azourki specimens the ones with lower scores (<50%, correct classification in both cases).

Regarding females, there were differences among all the variables of size and shape analysed (Table 3) with the exception of tail width (TW, Table 3). In this sex, the level of discrimination among populations exhibited a different pattern to males. The first two functions discriminated Outabati from the other ones, since individuals from this population had smaller size (Figure 4). Variation across the first axis represented 65.9% of the total variation observed and was mostly represented by the variable mSIZE (Table 4). The second discriminating function (19.5% variation) was mostly explained by the variables femur length (FL), trunk length (TRL), tibia length (TBL), head height (HH), and head width (HW).

Table 4. Summary of the two stepwise Canonical Discriminant Function Analysis (CDFA) performed on the linear measurements (including the multivariate size (mSIZE) and shape (remaining iso-corrected linear measurements)) and pholidosis. For each analysis the factor structure, eigenvalues, and partial and cumulative variation (in percentage) of the first three canonical discriminant functions (CDFs) is given. Analyses were made separately for males and females. More contributing variables (> ±0.50) are in bold. Variables that did not enter in the analysis due to their low contribution were indicated with "---".

	Males			Females		
Morphometric	CDF1	CDF2	CDF3	CDF1	CDF2	CDF3
SIZE	**1.03**	**0.76**	-0.04	**1.40**	0.01	-0.18
TRL	**-0.61**	0.00	**0.49**	---	---	---
PL	-0.10	0.28	**0.53**	---	---	---
HL	---	---	---	0.03	**0.46**	-0.03
HW	**0.57**	-0.30	**0.36**	**0.54**	**0.45**	-0.03
HH	**-0.36**	0.06	**0.41**	0.35	**0.84**	-0.14
TW	**0.81**	**-0.46**	**0.41**	**0.74**	**0.68**	**0.69**
FFL	---	---		**0.63**	0.16	**-0.51**
FL	0.17	**0.36**	**0.75**	**0.50**	**0.96**	-0.34
TBL	---	---	---	---	---	---
4TL	---	---	---	**0.90**	0.32	0.08
HFL	---	---	---	**0.76**	**0.51**	**0.77**
Eigenvalues	6.94	1.53	0.96	5.81	1.72	0.86
% explained	71.30	15.80	9.80	65.90	19.50	9.80
% cumulative	71.30	87.10	96.90	65.90	85.30	95.10
Scales						
VSN	**-0.45**	-0.28	**0.91**	0.25	**0.49**	0.21
FPN	**0.49**	**0.57**	0.17	---	---	---
Lam	**0.58**	**-0.49**	-0.13	0.28	-0.02	0.07
STSN	0.27	-0.32	-0.14	0.17	**-0.50**	0.13
GSN	**0.42**	0.34	-0.01	0.20	-0.01	**-0.41**
CSN	0.17	-0.20	0.31	**0.50**	0.18	**-0.45**
SCGN	**-0.42**	0.29	-0.14	-0.29	**0.50**	**-0.43**
SCSN	0.22	0.12	-0.08	-0.09	0.31	**0.41**
SLSN	**0.49**	-0.01	**0.49**	0.20	0.22	0.21
Eigenvalues	3.13	0.78	0.56	1.90	0.95	0.51
% explained	62.00	15.40	11.20	53.40	26.60	14.3
% cumulative	62.00	77.40	88.50	53.40	79.90	94.2

The third discriminant function (CDF3), which represents 9.8% of the variation was mostly explained by tail width (TW) and the length of the front limbs (FLL). The classification percentage calculated on the basis of the discriminant functions showed that Oukaimeden, Outabati and Jebel Azourki were the populations with a higher correct classification score (>80%), while Jebel Ayache and Tizin Tichka were the worse classified (<50%, Table 5). Jebel Sirwa had a classification score of 67%.

Table 5. Classification matrix based on the discriminant functions obtained of the analysis of the linear measurements. For each pair of populations the percentage (%) and frequency (N) of correctly classified individuals are in bold.

		Classification (% and N)									
Population	Sex	J. Sirwa		Oukaimeden		Tizin Tichka		Outabati		J. Azourki		J. Ayache	
J. Sirwa	Males	**100.0**	**13**	0	0	0	0	0	0	0	0	0	0
	Females	**66.7**	**6**	0	0	0	0	0	0	11.1	1	22.2	2
Oukaimeden	Males	0	0	**85.7**	**12**	14.3	2	0	0	0	0	0	0
	Females	0	0	**82.4**	**14**	17.6	3	0	0	0	0	0	0
Tizin Tichka	Males	8.3	1	16.7	2	**58.3**	**7**	16.7	2	0	0	0	0
	Females	0	0	33.3	4	**50.0**	**6**	0	0	0	0	16.7	2
Outabati	Males	0	0	0	0	16.7	1	**83.3**	**5**	0	0	0	0
	Females	0	0	0	0	20.0	1	**80.0**	**4**	0	0	0	0
J. Azourki	Males	10.0	1	0	0	10.0	1	0	0	**50.0**	**5**	30.0	3
	Females	4.8	1	0	0	0	0	0	0	**90.5**	**19**	4.8	1
J. Ayache	Males	12.5	1	0	0	0	0	0	0	37.5	3	**50.0**	**4**
	Females	25.0	3	0	0	16.7	2	0	0	25.0	3	**33.3**	**4**

In summary, some of the populations could be identified at the multivariate level on the basis of some distinct linear measurements (Figure 4). Regarding the Jebel Ayache and Jebel Azourki populations, these have very similar shape characteristics with the multivariate SIZE being the most distinctive character, particularly in males. The Jebel Ayache population has larger males (SVL mean 48.99 mm) with comparatively shorter and wider heads, while the population from Jebel Azourki has the largest females (SVL mean 51.79 mm) and a different shape of the head (longer and narrower heads). The Jebel Sirwa population has relatively smaller trunk length (TRL mean 21.64 mm), wider tails (TW mean 4.92 mm) and larger hind limb length (HLL). Regarding the southern populations, the one from Oukaimeden presents shorter hind limbs (HLL), while the one from Tizin Tichka did not have any distinctive characteristic, sharing with Outabati larger fourth toes and hind limbs (HTL and HLL, respectively). The Outabati population presents, in general, long, slender and flattened heads (HL mean 15.18 mm, HW mean 7.18 mm and HH mean 3.9 mm), and longer tibiae (TBL mean 5.68 mm; Table 1 and Figure 4).

Pholidosis

Analysis of scales revealed differences between populations and sexes, although interaction between the two factors was not significant (Table 3)**.** Populations differed in all the variables with the exception of the number of supralabial scales (SLSN; Table 3). Sexual differentiation in pholidosis was restricted to the number of ventral scales (VSN, Table 3) with a higher number in females than in males (Figure 5) and a similar pattern among populations (interaction LINEAGE*SEX not significant in any case, Table 3). In males, we observed differences among populations in the number of ventral scales (VSN), femoral pores (FPN),

lamellae (Lam) and number of gular scales (GSN, Table 3; Figure 5). However, the level of discrimination of the CDFA between populations is very low and the relationships among populations estimated in the body measurements analysis did not show the same pattern (Figure 6). In males, the first canonical function explains 62% of the total variation, with the main contribution of ventral (VSN), femoral (FPN), gular (GSN), supraciliar (SCGN) and supralabial (SLSN) scales and lamellae (Lam; Table 4).

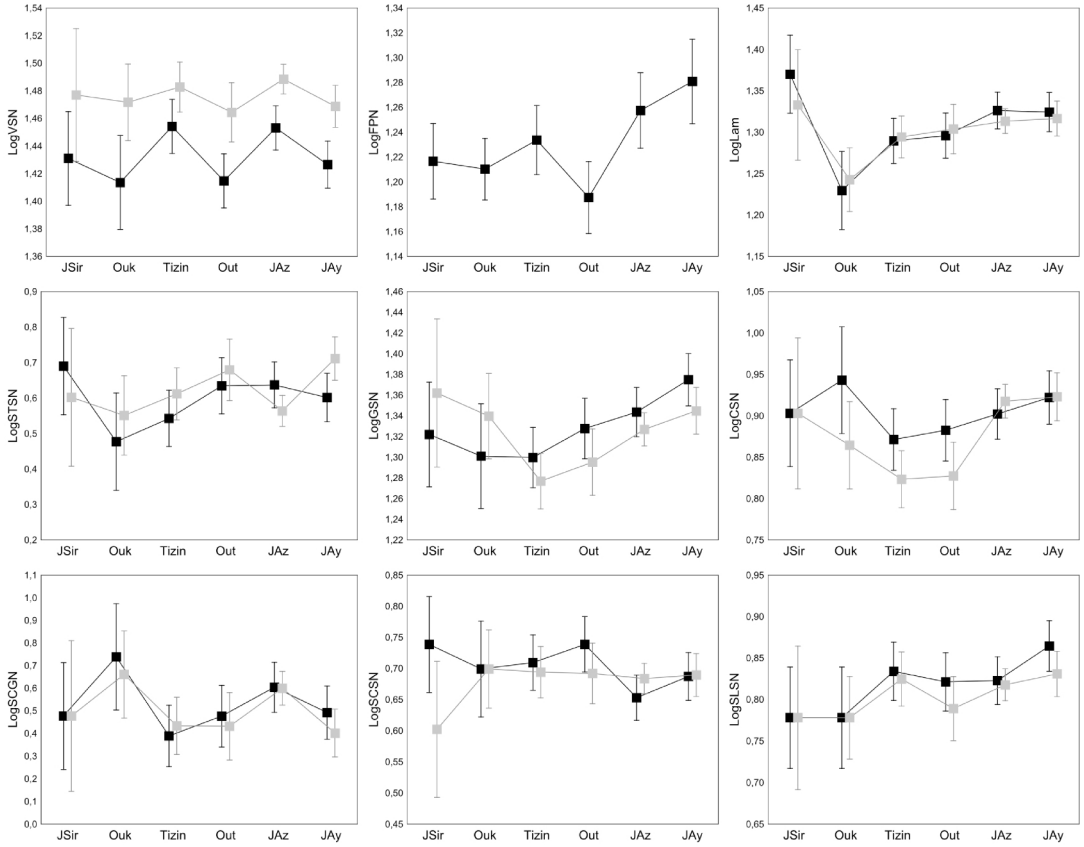

Figure 5. Variation in pholidotic variables (log-transformed values), of males (in black) and females (in grey) of the *A. andreanskyi* lineages, included in this study. For each population, mean values ± 95% confidence interval for the standard error is shown.

Regarding the second axis (15.4% of the variation) the main contributing variables were the number of femoral pores (FPN) and the number of lamellae (Lam; Table 4). The third discriminant function (11.2% of the variation) was mostly explained by the number of ventral scales (VSN) and the number of supralabial scales (SLSN; Table 4).

The degree of discrimination was low (Table 6). Oukaimeden was the best discriminated population (85.7%), followed by Jebel Sirwa and Jebel Ayache (61.5 and 62.5% variation, respectively). The remaining localities had discrimination scores lower than 40% (Table 6).

Table 6. Classification matrix based on the discriminant functions obtained of the analysis of the pholidotic variables. For each population the percentage (%) and frequency (N) of correctly classified individuals are in bold.

		Classification (% and N)											
Population	Sex	J. Sirwa		Oukaimeden		Tizin Tichka		Outabati		J. Azourki		J. Ayache	
J. Sirwa	Males	**61.5**	**8**	7.7	1	7.7	1	15.4	2	7.7	1	0	0
	Females	**44.4**	**4**	0	0	0	0	22.2	2	0	0	33.0	3
Oukaimeden	Males	0	0	**85.7**	**12**	7.1	1	7.1	1	0	0	0	0
	Females	11.8	2	**82.4**	**14**	5.9	1	0	0	0	0	0	0
Tizin Tichka	Males	25.0	3	16.7	2	**41.7**	**33**	8.3	1	8.3	1	0	0
	Females	0	0	16.7	2	**41.7**	**5**	33.3	4	8.3	1	0	0
Outabati	Males	16.7	1	0	0	16.7	1	**33.3**	**2**	16.7	1	16.7	1
	Females	0	0	20.0	1	80.0	4	**0.0**	**0**	0	0	0	0
J. Azourki	Males	40.0	4	0	0	20.0	2	10.0	1	**20.0**	**2**	10.0	1
	Females	4.8	1	4.8	1	19.0	4	0	0	**61.9**	**13**	9.5	2
J. Ayache	Males	0	0	0	0	0	0	38.0	3	0	0	**62.5**	**5**
	Females	8.3	1	8.3	1	0	0	0	0	25.0	3	**58.3**	**7**

In females, differences among populations were observed in the number of supratemporal (STSN), gular (GSN), and ciliar (CSN) scales, and supraciliar granules (SCGN; Table 3, Figure 5). CDFA also showed a low discrimination power between populations (Figure 6). The first function explains 53.4% of the total variation and is mainly explained by the number of collar scales (CSN), while the second discriminant function explained 26.6% of the variation being the number of ventral (VSN) and supratemporal (STSN) scales and the number of supraciliar granules (SCGN) the most contributing variables (Table 4).

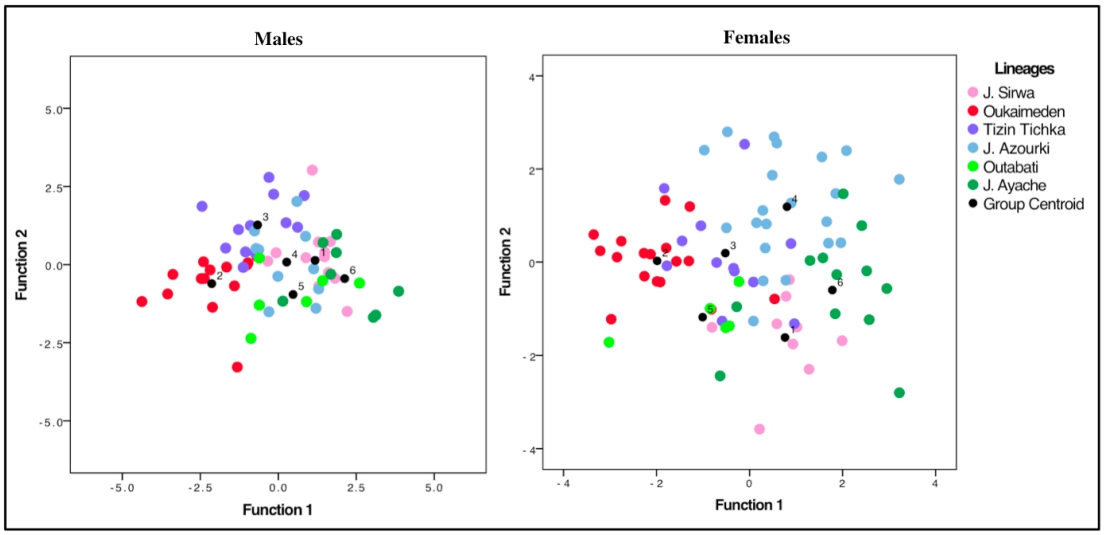

Figure 6. Canonical discriminate function analysis (CDFA) of pholidotic variables, for males (left) and females (right). Black circles represent the group centroids (1- J. Sirwa (pink), 2- Oukaimeden (red), 3- Tizin Tichka (dark blue), 4- J. Azourki (light blue), 5- Outabati (light green) and 6- J. Ayache (dark green)).

Overall, the correct classification scores are low (Table 6). The individuals from Oukaimeden were the only ones that show a relatively high level of correct classification (82%, Table 5),

followed by the individuals from Jebel Azourki and from Jebel Ayache (62% and 58%, respectively). The populations from Tizin Tichka, Jebel Sirwa and Outabati had classification scores lower than 45% (Table 6). It should be noted that none of the females from the Outabati population were correctly classified (Table 6), but this was the smallest group size assessed (only 5 females). Notably also the population with the largest number of analysed individuals (Oukaimeden) also had the highest percentage classified correctly.

Colour pattern

The analysis of males and females had a generally congruent pattern in both sexes (Figure 7), with three main groups: 1) Jebel Ayache and Outabati, 2) Tizin Tichka and Jebel Azourki, and 3) Jebel Sirwa and Oukaimeden.

In males, the first group, that includes the two most oriental populations, namely Outabati and Jebel Ayache, had, in general, a trend towards the absence of light dorsolateral lines (Wlline.1) and central dorsal line (CBL.1). The second group, formed by the central populations of Tizin Tichka and Jebel Azourki, tend to have black spots in the ventral head (HPV.3), two black dots in the anal plate (CD.2). The third group, formed by the occidental populations of Oukaimeden and Jebel Sirwa, showed a general trend towards a lack of black pigmentation in the ventral (VBP.1), head (HPL.1, HPV.1, HPD.1) and presence of continuous bright dorsolateral lines (Wllines.2).

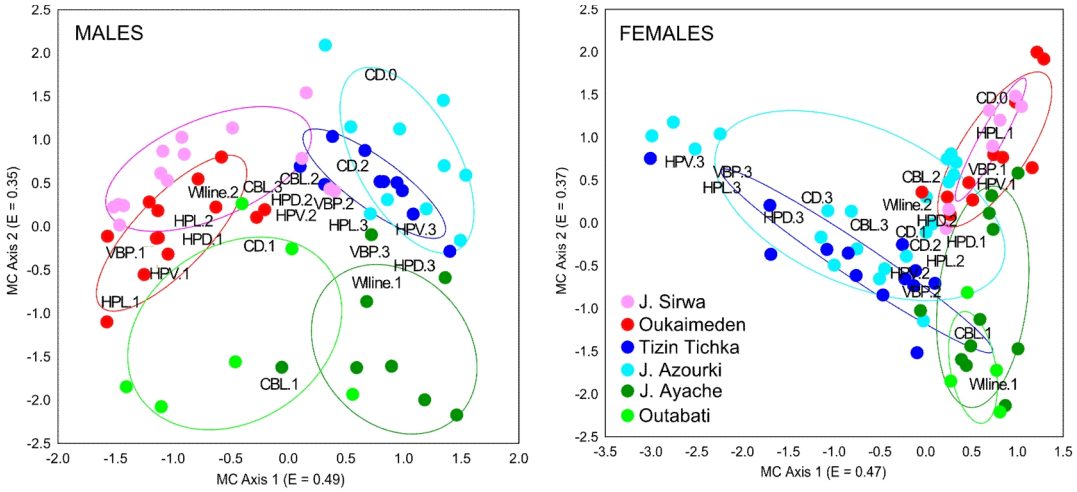

Figure 7. Multiple Correspondence analysis (MCA) of male (left) and female (right) colour pattern. Colour pattern acronyms are followed by states, as given in the text (e.g. 'HPL.2' indicates character HPL, with state two, 'presence of pigmented spots').

In females, the oriental group (Outabati and Jebel Ayache), tent to lack central dorsal line (CBL.1) and light dorsolateral lines (Wlline.1), and to have slightly or unspotted heads (HPD.1, HPV.1, Figure 7). The central group (Tizin Tichka and Jebel Azourki) had, in

general, a quite intense spotted pattern in ventral (HPV.3, VBP.3, CD3), lateral (HPL.3) and dorsal areas (HPD.3, HPD.2, CBL.3, CBL.2), continuous or discontinuous bright dorsolateral lines (Wlline.2), and more intense pigmentation in the anal plate (CD.2, CD.3). Finally, the occidental group (Oukaimeden and Jebel Sirwa), showed a trend towards an absence of pigmentation in the cloacal plate (CD.0), laterals of the head (HPL.1), and ventral body (HPV.1 and VBP.1).

Intra-lineage sexual dimorphism

Sexual dimorphism in size showed variation among populations. Males were on average larger than females in Oukaimeden and Jebel Ayache, while females were larger in the remaining populations (Table 1). Despite these differences, females from all populations had comparatively larger trunks than males, and this difference was significant in all cases, with the exception of Outabati and Jebel Ayache (Table 2).

Sexual dimorphism in shape was more accentuated in Jebel Sirwa, Jebel Azourki and Jebel Ayache (Table 2). In these populations, males had significantly longer limbs, wider tails and more robust heads than females (Table 2). On the other hand, Oukaimeden was the population with a lower degree of sexual dimorphism. The only difference, apart from the longer trunk in females, was the presence of longer pileus in males (Table 2). Finally, Tizin Tichka and Outabati showed an intermediate sexual dimorphism, with a tendency of males having more robust heads and longer hind limbs than females, but with no differences in tail width (TW) or forelimb length (FLL, Table 2).

Regarding pholidosis, males had developed femoral pores, generally absent or incomplete lines in females. Females had a higher number of ventral scales (VSN) than males in all populations with the exception of Oukaimeden, where the trend was not significant (Table 2). Again, Jebel Sirwa, Jebel Azourki and Jebel Ayache were more sexually dimorphic, although the pattern of sexual dimorphism was not consistent (Table 2). Thus, males had higher number of lamellas in Jebel Sirwa, more supratemporal scales (STSN) in Jebel Azourki and a larger number of supraciliar granules (SCG) in Jebel Ayache in comparison with their respective females (Table 2).

Finally, males and females also exhibited differences in colour pattern (Figure 8). Females had, in general, more uniform pattern than males. Males tend to have more pigmentation in the dorsum, and lines tend to be more discontinuous than in females. Moreover, while lateral dark bands tend to be reticulated in males, in females they tend to be uniform in coloration. Regarding the ventral region, males are generally more spotted than females. Moreover, when orange pigmentation is present, in males it tends to be present in all the ventral body, while in females, it is usually reduced to the cloaca, femoral region and ventral tail (Figure 8).

Genetic diagnosis

High genetic divergence values between the six lineages were previously demonstrated (Barata *et al.*, 2012a). Here we report the diagnosable positions in the mtDNA sequences analysed. All the lineages show a combination of unique differences in the fragments of 12S rRNA (Table 7) and ND4 (Table 8). Moreover, in Tizin Tichka, Jebel Azourki, Outabati and Jebel Ayache, some of those differences in ND4 translate into exclusive aminoacids (Table 9).

