Phylogenetics and Hybridization Assessment of *Acanthodactylus scutellatus* species group in North Africa

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Todas as correções determinadas pelo júri, e só essas, foram efetuadas.
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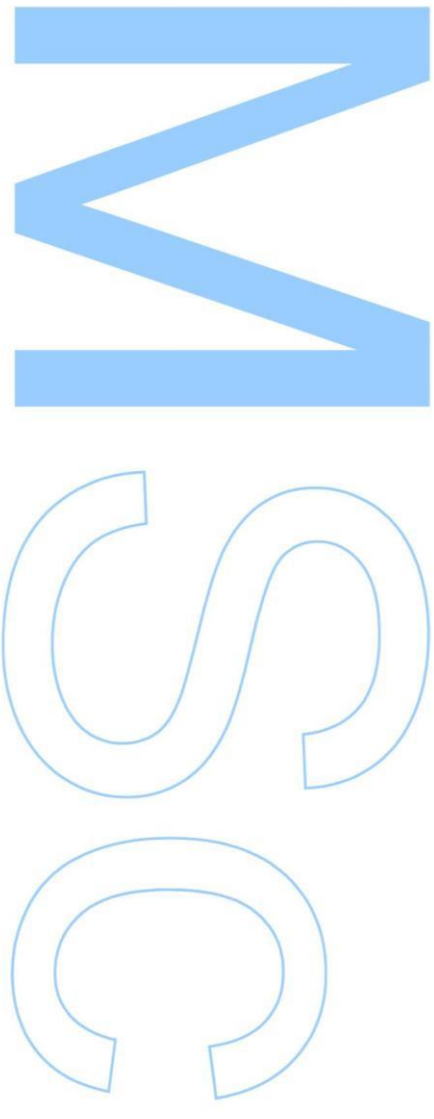

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Sumário

Eventos geológicos e paleoclimáticos são duas forças essenciais que atuam sobre os processos evolutivos na natureza. Estes são fenómenos fracamente estudados no Norte de África, apesar da sua grande diversidade de habitats, paisagens heterogéneas, e histórias climáticas e geológicas complexas. Os padrões atuais de biodiversidade do Sahara e do adjacente Sahel provavelmente resultaram de oscilações fortes no clima e paisagem. O complexo de espécies *Acanthodactylus scutellatus* inclui importantes elementos da herpetofauna de ecossistemas áridos no Norte de África, e bem adaptados a condições xéricas. Apesar da sua notável diversidade, a taxonomia dentro do complexo é controversa, e com a exceção de dados morfológicos, pouco é sabido acerca destes organismos. Observações de indivíduos com morfologia intermédia em áreas de simpatria sugerem hibridação entre estes taxa. Este estudo pretende inferir 1): relações filogenéticas dentro deste grupo e identificar as linhagens principais; e 2) o fluxo génico contemporâneo numa zona de contacto na Mauritânia. As análises filogenéticas basearam-se em 466 indivíduos sequenciados para ambos os genes mitocondriais 12S e Cyt-b (756 total bp) e para o gene nuclear C-mos (513 total bp). Um total de 208 indivíduos da zona de contacto foram genotipados para 15 microssatélites. Os resultados mostram que as linhagens principais recuperadas não coincidem completamente com a sistemática atual, sugerindo que taxonomia e sistemática necessitam de revisão. As análises de genotipagem confirmaram as linhagens históricas encontradas e mostram ausência de fluxo génico entre elas, não sendo detetados híbridos apesar da grande amostragem e vasta cobertura geográfica. A falta de fluxo de genes observado levanta questões acerca do potencial papel de barreiras reprodutivas e seleção do habitat na prevenção de hibridação. As ferramentas moleculares aplicadas permitiram maior compreensão sobre os limites definindo as espécies, relações evolutivas, história e diversidade dentro deste grupo, para além de contribuir para o conhecimento sobre fluxo de genes no Sahara-Sahel.

Palavras-chave: Grupo de espécies *Acanthodactylus scutellatus*, Norte de África, Sahara, Sahel, evolução, filogenia, análises de populações, estrutura genética, fluxo de genes

Abstract

Geological and paleoclimatic events are two main forces driving evolutionary processes in nature. These are poorly studied phenomena in North Africa, despite its great diversity of habitats, heterogeneous landscapes, and complex climatic and geological histories. Modern biodiversity patterns of the Sahara and the adjacent arid Sahel likely resulted from strong oscillations in climate and land-cover. *Acanthodactylus scutellatus* species group comprises important elements of the herpetofauna of arid ecosystems in North Africa, and well adapted to xeric conditions. Despite their remarkable diversity, the taxonomy within the complex is controversial, and with the exception of morphological data, little is known about these organisms. Observations of morphologically intermediate individuals in sympatry areas suggested hybridization between taxa. The present study aims to infer: 1) phylogenetic relationships within this group and identify major lineages; and 2) contemporary gene flow in a contact zone in Mauritania. Phylogenetic analyses were based on 466 specimens that were sequenced for both 12S and Cyt-b mitochondrial genes (756 total bp), and for the C-mos nuclear gene (513 total bp). A total of 208 individuals of the contact zone were genotyped for 15 microsatellites. Our results show that recovered major lineages do not match with current systematics, suggesting that taxonomy and systematics need revision. Genotyping analyses confirmed the historical lineages found in the studied contact zone and show absence of gene flow between them, with no hybrids detected despite the large sampling and broad geographic coverage. Lack of observed gene flow raises questions about the putative role of reproductive barriers and habitat selection in preventing admixture. Finally, the molecular tools applied allowed for insights on the species boundaries, relationships, history and diversity of this group, in addition to contributing to the knowledge about gene flow in the Sahara-Sahel.