Table 7. Diagnosable positions for each lineage (bold) from the fragment of 12S rRNA. The numbers of the positions correspond to the entire mitogenome of *P. muralis* (GenBank accession number NC_011607.1, Podnar *et al.*, 2009). Positions that are equal to the first line are represented by a ".".

	12S rRNA variable positions																						
Pop	**549**	**578**	**582**	**585**	**587**	**597**	**609**	**613**	**673**	**674**	**675**	**677**	**678**	**680**	**740**	**752**	**753**	**769**	**770**	**790**	**802**	**809**
Oukaimeden	.	.	.	.	T	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
J. Sirwa	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A
Tizin Tichka	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
Outabati	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.
J. Azourki	.	T	.	C	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.
J. Ayache	.	.	.	.	.	.	.	.	.	C	.	T	A	.	.	.	.	.	.	C	.	.

Table 8. Diagnosable positions for each lineage (bold) from the fragment of the ND4 gene and tRNA-His. The numbers of the positions are referent to the entire mitogenome of *P. muralis* (GenBank accession number NC_011607.1, Podnar *et al.*, 2009). Positions that are equal to the first line are represented by a ".".

	ND4 variable positions																						
Pop	**793**	**794**	**795**	**796**	**797**	**800**	**806**	**807**	**810**	**811**	**834**	**837**	**843**	**861**	**882**	**891**	**894**	**900**	**906**	**915**	**919**	**924**	**927**
Oukaimeden	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	C	.	.	.	G
J. Sirwa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
Tizin Tichka	.	.	.	.	.	.	.	G	.	.	T	T	.	.	.	.	.	.	.	.	.	.	.
Outabati	T	G	G	T	G	A	A	C	A	T	A	.	G	.	.	.	.	.	.	.	.	.	.
J. Azourki	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	T	.	.	.	T	.	.	.
J. Ayache	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	G	.

	ND4 variable positions																						
933	**960**	**964**	**991**	**1002**	**1011**	**1023**	**1035**	**1038**	**1044**	**1062**	**1069**	**1075**	**1080**	**1085**	**1095**	**1104**	**1105**	**1110**	**1119**	**1122**	**1125**
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.
.	.	.	T	.	A	.	.	.	.	.	.	.	.	.	.	.	.	C	T	.	.
C	.	.	.	.	.	.	G	.	.	.	C	.	.	.	C	.	.	.	.	.	G
.	.	G	.	G	.	G	.	.	.	.	.	.	.	T	.	.	.	.	.	G	.
.	.	.	.	.	.	.	.	.	.	G	.	C	.	.	.	.	C	.	.	.	.
.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.

	ND4 variable positions																						
1128	**1134**	**1152**	**1153**	**1164**	**1173**	**1179**	**1188**	**1194**	**1203**	**1209**	**1218**	**1230**	**1242**	**1248**	**1260**	**1263**	**1266**	**1268**	**1272**	**1275**	**1287**
.	.	.	.	G	C	.	.	.	.	.	.	.	T	.	G	.	.	.	.	T	.
G	.	.	.	.	.	.	G	.	.	G	.	G	.	.	.	.	.	.	.	C	.
.	.	.	.	.	.	.	.	C	.	.	C	.	.	T	.	.	G	.	C	.	.
.	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	G	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C
.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.

	ND4 variable positions																						
1291	**1293**	**1296**	**1308**	**1317**	**1321**	**1323**	**1339**	**1356**	**1359**	**1362**	**1368**	**1371**	**1374**	**1377**	**1378**	**1384**	**1386**	**1390**	**1398**	**1417**	**1422**
.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	C	.	.	.
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.
.	.	.	T	.	G	T	.	.	.	.	.	A	.	.	.	C	.	.	.	.	.
.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
A	.	.	.	T	.	.	.	C	C	T	C	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	G	.	G	.	G	.	.	.	.	T	.

Table 9. Diagnosable alterations in aminoacids (bold) for each lineage for the ND4 gene fragment. Proline (P), Tryptophan (W), Isoleucine (I), Cystine (C), Leucine (L), Valine (V), Phenylalanine (F), Threonine (T), Methionine (M), Serine (S) and Alanine (A). Positions that are equal to the first line are represented by a ".".

	ND4 aminoacids variable positions										
Pop	**2**	**3**	**18**	**44**	**59**	**94**	**99**	**161**	**168**	**178**	**197**
J. Sirwa	P	I	C	I	I	F	T	M	L	I	S
Oukaimeden	P	I	C	I	I	F	T	M	L	I	S
Tizin Tichka	P	I	C	I	I	**L**	T	**I**	L	**V**	S
Outabati	**W**	**C**	**W**	I	**V**	F	**M**	L	L	L	**T**
J. Azourki	P	I	C	I	I	F	T	M	**M**	I	S
J. Ayache	P	I	C	**L**	I	F	T	L	L	L	**A**

Bayesian species delimitation

When assuming six lineages, Bayesian species delimitation analysis strongly supports the guide tree, as found in previous studies (six clades), with speciation probability ≥ 0.99 on all nodes (Guide tree with posterior probability for presence of nodes:

((JSi, Ouk) 1.0, ((Tiz, JAz) 1.0, (Out, JAy) 0.995) 1.0) 1.0

We used only one guide tree, the one obtained in both mtDNA and nDNA phylogenetic analysis, since this resulting tree was very simple with no ambiguous relationships. Following Leaché & Fujita (2010) the use of random trees, with an artificial increase of sister species has a negative impact on the result.

Discussion

In recent years, studies involving cryptic diversity have increased, uncovering a "new" diversity previously unsuspected. This is happening mainly due to the implementation of genetic tools in taxonomy. It is clear that morphological differentiation is not an essential component of speciation, especially when considering only the part that falls within human perception (Fritz *et al.*, 2006). In such cases the results obtained in molecular studies may promote the more detailed assessment of other kinds of variation including morphological, ecological or behavioural that might have initially been overlooked (e.g. Bergmann & Russell, 2007; Funk *et al.*, 2011).

Despite the high genetic divergence previously found between the six analysed populations of *A. andreanskyi* reinforced by a combination of diagnostic positions in both 12S and ND4 sequences, and after a thorough observation of phenotypic variables, no simple diagnostic morphological characters supporting the genetic divergence were identified. However, the analysis of linear measurements and pholidotic characters did identified morphological variability among genetic lineages, although discrimination was limited by the high levels of intra-populational variation, and thus, had limited diagnostic value. Linear measurements separate the populations in two main groups, with the larger SIZE of the individuals from J.

Ayache and J. Azourki being the main contributing factor to this separation. However, the affinities among populations changed according to sex; so, while in males J. Sirwa is the most distinct population, in females, Outabati is the most differentiated one. Although this variation can be more related with a different degree of sexual dimorphism between populations, this could also be considered a characteristic of the lineages. That and the lack of geographic concordance, with a closer morphological similarity between Oukaimeden and Tizin Tichka on one side, and J. Sirwa J. Ayache and J. Azourki on the other, are the main contradictions with estimates of relationships based on genetic markers, and are related to the similarities in body size. Several studies have found a similar distinctive pattern of J. Sirwa regarding other nearby populations and rather a link with populations from Eastern Maghreb, and this may be a general biogeographic pattern (Fonseca *et al.*, 2009; Harris *et al.*, 2002; Lima *et al.*, 2009; Pinho *et al.*, 2007).

Regarding sexual dimorphism, in general, *A. andreanskyi* showed, in most of the populations analysed, a female-biased dimorphism regarding body size, with females having larger snout vent length and trunk length, and consequently a higher number of ventral scales than males. This trend, commonly observed in lacertids (see a review in Cox *et al.*, 2003) supports the fecundity advantage hypothesis (Cox *et al.*, 2003). According to this hypothesis, larger female body sizes would be favoured in species with short reproductive season, to maximize clutch success on each reproductive episode. This seems to be the case of *A. andreansky*i living in high mountain areas, where the harsh environmental conditions impose a long hibernation period of 6-8 months (Schleich *et al.*, 1996). The only sexually diagnostic character was the presence of enlarged femoral pores in males, much smaller in females. Femoral pores are known to be important in intraspecific communication, playing an important role in sex recognition, mating selection and territory marking (Gómez *et al.*, 1993; Kaliontzopoulou *et al.*, 2005; Martin *et al.*, 2007). Interestingly, femoral pores are completely absent in some females of the populations studied (Barata *et al.*, 2011). Regarding the other characters, males present, in general and for the same size, more robust bodies, bigger heads and larger limbs, that are advantageous in intersexual encounters, feeding and escaping from predators (Herrel *et al.*, 2001a; Herrel *et al.*, 1999; Herrel *et al.*, 2001b; Herrel *et al.*, 1996). However, populations from J. Ayache and Oukaimeden are unusual, with the former having larger males than females (SVL), and the latter not presenting differences in SVL between sexes. The degree of sexual dimorphism may reflect the competition and selective pressures acting on a population (Kaliontzopoulou *et al.*, 2007). However despite the greater human pressure observed in Oukaimeden (this locality has a ski station), *A. andreanskyi* is present in high densities (Busack, 1987 ; and pers. obs.). On the other hand, since sexual dimorphism in this population is low, we might expect segregation in diet or niche resources in order to reduce

intraspecific competition. Interestingly, the only quantitative study on diet composition in this species showed no differences in the diet of males and females in Oukaimeden (Carretero *et al.*, 2006b). Regarding habitat use, the populations analysed presented a wide variation of habitat characteristics including altitude (500 m variation), presence of water, different refuge availability and different spectrum of sympatric species. For example, in Oukaimeden, *Atlantolacerta* was found under small rocks while in other localities they chose the protection of spiky bushes (*Alyssum spinosum*, *Bupleurum spinosum*, *Cytisus balansae*; Rykena & Bischoff, 1992). The J. Ayache and J. Azourki populations were found in dry places, while in Oukaimeden individuals were found near a water source. Furthermore, Oukaimeden and J. Azourki had higher herpetofauna richness, including *Podarcis vaucheri*, *Tarentola mauritanica*, *Timon tangitanus*, *Natrix maura*, *Quedenfeldtia trachyblepharus*, *Scelarcis perspicillata* and *Vipera monticola*, while in other populations species richness was apparently lower. Unfortunately, as far as we know, there is no information regarding intraspecific microhabitat segregation. More studies need to be done to unveil what mechanisms are behind intraspecific segregation for resources in *A. andreanskyi*.

Although our analysis do show high morphological variability among some populations with a relatively good discrimination at the multivariate level, high levels of intraspecific variability increase the overlap among populations, entangling the finding of diagnosable characters that allow a simple morphological identification of the lineages. Similar results were found in another cryptic species complex, *Podarcis hispanica* (Kaliontzopoulou *et al.*, 2012) and might be partially due to the fact of having only one population analysed per genetic lineage. However, since almost all analysed populations of *A. andreanskyi* have been so far identified as distinct lineages, no other sampling methodology is currently possible.

As described in Barata *et al.* (2012a) *A. andreanskyi* has high levels of genetic diversity, and it is unsurprising that the stronger support came from mtDNA data, since this tool has been widely used to detect cryptic diversity in many species (Avise *et al.*, 1987; Wiens & Penkrot, 2002). The maternally inherited, non-recombinant characteristics of mtDNA imply relatively smaller population sizes and faster coalescence, especially in recent lineages when other nuclear or phenotypic characteristics may still not be fixed (Wiens & Penkrot, 2002). On the other hand, the recent proposed "unified species concept" (de Queiroz, 2007) and integrative taxonomy (Dayrat, 2005; Padial *et al.*, 2010), both suggest the combined use of multiple criteria to delimit species, although the continuity of the speciation process is well-known and criteria may not be achieved at the same time or order, so that the absence of one of the criteria does not provide strong evidence against the acceptance of the species (de Queiroz, 2007). Furthermore, the different speciation processes and the conditions where they take place influence the kind of divergence observed in different taxa - morphological similarity

can mask deep molecular divergence, or alternatively, fast and repeated phenotypic adaptive evolution can lead to considerable morphological variation within a single genetic unit. There is still subjectivity in how to interpret morphological data, and the same doubts, as with genetic distances; the limit between a population and an isolated lineage is always subject to discussion (Fujita *et al.*, 2012). Although, the preference for an integrative taxonomy is almost consensual, in cryptic species, morphology cannot detect the different lineages and in these cases it is possible that only multilocus data could be used to delimit taxa (Fujita *et al.*, 2012). This study also highlights another facet of "cryptic" taxa, in which lineages do have morphological differences, but for them to be identified based on these requires analysis of many individuals and characters, a situation similar to that observed in the *Podarcis hispanica* complex (Kaliontzopoulou *et al.*, 2012). Thus, while they might not be strictly "cryptic", from a practical point of view such forms essentially are until some simple diagnostic characters are identified.

Several authors (Morando *et al.*, 2003; Wiens & Penkrot, 2002) have argued that strong clade support; haplotype exclusivity and geographical concordance are good evidence of multiple species. In the case of *Atlantolacerta*, each genetic lineage corresponds to a different geographical locality isolated by unsuitable habitat between the different mountains, meaning that they are currently genetically isolated. In the estimate of relationships derived from mtDNA, the first and second assumptions, strong support and haplotype exclusivity were achieved with high divergence between all the lineages (Barata *et al.*, 2012a). Indeed, mtDNA divergence is far higher than that between accepted species of montane lacertids from the genus *Iberolacerta,* for example (Carranza *et al.*, 2004). Furthermore, the combination of nuclear information from five independent nuclear markers (concatenated tree and species tree) also recovers the six lineages, even if monophyly was not always achieved. The paraphyly between geographically closer populations (southern, central and northern) with the nuclear markers probably reflects incomplete lineage sorting. All six proposed lineages in the tree based on a coalescent analysis (Barata *et al.*, 2012a) and in the Bayesian species delimitation (Bpp) were strongly supported.

It has been argued that as the acquisition of multilocus data becomes more frequent, the use of coalescent-based species delimitation will improve taxonomic consistency and stability (Fujita *et al.*, 2012). These authors argue that a coalescent-based diagnosis, identifying populations that cluster as distinct lineages according to genetic analyses of multiple loci, is consistent with the International Code of Zoological Nomenclature. However, since such a view is contentious (e.g. Bauer *et al.*, 2011), we prefer to also note the diagnostic molecular characters that can be used to discriminate between populations, as well as the partially diagnostic morphological characters.

The type locality was given in 1929 by Werner as Tachdirt in the High Atlas. Since then, all publications regarding (*Atlantolacerta*) *andreanskyi* have considered this village of Tachdirt near Jebel Toubkal, as the type locality (Busack, 1987; Joger & Bischoff, 1989; Klemmer, 1969; Pasteur & Bons, 1960; Rykena & Bischoff, 1992; Saint Girons, 1953; Schleich *et al.*, 1996; Stemmler, 1972; Werner, 1929, 1931, 1935). Although Harvard University (which holds the type specimen) has a GPS location (31.1, -7.533) that is referent to a different Tachdirt, near Ouarzazate, the type locality was the one near Oukaimeden as shown in the species distribution map published by Werner two years later (1931) and confirmed by the limited description of the place (locality in the Imenin Valley, High Atlas with a nearby plateau at 2800 m of altitude, with the presence of *Quedenfeldtia trachyblepharus*, *Podarcis vaucheri*, *Timon pater* and *Bufo bufo*, Werner, 1929, 1931). Consequently, the lineage from Oukaimeden maintains the name *Atlantolacerta andreanskyi*, while we describe five new species corresponding to the remaining genetic lineages. Since our description relies on molecular characters, we assign a new paratype for Oukaimeden, from which genetic identification of this lineage was made.

Species description

Family **Lacertidae**

Tribe **Eremiadini** Shcherbak, 1975

Genus ***Atlantolacerta*** **(**Arnold *et al*. 2007)

(Werner, 1929, Malkmus, 1981, Schleich *et al.,* 1996): *Lacerta andreanskyi* Werner 1929;

(Mayer & Bischoff, 1996, Bischoff, 2005): *Teira andreanskyi* (Werner 1929);

(Arnold *et al*., 2007, Sindaco & Jeremcenko, 2008): *Atlantolacerta andreanskyi* (Werner 1929).

Conservation status: Near threatened**.** Listed as Near Threatened because, although its extent of occurrence is less than 20,000 km², it occurs in a habitat that is not under significant threat, and so it is probably not in decline (IUCN Red List, http://www.iucnredlist.org/details/61518/0). The current status takes into account that all the populations are part of one single species, and now clearly needs to be reassessed**.**

Restriction of *Atlantolacerta andreanskyi* (Werner 1929)

Name: *Atlantolacerta andreanskyi*

Atlas Dwarf Lizard

Tables 1, 2, 7 and 8. Figures 3, 4, 5, 6, 7 and 8A

MorphoBank: (to be added under acceptance).

Lacerta andreanskyi: Werner, 1929. Wissenschaftliche Ergebnisse einer zoologischen Forschungsreise nach Algerien. *Sitzungsberichte der Akademie der Wissenschaften, Abteilung* 1, 4-5. Type locality: Tachdirt, High Atlas, Morocco.

Lacerta andreanskyi: Klemmer, 1969 : 325; Bons, 1972 : 114; Malkmus, 1983 : 8; Joger & Bischoff, 1989: 100; Herrmann, 1991: 90; Rykena & Bischoff, 1992 : 339; Schleich *et al.*, 1996 : 403.

Lacerta andreanskii: Saint-Girons, 1992: 16.

Lacerta andreanszkyi: Werner, 1931 : 284; Busack, 1987 : 231; Bons & Geniez, 1996 :133; Harris *et al.*, 1998 :1947.

Teira andreanskyi: Mayer & Bischoff, 1996 : 169; Schlüter, 2003 : 99.

Atlantolacerta andreanskyi: Arnold *et al.*, 2007 : 63.

Lacerta (Atlantolacerta) andreanskyi; Sindaco & Jeremcenko, 2008 : 245.

Etymology: The lineage from Oukaimeden maintains the specific name "*andreanskyi*", since the species was initially described based on the description of a female specimen from Tachdirt (type locality) near Oukaimeden. The species epithet *andreanskyi* was dedicated to

the Hungarian botanist Baron Gábor Andreánzsky (1895–1967) that participated in the expedition during which the species was firstly observed (Werner 1929).

Specimens examined: 31 live specimens, 15 males and 16 females.

Holotype: MCZ 27391 (field number ZR27387), Harvard University (Catalogue number 153731). Female from above Tachdirt, 2500 m altitude, Morocco, collected in May 1928 by Dr. Gábor Andreánzsky (Werner, 1929).

Paratype: Adult female, collected in September 2009 in Oukaimeden plateau (31.20N 7.86W) by Mafalda Barata, Fátima Jorge, James Harris and Salvador Carranza. Museum code XXX (to be added under acceptance), and MorphoBank accession numbers: JX462077 (12S), JX462170 (ND4), JX461613 (PDC), JX461967 (ACM4), JX485224 (C-MOS), JX461781 (MC1R), JX461435 (RAG1).

Distribution: Type locality in Tachdirt, High Atlas Morocco. Two other populations from this lineage were found in Oukaimeden plateau (2600 m altitude, 31.20N 7.86W) where the paratype was collected, and other in Toubkal Mountain (2500 m, 31.20N, -7.87W).

Diagnosis: Small sized lizard (SVL between 35.7 and 47.5). Morphologically similar to the populations from Outabati and Tizin Tichka (Figure 4), but with comparatively shorter hind limbs (17.6 mm average, versus 19.7 mm and 20.4 mm of Tizin Tichka and Outabati respectively, Table 1, Figure 3) and shorter 4th toes (8.27 mm average, versus 9.07 mm and 9.13 mm of Tizin Tichka and Outabati respectively, Table 1, Figure 3). Low number of lamellae (13-22; Table 1); between none and 8 supraciliar granules between supraciliar and supraocular scales. Colour pattern resembling *Atlantolacerta tarrosoi* sp. nov.. Dorsal brownish, central dorsal line frequently continuous or discontinuous, in some cases practically absent; lateral bands limited in the upper side by continuous light lines that may fade as they get closer to the tail. Ventral greyish usually with orange iridescences in females. Orange ventral pigmentation mostly reduced to the cloaca, ventral tail, and femoral region in females, usually not present in males. All these morphological characteristics did, however, overlap with other populations, and thus they are not diagnostic. Molecular diagnosis based on the combination of several distinct nucleotides from a fragment of 12S rRNA and ND4 genes. Specimens from this lineage show a combination of two thymine (T) nucleotides in the positions 587 and 597 of the 12S rRNA fragment (Table 7) and the combination of exclusive nucleotides in eleven positions of the ND4 fragment; 861-A, 906-C, 927-G, 1104-C, 1164-G, 1173-C, 1242-T, 1260-G, 1275-T, 1377-T and 1390-C (Table 8).

Holotype description [Translation from the original in German, Werner 1929]. Female from Tachdirt, High Atlas, 2500 m, collected by Baron Gábor Andreánzsky in May 1928. (*Zootoca* sub genus), apparently, very similar to the subgenus *Lacerta vivipara,* but with the entire collar; ventral transverse rows without notches, front and hind limbs not touching each other

when plied towards each other close to the body. Dorsal scales in 37 longitudinal series. Ventralia with six longitudinal lines and 32 transversal rows. A curved row of six preanal scales. Dorsal scales of the tail slightly keeled. Twenty-six scales around the tail in the sixth ring of the tail following cloaca.

Gular scales in 20 transverse rows until the collar, six small collar scales; first and fourth supraocular scales smaller, no granules between supraciliar and supraocular scales; occipital and interparietal scales separated by parietal (maybe individual variation); nostril between two nasals, touching the rostral; four supralabials before the subocular, narrowing down. Succesion of two lines of supratemporalia scales; a large tympanic scale; massetericum small; temporal scales small, smooth and polygonal. Five to eight femoral pores; pterygoid teeth indistinguishable; 18 lamellae under the fourth toe.