Keywords: *Acanthodactylus scutellatus* species group, North Africa, Sahara, Sahel, evolution, phylogeny, population analyses, genetic structure, gene flow

Index

Figure Index

Table Index

1. Introduction

1.1. How past history shaped genetic structure - A few insights

1.1.1. Isolation and vicariance

Research in Evolutionary Biology grants deep knowledge about biodiversity patterns and processes. The current biodiversity patterns emerge from interactions between the processes of range shift, extinction, and speciation. The rate and duration of speciation - the evolutionary process through which new species arise from existing ones - result from a balance between numerous intrinsic and extrinsic factors (Butlin *et al*. 2012). Several forces and mechanisms act on speciation and evolution in general, such as gene flow, natural selection and genetic drift (Slatkin 1987; Butlin *et al*. 2012).

Gene flow is the exchange of genes as consequence of the movement and breeding between individuals (Slatkin 1987). It may act as a force constraining evolution, since it can preclude local adaptation of populations to their environment, or facilitating evolution by disseminating new genes through different populations (Slatkin 1985; Slatkin 1987). Gene flow can be a powerful evolutionary force (Slatkin 1987). If dispersal and gene flow are prevented, there is opportunity for differentiation. This is precisely what characterizes the allopatric mode of speciation, where the interruption of dispersal by a barrier allows isolation between populations, increases genetic differentiation and facilitates independent evolutionary processes, with eventually formation of distinct species (Slatkin 1987; Abbott *et al*. 2013). Vicariance, one of the main mechanisms of speciation in allopatry, occurs when the emergence of a barrier to gene flow creates a separation of populations within the range of an ancestral taxon (Crisp *et al*. 2010).

Climate has been one of the major drivers of change in biodiversity patterns and processes (Hewitt 2004). The strong and global climatic oscillations of the Quaternary, which began 2.4 Myr ago, caused massive changes in species distributions and evolutionary processes, inducing extinction in some populations and survival in refugia in others (Hewitt 2000). This period was characterized by alternation of glacial and interglacial cycles, translated in temperature shifts of great magnitudes, which lead to expansions and contractions in species ranges. A common pattern for species inhabiting temperate regions is that contractions mostly occurred during cold periods, following the spread of the polar ice sheets, and subsequent expansions occurred during warmer periods (Hewitt 1999). However, species occurring in other regions of the planet experienced these fluctuations in different ways. For instance, while temperate regions and tropical rainforests were reduced during the ice ages, savannahs and deserts were extended (Fig.1; Hewitt 2000; Hewitt

2001). One global feature is that in every region, species responded to the climatic changes by trying to track the shifting suitable environments (Hewitt 1999). The permanence of populations in disjoint refugia contributed to the formation of divergent genetic lineages, with multiple degrees of isolation (Taberlet *et al*. 1998; Gómez & Lunt 2007). Divergence occurred due to the lack of gene exchange and also because of potential differences in selective pressures between refugia. The occurrence or absence of gene flow over time would depend on factors such as the intrinsic dispersal capacity of the organisms, their suitable habitat, and the presence of geographical barriers (Hewitt 1999).These phenomena are particularly well studied in Europe (Taberlet *et al*., 1998; Hewitt, 1999, 2001; Weiss and Ferrand, 2007; Sommer and Zachos, 2009). During the Pleistocene period, the South of the European continent was more able than other regions to provide suitable habitats, owing to its high variability in topography and climate. The mountains provided great geographical substructure, and allowed for altitudinal shifts in species ranges (Hewitt 1999). These regions offered opportunities for the isolation, survival and divergence of lineages without large geographical shifts (Hewitt 2001). The Southern peninsulas (Iberian, Italic, and Balkans) constituted the major refugia during the last ice age for most species. The