Total length, with tail, 95 mm, without tail 47 mm (regenerated tail), head: 8 mm length, 5 mm width, and 3.5 mm height. Front limb 12 mm, hind limb 17 mm, fourth toe 7 mm.

Light brown coloration, with a dark brown side band from the edge of the eye until the tail. This stripe is limited by two yellow lines that are in the bottom, one is limited by a dark line. Dorsum with a central line formed by dots and two more limiting the upper bright lateral lines.

Supralabials with a dark spot; ventralia with a monochromatic green colour (preserved animals).

Paratype description (Figure 8A)**:** Adult female with a snout-vent length (SVL) of 46.61 mm, trunk length (TRL) of 25.21 mm, head length (HL) of 14.88 mm, head width (HW) of 5.84 mm, head height (HH) of 3.44 mm, pileus length (PL) of 8.93 mm, tail width (TW) of 3.60 mm; toes of fore and hind limbs slightly touching each other when limbs are plied towards each other along the body; femur length (FL) of 7.76 mm, tibia length (TBL) of 5.28 mm, fourth toe length (4TL) of 8.54 mm, forelimb length (FLL) of 11.74 mm and hind limb length (HLL) of 17.04 mm. Complete collar with 8 scales; gularia with 25 scales. Dorsal scales smooth, not keeled, disposed in 33 longitudinal lines counted in the midbody, and 104 transversal rows. Ventral scales arranged in six longitudinal lines and 30 transversal rows. Anal plate surrounded by eight preanal scales; dorsal scales of the tail slightly keeled with 24 scales around the seventh ring following the cloaca opening. Four preocular scales, first and fourth smaller. Supraciliar and supraocular scales separated by a continuous line of 8 (left) and 9 (right) supraciliar granules; interparietal and occipital in contact; supranasals widely in contact between them and with the rostral scale. Four supralabial scales before subocular narrowing down, with an additional smaller scale in an upper position between the second and third left side supralabials. 5th supralabial (subocular) touches eye. A line of five supratemporal scales, the first one larger; a single large tympanic scale; masseteric scale small

and undifferentiated; temporal scales smooth and slightly larger than the rest. No developed femoral pores. 21 lamellae under the fourth toe.

Coloration (live specimen) dorsal brownish. Two lateral dark side bands extending from the nostril to the hind limbs, reaching the tail; side bands upper and lower limited by thin continuous light lines, not present in the tail. Highly discontinuous line of dark points limiting the upper side of the light lines. Dorsal central dark spotted line progressively more continuous as it gets closer to the tail. Regenerated section of the tail with uniform brownish colour. Ventral greyish, with yellowish coloration in the lower part of the venter; that disappeared when preserved in ethanol; no spots in the trunk nor the head, with the exception of a few dark spots in the inframaxilar scales and in the ventral scales. A single dark dot in the anal plate. Unregenerate part of tail with dark spots.

Population variation and sexual dimorphism: Morphological variation within this population is detailed in Table 1. *A. andreanskyi* has the lowest degree of sexual dimorphism in both size and shape. Males slightly larger than females on average (mean SVL males 42.02 mm and females 41.98 mm). Females with longer trunks and shorter pileus than males (Table 6). Sexual dimorphism in pholidosis almost absent, restricted to the presence of developed femoral pores in males. Moderated sexual dimorphism in coloration. Dorsal body brownish with a black central line, usually discontinuous becoming more continuous as gets closer to the tail, occasionally absent or well defined; two dark lateral bands, usually marbled in males and uniform in females. Bands limited pale light lines, usually more discontinuous in males than in females, that tends to disappear as they get closer to the tail. These lines are generally upper limited by a discontinuous line of black spots, more defined between the forelimb, and usually less evident in females. Ventral greyish with orange iridescences in females, generally absent in males. Ventral slightly spotted, limited, if existing, to the first and second external pair of ventral lines of scales; females with intense orange ventral pigmentation in the venter, cloaca, femoral regions and ventral tail; usually not present in males.

Remarks: Individuals from Oukaimeden were mostly found under rocks near the water, while in Toubkal, they were mostly living nearby thorny bushes that were used as refuge. Other sympatric species observed in Oukaimeden were *Natrix maura*, *Timon tangitanus*, *Podarcis vaucheri*, *Scelarcis perspicillata,* and *Quedenfeldtia trachyblepharus* (Carretero *et al.*, 2006a). Details on the reproductive biology of Oukaimeden population were analysed in Busack (1987).

Description of *Atlantolacerta tarrosoi* sp. nov.

Tables 1, 2, 7 and 8. Figures 3, 4, 5, 6, 7 and 8B

Morphobank: (to be added under acceptance).

Etymology: The species name *tarrosoi* was dedicated to the Portuguese biologist Pedro Tarroso that helped with fieldwork and found the first specimen in this locality

Specimens examined: 22 live specimens, 13 males and 9 females.

Holotype: Adult female, High Atlas Mountains, Jebel Sirwa, sampled in May 2008 by Mafalda Barata, Ana Perera Daniele Salvi, Pedro Sousa, Sónia Ferreira and D. James Harris. Museum code XXX (to be added upon acceptance). GenBank accession number: not sequenced.

Distribution: Jebel Sirwa Mountain, High Atlas, Morocco, in a plateau, at 2561 m altitude (30.77N, 7.65W). Klemmer (1969) mentioned *A. andreanskyi* from the volcano Siroua (Jebel Sirwa), which presumably correspond to this new species.

Diagnosis: Small sized lizards (42.24 mm average SVL in males and 45.51 mm in females), with similar size to Oukaimeden, Outabati and Tizin Tichka; males from Jebel Sirwa being distinguished from the others by their wider tails and shorter trunks (Table 1, Figure 4). Large number of scales under the fourth toe (21 to 25). Colour pattern resembling *A. andreanskyi*, but generally with less pigmented ventral body. Dorsal brownish, central dorsal line generally present and continuous; lateral bands limited in the upper side by continuous light lines that may fade as they get closer to the tail. Ventral greyish with orange iridescences in females, rarely present in males. Orange ventral pigmentation, mostly reduced to the cloaca, ventral tail, and femoral region in females, usually not present in males, exceptionally in the gular. All these morphological characteristics did, however, overlap with other populations, and thus they are not diagnostic. Molecular diagnosis based on the combination of several distinct nucleotides from a fragment of 12S rRNA and ND4 genes. Specimens from this lineage show a combination of exclusive nucleotides in three positions in the 12S rRNA: 549-A, 802-C and 809-A (Table 7) and a combination of eleven different nucleotides in ND4 fragment: 882-C, 991-T, 1011-A, 1110-C, 1119-T, 1128-G, 1188-G, 1209-G, 1230-G, 1275-C and 1398-G (Table 8).

Holotype description: Adult female with a snout-vent length (SVL) of 45.1 mm, trunk length (TRL) of 29.4 mm, head length (HL) of 15.9 mm, head width (HW) of 6.3 mm, head height (HH) of 4.6 mm, pileus length (PL) of 10.0 mm, tail width (TW) of 4.7 mm, femur length (FL) of 7.9 mm, tibia length (TBL) of 5.7 mm, fourth toe length (4TL) of 8.8 mm, forelimb length (FLL) of 13.0 mm and hind limb length (HLL) of 21.7 mm. Complete collar with 8 scales; gularia with 24 scales. Dorsal scales smooth, not keeled, disposed in 39 longitudinal lines counted in the midbody, and 111 transversal rows. Ventral scales arranged in 6 longitudinal lines and 32 transversal rows. Anal plate surrounded by five preanal scales; dorsal scales of the tail slightly keeled with 26 scales around the seventh ring following the cloaca opening. Four preocular scales, first and fourth smaller. Supraciliar and supraocular

scales separated by a discontinuous line of 3 (left) and 3 (right) supraciliar granules; interparietal and occipital in contact; supranasals slightly in contact between them and widely contacting the rostral scale. Four supralabial scales before subocular narrowing down; subocular touching eye. A line of five supratemporal scales, the first one larger; a single large tympanic scale; large masseteric scale, in the left side of the head in contact with the first supratemporal; temporal scales smooth and slightly larger than the rest. Ten slightly developed femoral pores. Twenty-one lamellae under the fourth toe.

Coloration (live specimen) dorsal brownish. Dorsal central dark spotted line progressively more continuous from the snout to the tail. Two lateral dark bands with spots that extend from the nostril to the hindlimbs, partially reaching the tail; upper side bands are limited by a thin continuous light line, not present in the tail. These lines are generally limited by a discontinuous line of dark points, more obvious in the anterior part of the dorsum. Ventral greyish, with orange coloration in the posterior venter, specially in the cloaca, ventral hind limbs and tail, visible in live specimen but that disappeared when preserved in ethanol; trunk with black spots in the lower venter, preanal scales, and tail, and few spots in the chest, sides of the gular and inframaxilar scales. A single dark blotch in the anal plate. The specimen presents several mating bites in both sides of the venter.

Population variation and sexual dimorphism: Morphological variation within this population is detailed in Table 1. Accentuated sexual dimorphism in body size and shape, but reduced in pholidosis. Larger females with comparatively larger trunks (females average was 25.7 mm and males 21.6 mm); males with more robust heads, longer limbs and wider tails (Table 6). Females with a higher number of ventral scales (30 in females versus 26.6 in males on average); males with higher number of lamellae under the fourth toe (22.29 in males, 20.6 in females on average). High variability in coloration. Dorsal body brownish with a black continuous central line, occasionally discontinuous becoming more defined as getting closer to the tail, especially in males; two dark marbled side bands more uniform in females, limited by two, generally continuous, light lines more evident between the forelimbs, occasionally pale and discontinuous in males; in females, they tend to be more defined. Light lines frequently limited by a discontinuous series of black spots, more obvious between forelimbs; in females, spotted line sometimes substituted by a continuous dark line. Ventral greyish generally not spotted or with black spots limited to the cloaca and the two external lines of ventral scales, occasionally extending to all the ventral body; orange iridescence usually absent in males, but frequent in females; orange pigmentation in females generally common in ventral tail, cloaca and femoral regions, rarely extending to all the body; in males usually absent, occasionally present in the gular and ventral tail. Some males with blue blotches in the ventro-lateral area, never present in females.

Remarks: Animals were found under rocks and bushes in a humid place; other species including *Quedenfeldtia trachyblepharus* and *Podarcis vaucheri* were found only a few meters away.

Description of *Atlantolacerta salvii* sp. nov.

Tables 1, 2, 7 and 8. Figures 3, 4, 5, 6, 7 and 8C

Morphobank: (to be added under acceptance).

References: No references found about this location.

Etymology: The species name *salvii* is dedicated to Daniele Salvi an Italian biologist that helped with fieldwork.

Specimens examined: 24 live specimens, 12 males and 12 females.

Holotype: Adult male, High Atlas Mountains, Tizin Tichka, sampled in September 2009 by Mafalda Barata, Fátima Jorge, D. James Harris and Salvador Carranza and in April 2010 by Mafalda Barata, Dianna Steiner, Ana Perera, D. James Harris and Daniele Salvi. Museum code (to be added upon acceptance). GenBank accession number: JX462059 (12S), JX462193 (ND4).

Distribution: Tizin Tichka Mountain, High Atlas, Morocco, at 2800 m altitude (31.30N, 7.41W).

Diagnosis: Small sized lizards (SVL between 36.9 and 48.3 mm). Morphologically similar to the populations from Outabati and Oukaimeden (Table 1, Figure 3, Figure 4), but with relatively shorter forelimbs (Table 1, Figure 3), and a larger average number of femoral pores (FPN, Table 1). Colour pattern similar to *A. kaliontzopoulouae* sp. nov.. Dorsal brownish, central dorsal line discontinuous or continuous generally present; lateral bands limited in the upper side by continuous light lines that may fade as they get closer to the tail. Ventral greyish with orange iridescences; black spots more common in males, generally present in the gular, chest, and venter, exceptionally distributed along all the ventral body. Intense ventral orange pigmentation, more common in females, usually reduced to the ventral tail, cloaca, venter and femoral region. All these morphological characteristics did, however, overlap with other populations, and thus they are not diagnostic. Molecular diagnosis based on the combination of several distinct nucleotides from a fragment of 12S rRNA and ND4 genes. Combination of exclusive nucleotides in three positions in the 12S rRNA fragment; 609-T, 752-A and 769-A (Table 7) and a combination of eighteen different nucleotides in the ND4 fragment; 807-G, 834-T, 837-T, 933-C, 1035-G, 1069-C, 1095-C, 1125-G, 1194-C, 1218-C, 1248-T, 1266-G, 1272-C, 1308-T, 1321-G, 1323-T, 1371-A and 1384-C (Table 8). The combination of some of these nucleotides resulted in three exclusive aminoacids, a leucine (L) instead of a phenylalanine (F) in position 94, an isoleucine (I) instead of a methionine (M)

or a leucine (L) in position 161 and a valine (V) instead of a isoleucine (I) or a leucine (L) in position 178 (Table 9).

Holotype description: Adult male with a snout-vent length (SVL) of 45.48 mm, trunk length (TRL) of 22.54 mm, head length (HL) of 15.31 mm, head width (HW) of 5.67 mm, head height (HH) of 4.07 mm, pileus length (PL) of 9.94 mm, tail width (TW) of 3.67 mm, femur length (FL) of 8.03 mm, tibia length (TBL) of 5.67 mm, fourth toe length (4TL) of 9.07 mm, forelimb length (FLL) of 12.69 mm and hind limb length (HLL) of 18.28 mm. Complete collar with seven scales; gularia with 21 scales. Dorsal scales smooth, not keeled, disposed in 36 longitudinal lines counted in the midbody, and 107 transversal rows. Ventral scales arranged in six longitudinal lines and 28 transversal rows. Anal plate surrounded by five preanal scales; dorsal scales of the tail slightly keeled with 30 scales around the seventh ring following the cloaca opening. Four preocular scales, first and fourth smaller. Supraciliar and supraocular scales in contact; supraciliar granules not present; interparietal and occipital in contact; supranasals widely in contact between them and with the rostral scale. Four supralabial scales before subocular narrowing down; subocular touching eye. A line of five supratemporal scales, the first one larger; a single large tympanic scale; masseteric scale undifferentiated; temporal scales smooth and slightly larger than the rest. Sixteen developed femoral pores. 23 lamellae under the fourth toe.

Coloration (live specimen) dorsal brownish; Two dorsal brownish reticulated side bands with light dots and black blotches extending from the nostril to the hindlimbs, partially reaching the tail; lateral bands limited by continuous lighter lines, that vanishes when reaching the tail. These lines are bounded on the outside by a discontinuous line of dark points. Dorsal central black spotted line progressively more continuous from the snout to the tail. Black dots in the pileus. Ventral grey with an orange iridescence tonality, only evident in the live specimen, with black spots in the inframaxilar scales, chest, lower venter, preanal scales, and tail. A single dark blotch in the anal plate.

Population variation and sexual dimorphism: Morphological variation within this population is detailed in Table 1. Moderate sexual dimorphism in size and shape. Females larger than males (46 mm average in males and 48.3 in females). Males with more robust heads and longer tibiae than females (Tables 1 and 6); females with comparatively longer trunks (22.5 mm average in males and 26.8 in females). Sexual dimorphism in pholidosis limited to a higher number of ventral scales in females (20-30 in males and 29-32 in females. Dorsal body brownish, with a black central discontinuous line that becomes more defined closer to the tail; in females frequently continuous. Dark bands along the lateral body, reticulated in males and more uniform in females; bands limited by two continuous light lines, more obvious between the forelimbs, and more defined in females; upper side of the

light lines frequently limited in males by a discontinuous line of black spots, more intense between the forelimbs; in females, spotted lines usually blurred or absent, with a faded dark line instead. Ventral greyish with orange iridescences, very frequent in females, rarely in males; usually females with intense orange pigmentation in the ventral tail, venter, cloaca and femoral regions, normally absent in males. Ventral black spots in gular, chest, cloaca, that sometimes extent to the whole ventral body in males, rarely in females. Anal plate sometimes with multiple spots.

Remarks: Animals were found near a stream, mostly near thorny bushes or under rocks.

Description of *Atlantolacerta kaliontzopoulouae* sp. nov.

Tables 1, 2, 7 and 8. Figures 3, 4, 5, 6, 7 and 8D

Morphobank: (to be added under acceptance).

References: There is no reference about this location, Rykena & Bischoff (1992) mentioned two specimens of *A. andreanskyi* (NMB13469, MNHP1939-156) that were collected in Jebel Tarkedit (31.53N, 6.40W; near J. Azourki).

Etymology: The species name *kaliontzopoulouae* is dedicated to the Greek biologist Antigoni Kaliontzopoulou.

Specimens examined: 31 live specimens, 10 males and 21 females.

Holotype: Adult male from Jebel Azourki, High Atlas, Morocco, sampled in May 2010 by Mafalda Barata, Dianna Steiner, Ana Perera, D. James Harris and Daniele Salvi. Museum code (to be added upon acceptance). GenBank accession number: not sequenced. MorphoBank accession number: (to be added upon acceptance).

Distribution: Jebel Azourki Mountain, High Atlas, Morocco, at 2796 m altitude (31.76N, 6.29W).

Diagnosis: This population, together with *A. carreteroi* sp. nov. has the largest individuals of the genus, with males reaching up to 52.4 and females 58.9 mm SVL (Table 1). It differs from *A. carreteroi* sp. nov. by the shape of the head, thinner and longer in the former. General colour pattern resembling *A. salvii* sp. nov.. Dorsal brownish, central dorsal line generally discontinuous or not present; lateral bands limited in the upper side by discontinuous light lines that may fade as they get closer to the tail, sometimes almost absent in males. Ventral greyish with orange iridescences; black spots more accentuated in males, generally present in the gular, chest, and venter, exceptionally distributed along all the ventral body. Intense ventral orange pigmentation, more common in females, in the ventral tail, cloaca, venter and femoral region. In males, ventro-lateral scales with blue dots occasionally. All these morphological characteristics did, however, overlap with other populations, and thus they are not diagnostic. Molecular diagnosis based on the combination of several distinct nucleotides

from a fragment of 12S rRNA and ND4 genes. Specimens from this lineage show a combination of exclusive nucleotides in four positions in the 12S rRNA fragment; 578-T, 585-C, 673-T and 680-A (Table 7) and a combination of fifteen different nucleotides in the ND4 fragment; 837-C, 891-T, 915-T, 1062-G, 1075-C, 1105-C, 1179-G, 1218-T, 1287-C, 1291-A, 1317-T, 1356-C, 1359-C, 1362-T and 1368-C (Table 8). The combination of some exclusive nucleotides resulted in one exclusive aminoacid, a methionine (M) instead of a leucine (L) in position 168 (Table 9).

Holotype description: Adult male with a snout-vent length (SVL) of 44.59 mm, trunk length (TRL) of 22.83 mm; head length (HL) of 16.11 mm, head width (HW) of 6.03 mm, head height (HH) of 4.18 mm and pileus length (PL) of 10.32 mm; tail width (TW) of 5.13 mm; fore and hind limbs widely in contact when plied towards each other along the body, femur length (FL) of 8.76 mm, tibia length (TBL) of 5.76 mm, fourth toe length (4TL) of 9.43 mm, forelimb length (FLL) of 15.34 mm and hind limb length (HLL) of 22.47 mm.

Complete collar with eight scales; gularia with 20 scales. Dorsal scales smooth, not keeled, disposed in 42 longitudinal lines counted in the mid body, and 106 transversal rows. Ventral scales arranged in 6 longitudinal lines and 28 transversal rows. Anal plate surrounded by six preanal scales; dorsal scales of the tail slightly keeled with 32 scales around the seventh ring following the cloaca opening. Four preocular scales, first and fourth smaller. Supraciliar and supraocular scales separated by a continuous line of four supraciliar granules; interparietal and occipital in contact; supranasals slightly in contact between them and widely contacting the rostral scale. Four supralabial scales before subocular narrowing down; subocular touching eye. A line of five supratemporal scales, the first one larger; a single large tympanic scale; masseteric scale undifferentiated; temporal scales smooth and slightly larger than the rest. Seventeen developed femoral pores. 21-22 lamellae under the fourth toe.

Coloration (live specimen) dorsal brownish; Two dorsal lighter brown reticulated side bands spotted with light dots that extent from the nostril to the hind limbs, partially reaching the tail; dark sidebands limited upwards by a lighter line, continuous in the anterior dorsum and that vanishes when reaching the tail. These lines are out bounded by a discontinuous line of dark points, more evident between the forelimbs. Discontinuous dorsal central black spotted line only visible in the anterior third part of the dorsum. A few black dots in the pileus and supralabial scales. Ventral grey, with black spots in the inframaxilar scales, sides of the gular, chest, lower venter, preanal scales, and tail. A single dark blotch in the anal plate. Tail regenerated; regenerated part uniformly brownish (Fig. 8D).

Population variation and sexual dimorphism: Morphological variation within this population is detailed in Table 1. Population with accentuated sexual dimorphism in size and

shape, similarly to the one described for *A. tarrosoi* sp. nov.. Females with comparatively larger trunk than males (30.9 mm in females and 24.6 mm in males); males with more robust heads, longer limbs and wider tails (Table 6). Low dimorphism in pholidosis; females with 27-35 ventral scales, males with 26-31; males with 3-6 supratemporal scales, females with 2-5. Moderated sexual dimorphism in coloration. Dorsal brownish frequently with black spots, sometimes relatively abundant in males, but usually absent in females. Continuous dorsal central line usually not present, although frequently black spots might form a discontinuous central line that becomes more continuous as it gets closer to the tail, generally extending to it; occasionally, this line becomes continuous in females. Two marbled dark lateral bands in males, tending to be more uniform in females, sometimes with pale dots; light lines limiting them absent or reduced to a few discontinuous light spots in males; in females these lines are usually pale and continuous in the anterior part of the dorsum, tending to disappear as they get closer to the tail, although exceptionally they can be well defined and continuous. Ventral body greyish, with high variation in the degree of ventral pigmentation, generally spotted in the cloaca, chest, outer ventral rows and venter, mostly extending to all ventral body; exceptionally, not spotted. Orange iridescence in the ventral side of the body, very common in females, rare in males. Intense orange ventral pigmentation in cloaca, venter and femoral region, in females, generally absent in males; when existing limited to the gular. In males, ventro-lateral scales with blue dots present occasionally.

Remarks: Several specimens from this population were infected with mites, generally located on both sides of the cloaca and behind the front and hind limbs. All specimens were found near thorny bushes and under rocks. Several other reptile species were found nearby, including *Podarcis vaucheri, Tarentola mauritanica, Quedenfeldtia trachyblepharus, Vipera monticola* and *Timon tangitanus.*

Description of *Atlantolacerta martinezfreiriai* sp. nov.

Tables 1, 2, 7 and 8. Figures 3, 4, 5, 6, 7 and 8D

Morphobank: (to be added under acceptance).

References: There is no reference about this location.

Etymology: The species name *martinezfreiriai* is dedicated to the Spanish biologist Fernando Martínez-Freiria that was present in the expedition when this population was found.

Specimens examined: 11 live specimens, 6 males and 5 females.

Holotype: Adult male, Outabati, High Atlas Mountains, sampled in May 2011 by Mafalda Barata, D. James Harris, Daniele Salvi and Fernando Martínez-Freiria. Museum code (to be added upon acceptance). GenBank accession number: not sequenced.

Distribution: Outabati Mountain, High Atlas, Morocco, at 2441 m altitude (32.17N, -5.33W).

Diagnosis: Smallest sized population (SVL between 40.36 and 46.26 mm). Morphologically similar to the populations from Tizin Tichka and Oukaimeden, but with relatively longer, narrower and thinner heads, and larger hind limbs. Males with reduced number of femoral pores (17-21). Colour pattern resembling *A. carreteroi* sp. nov., with a central dorsal line generally absent, sometimes discontinuous, but never complete; absent or discontinuous light dorsolateral lines, and ventral body slightly spotted. All these morphological characteristics did, however, overlap with other populations, and thus they are not diagnostic. Molecular diagnosis based on the combination of several distinct nucleotides from a fragment of 12S rRNA and ND4 genes. Specimens from this lineage show a combination of two exclusive nucleotides: a thymine (T) and an adenine (A) in the positions 674 and 770 from 12S rRNA fragment (Table 7) and a combination of twenty one different nucleotides in the ND4 fragment with a first block of ten exclusive nucleotides from the position 793 to the position 843: 793-T, 794-G, 795-G, 796-T, 797-G, 800-A, 806-A, 807-C, 810-A, 811-T, 834-A, 843-G, 964-G, 1002-G, 1023-G, 1085-T, 1122-G, 1134-C, 1152-G, 1293-G and 1378-A (Table 8). The combination of some the exclusive nucleotides resulted in six exclusive aminoacids, a tryptophan (W) instead of a proline (P) in the second position, a cysteine (C) instead of a isoleucine (I) in third position, a tryptophan (W) instead of a cysteine (C) in eighteenth position, a valine (V) instead a isoleucine (I) in fifteenth nine position, a methionine (M) instead of a thymine (T) in the 99th position and a timine (T) instead of a serine (S) in 197th position (Table 9).

Holotype description: Adult male with a snout-vent length (SVL) of 41.95 mm, trunk length (TRL) of 21.21 mm, head length (HL) of 15.58 mm, head width (HW) of 5.5 mm, head height (HH) of 3.72 mm and pileus length (PL) of 9.75 mm, tail width (TW) of 3.92 mm; fore and hind limbs widely in contact when plied towards each other along the body, femur length (FL) of 8.07 mm, tibia length (TBL) of 5.63 mm, fourth toe length (4TL) of 8.83 mm, forelimb length (FLL) of 12.39 mm and hind limb length (HLL) of 20.89 mm. Complete collar with seven scales; gularia with 26 scales. Dorsal scales smooth, not keeled, disposed in 41 longitudinal lines counted in the mid body, and 116 transversal rows. Ventral scales arranged in 6 longitudinal lines and 26 transversal rows. Anal plate surrounded by five preanal scales; dorsal scales of the tail slightly keeled. Four preocular scales, first and fourth smaller. Supraciliar and supraocular scales separated by a continuous line of four and five (left and right sides, respectively) supraciliar granules; interparietal and occipital in contact. Supranasals slightly in contact between them and widely contacting the rostral scale. Four supralabial scales before subocular narrowing down, with an additional smaller scale in an

upper position between the third and fourth supralabials; Subocular touching eye. A line of five supratemporal scales, the first one larger; a single large tympanic scale; masseteric scale undifferentiated; temporal scales smooth and slightly larger than the rest. 18-20 developed femoral pores. 22-23 lamellae under the fourth toe.

Coloration (live specimen) dorsal brownish pigmented with few black spots. Two dorsal dark reticulated side bands that extent from the nostril to the hind limbs, partially reaching the tail; lighter lines surrounding the side bands absent. Dorso-lateral discontinuous line of black dots only present between the forelimbs. Central black spotted line highly discontinuous, that vanishes completely between the hind limbs. A few black dots in the pileus, more abundant in the supralabial scales. Ventral greyish with a bright orange pigmentation in the gular and ventral extending to the tail; this pigmentation disappeared when preserved in ethanol. Abundant black spots in inframaxillar, gular, chest, venter, cloaca and tail. A single dark blotch in the anal plate. Tail autotomized (Figure 8D).

Population variation and sexual dimorphism: Morphological variation within this population is detailed in Table 1. Moderate degree of sexual dimorphism in size and shape (Table 6). Females larger than males on average, but males with comparatively more robust heads and longer hindlimbs (HFL and FL). No significant differences in trunk length or in tail width. Subtle sexual dimorphism in pholidosis, with the exception of the number of ventral scales, higher in females. Moderate sexual dimorphism in colour pattern. Dorsal body brownish; with few random black spots in males, generally not present in females; central dorsal line generally absent in both sexes, occasionally a discontinuous line of black spots that can become continuous as it gets closer to the tail; not complete in any case. Dark bands along the laterals of the body, reticulated in males and more uniform in females, limited by lighter lines; in males, the upper one generally reduced to a discontinuous line of pale dots, more evident between forelimbs; in females, generally well defined, continuous and brighter. Externally to them, presence of a discontinuous line of black dots in males; in females, spotted line usually blurred or absent, with a faded dark line instead. Ventral greyish, males frequently spotted in the outer lines and venter, occasionally in all the ventral body; females with spots reduced, if existing, to the chest and venter. Males with occasional orange pigmentation in the gular, chest, and cloaca, sometimes extending to the whole ventral body; in females, intense orange pigmentation limited to the ventral tail, cloaca and femoral region, eventually reaching the lower venter.

Remarks: All specimens were found near thorny bushes in a valley on the banks of a dry stream.

Description of *Atlantolacerta carreteroi* sp. nov.

Tables 1, 2, 7 and 8. Figures 3, 4, 5, 6, 7 and 8F

MorphoBank: (to be added under acceptance).

References: Rykena & Bischoff (1992) mentioned three specimens of *A. andreanskyi* from J. Ayache (NMB13468, MNHP1939-155) (ZFMK49736, Joger & Bischoff, 1989) that "could be distinct from *L. andreanskyi* on subspecific or specific level", but without further details.

Etymology: The species name *carreteroi* is dedicated to the Spanish biologist Miguel Carretero.

Distribution: Jebel Ayache Mountain, High Atlas, Morocco, 3043 m altitude (32.54 N, 4.79W).

Specimens examined: 25 live specimens, 13 males and 12 females.

Holotype: Adult male, High Atlas Mountains, Jebel Ayache, sampled in September 2009 by Mafalda Barata, Fátima Jorge, Salvador Carranza and D. James Harris and in April 2010 by Mafalda Barata, Dianna Steiner, Ana Perera, D. James Harris and Daniele Salvi. Museum code (to be added upon acceptance). GenBank accession numbers: JX462102 (12S), JX462175 (ND4), JX461611 (PDC), JX461965 (ACM4), JX485270 (C-MOS), JX461779 (MC1R), JX461433 (RAG1).

Diagnosis: This population, together with the one analysed from *A. Kaliontzopoulouae* sp. nov. has the largest individuals (largest male 51.7 mm, female 52.4 mm). It differentiates from *A. kaliontzopoulouae* sp. nov. by a relatively more rounded head shape and shorter neck (Table 1, Figure 2). It also presents the most pronounced sexual dimorphism. Colour pattern resembling *A. martinezfreiriai* sp. nov., with a central dorsal line generally absent, sometimes discontinuous, but never complete; absent or discontinuous light dorsolateral lines, and ventral body slightly spotted. However, individuals from *A. carreteroi* sp. nov. have more spotted dorsum, and an almost total absence of dorsolateral lines. However, morphological characteristics were highly overlapping with the ones from other species described, and thus no diagnostic morphometric or pholidotic characters could be defined. Molecular diagnosis based on the combination of several distinct nucleotides from a fragment of 12S rRNA and ND4 genes. Specimens from this lineage show a combination of exclusive nucleotides in four positions: 674-C, 677-T, 678-A and 790-C from the 12S rRNA fragment (Table 7) and a combination of eight different nucleotides in ND4 fragment in the positions: 919-C, 924-G, 1038-G, 1203-C, 1368-G, 1374-G, 1378-G and 1417-T (Table 8). The combination of some of these nucleotides resulted on two exclusive aminoacids, a leucine (L) instead of an isoleucine (I) in the forty-fourth position and an adenine (A) instead of a serine (S) or a timine (T) in the 197th position (Table 9).

Holotype description: Adult female with a snout-vent length (SVL) of 34.68 mm, trunk length (TRL) of 26.15 mm, head length (HL) of 12.04 mm, head width (HW) of 5.87 mm, head height (HH) of 3.92 mm, pileus length (PL) of 8.52 mm, tail width (TW) of 3.54 mm, femur length (FL) of 6.46 mm, tibia length (TBL) of 5.14 mm, fourth toe length (4TL) of 9.13 mm, forelimb length (FLL) of 10.83 mm and hind limb length (HLL) of 16.47 mm. Complete collar with eight scales; gularia with 23 scales. Dorsal scales smooth, not keeled, disposed in 40 longitudinal lines counted in the mid body, and 110 transversal rows. Ventral scales arranged in 6 longitudinal lines and 31 transversal rows. Anal plate surrounded by five preanal scales; dorsal scales of the tail slightly keeled with 29 scales around the seventh ring following the cloaca opening. Four preocular scales, first and fourth smaller. Supraciliar and supraocular scales separated by a continuous line of three supraciliar granules; interparietal and occipital in contact; supranasals widely in contact in their upper part and widely contacting the rostral scale. Four supralabial scales before subocular narrowing down; subocular touching eye. A line of five supratemporal scales, the first one larger; a single large tympanic scale; masseteric scale undifferentiated; temporal scales smooth and slightly larger than the rest. Nine femoral pores undeveloped. Twenty lamellae under the fourth toe.

Coloration (live specimen) dorsal brownish; dark bands along the laterals of the body, that extent from the nostril to the hindlimbs, partially reaching the tail; side bands limited by a light line, more evident between the forelimbs and that extents to the tail. These lines are limited in both sides by a highly discontinuous line of black spots. Discontinuous dorsal central black spotted line that becomes more continuous as it gets closer to the tail. Pileus and supralabial scales occasionally pigmented with black dots. Ventral grey, with a few black spots in the inframaxilar scales, chest, lower ventral, preanal scales, and tail. A single dark dot in the anal plate (Figure 8F).

Population description and sexual dimorphism: Morphological variation within this species is detailed in Table 1. Population with accentuated sexual dimorphism in size and shape. Males with larger bodies, and comparatively more robust heads, longer limbs and wider tails; trunk length similar in both sexes. Moderate sexual dimorphism in pholidosis; on average, females with a higher number of ventral scales and males a higher number of gular scales. Moderate sexual dimorphism in coloration. Dorsal brownish with black spots, sometimes quite abundant. Dorsal central line not present, although occasionally black spots might form a discontinuous central line, that becomes more continuous as it gets closer to the tail, extending over it. Two marbled dark lateral bands with lighter lines limiting them absent or reduced to a few discontinuous light spots in males; in females these lines are pale and continuous in the anterior part of the dorsum, tending to disappear as they get closer to the tail. Ventral body greyish, frequently spotted in the cloacal area, chest, outer ventral rows and

venter, although occasionally they extent to all ventral body. Males sometimes exhibit orange pigmentation in the cloaca, gular or chest, occasionally extending to all the ventral body, sometimes reaching the infralabial and supralabial scales. Females also present intense orange pigmentation, but it is limited to the ventral tail, cloaca and femoral regions. Some males with blue blotches in the ventro-lateral area, not present in females.

Remarks: The population was found near thorny bushes in a dry area on the top of a mountain ridge. *Vipera monticola* was observed nearby.

The present taxonomic revision has enormous implications for the conservation status of *Atlantolacerta* as the previous species, already with a small distribution, is now divided into 6 different species with very restricted areas. There is now only one known population for each newly described species. Furthermore, the mountain habitat is difficult to sample and the possibility of other cryptic forms occurring is high. Additionally, this work has implications for the study of other mountain species in the region, especially "cryptic" ones, since cryptic diversity might have been overlook due to a lack of morphological variation. The processes that promoted this kind of speciation, most probably have implications for other species living in similar habitats, and thus further attention to other high altitude Atlas Mountain endemics is warranted.

Figure 8. Representative specimen from each lineage, J. Sirwa (A), Oukaimeden (B), Tizin Tichka (C), Outabati (D), J. Azourki (E) and J. Ayache (F).

NOTE: The present study in not yet published and for that reason, the proposed names for the new species were submitted for revision as all the information in this chapter.

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CHAPTER 4.

PHYLOGENETIC RELATIONSHIPS OF SOME *CHALCIDES* (LAURENTI, 1768) SPECIES

Ana Perera, Sidi Yahia, 2007

ARTICLE 4.

Barata M. Geniez P. Carranza S. and Harris D.J. (in preparation) **Complex estimates of phylogenetic relationships between three species of *Chalcides* skinks from Morocco.**

Complex estimates of phylogenetic relationships between three species of *Chalcides* skinks from Morocco

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Abstract

Chalcides is a widespread genus that comprises 24 species. All the species from this genus present a very similar morphology, being most of them identify only by differences in colour pattern. Although several authors have successively revised the taxonomy, it is still difficult to delimit the species based on morphological characterization. This study pretends to clarify the relationships between three endemic species from Morocco, *C. polylepis*, *C. montanus* and *C. manueli*. The genetic analysis of a nuclear gene, MC1R excluded the previous proposed hypothesis that *C. montanus* was receiving mtDNA from *C. polylepis*. Furthermore, mtDNA and MC1R recovered a complex phylogeny that questions the existence of the three species, as they are presently known. *Chalcides polylepis* samples were divided into two groups, one that are monophyletic and basal to the tree and a second one that group with *C. montanus* and *C. manueli,* in the other hand, *C. montanus* samples are also divided into two clades. This result unveils the uncertainty in the taxonomy of *Chalcides* and the problematic of using only morphology in taxonomy. However the difficulties to find these animals limited the results and an extra effort should be done in order to cover the distribution of the species.

Keywords: North Africa - *Chalcides montanus* - *Chalcides polylepis* - *Chalcides manueli* - mtDNA - nuclear DNA – phylogeography

Introduction

Chalcides, a genus of the family Scincidae, is a group of elongated lizards that often present reduced legs, as an adaptation to their subterranean lifestyles. This genus has around 24 species, many of which (eleven species of which seven are endemics) occur in Morocco and surrounding areas (Carranza *et al.* 2008). However, the genus has a wide distribution including southern Europe and an extension to the east, including the countries Somalia, Kenya, Turkey, Iraq, Arabia, Iran and Pakistan (Carranza *et al.* 2008). Since visual communication is not very important due to its subterranean life, morphological differentiation may be limited, and the apparent similarity due to parallel evolution, probably, promoted by environmental constraints. Perhaps as a result, several species are morphologically similar and difficult to identify (Schleich *et al.* 1996). Thus although the taxonomy has been successively revised by several authors (Boulenger 1887; 1890; 1896; 1898; Boulenger 1920; Lanza 1957; Pasteur 1981; Caputo 1993; Mateo *et al.* 1995; Greenbaum 2005; Greenbaum *et al.* 2006), it is still difficult to delimit the species based on morphological characterization. It is therefore a group in which genetic data should be particularly useful for delimiting species and estimating relationships. In 2008 Carranza *et al*. published a phylogeny of *Chalcides* (including what was regarded at that time as *Sphenops*) based on two mitochondrial genes (12S rRNA and Cytocrome *b*) and showed that the genus *Chalcides* contained four genetically well differentiated groups. Several species, such as *C. ocellatus*, demonstrated considerable interspecific variation and later (Lavin and Papenfuss 2012) have confirmed this. A similar pattern of high diversity within *Chalcides* species was also found in the Canary Islands (e.g. Pestano and Brown 1999). Regarding the *Chalcides* from Morocco, the relationships estimated by Carranza *et al.* (2008) were particularly unexpected. *Chalcides lanzai*, sometimes previously considered a subspecies of *C. montanus*, was confirmed to be unrelated to this species. Furthermore, specimens of *C. montanus*, from a single locality, fell within a diverse clade of *C. polylepis*, rendering this latter species paraphyletic. The later group was then closely related to *C. manueli*, which diverged around 3.2 mya, followed soon after by divergence of major lineages within *C. polylepis*. Carranza *et al.* (2008) hypothesised a possible introgression event, with *C. montanus* receiving mtDNA from *C. polylepis*, but that further data from mtDNA and nuclear markers would be needed to confirm this.

This work is focused in the relations between the three species belonging to the Western clade of *Chalcides*, *C. polylepis* Boulenger 1890 , *C. montanus* Werner 1931 and *C. manueli* Hediger 1935. *Chalcides polylepis* is the most widespread of the three species, found in the High Atlas, Middle Atlas and Tangiers, occurring at an altitude up to 1950 m a.s.l. (Bons and Geniez 1996). It is larger than *C. montanus* and *C. manueli*, but smaller than *C. ocellatus*. The colour can range from yellowish brown to black and the body has parallel longitudinal and

transversal lines formed by white ocelli. The central spots of the ocelli are often round (Schleich *et al.* 1996). Some individuals are considered to be morphologically similar to *C. montanus* (Schleich *et al.* 1996). *Chalcides montanus* is present in the High and Middle Atlas Mountains between altitudes of 1500 and 2830 m a.s.l. (Bons and Geniez 1996). It is a relatively small *Chalcides* with white parallel lines on the neck and light yellow in the venter (Schleich *et al.* 1996). The juveniles are easily recognized due to its orange tail, which sometimes perseveres in adults (Bons and Geniez 1996). Concerning *Chalcides manueli*, Bons and Geniez (1996) reported the distribution of this species at the base of the Western slopes of the High Atlas in the Atlantic coast between Essaouira and Agadir, however, Carranza *et al*. (2008) included samples from Sidi Ifni, slightly to the South of the previously known distribution range. First described by Hediger (1935) as a subspecies of *Chalcides ocellatus*, it was considered a full species based on several morphological differences and pigmentation (Caputo and Mellado 1992). This species is characterized by its uniform brown colouring and absence of ocelli (Schleich *et al.* 1996). It is listed as vulnerable on the IUCN red list because of its small and fragmented distribution, and apparent population reductions (Joger *et al.* 2007).

With this study we aim to unravel the evolutionary processes that are occurring between *C. polylepis* and *C. montanus,* and test the hypothesis of *C. montanus* had received mitochondrial DNA from *C. polylepis* through introgression, as proposed by Carranza *et al.* (2008). We also intend to assess variation across the range of *C. manueli* and to further assess relationships between the species

Material and Methods

In the field, specimens were captured by hand from under rocks. Tail tips where collected and stored in 96% ethanol and, after this, individuals were released in the same place where they were caught. Additional samples used came from the Herpetological Collection of the Ecologie et Biogéographie des Vertébrés team of EPHEUMR 5175, Université de Montpellier, France (Table 1). The identification of *Chalcides* specimens were done in the field and confirmed by Philippe Geniez, in the field or using photographs (Fig. 5).