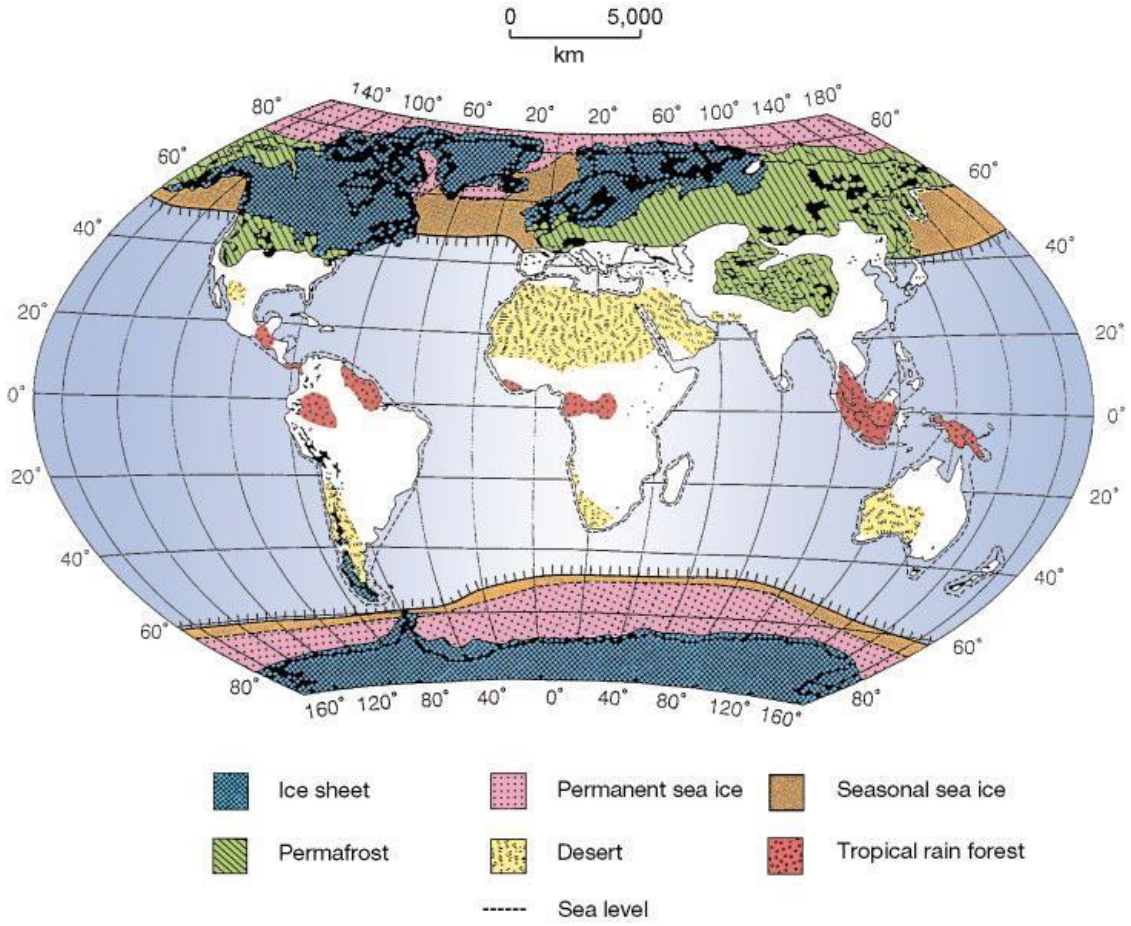

Fig. 1 - Representation of the desert and tropical rain forest habitats, lowered sea level and extent of ice and permafrost at 20 000 yr BP (Hewitt 2000).

populations from those refugia served as sources for future radiations or expansions, or as long-term relicts (Weiss & Ferrand 2007).

Geological events are other strong drivers of isolation processes, as they may form biogeographical barriers that are difficult to overcome (Lieberman 2005). A good example is the formation of the Strait of Gibraltar, about 5.3 Myr ago. This event created a barrier to gene flow between North Africa and the Iberian Peninsula, although the Strait presented distinct degrees of permeability between taxa. For many of them, it led to a profound divergence between populations in both continents (e.g., herpetofauna, Busack 1987; Fonseca *et al*. 2009; plants, Lavergne *et al*. 2013) while other taxa have crossed the Strait of Gibraltar since the re-opening, evidencing a barrier permeable to migration in this region (e.g., mammals, (Cosson *et al*. 2005); amphibians, (Recuero *et al*. 2007); insects, (Habel *et al*. 2012); plants, (Lavergne *et al*. 2013); reptiles, (Stuckas *et al*. 2014)). The uplift of mountains is another obvious event with great potential to drive isolation and vicariance. For example, a crucial role of the Atlas Mountains uplift was shown in *Mauremys leprosa* (Fritz *et al*. 2006) and *Agama impalearis* (Brown *et al*. 2002), with distinct clades occurring on different sides of the mountain chain.

1.1.2. Gene flow, range expansion and secondary contact

When barriers to gene flow cease to exist or are overcome, previously isolated populations may enter in contact, with strong genetic consequences (Abbott *et al*. 2013). If the breakdown of barriers happens due to climatic fluctuations, there may be successive cycles of contractions and expansions, with populations and lineages repeatedly experiencing divergence followed by gene flow (Hewitt 2001; Sommer & Zachos 2009). During a range expansion, individuals are subjected to selection in new environments and adaptive events (Hewitt 2000), and to the loss of genetic diversity (Excoffier *et al*. 2009). The range expansion implies a succession of founding events, resulting in a chain of bottlenecks: diversity declines over the expansion route, with alleles being lost and homozygosis increasing (Excoffier *et al*. 2009). An outcome of such processes is that usually higher diversity is retained near the refugial regions (Hewitt 2000).

Patterns of expansion from particular refugia can be contrasting, and rates of colonization may differ (Fig. 2; Hewitt, 2004). But congruence is also frequent, and as a result, suture zones emerge. These are bands of geographical overlap between major biotic assemblages, and where gene flow can occur (Taberlet *et al*. 1998). Suture zones appear because after expansion from separate refugia, divergent lineages can meet and experience what is called secondary contact. If this contact is followed by gene flow between the

overlapping lineages, the contact zone becomes a hybrid zone. The complexity of the divergence processes is obvious when considering hybrid zones, where genetic diversity exhibits different levels of isolation and divergence between taxa (Hewitt 2001). For example, considering the Iberian Peninsula, the two mitochondrial lineages of the salamander *Chioglossa lusitanica* exchange genes in a hybrid zone near the Mondego River. Genetic data suggest they would have existed in discontinuous isolation during the Pleistocene, being this complex history likely responsible for the incomplete isolation, despite the differences in ecology and the different climatic conditions these evolutionary units face (Sequeira *et al*. 2005). Numerous other examples are known for the Iberian Peninsula that also reflect complex evolutionary dynamics and differential introgression across markers in hybrid zones (Gómez & Lunt 2007; Weiss & Ferrand 2007).

1.1.3. Hybridization

Hybridization occurs when genetically distinct groups of organisms interbreed, resulting in the production of viable individuals. Hybridization processes were seen for a long time as rare and mostly deleterious at the species level, given that the biological species concept implies the existence of isolating mechanisms, acting as reproductive barriers (Mallet 2005). As lineages diverge, reproductive isolation is expected to evolve, through

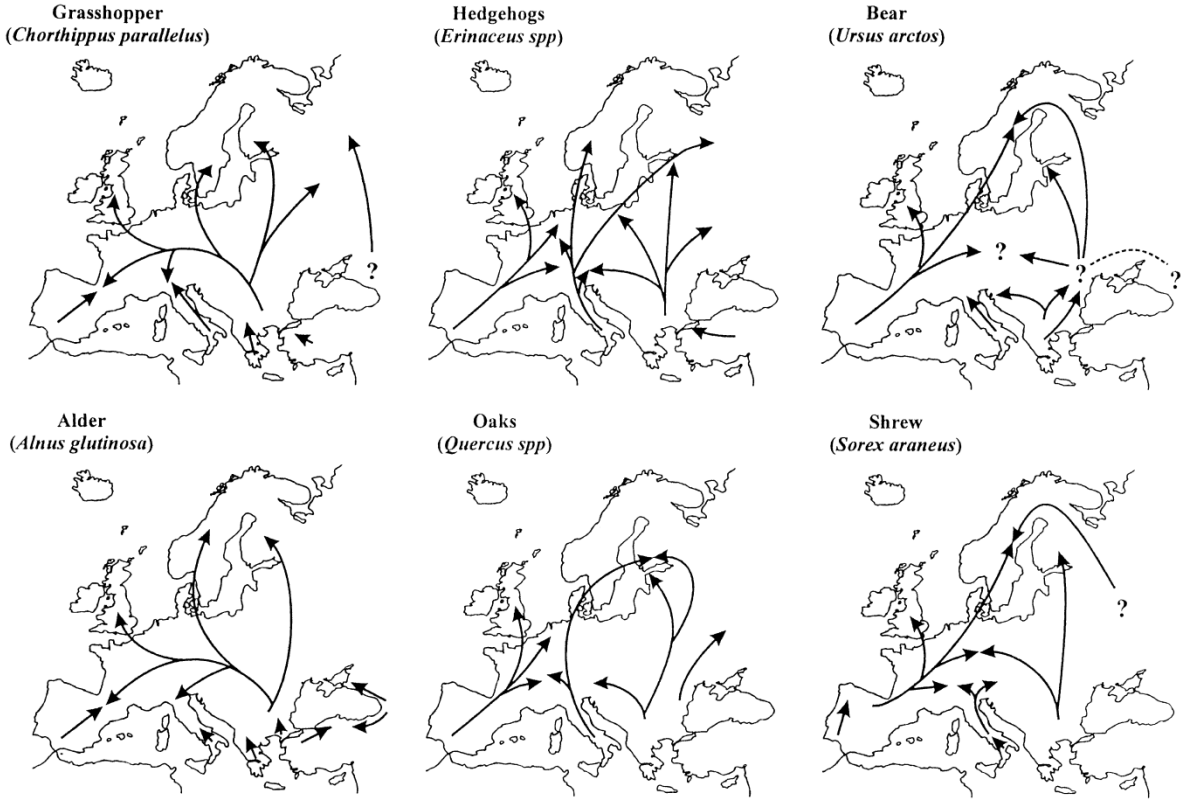

Fig. 2 - Examples of postglacial colonization patterns in Europe (Hewitt 2001).

either prezygotic or postzygotic barriers, preventing the formation of hybrids or resulting in sterility and unviable hybrids (Butlin *et al*. 2012). Reproductive isolation is indeed verified in many cases. Nevertheless, there is nowadays abundant genetic data showing that hybridization between species is common, which is not difficult to accept if one keeps in mind that man-defined species boundaries do not always reflect the true nature of the focused units, and that speciation is usually a gradual process (Mallet 2008). Complete isolation may need time to evolve, and admixture can be possible until then (Mallet 2005; Abbott *et al*. 2013). For example, frequent hybridization was shown between *Vipera aspis* and *V. latastei*, evidencing a still incomplete reproductive isolation. In this case, the barrier to admixture is achieved mostly through ecological segregation: hybridization was detected only in habitats that are suboptimal for the parental taxa (Tarroso *et al*. 2014), and these viper's ranges in the contact zones is determined by different patterns of habitat selection (Martínez-Freiría *et al*. 2008) and local adaptations (Martínez-Freiría *et al*. 2009).

Hybrids between species were also believed to be often less fit than individuals from the parental species, with strong selection against them (Mallet 2005). Studies in recent years challenged those older views of the subject, proving that not only hybridization can be widespread but that it also may have a positive impact on evolution (Seehausen 2004; Mallet 2005; Abbott *et al*. 2013). Hybridization induced by environmental change may be a widespread phenomenon in contact zones between populations or lineages. It may decrease diversity due to genome replacement, and as a result, reduce fitness. However, hybridization should not be seen as an always harmful process (Seehausen 2004; Abbott *et al*. 2013). It may also provide genetic diversity, raising the evolutionary potential of populations and easing adaptation to new environmental conditions (Hoffmann & Sgrò 2011). For example, hybridization had an adaptive role in *Senecio* species by allowing a complex trait to be regained, providing the possibility of its re-emergence without requiring the accumulation of multiple mutations (Rieseberg 2009). Also, Becker *et al*. (2013) reported evidence for hybridization enabling fast adaptation to climate change in plants of the genus *Pachycladon,* during the Last Glacial Maximum. Gene flow would have conferred higher chemical defences in hybrids against pathogens and herbivores, promoting long-term survival (Becker *et al*. 2013).