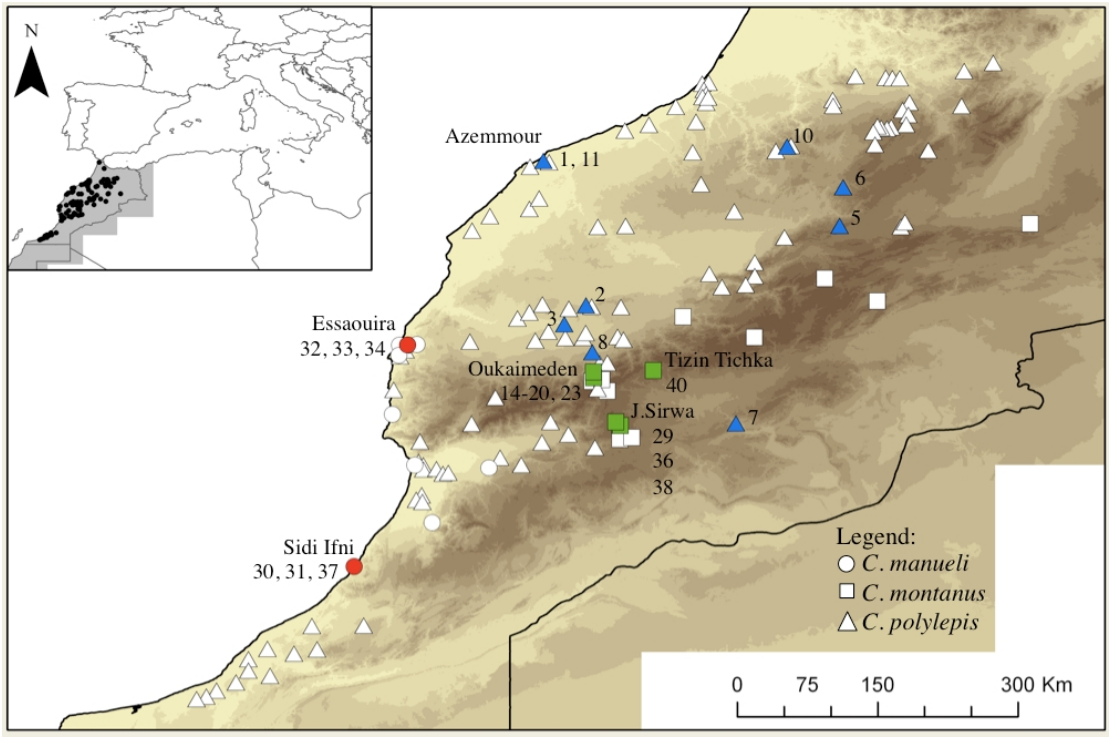

Figure 1. Distribution map of *Chalcides* samples. The white symbols represent the distribution from Bons and Geniez (1996) and the coloured symbols are the locations of the samples used in this study. Triangles are *C. polylepis*, squares are *C. montanus* and circles are *C. manueli*. Codes and colours are the same present in table 1, tree (Figure 2) and network (Figure 3).

From DNA extraction to sequences

Genomic DNA was extracted from tails using standard high-salt protocols. PCR was used to amplify portions of two mitochondrial DNA sequences, 12S rRNA and Cytocrome *b* (cyt*b*) and the partial nuclear protein-coding gene melanocortin receptor 1 (MC1R). The primers used were, for 12S rRNA, 12Sa and 12Sb (Kocher *et al.* 1989), S1F and cyt*b*2 (Carranza *et al.* 2008) for cyt*b*, and MC1RF and MC1RR (Pinho *et al.* 2010) for MC1R. The amplification conditions were the same as those indicated in the relevant references. PCR products were purified using ExoSAP-IT and resulting amplified fragments were sequenced on a 310 Applied Biosystem DNA Sequencing Apparatus.

Phylogenetic analysis

Sequences were aligned for each gene independently using the online version of MAFFT v.6 (Katoh *et al.* 2002) with default parameters (gap opening penalty = 1.53, gap extension = 0.0) and FFT-NS-1 algorithm. Haplotype sequence divergence (*p*-uncorrected distance) was estimated in Mega v.3.0 (Kumar *et al.* 2004) only for 12S and cyt*b* fragments. The most appropriated evolutionary model of sequence evolution was calculated for each gene fragment using jModel test (Posada 2008) under the Akaike information criteria following Posada and Buckley (2004).

Table 1. Samples used in this study. Code, species group, locality and GPS location, GenBank code (to add under acceptance).

Sample code	Species	Locality	Lat	Long	GenBank acession nº 12S / cytb / MC1R
21	*Chalcides montanus*	Oukaimeden	31.201	-7.865	
19	*Chalcides montanus*	Oukaimeden	31.201	-7.865	
23	*Chalcides montanus*	5Km N Oukaimeden dir. Marrakech	31.256	-7.867	
16	*Chalcides montanus*	Oukaimeden	31.201	-7.865	
20	*Chalcides montanus*	Oukaimeden	31.201	-7.865	
18	*Chalcides montanus*	Oukaimeden	31.201	-7.865	
15	*Chalcides montanus*	5Km N Oukaimeden dir. Marrakech	31.256	-7.867	
14	*Chalcides montanus*	5Km N Oukaimeden dir. Marrakech	31.256	-7.867	
17	*Chalcides montanus*	5Km N Oukaimeden dir. Marrakech	31.256	-7.867	
3	*Chalcides polylepis*	10 Km NW Marrakech	31.717	-8.147	
8	*Chalcides polylepis*	18 Km Marrakesh	31.447	-7.877	
1	*Chalcides polylepis*	Azemmour	33.285	-8.348	
2	*Chalcides polylepis*	25 Km N Marrakesh	31.899	-7.939	
11	*Chalcides polylepis*	Azemmour	33.285	-8.348	
4	*Chalcides polylepis*	Medium Atlas	Unknown		
5	*Chalcides polylepis*	5km E Sidi Yahya	32.662	-5.499	
6	*Chalcides polylepis*	5km Wazrou	33.032	-5.464	
7	*Chalcides polylepis*	Sidi Azigza	30.766	-6.496	
9	*Chalcides polylepis*	Medium Atlas	Unknown		
10	*Chalcides polylepis*	Oulmes	33.422	-6.005	
30	*Chalcides manueli*	Sidi Ifni	29.387	-10.168	
31	*Chalcides manueli*	Sidi Ifni	29.387	-10.168	
37	*Chalcides manueli*	Sidi Ifni	29.387	-10.168	
32	*Chalcides manueli*	Essaouira	31.516	-9.654	
33	*Chalcides manueli*	Essaouira	31.516	-9.654	
34	*Chalcides manueli*	Essaouira	31.516	-9.654	
29	*Chalcides montanus*	Jebel Sirwa	30.743	-7.610	
38	*Chalcides montanus*	Jebel Sirwa	30.743	-7.610	
36	*Chalcides montanus*	Jebel Sirwa	30.777	-7.653	
40	*Chalcides montanus*	Tizin Tichka	31.270	-7.292	
outgroup	*Chalcides lanzai*	Azrou	33.493	-5.148	

We performed Maximum Likelihood and Bayesian analysis for mtDNA and nDNA dataset separately. Likelihood (ML) analysis was executed using RaxML (Stamatakis 2006). Bootstrapping (1000 pseudo-replicates) was used to evaluate the stability of nodes of the ML tree (Felsenstein 1985).

Bayesian inference (BI) was performed using MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001) for concatenated data with partitions (mtDNA), using the most appropriate models for each gene fragment. All analysis started with randomly generated trees and ran for 20 million generations, saving one tree in each 1000 generations. Two independent replicates were conducted and inspected for consistency to check for local optima (Huelsenbeck and Bollback 2001). After assurance that the log-likelihood achieved stationarity (as plotted against generations), the first 20% of obtained trees were discarded as a burn-in and a 50% majority rule consensus tree was then produced from the posterior distribution of the trees and posterior probabilities calculated as the percentage of a sampled tree recovering any particular

clade (Huelsenbeck and Ronquist 2001). Nodes that received ML bootstrap support values ≥ 70% and posterior probability (pp) values ≥ 0.95 were considered strongly supported (Wilcox *et al.* 2002; Huelsenbeck and Rannala 2004). Trees were visualized using the FigTree v1.3.1 (Rambaut 2008).

Nuclear Network

SEQPHASE (Flot *et al.* 2010) was used to convert the input files, and the software PHASE v2.1.1 to resolve phased haplotypes (Stephens and Donnelly 2003). Default settings of PHASE were used except for phase probabilities that were set as ≥ 0.7 (Harrigan *et al.* 2008). All polymorphic sites with a probability of < 0.7 were coded in both alleles with the appropriate IUPAC ambiguity code.

The genealogical relationships between the populations were assessed with haplotype networks for the individual nuclear gene, MC1R, constructed using statistical parsimony (Templeton *et al.* 1992) implemented in the program TCS v 1.21 (Clement *et al.* 2000) with a connection limit of 95%. This analysis was made with the phased sequences. Haplotypes were colored taking into account the population of origin.

Results

A fragment of 786 base pairs of concatenated mtDNA, (12S rRNA 388 bp and Cyt*b* 397 bp) was obtained from 33 *Chalcides* specimens: 13 *C. montanus*, 11 *C. polylepis* and 6 *C. manueli* for the ingroup, and 3 *C. lanzai* that were used as outgroups (Table 1).

A fragment of 695 base pairs of the MC1R gene was obtained from 25 *Chalcides*: 9 *C. montanus*, 5 *C. polylepis* and 7 *C. manueli* for the ingroup, and 3 *C. lanzai* that were used as outgroups (Table 1). The models selected were: 12S rRNA – HKI+I+G, cyt*b* – GTR+G and MC1R – HKI+I+G.

Phylogenetic analyses

Both mitochondrial and nuclear phylogenies (ML and BI analysis) support the existence of two main groups, the samples belonging to *C. polylepis* from the East (5, 6, 7 and 10) and a big group that comprises all the other samples. However, the nuclear network supports the existence of smaller groups inside the big group: samples identified as *C. manueli* (Essaouira and Sidi Ifni, samples from Oukaimeden and Marrakesh area (*C. montanus* from Oukaimeden and *C. polylepis* 8) and samples from J. Sirwa, Tizin Tichka and *C. polylepis* 8 (*C. montanus* from J. Sirwa and Tizin Tichka).

Mitochondrial DNA

The mitochondrial phylogeny (Fig. 2), besides the early diverging group formed by the East *C. polylepis* samples, split the big group (containing all the other samples) in two smaller clades also with a good support. One clade includes the samples morphologically classified as *C. manueli* from Sidi ifni and *C. montanus* from J. Sirwa and the other comprises all the other samples: *C. montanus* from Oukaimeden, *C. manueli* from Essaouira and *C. polylepis* from the coast (1 and 11) and from the area north of Oukaimeden (2, 3 and 8).

The divergence between the three mitochondrial groups is: 3.9% and 7.1% between the first and the second groups, 5% and 7.5% between the first and the third and 4% and 7.4% between the second and the third, for 12S and cyt*b* (Fig. 2).

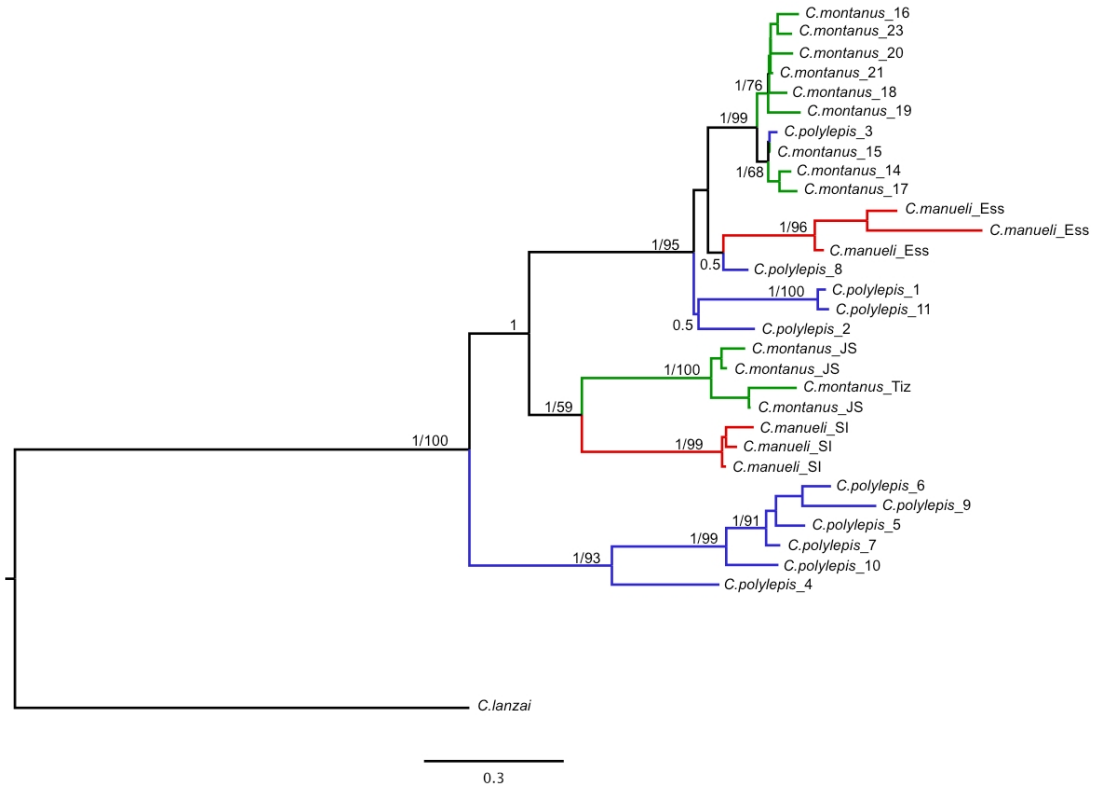

Figure 2. Tree resulting from partitioned Bayesian analysis from mitochondrial DNA (12S rRNA and cyt*b*). The partitions used the models described in the text. Bayesian posterior probabilities (0-1) and bootstrap values (> 50 %) for ML (1-100) are indicated near the branches. The trees were rooted using 3 sequences of *Chalcides lanzai*. The colours of the branches correspond to the previous morphological identified species and are the same used in the map (Fig. 1) and in the network (Fig. 3): blue – *C. polylepis*, green – *C. montanus* and reed – *C. manueli.* The names of the samples represent the species and specimen code (table 1) or locality: Ess – Essaouira, SI – Sidi Ifni, JS – J. Sirwa, Tiz – Tizin Tichka.

Nuclear DNA

The nuclear phylogeny (Fig. 3), besides the early diverging group formed by the East *C. polylepis* samples, supports the existence of two other small groups (Bayesian posterior probabilities and bootstrap values > 50): the samples from *C. manueli* (Essaouira and Sidi Ifni), and the samples from *C. montanus* from Oukaimeden area and *C. polylepis* 8.

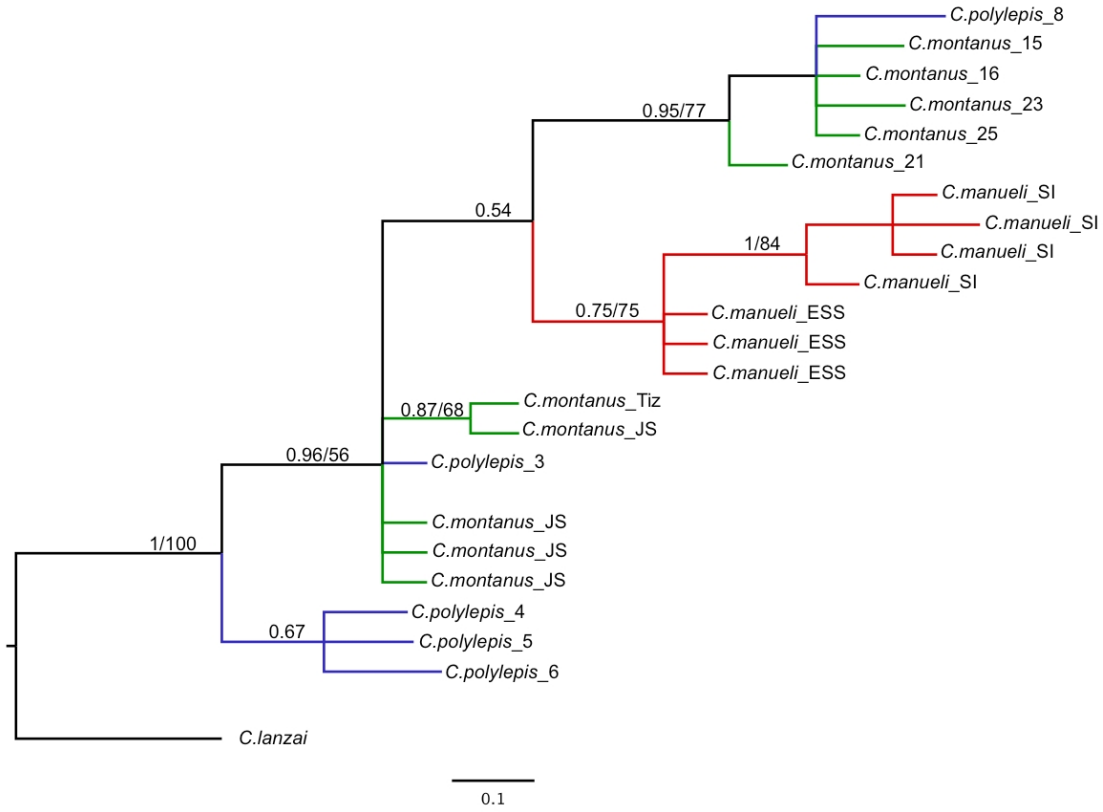

Figure 3. Tree resulting from partitioned Bayesian analysis from nuclear DNA (MC1R). The partitions used the models described in the text. Bayesian posterior probabilities (0-1) and bootstrap values (> 50 %) for ML (1-100) are indicated near the branches. The trees were rooted using 3 sequences of *Chalcides lanzai*. The colours of the branches correspond to the previous morphological identified species and are the same used in the map (Fig. 1), in mtDNA tree (Fig. 2) and in the network (Fig. 4): blue – *C. polylepis*, green – *C. montanus* and reed – *C. manueli.* The names of the samples represent the species and specimen code (table 1) or locality: Ess – Essaouira, SI – Sidi Ifni, JS – J. Sirwa, Tiz – Tizin Tichka.

Nuclear DNA - Network

The nuclear network (Fig. 4) shows a slightly different pattern where it is possible to see some isolation between four small groups. The previous mentioned samples of *C. polylepis* from the East Morocco, the samples from Essaouira and Sidi Ifni (morphologically identified as *C. manueli*), the samples from Oukaimeden and Marrakesh area (*C. montanus* and *C. polylepis* 8) and the samples from J. Sirwa and Tizin Tichka (*C. montanus*) that are grouped with the sample 3 (*C. polylepis*).

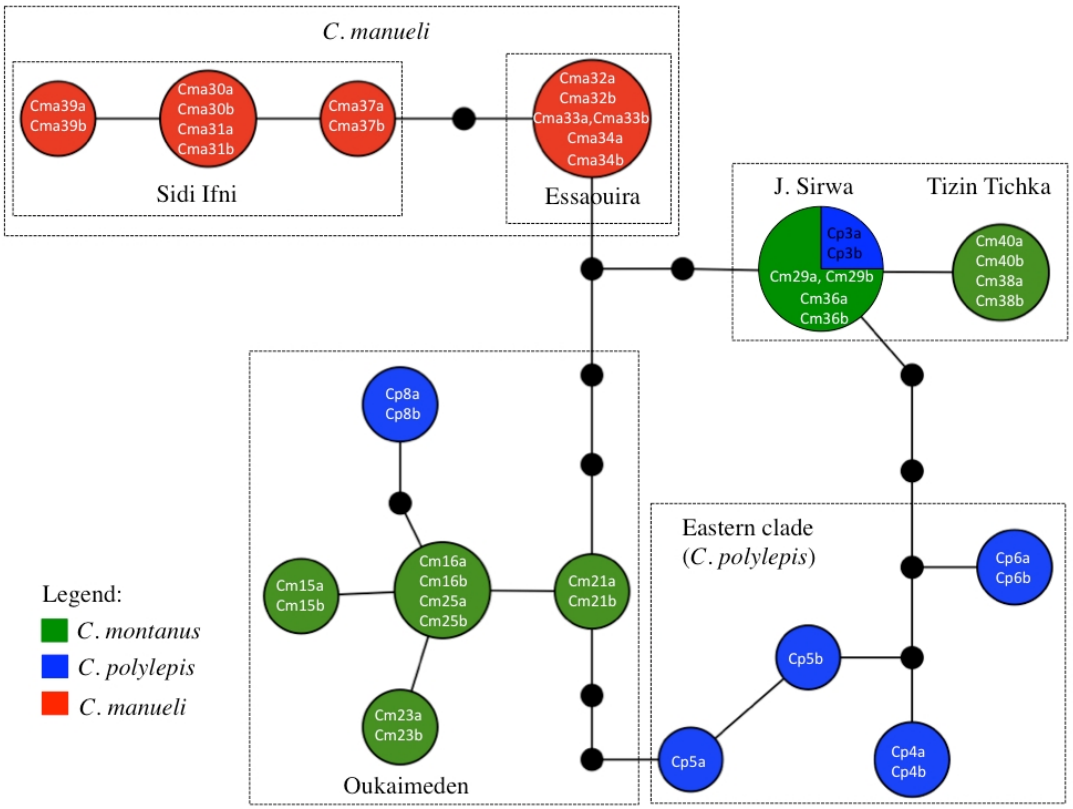

Figure 4. Parsimony network corresponding to MC1R gene fragment. The colours used were the same as used in the map (Fig. 1) and in the trees (Fig. 2 and 3), *C. montanus* (green), *C. polylepis* (blue) and *C. manueli* (red). Lines represent a mutation step; circles represent haplotypes and dots missing haplotypes. Size of the circles is proportional to the number of alleles. Dotted squares identify the groups; an exception is sample Cp3 (black) that is from Oukaimeden area (Marrakesh) and not from J. Sirwa.

Discussion and Conclusions

Both, mtDNA and nDNA support the existence of two main groups: an early diverging group that include the samples of *C. polylepis* from Eastern Morocco and other big group that include all the remaining samples.

Besides the early diverging group of *C. polylepis* from Eastern Morocco (that includes samples from the area of the type locality, Fes) mtDNA presents two other well supported clades that are geographically isolated by the High Atlas and Anti Atlas Mountains. A clade that include samples from localities in the south of the Anti Atlas: Sidi Ifni (*C. manueli*) and J. Sirwa (*C. montanus*) and a third clade that include the samples from localities in the north of High Atlas: Oukaimeden and Marrakesh area (*C. montanus* and *C. polylepis* 2, 3 and 8), Azemmour (*C. polylepis* 1 and 11) and Essaouira (*C. manueli* type locality). The nuclear marker MC1R does not provide further support, for the same clades, besides the first split between *C. polylepis* from Eastern Morocco and all other samples. Nevertheless, and contrasting with mtDNA results, it groups all the samples identified as *C. manueli* (Essaouira and Sidi Ifni), and groups the samples 8 of *C. polylepis* from Marrakesh area with samples

from J. Sirwa (*C. montanus*) instead of with the samples from Oukaimeden (*C. montanus*).