Past climatic changes shaped contact zones and species´ range shifts, consequently modifying reproductive isolation and gene flow among populations (Hewitt 2000). As a result, these phenomena had a strong influence in evolution (Hoffmann & Sgrò 2011). Studying situations where genetically different populations meet, and potentially exchange genes, allows understanding the genetic cohesiveness of evolutionary units, which can be extremely important from either an evolutionary, systematic or conservation point of view.

1.2. Biodiversity dynamics in North Africa and the Sahara-Sahel

North Africa is a region of great biogeographic interest, due to its diversity of habitats, heterogeneous landscapes and complex climatic and geological histories (Le Houérou 1997; Sayre *et al*. 2013). The age of some geological events is known, such as the formation of the Strait of Gibraltar at the end of the Messinian Salinity Crisis, about 5.3 Ma ago (Krijgsman *et al*. 1999; Duggen *et al*. 2003). North Africa includes two of the main ecoregions of the continent, the Sahara desert and the adjacent arid Sahel. The Sahara is the widest warm desert of the planet, covering, including the Sahel, approximately 11,230,000 km^2 (Olson *et al*. 2001). Calling the Sahara a "desert" may be an unrealistic oversimplification, considering the diversity in climate and landscapes it currently exhibits since historical times (Le Houérou 1997). Together, Sahara and Sahel are characterized by high diversity of topographic features and a heterogeneous climate, due to significant spatial variability in both rainfall and temperature. The boundary between them establishes the transition between the Palaearctic and Afro-Tropical biogeographic realms (Olson *et al*. 2001). As a result, there is great latitudinal variation in species distributions and high local biodiversity (Dumont 1982, Le Houérou 1992). The Sahara-Sahel covers over ten countries, being many of them rated as low development and characterized by long-term political instability. These features make field surveys, trans-border research and conservation scheduling hard to achieve (Brito *et al*. 2014).

Deserts are classified according to an aridity index (average annual precipitation/potential evapo-transpiration), being hyperarid the areas with an index below 0.05, while arid regions present an index ranging between 0.05 and 0.20 (Ward 2009). Both these types of areas are usually pictured as quite homogeneous, bare, and with low diversity comparing to other regions. For this reason, they tend to draw less scientific attention (Durant *et al*. 2012). However, as sharp ecological gradients are created by climatic extremes, these are perfect areas to investigate the effects of extreme environments on biodiversity patterns. They possess locally endangered micro-hotspots of biological diversity (Dumont 1982; Davies *et al*. 2012). Organisms occupying those regions have evolved exclusive adaptive features to deal with the severe environmental conditions, such as scarcity and unpredictability in water and food resources, as well as extreme solar radiation and temperatures (see Brito *et al*. 2014 for a review). Many species tend to have patchy distributions, with range boundaries under tough climatic control. Also, the adaptive processes of organisms to such extreme environmental conditions lead to rather high rates of endemism (Ward 2009).

Data suggest the desert conditions in the Sahara began about 7 Myr ago (Schuster *et al*. 2006; Zhang *et al*. 2014). The region suffered, and apparently still suffers, strong fluctuations in climate (Fig. 3). Since the Pliocene, between 5.3 and 2.5 Mya ago, numerous dry-wet cycles occurred in the Sahara-Sahel. It is recognized that both regions experienced at least eight to ten wet and dry periods over the past 125,000 years (Le Houérou 1997). The shifts in climate during the Pleistocene and Holocene caused great changes in geomorphic processes, followed by modifications in fauna and flora. During the Pleistocene, the Sahara mountains exhibited a temperate climate flora (Le Houérou 1997). It is known that during the humid period, the Sahara had a dense palaeoriver network, and multiple basins supported large lakes. Tectonic activity played an important role in northern basins, leading to river diversion and transference of aquatic biota between basins (Drake *et al*. 2011). Between around 6 and 5,000 years ago, this wet period came to an end, and aridity started to increase significantly. As a consequence, there were dramatic changes such as

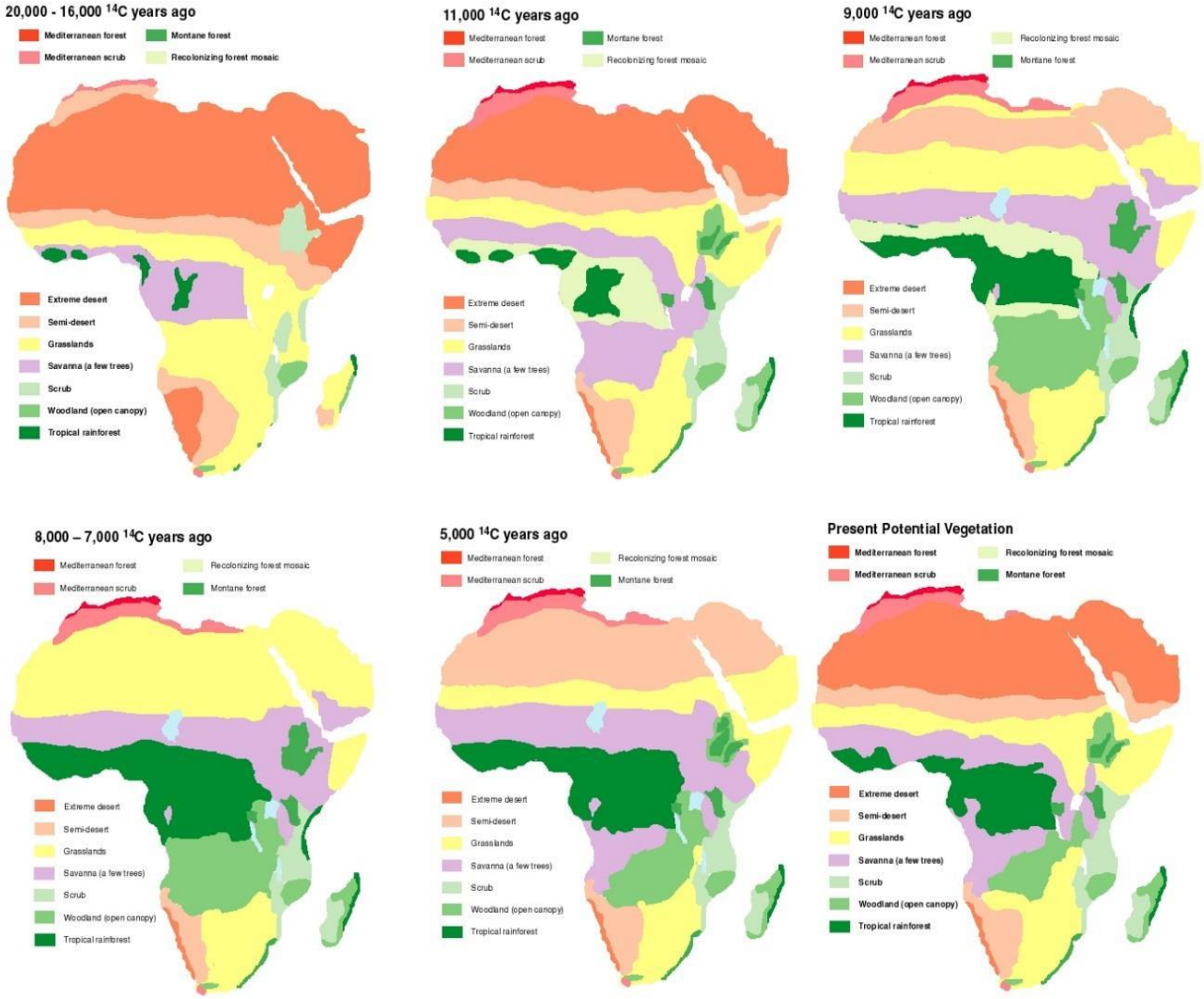

Fig. 3 - Temporal oscillations of the African habitats between the Last Glacial Maximum and the present. Adapted from Adams and Faure (2004).

the disappearance of the mesic vegetation communities, and the decrease in the lake levels. Modern biodiversity patterns in the Sahara-Sahel are the result of these oscillations in climate and huge shifts in land-cover (see Brito *et al*. 2014 for a review).

1.2.1 Evolutionary processes in the Sahara-Sahel

The knowledge about evolutionary processes in the Sahara-Sahel is very scarce. Phylogeographic studies reveal that diversification and speciation events occurring in the region are probably due to the spatial and temporal variation of the desert extent (Brito *et al*. 2014). While the limits of the Sahara moved significantly southwards during the Quaternary, the northward limit seem to have retained approximately the same position (Le Houérou 1997). The emergence of the Sahara itself likely created vicariance between lineages located at North and South, influencing diversification processes for numerous species and causing allopatry. There are a few studies showing concordance between the evolution of clades and the history of the Sahara. For example, Carranza *et al*. (2008) reported coherence between the origin and divergence dates of skinks, and the age of the Sahara. The palaeoclimatic fluctuations verified after the Sahara formation are thought to have occurred at cycles of around 100 000–20 000 years, during the last million years (Le Houérou 1997). These fluctuations would have deeply shaped the range of desert and savannah environments, as well as constrained the distribution and genetic structure of their species (Brito *et al*. 2014). The cycles of population contraction and expansion resulted in contrasting patterns. On the one hand, unsuitable climatic periods led to increased divergence in the absence of gene flow between refugia, facilitating speciation and morphological evolution (e.g.,Guillaumet *et al*. 2008). On the other hand, suitable climatic periods led to dispersal along geographical corridors, promoting gene flow (e.g., Gaubert *et al*. 2012). Assuming that adaptation processes were not the principal mechanisms driving speciation in the Sahara-Sahel, the intraspecific divergence emerged mainly through vicariance. The nature and time of vicariant events vary in their effects on distinct taxa, depending on their habitat requirements. Allopatry would have a strong role by interrupting gene flow and originating evolutionarily independent lineages, or eventually new species (Brito *et al*. 2014). For example, Douady *et al*. (2003) suggested an important role of the aridification in creating vicariance and diversification in elephant shrews. Gonçalves *et al*. (2012) identified cryptic diversity in North African lizards from the genus *Agama* and gave insights about the colonization and diversification of the genus in the Sahara. However, all these phenomena are still poorly studied in the Sahara-Sahel, and even less for species that are truly adapted to its conditions. High diversity was also reported within arid-adapted

species of the genus *Stenodactylus*; the same study pointed out geological events and climatic instability as main drivers of divergence (Metallinou *et al*. 2012). Studies focusing on modern timescales granted novel perspectives. Habel *et al*. (2012) reported a surprising lack of differentiation in a butterfly species in North Africa, which are restricted to isolated forest habitats within the oases, surrounded by highly unfavourable desert environment. The results suggested a recent character of the oases colonization, probably combined with similarity in the environments across oases. Velo-Antón *et al*. (2014) identified a recent metapopulation system of *Crocodylus suchus* across Mauritanian mountains, formed when these mountains were colonized from southern ranges during the last humid phase of the Sahara. The examples above illustrate part of the little knowledge existing about divergence processes across a range of species with different ecologies, and exhibiting different patterns. The Sahara-Sahel is a good model system to study the effects of extreme climate shifts on biodiversity dynamics. Despite the effort in recent years to know more about such historical gene flow in this region, studies on modern patterns and processes have been largely neglected. There is scarce knowledge about the role of landscape features and climate in determining the extent of connectivity, gene flow, and ranges. Understanding patterns of gene flow and contact zone dynamics in desert environments will require molecular studies and integrative landscape models. With these advances it should be possible to reinforce the knowledge on adaptation and evolutionary mechanisms to extreme arid conditions (Brito *et al*. 2014).