The present results show a complex evolutionary history including the three species of *Chalcides* studied. The patterns observed in the present, probably reflect an intricate process of migrations and isolation and local/ecological adaptations promoted by climatic oscillations and the presence of the Atlas Mountains acting both as diversification promoter and as a geographic barrier (as proposed for other species; Brown *et al.* 2002; Fritz *et al.* 2005).

The nuclear results of our study exclude the possibility of *C. montanus* receiving mitochondrial DNA from *C. polylepis* through introgression, as Carranza *et al.* (2008) proposed, since at least two nuclear sequences from *C. polylepis* are grouped with *C. montanus* (3 and 8; the same samples used by Carranza *et al.* 2008).

Other possible explanation for the phenotype exhibited by samples 3 and 8 is that the samples of *C. polylepis* from Marrakesh area (and from the coast) be a lowland form of *C. montanus* as proposed by Carranza *et al* (2008), however more samples are needed to confirm this hypothesis.

The genus *Chalcides*, remains unresolved and the doubts raised from the genetic analysis of a few samples from the three species probably reflects what is happening in most of the other *Chalcides* species. One cause of the confusion in *Chalcides* taxonomy should be promoted by the morphological similarities between them, but the genetic analysis showed that the solution would be more complex than that. As in our work, Brown *et al.* (2012) shows that the morphological characters used to delimit species in the genus *Chalcides* are not appropriate. The digit number, used to delimit several species of *Chalcides*, was showed to be of no use in *C. mionecton*, as all the other external morphological characteristics (Brown *et al.* 2012).

This work shows the importance and need of new studies within *Chalcides* genus, since there are several fundamental questions open. Despite *Chalcides* species are not easy to sample, further sampling and the analysis of different nuclear markers will be especially useful to understand the relationships between the species complex recovered: *C. montanus*, *C. manueli* and *C. polylepis*.

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CHAPTER 5.

NEW OBSERVATIONS OF AMPHIBIANS AND REPTILES IN MOROCCO

Mafalda Barata, Jebel Awlime, 2011

ARTICLE 5.

Barata M. Perera A. Harris D.J. Van Der Meijden A. Carranza S. Ceacero F. García-Muñoz E. Gonçalves D. Henriques S. Jorge F. Marshall J.C. Pedrajas L. and Sousa P. 2011. **New observations of amphibians and reptiles in Morocco, with a special emphasis on the Eastern Region**. *Herpetological Bulletin*, 116: 4-14.

New observations of amphibians and reptiles in Morocco, with a special emphasis on the Eastern Region

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Abstract

This study reports the observations of 54 species of amphibians and reptiles obtained during four field surveys to Morocco, including the southern and south-eastern regions. Our records reveal a notable expansion of the current distribution range for several species especially in the eastern part of the country, highlighting the need for more intensive sampling within this region.

Morocco is one of the most biodiverse regions in North Africa (Bons and Geniez 1996). It covers a total area of more than 450,000 km^2 (Schlüter 2006) and has a Mediterranean and sub-saharan climate with mean annual precipitation ranging from 300 to 600 mm (Michard *et al.* 2008). Morocco shares similar topographic characteristics with Algeria and Tunisia and together they constitute the western Maghreb. However, Morocco differs by its greater geological complexity and higher elevation (Michard *et al.* 2008), with several mountain systems reaching more than 3000 m a.s.l., including the highest peak in north Africa (Jebel Toubkal, 4167 m a.s.l.). Moreover, its proximity to Europe (actually separated by only 14 km) and its contact during the Messinian stage of the late Miocene (5-6 Mya, Hsu *et al.* 1973) is fundamental in explaining the richness of amphibians and reptiles of both African and European origins and its high number of endemisms (Bons and Geniez 1996). In 2006, 12 species of amphibians and 95 species of non-marine reptiles were recognized (Cox *et al.* 2006). Although this country is one of the best sampled areas of the western Maghreb (Bons and Geniez 1996; Real *et al.* 1997; Fahd and Pleguezuelos 2001; Brito 2003; Crochet *et al.* 2004; Guzman *et al.* 2007; Harris *et al.* 2008; Pleguezuelos *et al.* 2008; García-Muñoz *et al.* 2009; Ceacero *et al.* 2010; Harris *et al.* 2010), there are still some regions in south and south-eastern Morocco that have been poorly surveyed (Bons and Geniez 1996).

This study compiles the records from three surveys performed in May 2008 and May and July 2009 to the central and western Morocco, and one survey in September 2009 to the southern (Souss-Massa-Drâ and Guelmim-Es Mara) and south-eastern (oriental and Méknes-Tafilalet) provinces. In total, 342 records of 54 species of amphibians and reptiles from 97 localities were reported (Fig. 1). All specimens found were located with GPS and were identified using morphological characteristics and using the most updated taxonomy. Detailed information on the species per locality is given in Table 1, and those species whose records are particularly interesting or that have been object of recent taxonomical changes are discussed.

AMPHIBIA

ORDER ANURA

Family Bufonidae

Bufo mauritanicus Schlegel 1841 was reported in 17 localities (17, 26, 31, 33, 37, 39, 43, 46, 47, 49, 50, 51, 58, 65, 74, 86 and 97, Table 1). Although considered incertae sedis by Frost et al. (2006), it clearly belongs to the *Amietophrynus* clade (Harris and Perera 2009). This species, one of the most abundant in Morocco (Bons and Geniez 1996), was reported in 17 localities mostly associated to wet central regions. However, the finding of individuals further South, in Foum Zguid (locality 26), Bouanane (locality 58) and Ich (locality 74), confirm the

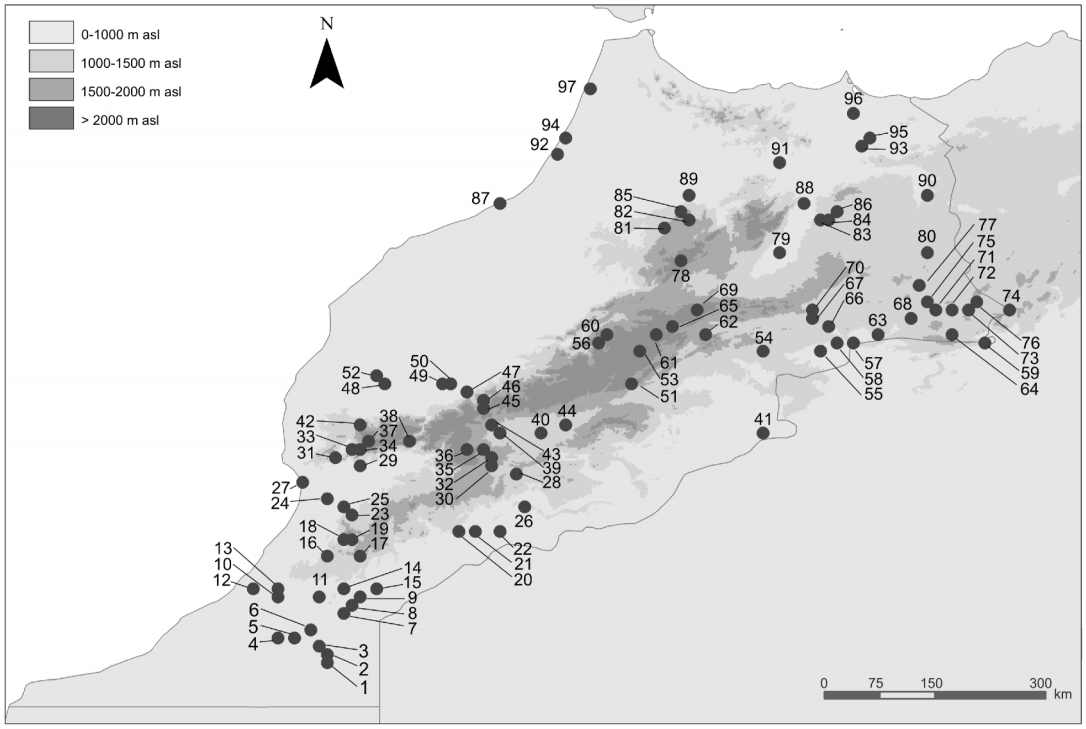

Figure 1. Map of Morocco with the distribution of the sampling localities presented in this study.

existence of isolated populations in the fringes of the Sahara (Bons and Geniez 1996; Schleich *et al.* 1996; Brito 2003; Guzman *et al.* 2007). Despite its wide distribution, *B. mauritanicus* exhibits low levels of genetic variation, indicating a recent post-glacial expansion into this region (Harris and Perera 2009).

Pseudepidalea viridis (Laurenti 1768). Localities 24, 25, 29, 56, 59 and 64 (Table 1). Historically included as a member of the genus *Bufo* prior to Frost *et al.* (2006), and considered by some authors as *P. boulengeri* (Frost *et al.* 2006 but see Speybroeck *et al.* 2010), this species is abundant and widespread (Bons and Geniez 1996), being able to penetrate more than other toads into desert areas.

Family Ranidae

Pelophylax saharicus (Boulenger 1913). Localities 4, 17, 19, 26, 31, 51, 65 and 66 (Table 1). Previously considered *Rana saharica,* but recently reassigned to the genus *Pelophylax* (Frost *et al.* 2006; Speybroeck *et al.* 2010), it displays enormous morphological variation (Bons and Geniez 1996; Schleich *et al.* 1996) but minimal mtDNA sequence variation within Morocco (Harris *et al.* 2003).

REPTILIA

ORDER TESTUDINES

Family Geoemydidae

Mauremys leprosa (Schweigger 1812). Localities 52 and 95 (Table 1). The study published by Fritz *et al.* (2006) propose a reduction in the number of existing subspecies to two, *M. l. leprosa* (Schweigger 1812) and *M. l. saharica* Schleich 1996 distributed across North and South of Morocco respectively and separated by the Atlas mountains.

ORDER SQUAMATA

Family Agamidae

Trapelus mutabilis Merrem 1820. Localities 1, 3, 5, 6, 7, 9, 14, 20 and 73. All localities reported belong to the southern province of Guelmim-Es-Mara with the exception of a single individual found in the Oriental province (locality 73).

Uromastyx acanthinura Bell 1825. Localities 2, 3, 4, 6, 7, 8, 9, 10, 11, 15, 21, 28, 57, 75 and 76. This species, endemic to North Africa was reported in two new localities in the Oriental province, expanding northwards the distribution of the species in the area (localities 57, 75 and 76).

Family Chamaeleonidae

Chamaeleo chamaeleon (L. 1758). Localities: 33, 42 and 66. Individuals found in Ksar Morhel (locality 67) indicate, for the first time, the presence of this species in the southern area of the Oriental province (Bons and Geniez 1996). In total, three individuals, a male and two females (one of them gravid) were found. With the finding of an eastern Mediterranean haplotype in Tunisia and other distinct haplotypes in western Morocco, Dimaki *et al*. (2008) suggest the existence of a phylogeographic break in north-western Africa.

Family Phyllodactylidae

Tarentola mauritanica (L. 1758). Localities 34 and 52. Recent molecular studies show the complexity of this group, with multiple highly divergent genetic lineages across Morocco (Harris *et al.* 2004; Rato *et al.* 2010) that do not match current subspecific taxonomy.

Tarentola deserti Boulenger 1891. Localities 57, 58, 59, 71 and 76 (Table 1 and Fig. 2A). Fieldwork in the Oriental province resulted in new locations (localities 57, 58, 71 and 76) linking the two known distribution areas for this species in Morocco: the triangle Tinerhir-Boudenib-Taouz, that holds the bulk of the distribution (Bons and Geniez 1996) and the

isolated localities in Figuig (locality 59 and province Bons and Geniez 1996). All specimens were confirmed genetically (Perera, pers. comm.).

Ptyodactylus oudrii Lataste 1880. Localities 30, 57 and 65. A recent study concerning the genetic variation of the fan-footed gecko in Morocco reported very high divergence levels among the populations from eastern Atlas, western Atlas and Anti Atlas, suggestive of cryptic species (Perera and Harris 2010). New records in Beni Yatti (locality 57) expand its distribution more than 50 km eastwards.

Family Sphaerodactylidae

Quedenfeldtia trachyblepharus (Boettger 1874). Locality 35. This Moroccan endemism can be found at altitudes up to 4000 m (Bons and Geniez 1996). Individuals from Jebel Sirwa region, considered as "indeterminated" by Bons and Geniez (1996) were confirmed as *Q. trachyblepharus* (Locality 35).

Quedenfeldtia moerens (Chabanaud 1916). Localities 12, 34, 37, 38, 53 and 61. This endemism, not so restricted to high altitudes as *Q. trachyblepharus* (10-2700 m altitude), is widely distributed across the High Atlas, Anti Atlas, Jebel Ouarkik and near the Middle Atlas, reaching coastal habitats (Bons and Geniez 1996). "Indeterminate" individuals from Agoudal (Bons and Geniez 1996) were identified as *Q. moerens* (locality 53).

Stenodactylus sthenodactylus (Liechtenstein 1823). Locality 64 (Table 1 and Fig. 2B). The finding of two individuals in Jboub Zoulai, more than 150 km from other known localities in Morocco (Bons and Geniez 1996) suggest a possible relationship with the closer Algerian populations (Sindaco and Jeremcenko 2008).

Saurodactylus mauritanicus (Duméril and Bibron 1836). Localities 91 and 96 (Table 1). This small gecko is distributed across northeast Morocco and North of Algeria (Sindaco and Jeremcenko 2008). The finding of an individual near Irhoudane (Locality 91) expands 70 km southwest the current known distribution for *S. mauritanicus* in Morocco.

Saurodactylus fasciatus Werner 1931. Locality 89 (Table 1). This endemism, associated to stony areas in North and West of the Atlas system and southwest of the Rif, has a distribution limited to less than 40 localities across its range (Bons and Geniez 1996; Harris *et al.* 2008; Harris *et al.* 2010). This new observation expands South the distribution of the eastern populations by 20 km.

Family Lacertidae

Scelarcis perspicillata (Duméril and Bibron 1839). Localities 34 and 78. This climbing lizard extends across the Middle and High Atlas regions, mostly associated to water sources and abundance of cliffs or rocks. Although there are three described subspecies (*S. p. perspicillata* (Duméril and Bibron 1839), *S. p. chabanaudi* (Werner 1931) and *S. p. pellegrini* (Werner 1929)) recognisable by their colour pattern, molecular studies do not show direct congruence between external pattern and genetic lineages (Harris *et al.* 2003; Perera *et al.* 2007). The finding of individuals identified morphologically as *S. p. pellegrini* in Tasguint (locality 34) expands its current known distribution 40 km westwards in the High Atlas. The species was found coexisting with *Q. moerens* and *T. mauritanica*.

Acanthodactylus erythrurus lineomaculatus (Duméril and Bibron 1839). Localities 94 and 97. Recent molecular analyses do not support the specific differentiation of *A. e. lineomaculatus* (Duméril and Bibron 1839) and *A. e. belli* Gray 1845, indicating that both morphotypes are probably ecotypical adaptations to different habitats (Fonseca *et al.* 2009).

Acanthodactylus boskianus (Daudin 1802). Localities: 1, 2, 64, 66, 70 and 73. This survey to the oriental province recorded two new localities, in Jboub Zoulai (locality 64) and Bouarfa (locality 73).

Acanthodactylus pardalis complex: Localities 24 and 71. Two new localities for this group were found, one locality with several individuals identified as *A. busacki* Salvador 1982 in Imi Mqoum (locality 24) and another in Bouarfa (locality 71) where individuals were identified as *A. pardalis* Lichtenstein 1823, although this appears genetically to be a species complex (Fonseca *et al.* 2008).

Family Scincidae

Chalcides ocellatus (Forskål 1775). Localities 4, 72, 74, 77, 80, 88 and 90 (Table 1 and Fig. 2C). Individuals from the South were identified as *C. o. ocellatus* (Forskål 1775) (locality 4), although specimens observed in the Oriental Province (localities 72, 74, 77 and 80) could not be identified as belonging to the subspecies *C. tiligugu* (Gmelin 1789) or *C. o. subtypicus* Werner 1931. Recent studies show high genetic divergences between the southern and northern subspecies (Kornilios *et al.* 2010), although more studies are needed to confirm this differentiation.

Chalcides manueli Werner 1931. Locality 35. The range of this endemic skink, known only from 8 different localities (Bons and Geniez 1996), four of them near Essaouira, was considerably extended to the east with its recent finding in Jebel Sirwa (Harris *et al.* 2010). Locality 35 confirms the existence of the species in the area. The specimens found were first identified as *C. montanus* (also reported for this area*)* because of the stripped coloration very different from the homogeneous pattern typical for *C. manueli* (Bons and Geniez 1996). However, despite the morphology patterns observed, individuals were confirmed genetically as *C. manueli* using DNA sequencing (Barata, pers. comm.).

Family Trogonophidae

Trogonophis wiegmanni Kaup 1830. Localities 37, 74, 82 and 93 (Table 1 and Fig. 2D). This endemism to the Maghreb is distributed across the humid, semihumid, arid and semiarid climates (Bons and Geniez 1996) previously suggested to not exceed 1900 m altitude (Bons and Geniez 1996). Two subspecies are recognized, *T. w. wiegmanni* (Kaup 1830) in the western, and *T. w. elegans* (Gervais 1835) in the eastern region, morphologically distinguishable and genetically distinct (Mendoça and Harris 2007). The finding of an adult in Jebel Awlime (locality 37), at 2084 m altitude, represents a new high altitude record for this species. On the other side, the finding of an individual in Ich oasis (locality 74) indicates for the first time the presence of this species in the south of the oriental province geographically well separated from other Moroccan populations, but close to western Algerian ones (Sindaco and Jeremcenko 2008).

Family Leptotyphlopidae

Leptotyphlops macrorhynchus (Jan 1861). Locality 57 (Fig. 3E). With only 11 localities reported for this species in Morocco, this is one of the rarest snakes in the country. An individual was found in Beni Yatti, 65 km northeast of the previous known distribution range (Bons and Geniez 1996).

Family Colubridae

Scutophis moilensis (Reuss 1834). Localities 6, 43, 54, 55, 63 and 68 (Table 1 and Fig. 3F). New localities extend the range across the South of the Oriental province, being found between Boudenib and Figuig where it was previously thought to be absent (Bons and Geniez 1996).

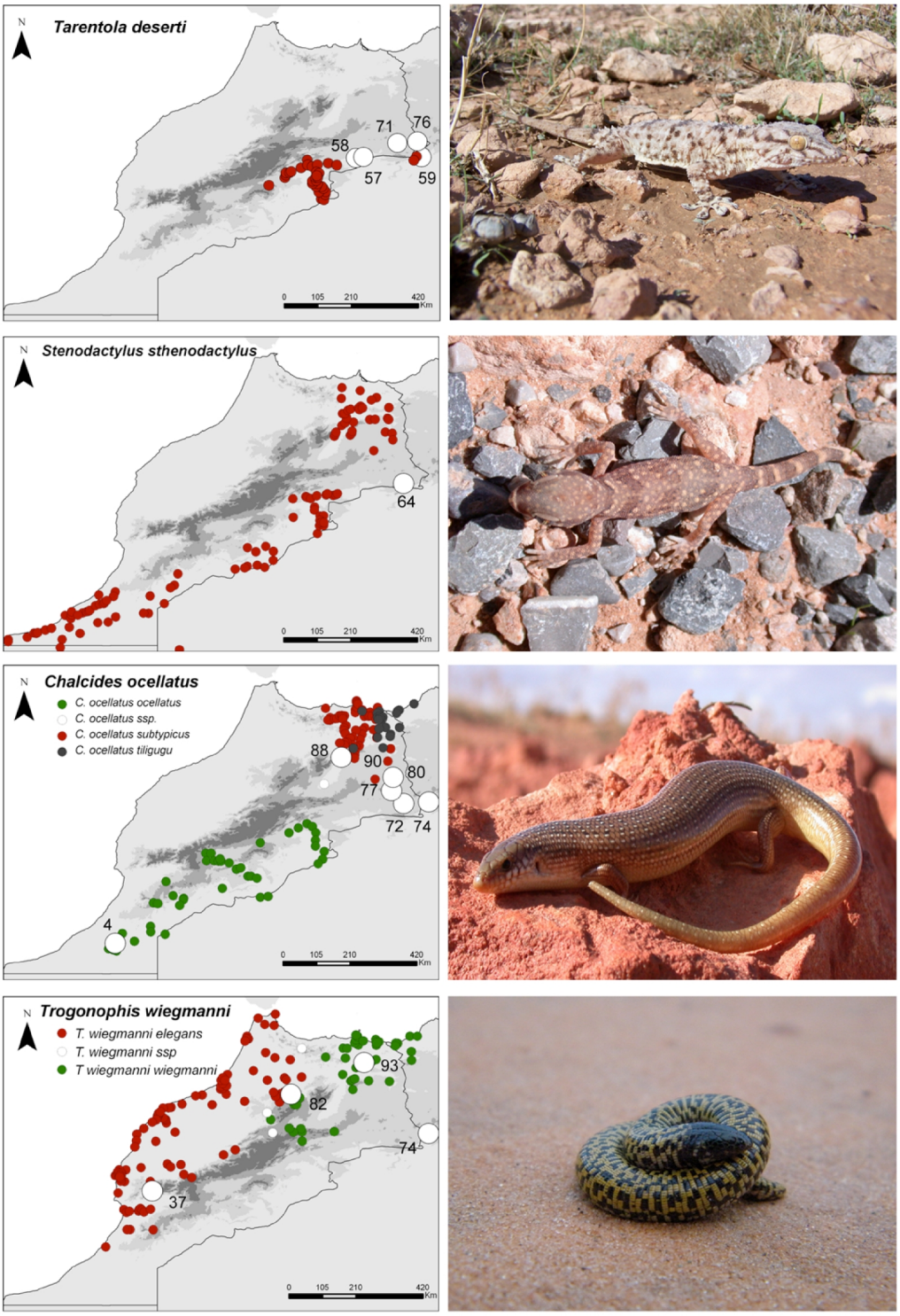

Figure 2. Distribution map and photographs of A) *Tarentola deserti*, B) *Stenodactylus sthenodactylus,* C) *Chalcides ocellatus*, D) *Trogonophis wiegmanni*. Colour dots represent published observations (Bons and Geniez 1996; Guzman *et al.* 2007; Harris *et al.* 2008; Harris *et al.* 2010) and white dots show new localities included in this study.