1.3. The lizards of the genus *Acanthodactylus*

The genus *Acanthodactylus* is composed by eight species groups or complexes: *A. boskianus, A. cantoris, A. erythrurus, A. micropholis, A. opheodurus, A. pardalis, A. scutellatus* and *A. tristami* (Harris & Arnold 2000). Commonly known as fringe-toed lizards or spiny-footed lizards, they form a clade of small ground-dwelling lizards occurring mostly on sandy ground in arid regions (Harris & Arnold 2000; Crochet *et al*. 2003). The genus has the particularity of being currently the most specious of the Lacertidae family, including more than 30 species. It is widely distributed, occurring in the Iberian Peninsula, south of the Mediterranean Basin, Sahara-Sahel, Arabian Peninsula, and India (Crochet *et al*. 2003). These lizards are conspicuous, diurnal and important elements among the vertebrates in deserts and arid ecosystems in North Africa. Despite their pronounced diversity, the knowledge about most of the species is still scarce and their taxonomy is still partially unresolved (Harris & Arnold 2000; Crochet *et al*. 2003).

The monophyly of this group was shown (Fig. 4) in a phylogeny established with

mitochondrial DNA sequences (Harris & Arnold 2000). The genus is believed to have emerged from mesic ancestors: after invading Africa, they would have originated more xeric forms in the Ethiopian region. These would have later radiated in North Africa, leading to the modern dry-adapted forms, capable of tolerating high temperatures and equipped with fringed-toes ideal for soft-sand habitats (Arnold 1989). The systematics has been based mainly on a large range of external morphological traits, osteological characters, hemipenises morphology, and in a smaller extent, on biochemical data (Bons 1959;