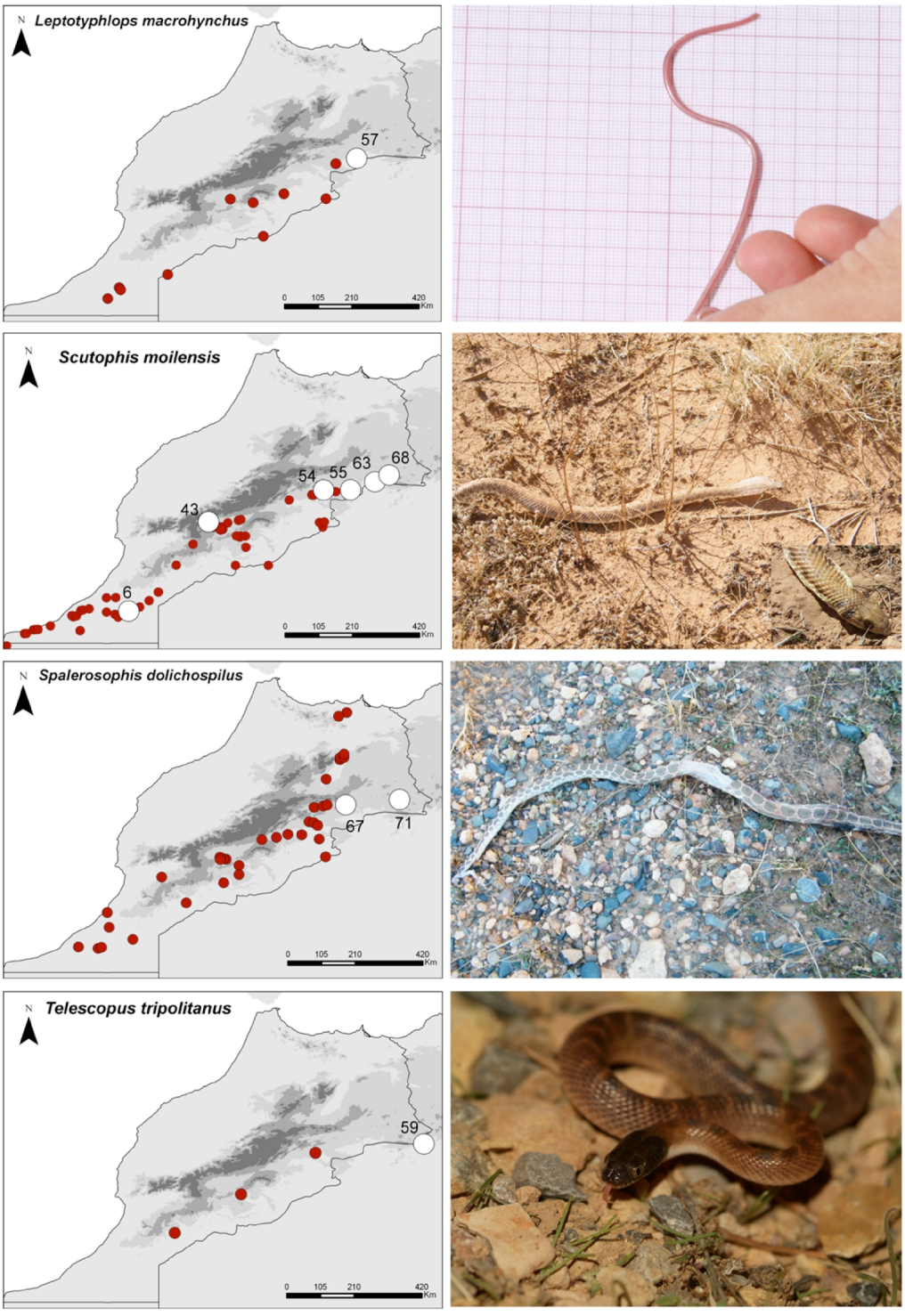

Figure 3. Distribution map and photographs of E) *Leptotyphlops macrohynchus*, F). *Scutophis moilensis*, G) *Spalerosophis dolichospilus,* H) *Telescopus tripolinanus*. Colour dots represent published observations (Bons and Geniez 1996; Guzman *et al.* 2007; Harris *et al.* 2008; Harris *et al.* 2010) and white dots show new localities included in this study.

Psammophis schokari (Forskål 1775). Localities 2, 6, 19, 36, 55, 58, 79, 83 and 84. Although various colour patterns exist (Bons and Geniez 1996) these do not show corresponding mtDNA genetic differentiation within Morocco (Rato *et al.* 2007).

Spalerosophis dolichospilus (Werner 1923). Localities 67 and 71 (Fig. 3G). This snake is restricted to the pre-Saharan regions of Morocco, Algeria and Tunisia (Pasteur 1967; Bons and Geniez 1996). Two new records in Ait Yakoub (locality 67) and Bouarfa (locality 71) represent the first two observations of this species on the oriental province and expand its known distribution considerably in Morocco.

Telescopus tripolitanus (Werner 1909). Locality 59 (Fig. 3H). Previously named *Telescopus dhara* (Crochet *et al.* 2008) it was discovered for the first time in Morocco only in 1989 (Böhme *et al.* 1989). It remains one of the least reported snakes in Morocco, with only 5 known localities restricted to the Moroccan pre-Sahara (Bons and Geniez 1996). One individual, with black head and light brownish/orange colour and darker bands across its body (Fig. 2) was found in Figuig during a crepuscular survey.

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Appendix 1. Localities sampled in this study. For each locality, GPS coordinates (WGS84 decimal degrees) and list of the species found is given.

Local nº	Locality	Latitude	Longitude	Species found
1	20 km north Zag	28.21	-9.30	*A. boskianus, T. mutabilis*
2	5 km south Tistguezzemtz	28.29	-9.34	*A. boskianus, C. cerastes, P. schokari,U. acanthinura*
3	Tistguezzemtz	28.41	-9.41	*A. impalearis, C. cerastes, T. mutabilis, U. acanthinura*
4	Aouinet Torkoz	28.53	-9.86	*C. ocellatus, H. algirus, M. guttulata, P. saharicus, S. boulengeri, U. acanthinura*
5	Between Aouinet Torkoz and Tadachacht	28.49	-9.65	*T. mutabilis*
6	Near Assa	28.57	-9.50	*A. impalearis, C. cerastes, S. moilensis, P. schokari, T. mutabilis, U. acanthinura*
7	Near Tanezida	28.77	-9.11	*T. mutabilis, U. acanthinura*
8	Between Tanezida and Foum el Hassane	28.89	-8.99	*U. acanthinura*
9	Foum el Hassane	28.99	-8.91	*T. mutabilis, U. acanthinura*
10	5 km north Taourirt Doubiane	28.98	-9.90	*U. acanthinura*
11	Near Taghjicht / Bouizakarne	29.05	-9.35	*U. acanthinura*
12	Gorges near Guelmin	29.07	-10.25	*E. algeriensis, Q. moerens*
13	N1 Ouaoutelt	29.09	-9.89	*S. brosseti*
14	Bouizakarne	29.11	-9.14	*T. mutabilis*
15	Between Tizgui and Icht	29.07	-8.70	*U. acanthinura*
16	Kerdous	29.55	-9.33	*A. impalearis*
17	10 km north Aguerd Imelal	29.54	-8.87	*B. mauritanicus, P. saharicus*
18	Near Aimou road	29.65	-9.06	*A. impalearis*
19	3 km north Ayerd	29.67	-8.96	*A. impalearis, P. schokari, P. saharicus, S. brosseti*
20	2 km west Akka Iguirene	29.76	-7.73	*T. mutabilis*
21	Kasba El Joua	29.85	-7.47	*A. impalearis, U. acanthinura*
22	N12 Mrimina	29.81	-7.20	*N. maura*
23	4 km north Ifrhel	29.96	-9.01	*A. impalearis, S. brosseti*
24	4 km north Imi Mqoum	30.18	-9.28	*A. busacki, P. viridis, S. brosseti, S. sphenopsiformis*
25	Ait Baha Barragem	30.06	-9.12	*A. impalearis, P. viridis*
26	Foum Zguid	30.09	-6.88	*B. mauritanicus, P. saharicus, T. boehmei*
27	Agadir	30.42	-9.61	*A. aureus, S. sphenopsiformis*
28	North Foum Zguid	30.49	-7.00	*U. acanthinura*
29	Taurodant-Tasguint	30.63	-8.91	*P. viridis*
30	Tazenakht	30.63	-7.27	*P. oudrii*
31	10 km South Argana	30.74	-9.18	*B. mauritanicus, P. saharicus*
32	N10 to Anezal	30.71	-7.29	*M. mauritanica*
33	Near Argana	30.84	-8.99	*B. mauritanicus, C. chamaeleon*
34	Tasguint	30.78	-8.86	*Q. moerens, S. perspicillata, T. mauritanica*
35	Road to Jbel Siroua	30.79	-7.59	*H. meridionalis, A. erythrurus, C. manueli, A. andreanskyi, T. tangitanus, P. vaucheri, Q. trachyblepl*
36	West Anezal	30.78	-7.37	*P. schokari*
37	Jbel Aoulime	30.89	-8.81	*A. impalearis, B. mauritanicus, Q. moerens, T. wiegmanni*
38	Tasguint	30.91	-8.31	*Q. moerens*
39	Near Agadir	30.97	-7.22	*B. mauritanicus, C. cerastes, M. mauritanica*
40	N10 North Ouarzazate	30.98	-6.74	*M. guttulata*
41	Jboub Zoulal	31.01	-4.00	*M. rubropunctata*
42	Talaint N8	31.10	-8.94	*C. chamaeleon, S. brosseti*
43	Afela n'lsly	31.07	-7.26	*B. mauritanicus, S. moilensis*
44	Skoura N10	31.10	-6.43	*M. cucullatus*
45	Taddert	31.30	-7.41	*A. andreanskyi, P. vaucheri*
46	N9 to Ait Mannsour	31.39	-7.40	*B. mauritanicus*
47	Tasrhimout	31.55	-7.60	*B. mauritanicus*
48	Mzouda N8	31.58	-8.55	*A. erythrurus, C. mionecton, S. brosseti*
49	N9 south Marrakech	31.59	-7.92	*B. mauritanicus*
50	Oulad el Guern	31.58	-7.82	*B. mauritanicus*
51	Gorges du Todra	31.59	-5.59	*B. mauritanicus, P. saharicus*
52	Near Sidi-Chikér	31.75	-8.74	*C. polylepis, M. leprosa, S. brosseti, T. mauritanica*
53	Agoudal	31.97	-5.49	*A. erythrurus, T. tangitanus, P. vaucheri, Q. moerens*
54	Near Tazzouguert	31.97	-4.02	*S. moilensis*
55	Belibilia	31.98	-3.27	*S. moilensis, P. schokari*
56	Road to Imilchil	32.10	-5.95	*P. viridis*
57	Beni Yatti	32.09	-3.10	*L. macrorhynchus, P. oudrii, T. deserti, U. acanthinura*
58	Near Bouanane	32.11	-2.88	*B. mauritanicus, P. schokari, T. deserti, T. tripolitanus*
59	Figuig	32.11	-1.25	*P. viridis, T. deserti, T. tripolitanus (T. dhara)*
60	Jebel Morrik	32.18	-5.88	*P. vaucheri*
61	Road from Imilchil to Rich	32.17	-5.34	*P. vaucheri, Q. moerens*
62	Rich	32.22	-4.68	*H. hippocrepis*
63	Near Ain Chair	32.20	-2.59	*S. moilensis*
64	Jboub Zoulai	32.24	-1.72	*A. boskianus, P. viridis, S. sthenodactylus*
65	Jbel Aderdouz	32.26	-5.15	*B. mauritanicus, H. hippocrepis, N. maura, P. algirus, P. oudrii, P. saharicus*
66	Ksar Morhel	32.25	-3.18	*A. boskianus, C. chamaeleon, P. saharicus*
67	Ait Yakoub	32.36	-3.44	*Spalerosophis dolichospilus*
68	N10 to Mengoub	32.39	-2.19	*S. moilensis*
69	Cirque de Jafar	32.54	-4.79	*A. andreanskyi*
70	Talsint	32.49	-3.41	*A. boskianus, A. impalearis, N. maura*
71	N17 to Bouarfa	32.51	-1.93	*A. pardalis, S. dolichospilus, T. deserti*
72	N17 to Jboub Zoulai	32.48	-1.72	*C. ocellatus*
73	N17 to Bouarfa	32.51	-1.50	*A. boskianus, T. mutabilis*
74	Ich	32.52	-1.01	*A. impalearis, B. mauritanicus, C. ocellatus, T. wiegmanni*
75	Bouarfa	32.57	-2.02	*A. impalearis, U. acanthinura*
76	N18 to El Mlalih	32.56	-1.37	*C. cerastes, T. deserti, U. acanthinura*

77	Bouarfa	32.84	-2.07	*C. ocellatus*
78	Lake Aguelmame Sidi Ali	33.07	-5.01	*T. tangitanus, N. maura, P. vaucheri, S. perspicillata*
79	Teggour	33.24	-3.83	*P. schokari*
80	N17 to Tendara	33.21	-2.02	*C. ocellatus*
81	R707 to Ifrane	33.54	-5.16	*C. lanzai*
82	Imouzzer Kandar	33.63	-4.90	*A. erythrurus, T. tangitanus, P. vaucheri, P. algirus, T. wiegmanni*
83	Bouloutane	33.58	-3.33	*A. impalearis, P. schokari*
84	Bouloutane	33.57	-3.21	*P. schokari*
85	Imouzzer Kandar	33.66	-5.04	*T. tangitanus, P. vaucheri*
86	El Hamar	33.71	-3.05	*B. mauritanicus*
87	Between Casablanca and Rabat	33.78	-7.23	*E. algeriensis, N. maura*
88	N15 to Zerzaia	33.78	-3.48	*C. ocellatus, E. algeriensis*
89	Sefrou	33.85	-4.86	*S. fasciatus, T. graeca*
90	Near Ain Benimathar	33.89	-2.02	*C. ocellatus*
91	N6 to Irhoudane	34.25	-3.85	*S. mauritanicus*
92	A1 to Akbate	34.43	-6.52	*H. hippocrepis*
93	N6 to Moulay Bagdad	34.52	-2.84	*T. wiegmanni*
94	El Behara	34.65	-6.41	*A. e. lineomaculatus*
95	N7 to Moulay Bagdad	34.57	-2.73	*M. leprosa*
96	N19 to Oulad Bouihia	34.94	-2.88	*S. mauritanicus*
97	Larache	35.17	-6.12	*A. e. lineomaculatus, B. tingitanus, B. mauritanicus, M. cucullatus, T . graeca*

CHAPTER 6.

GENERAL DISCUSSION

Mafalda Barata, Morocco, 2009

"One thing only I know, and that is that I know nothing."
Socrates

6. General discussion

The general objective of this thesis was to investigate the evolutionary patterns, diversity and phylogenetic relations within endemic reptiles species from high altitude in the Atlas Mountains, Morocco (North Africa). In recent years, several studies revealed patterns of biodiversity and evolution history of various reptiles from Morocco (e.g. Carranza *et al.* 2002; Perera *et al.* 2007; Fonseca *et al.* 2008; Rato and Harris 2008; Fonseca *et al.* 2009; Perera and Harris 2010a). Nevertheless, the study of high altitude species represent a challenge, as these areas are difficult to access and consequently, a greater amount of time and planning is needed to sample the species. The Atlas Mountains are not an exception, and although some high altitude mountains are easily accessed like Oukaimeden, access to others demands a careful planning and implies difficult trekking or even climbing. Apart from that, the low density of some species and/or their secretive nature, for example *Chalcides* spp., is a considerable obstacle for sampling and therefore for the study of the species.

6.1. Sampling high altitude reptiles in Morocco

Sampling the known distribution of the chosen high altitude reptiles in Morocco was the first task in this work. The difficulty to sample in high altitude is reflected in a paucity of historical records for many species, which in turn limits methodologies such as GIS modelling.

Sampling campaigns at high altitudes are very taxa specific because there are only a few reptile species living in these extreme environments. Furthermore, since species are not completely isolated and/or had dubious relationships with other lowland species in the study area, it was necessary also to extend the study to those (lowland) species, in order to clarify their relationships and evolutionary history. This study include all montane endemic reptiles of the Atlas Mountains (*Atlantolacerta andreanskyi*, *Quedenfeldtia trachyblepharus* and *Chalcides montanus*) with the exception of the extremely secretive *Vipera monticola* (Bons and Geniez 1996). These species are representatives of three diverse reptile groups: lacertids, geckos and skinks.

Atlantolacerta andreanskyi is a high altitude endemic only known from the higher peaks of High Atlas Mountains from 2400 to 3500 m. It was previously known from 22 geographic localities, but half of these are in the Oukaimeden and Toubkal area. The species has no known close relatives (Fu 2000; Arnold *et al.* 2007).

In the present study 8 localities for *A. andreanskyi* were found and sampled, some of them being in areas from where the species was not cited before(Harris *et al.* 2010).

These 8 localities cover all the distribution area of *A. andreanskyi*. As the populations are isolated in the peaks of the mountains, the distribution is not continuous; all the known

populations are isolated units. However, and despite the various attempts that took place, for sampling in the southernmost locality, Jebel Awlime, only three specimens were obtained from this location. This fact limited some of the genetic, and particularly the morphological analyses. Initially was thought that the species presence was associated with running water, due to conditions at Oukaimeden, but later three populations were found in mountain peaks without any water course: J. Awlime, Outabati and J. Ayache. In J. Awlime there was still some ice in May. Presence of this specie was found to be highly related with the presence of spiny bushes that are used as shelter, although in some cases *A. andreanskyi* were also found under rocks, for example in Oukaimeden. As a high altitude endemic, *A. Andreanskyi* inhabits places where, normally, there are few other reptile species; only *V. monticola* and *Q. trachyblepharus* are commonly found at such altitudes. However in some of the places, as J. Azourki and Oukaimeden, several other species were found including *Natrix maura*, *Scelarcis perspicillata*, *Timon pater*, *Tarentola mauritanica* and *Podarcis vaucheri*.

The genus ***Quedenfeldtia*** (Boettger, 1883) is represented by two species: *Quedenfeldtia trachyblepharus and Quedenfeldtia moerens.*

Quedenfeldtia trachyblepharus it is the only species found at the highest peaks of the Atlas Mountains, above 4000 m. Again, most of the cited localities are confined to the Toubkal and Oukaimeden area, however the limited distribution include some localities to the East (Aguelmous and J. Azourki) and to the South (J. Sirwa) and southwest (J. Awlime), but the distribution is not continuous showing a pattern of isolated areas (Bons and Geniez 1996).

Quedenfeldtia moerens is not endemic to high altitudes, even though it was found at almost 3000 m (a.s.l.), it is also found at sea level (Bons and Geniez 1996). It has a more continuous and comprehensive distribution, reaching from the southern limits of Morocco until the limit between the High and Middle Atlas (Bons and Geniez 1996).

In the present study 14 localities of *Quedenfeldtia moerens* and 4 of *Quedenfeldtia trachyblepharus* were sampled. Records from some of these localities were previously identified only as "*Quedenfeldtia* sp.". The fieldwork conducted allowed us to improve the distribution known for both species, for example the record of *Q. trachyblepharus* in Jebel Sirwa and the record of *Q. moerens* in Agoudal locality (Harris *et al.* 2010; Barata *et al.* 2011).

Both of these species are, generally, found on large rocks surrounded by some vegetation and comprise relatively dense populations. Although the genus was considered monotypic for a long time (Arnold 1990), the colour patterns of both species are very different and easy to identify in the field. The differences between their distributions suggest that they have different ecological requirements and the result of the ecological niche modelling corroborate that hypothesis (Barata *et al.* 2012b).

The genus ***Chacides*** has eleven species in Morocco (seven endemics) three of which were included in the present study: ***Chalcides montanus***, ***Chalcides polylepis*** *and* ***Chalcides manueli.***

Chalcides montanus is a montane skink endemic to the High Atlas Mountains. This specie was recorded for only 10 localities (Bons and Geniez 1996; Gamble *et al.* 2008; Gamble *et al.* 2010). It presents very low densities and is difficult to find since they are rarely seen on the surface, most specimens were collected under rocks. This species is more closely related with *C. polylepis* and *C. manueli,* all of which were first identified as *C. ocellatus* by Hediger (1935).

Chalcides polylepis is a much larger member of the genus. It is found from the sea level to 1950 m in the west size of the Atlas and has also been recorded from some localities in western Sahara (Bons and Geniez 1996). Is a diurnal species normally found under rocks and stones and in thickets of dense vegetation.

Chalcides manueli, another endemic skink from Morocco, is found only in lowland habitats. It was previously only cited for 8 localities in the occidental base of the High Atlas Mountains, between Dar Mzoudi, Tarudant to Essaouira and Agadir (Bons and Geniez 1996) and Sidi Ifni (Bons and Geniez 1996; Carranza *et al.* 2008).

Due to the difficulties to find these animals, the number of samples obtained during this study, was very limited, and as a consequence the genetic study was based on few samples.

This work brings new contributions to the knowledge of the reptiles from the Atlas Mountains. During this work the samples of the target species were amplified as the several other species that could be used in future works (Barata *et al.* 2011). In some cases the limits of the distributions were expanded and the knowledge about their habitats increased.

6.2. Identifying levels of genetic variation and detecting cryptic diversity

In recent years, as the study of Morocco herpetofauna has increased, high levels of genetic diversity have been reported in many diverse groups (Brown *et al.* 2002; Harris *et al.* 2004a; Harris *et al.* 2004b; Pinho *et al.* 2007; Barata *et al.* 2008; Rato and Harris 2008; Perera and Harris 2010b; Kaliontzopoulou *et al.* 2011). In some of the cases this variability refers to cryptic diversity, since no obvious morphological differences were found between highly divergent genetic lineages (e.g. Perera and Harris 2010b). As regards phylogenetic diversity within the montane herpetofauna, prior to this thesis, nothing had been done in North Africa. However, not far away, European montane herpetofauna was known to have survived the last glacial maxima through limited altitudinal range shifts, in opposition to the classic larger contraction and recolonization patterns observed in lowland species (Mouret *et al.* 2011).

Accordingly montane lizards such as *Iberolacerta bonnali* have minimal mtDNA diversity, and its phylogeographic patterns reflect colonization history rather than current habitat (Mouret *et al.* 2011). On the other hand, to the South, in the African tropics, there was greater climatic buffering during the Pleistocene, allowing the prevalence of speciation through ecological diversification (Fjeldsa and Lovett 1997), in other words, the present distribution area of the species represent the remnants of originally larger distribution ranges that have been reduced due to environmental changes – "paleoendemics". The persistence of these "palaeoendemics" in stable refugia may also have retained biodiversity at greater levels than at higher latitudes. This seems to be the case for example in East African forest chameleons (Tolley *et al.* 2011). In this scenario, further complexities are likely; within networks of refugia species may still undergo some cycles of fragmentation and admixture, leading refugia to be "melting pots" ratter than hotspots of diversity (Canestrelli *et al.* 2010; Canestrelli *et al.* 2012). An alternative hypothesis however is that much of the diversity is more recent, as species adapted to exploit novel niches, resulting in shallower radiations (e.g. Blackburn and Measey 2009). The lineages found in *Quendenfeldtia* and *Atlantolacerta* can be considered Paleoendemics because they are clearly very old and their present limited distribution, probably, represents the effect of climate changes in the original larger distribution ranges (Barata *et al.* 2012a; Barata *et al.* 2012b). The variation observed in North Africa high altitude reptiles seems to reflect the events that occur in southern Africa better than southern European refugia.

Studies focusing in cryptic diversity are increasing, uncovering an additional diversity that had been previously unsuspected. The use of genetic tools in taxonomic studies is primarily responsible for unveiling this "new kind" of diversity. In most cases the results obtained from molecular studies have promoted the assessment of other kinds of variation such as morphologic, ecologic or behavioural that was not obvious at first sight (e.g. Bergman *et al.* 2007; Funk *et al*. 2011).

The, previous mentioned, cryptic diversity led to the definition of "cryptic species", species based on extreme genetic diversity supported, in some cases, by morphological, ecological, chemical or behaviour shallow diversity. Studies on cryptic diversity have increase exponentially in the last decades mainly because of the availability of DNA sequences. This classification of species is still very controversial, especially when there is no other detected diversity besides the genetic (Bickford *et al.* 2006).

A common assumption is that cryptic species are very recent formed species where morphological traits or other diagnosable characteristics had not yet time to diverge. While this can be true in some cases, several cryptic species hide an ancient origin (Bickford *et al.* 2006). Although, there is not a particular speciation mechanism that promote cryptic

diversity, Hoskin *et al*. (2011) demonstrated that responses to past climate change affected both morphological and genetic divergence in a tropical frog. Therefore different scenarios result in different levels of variation between the lineages. In contrast, strong divergent natural or sexual selection, are believed to be the principal factors that promote rapid morphological divergence with low genetic variation. This phenomenon is well known in species like the African cichlid fishes, where several microhabitats in a lake promoted morphological variation (Bickford *et al.* 2006).

In the present study the levels of isolation and divergence found between high altitude lineages are generally superior to lowland taxa, this is well exemplified in *Quedenfeldtia* genus. *Quedenfeldtia moerens* has a wide distribution and show a genetic diversity pattern typically observed in lowland species, with high levels of diversity extended across the distribution. Northern and southern populations have some differences in nuclear markers that were already fixed, however, mtDNA revealed potential introgression. The two genetic clades recovered inside this species, North and South, were significantly divergent in mtDNA markers (8.7%, ND4), and even the southern clade was subdivided in two subclades geographically concordant with 5.3% divergence. The North and South clades showed some morphological variation, especially in colour pattern, detected in statistical analysis but insufficient to be used as identification parameter in the field (Barata *et al.* 2012a).

In contrast with what was observed in *Quedenfeldtia moerens, Q. trachyblepharus* and *A. Andreanskyi* have a very limited distribution restricted at high altitude (above 1400 m and 2400 m, respectively) and show a pattern of fragmentation with highly divergent lineages (ND4 *p*-distance uncorrected: 9.7% in *Q. trachyblepharus* and from 7.7% to 16.5%, in *A. andreanskyi*) and very low variation inside each clade (around 1%) (Barata *et al.* 2012a; Barata *et al.* 2012b). This variation in diversity seems to indicate that rather than a pattern of expansion from a single primary refugium, as is often observed in European herpetofauna (e.g. Rowe *et al.* 2006), in these species multiple small but distinct refugia existed during the last glaciations leading to maintenance of high genetic diversity between lineages but with limited variation within them.

Although high mitochondrial DNA variation was observed between populations/lineages, nuclear markers did not recover the same level of diversity. Even that if the combined nuclear markers recovered the same lineages as the mitochondrial, monophyly were not always achieved in geographic close populations and individual markers did not show fixed differences. Probably this is the result of the larger effective population size of the nuclear DNA when compared to the effective population size of mitochondrial DNA and of the consequent stronger effect of the incomplete lineage sorting at each single nuclear loci (Funk and Omland 2003). Additionally, the evolutionary rate of the genetic markers may fluctuate

depending on several factors that differ with the history of the species.
Regarding morphology, both *Quedenfeldtia* and *Atlantolacerta*, were demonstrated to be quite conservative, and although statistical analysis supported the existence of some differences, no diagnosable characteristic were found for the deep genetic lineages within the present described species (Barata *et al.* 2012a; Barata *et al.* 2012b).

The recently proposed "unified species concept" (de Queiroz 2007) and the integrative taxonomy approach (Padial *et al.* 2010), suggest the combined use of multiple criteria to delimit species. However, the continuity of the speciation process and the fact that the differentiation of characters is not achieved at the same time or order is well known, and consequently the absence of one of the criteria does not invalidate a species hypothesis under the "unified species concept" (de Queiroz 2007). This reflects the different and variable evolutionary processes acting to promote speciation, which not always follows the same roles. For example, fast adaptive radiations can result in morphologically divergent species with low levels of genetic differentiation (e.g. Cunha *et al.* 2005). In opposition, genetic drift could promote rapid genetic differentiation despite morphological stability (Sturmbauer and Meyer 1992). Moreover, only the absence of all of those criteria should be considered strong evidence against the hypothesis that two populations (or groups of populations) represent different evolving lineages (de Queiroz 2007). Goldstein and de Salle (2011) suggested to not recognize species unless they have morphological diagnosable characteristics. However, reproductive isolation can be achieved with the evolution of other diverse characteristics including bioacoustics, chemical cues or behaviour (Funk *et al.* 2011) and morphological differences can exist in populations that are not reproductively isolated.
Another widely discussed issue in species delimitation is the level of sequence divergence necessary to consider that two clades are different species. Despite the idea of a "barcoding gap" (Hebert *et al.* 2004) it is widely accepted that different species can have very different rates of evolution – obviously given different generation times, metabolic rates, repair enzyme efficiencies and so forth. Some authors have suggested that comparisons can be drawn with closely related taxa – if other similar accepted species show a similar degree of genetic divergence then this can be considered a reasonable benchmark for designating new forms (Speybroeck *et al.* 2010).
Delimiting allopatric cryptic species, in opposition to sympatric cryptics, is often more controversial because it is difficult to determine some criteria that are considered limiting for reproductive isolation or lineage status. The degree of morphological divergence to delimit species can be as debateable as that for genetic studies, and allopatric cryptic species can be a challenge to identify in the field (Fujita *et al.* 2012), especially in new localities. The same authors suggested that coalescent theory could objectively identify cryptic species (allopatric

or not) using genetic data than morphological information for reproductive potential or gene flow. Coalescent based-methods use multilocus data to test alternative hypotheses of speciation and allow for species tree discordance. Delimiting species based primarily on molecular information is a recent and emerging practice, particularly for vertebrates but essential for cryptic species. However, an integrative approach should be used to investigate diversity when feasible (Fujita *et al.* 2012). In the specific example of our work, *Atlantolacerta* spp., were not found any morphologic diagnosable character for field identification of the six proposed new species, however, their deep genetic differentiation seems to indicate an old divergence (Barata *et al.* 2012b).

Regarding conservation, cryptic species (specially allopatric) are of particular concern. If species with a limited distribution, such as high altitude endemics from Morocco, may be actually composed of multiple species with even smaller distributions, then these species will have even greater conservation priority. For example *Atlantolacerta* lineages in most of the cases, as far as we know, are limited to a single locality surrounded by unfavourable habitat and each lineage of *Quedenfeldtia trachyblepharus* is limited to only a few localities. Such isolated populations on high mountains have been identified as those of greatest concern under global warming scenarios (Pounds *et al.* 1999).

Determination of the time of speciation events is important to understand the evolutionary biogeography of species (Brown *et al.* 2008). However, it is difficult to estimate ages in phylogenies without several sources of error, especially if not enough calibration points information are available (Brown *et al.* 2008). During the Miocene tectonic activity in the region was intense and included the uplift of the Atlas Mountains that occurred around 9.0 Mya (Gómez *et al.* 2000; Babault *et al.* 2008). At more or less the same time *Podarcis* invaded North Africa (7.5 ± 1.2 Mya, Carretero 2008) and the European montane species within *Iberolacerta* started to fragment (6.1 Mya, Arribas and Carranza 2004). Although the differences between the time estimates for lineages of *Quedenfeldtia* (15-17 Myr, Gamble *et al.* 2010) and the North and South groups of *Atlantolacerta (*7.6± 4.3-11.9 Myr, this study), the divergence were more or less the same (±14% for mtDNA). Clearly these lineages are pre-Pleistocenic and, as found in Central African chameleons (Tolley *et al.* 2011) can be considered paleoendemics. However, without better calibration points it is difficult to date the split of the lineages more precisely than this.

The different ecological occupation of the species of *Quedenfeldtia* and *A. andreanskyi* can result from several historical events and/or different constraints. One possibility, in *Quedenfeldtia* genus, is that the two species split and adapted to different environmental conditions, temperate and drier climate in *Q. moerens* and high and humid mountains in *Q.*

trachyblepharus. After the last glacial maxima, when the temperature increased, *Q. moerens* expanded its distribution upwards, forcing *Q. trachyblepharus* to move to higher altitudes. In the case of *Atlantolacerta*, the low temperatures of the glacial maxima probably force lineages to disperse and survived in several lowland refugia, when temperature increase they expand to high altitudes. Probably, some of the population, where in contact more recently than others. The time estimation, with the know limitations, suggests that the six lineages diverged later, probably during the Quaternary Glaciations (4.3 ± 3; 2.4 ± 2; 2.9 ± 2 Mya).

The existence of six evolving lineages within *Atlantolacerta* spp., with high level of cryptic diversity, is supported by a set of tools evaluated together. The isolation and unique populations nature of those lineages requires their status elevation to species. Consequently, the description of 6 species inside *Atlantolacerta* genus, alert to possible diversity underestimation and to their potential endangered status.

6.3. Clarifying the relation between *Chalcides montanus*, *Chalcides polylepis* and *Chalcides manueli*

Chalcides is a genus of skinks composed mostly of elongated species that do not present great morphological variation, and that can be difficult to identify in the field (Schleich *et al.* 1996) This can be a result of parallel evolution due to the environmental constraints of a subterranean lifestyle, where species predominantly use chemical cues to communicate rather than visual signs. Unsurprisingly therefore, the morphology-based taxonomy shows some inconsistencies when compared with the genetic results (Carranza *et al.* 2008 and this thesis).
The present genetic study recovers two main results involving *C. polylepis, C. montanus* and *C. manueli*. The division of *C. polylepis* in two divergent clades is supported by analysis of mtDNA and a nuclear marker (MC1R). One clade is composed by the northern samples from the area of the Middle Atlas (including the area of the type locality – Fes) and the second include the samples from the area of Marrakesh and some from the coast near Azemmour grouped with the samples of *C. montanus* and *C. manueli*. The results from this study do seem to exclude the hypothesis proposed by Carranza *et al.* (2008), of *C. montanus* receiving mitochondrial DNA from *C. polylepis* through introgression, as the nuclear marker recover the same result as mtDNA with the same sample used by Carranza *et al.* (2008). Another possibility is that this second clade of *C. polylepis* (samples from Oukaimeden area) be a lowland form of *C. montanus* (also proposed by Carranza *et al.* 2008), but if so, this implies considerable morphological changes in a short evolutionary time period. This last hypothesis seems more likely to be close to the real scenario, however a detail morphological study should be done with access to more samples.

Our study also shows a complex paraphyly between the samples of *C. montanus* and *C. manueli*. If in the one hand the mtDNA analysis recovered similarities between the samples of *C. manueli* from Essaouira (type locality) and the samples of *C. montanus* from Oukaimeden area, and the samples of *C. manueli* from Sidi Ifni seems to be closer to the samples of *C. montanus* from J. Sirwa and Tizin Tichka. In the other hand, the nuclear marker analysed (MC1R) recovered a *C. manueli* clade composed by the samples from Essaouira and Sidi Ifni and two clades of *C. montanus*, Oukaimeden area and J. Sirwa (and Tizin Tichka). This may suggest a recent contact and change of alleles between *C. montanus* from J. Sirwa and *C. manueli* from Sidi Ifni that did not had time to fix changes in the nuclear markers.

The mitochondrial clades are, in some way, geographically concordant since Essaouira and Oukaimeden are in the north of High Atlas and Sidi Ifni, J. Sirwa and Tizin Tichka are in the south of Anti Atlas, while the first divergent clade (*C. polylepis*) is in the northeast of the High and Anti Atlas Mountains. Probably, the pattern observed between these tree species of *Chalcides* is a result of a complex process of migrations and isolation and local/ecological adaptations promoted by past climatic oscillations and the presence of the Atlas Mountains acting both as diversification promoter and as a geographic barrier, as already proposed for other species (Brown *et al.* 2002; Fritz *et al.* 2005).

However, with more samples and additional markers the estimate of relationships became much more complex, the result of the present study raises the question, if *C. manueli* really is a different species from *C. montanus*.

These results highlight the complexity of the relationships between these three species, as they are currently accepted. However, the sampling was very limited and there is a large area from the *C. polylepis* and *C. montanus* distribution that still need to be sampled and assigned to clades, in order to really understand how these diverse populations are interrelated.

The results obtained in the present study, once more, highlight the problems of species description based only in one data source, in this case morphology, that proved to be inadequate to use in the taxonomy of this genus (this work; Brown *et al.* 2012). As was previously mentioned, an integrative methodology should be used when possible, as clearly results from different sources of information will not always be the same.

6.4. Final Remarks

In a recent study, Mora *et al.* (2011) highlighted the importance of knowing the number of species on our planet; the same authors estimated that 86% of species on Earth await formal description. This issue is motivated not only by scientist's curiosity, but also by the need to have a reference to the present and future loss of diversity in an attempt to reverse that

tendency by the implementation of appropriate conservation measures. It is likely that cryptic diversity are highly represented in that percentage of species waiting to be described, as taxonomy have been based in morphology for centuries. Cryptic species are widespread, both geographically and across Metazoan taxa (Pfenninger and Schwenk 2007). On the one hand, there are several reported situations where morphological distinctiveness was not supported by high genetic divergence, as in Malagasy frogs (Vieites *et al.* 2009) and African cichlids (Salzburger and Meyer 2004) and in these cases species descriptions were well accepted by taxonomists. On the other hand, for cryptic species, when speciation is not accompanied by morphological change, species descriptions are often a controversial issue. The problem of delimiting species only with genetic data or even only with mtDNA is also well discussed and the problems are well known (Pinho *et al.* 2008); however the use of morphological variation as the only support to elevate populations to different species should be also tested. The unexpected results obtained in the *Chalcides* species highlight the weakness of using only one tool in taxonomy, especially in this case when only colour pattern differences was considered enough to recognize different species. As evolution and speciation are complicated processes, an integrative taxonomy (Padial *et al.* 2010) should be used to delimit species but always bearing in mind that the absence of one of the criteria does not constitute strong evidence against the acceptance of a distinct species (de Queiroz 2007).

Cryptic species, besides being very interesting in the way that they shed light on diversity and speciation processes, deserve special consideration in conservation planning. Species that have limited distributions might be in fact a complex of cryptic species that are, each of them, even more rare and endangered species. Due to the particularities of each species biology and ecological requirements, they may need different conservation approaches (Schonrogge *et al.* 2002). When cryptic species inhabit high altitude regions, as in the case of *Atlantolacerta* spp. and *Quedenfeldtia trachyblepharus*, there are other concerns adding to the previous ones. They are particularly sensitive to climatic fluctuations due to the fact that their small window of tolerances to temperature and elevation ranges can restrict their ability to persist in, or disperse across, different habitats (Janzen 1967; Ghalambor *et al.* 2006; Deutsch *et al.* 2008; McCain 2009).

The present study showed how different groups as lacertids, geckos and skinks show similar patterns of cryptic diversity and how cryptic diversity can be misidentified or overlooked. All of them are endemic to Morocco with a limited distribution, and their similar morphology hides complex patterns of diversity. Clearly they exemplify, as more investigation in cryptic diversity is needed to assess the real biodiversity on Earth.

6.5. Future perspectives

As so often occurs in scientific works, questions bring more question rather than just answers. This study is not an exception and there are some issues that it would be interesting to see explored in the near future.

6.5.1. *Quedenfeldtia* spp.

Confirmation of *Q. trachyblepharus* type locality

Quedenfeldtia trachyblepharus was described for Jebel Hadid in the coast, near Essaouira. Other authors (J. Bons; Hoogmoed 1974), actively sought for the species in that location without ever having found it, so that Arnold (1990) suggested that it could be a label error in the holotype collected by C. Von Fritsch and J. Rein. During the fieldwork for this study, we also looked for *Quedenfeldtia* in Jebel Hadid without finding the species. Possibly the type locality was Oukaimeden, as it is a well-known locality with easy access and most of the descriptions of *Q. trachyblepharus* are from there (Arnold 1990; Bons and Geniez 1996; Schleich *et al.* 1996). Although this was already suggested in Article 1, here we reinforce that this issue should be enlightened in order to simplify the available information in the future, and since *Q. trachyblepharus* is only found at much higher altitudes (1200-4000 m), the type locality does seem to be erroneous. On the other hand, range extensions of reptile species are common in Morocco, and the possibility that the species remains in Jebel Hadid without having been found in recent times remains open.

Increase the sampling of *Q. moerens* from southern localities

In the present study, the southern limit for the *Q. moerens* samples were Guelmin, however, several localities for the species were cited more to the south. After an intensive field work in the southern part of Morocco, we could not find specimens in this area. Furthermore, this region is extremely dry and desertic, and so if populations exist here, they will almost certainly be very small and completely isolated. For future fieldwork it would be interesting to know if the species is still present in this southern region and if so, what the genetic variation between them and the analysed samples.

New *Q. trachyblepharus* localities means new diversity?

After finishing the *Quedenfeldtia* manuscript, in a later fieldtrip aiming to sample *A. andreankyi*, two more localities for *Q. trachyblepharus* were found. These localities are geographically more distant than Oukaimeden and J. Sirwa and this may represent more long time isolation. One population is in the north, J. Azourki and the second more to the west, J. Awlime. A preliminary analysis revealed that J. Azourki seems to be genetically different

from the analysed populations and samples from J. Awlime were not amplified possibly due to a mutation in the binding zone of the primers. These preliminary results indicate that possibly there are more isolated populations in high altitude mountains.

Possible contact zone between *Q. trachyblepharus* and *Q. moerens*

In J. Awlime Mountain, for the first and only time in our work in the field, the two species of *Quedenfeldtia* were observed in the same area. *Quedenfeldtia moerens* were found from the 1478 m to 2529 m and *Q. trachyblepharus* were found from the 2659 m to the top at 2871 m. This possible contact zone should be confirmed and can reveal interesting facts about the species, like the hypothesis of *Q. moerens* limited the distribution of *Q. trachyblepharus* to the high altitudes.

6.5.2. *Atlantolacerta* spp.

After the unexpected results revealed by this study, several questions arise:

Does the J. Awlime population (the one with only 3 samples) have the same pattern as the remaining populations? An extra effort to increase the sampling in this area should be done in order to get more complete results to nuclear analysis and morphology.

Are there still more unsampled lineages? If so, are they as divergent as those presently known?

Toubkal and Oukaimeden where the populations/lineages that show low divergence between them and recently specimens where observed on the suitable high-altitude habitat existent between the two localities (Martinez-Freiria, personal observation). What patterns will be observed in the Toubkal area if an intensive sampling in the area shows continuity in the distribution?

6.5.3. *Chalcides* spp.

Due to the enormous difficulties already mentioned to sample this animals, the samples were limited, however they were enough to highlight the need of restructuration of the entire *Chalcides* taxonomy. An intensive and directed sampling should be done in Morocco to really understand the taxonomic organization of the genus and their evolutionary patterns. The results obtained in this work probably are just the tip of the iceberg in respect to uncovering the evolutionary history of the *Chalcides* genus.

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