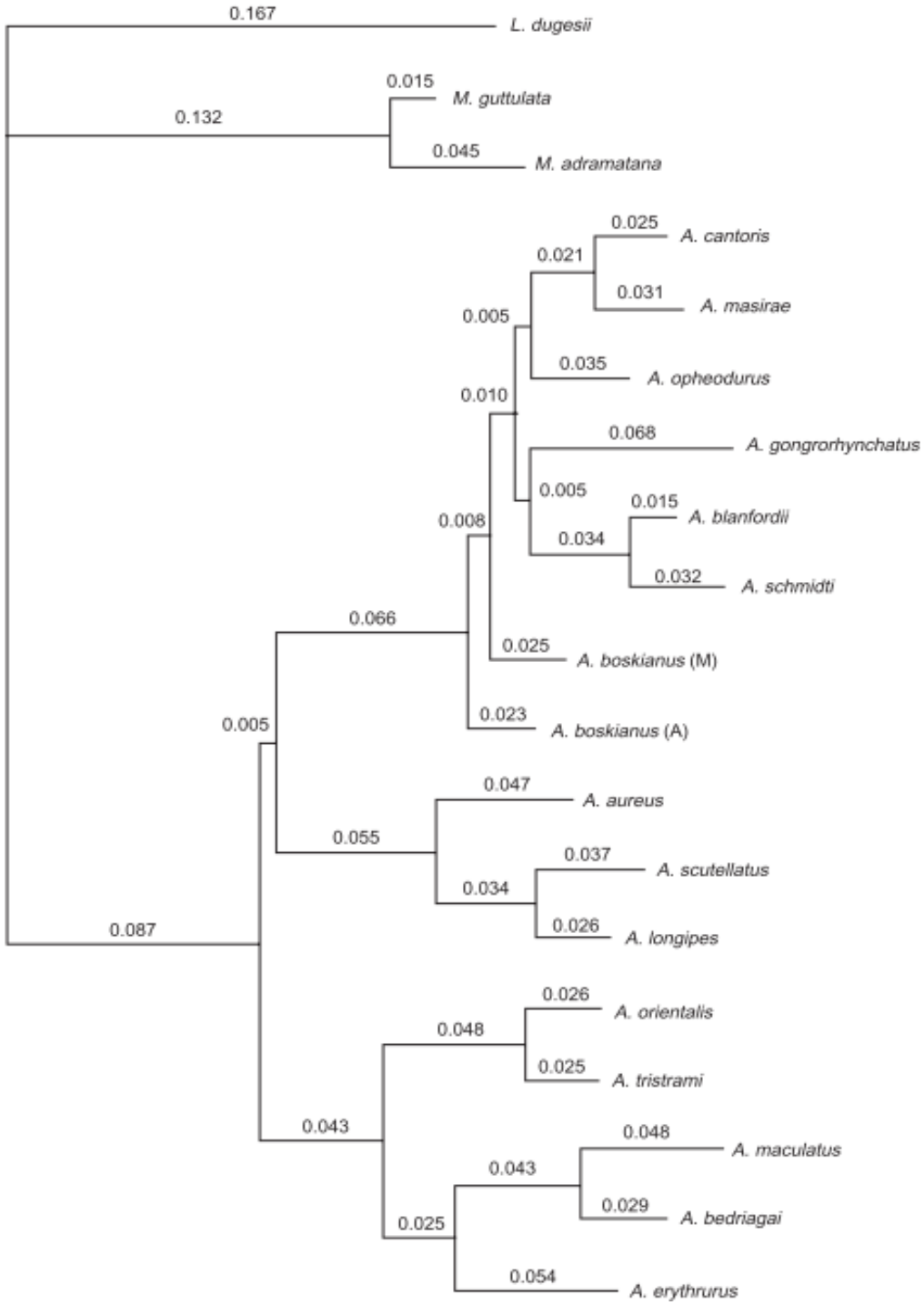

Fig. 4 - Mitochondrial phylogeny of the Acanthodactylus genus, estimated with Maximum Likelihood (Harris et al. 2000).

Salvador 1982; Arnold 1983; Blanc & Cariou 1987; Mellado & Olmedo 1990; Harris & Arnold 2000; Crochet *et al*. 2003; Fonseca *et al*. 2009). Conflicting evidence emerge from the analysis of morphology, mostly due to the lability of external characters (Mellado & Olmedo 1990). On the opposite, hemipenial characters have been suggested to be more prone of retaining changes, although they can also be labile because certain changes can evolve independently multiple times. Still, described hemipenial differences in *Acanthodactylus* were claimed to possibly act as isolating mechanisms: if incompatibility in genitalia evolved, interspecific mating could be prevented. Indeed, significant differences in hemipenial morphology can be found between *Acanthodactylus* species that are externally very similar (Arnold 1983). This could be an important mechanism in contact zones, considering males show a weak courtship behaviour and conspecific males can be difficult to discriminate from others (Harris & Arnold 2000). Even if discerning between species complexes was proved to be rather easy, within complexes the external characters are too variable to allow a clear and unequivocal distinction between taxa (Mellado & Olmedo 1990). Molecular data suggested that the use of morphology based on outgroup comparisons led to incorrect inferences about the character polarities within the genus (Harris & Arnold 2000). Morphological characters exhibit high intraspecific diversity, and diagnosable traits appear in confused combinations between adjacent populations. Despite the intraspecific morphological variability in this group, another approach that has been used is to discriminate between species given the coexistence in sympatry of different forms, based on the idea that if different groups can coexist in sympatry and remain distinct, they must be considered valid species (Crochet *et al*. 2003).

Early studies using allozyme frequencies detected high genetic variability in some *Acanthodactylus* species, and questioned if this level of diversity may be an adaptive strategy to unstable environments (Blanc & Cariou 1980; Blanc & Cariou 1987). The same authors also found large genetic distances, as well as conflicts between morphological and biochemical data (Blanc & Cariou, 1980, 1981, 1987). More recent data suggest there were repeated adaptations to similar environments in the *Acanthodactylus* species, with comparable and strong selective pressures moulding the evolution in this genus (Harris & Arnold 2000). The results of a study for the *A. erythrurus* group suggested that the habitats occupied by the species belonging to this group experienced phases of expansion and contraction, linked to rapid climate changes, and probably resulting in complex micro-evolutionary processes. Researching contact zones was recommended in order to provide a better understanding of the evolution of these organisms (Fonseca *et al*. 2009).

Few data are available on contact zones in genus *Acanthodactylus*. Morphologically intermediate individuals were interpreted and reported as hybrids between *A. pardalis* and other species of the same complex, in Tunisia (Blanc & Cariou, 1981; Blanc & Cariou, 1987).

Fonseca *et al*. (2009) refer that concerning the *erythrurus* group, although *A. lineomaculatus* and *A. e. atlanticus* are genetically distinct, previous morphological analyses suggested that they may hybridize. These putative cases of hybridization were not verified through molecular methods and the uncertainties persist.

Even being a significant improvement from morphological analyses alone or allozyme studies, the only molecular phylogeny of genus *Acanthodactylus* to date provided incomplete data. The study reflected only maternal inheritance, and covered only 15 species, being 14 of them represented only by one individual (Harris & Arnold 2000; Fig. 4); besides lacking the inclusion of the remaining species, such poor sampling precludes the detection of cryptic diversity. Fieldwork presents issues in many of the countries where these organisms occur, but further sampling is needed, as well as more robust analyses including nuclear markers. Despite the limitations in applying molecular tools for species delimitation in the precedent decades, the current accessibility of such methods should make their use mandatory to clarify the species boundaries and relationships within the genus.

1.3.1. The *Acanthodactylus scutellatus* species-complex

The species grouped in the *Acanthodactylus scutellatus* complex are almost all found in sandy habitats, differing in their specific habitat preferences, which can go from sand banks on rocky substratum to moving dunes. In what regards geography, they exhibit a wide range (Fig 5.), from the Sahara in the west to the Northern Arabian Peninsula, Israel and Iraq in the east (Crochet *et al*. 2003).

Although the limits of the *scutellatus* group are consensual, no single revision agrees on the taxonomy of its taxa. In 1921, Boulenger considered merely the species *Acanthodactylus scutellatus*, with some “varieties” within it. From those varieties, Bons and Girot (1964) designated four species: *A. dumerili, A. inornatus, A. longipes, and A. scutellatus*. Four non-equivalent species were defined later by Salvador (1982): *A. dumerili* (lumping populations of *A. dumerili* and *A. inornatus)*, *A. longipes*, *A. scutellatus*, and *A. aureus* (previously considered a subspecies of *A. inornatus)*. He also synonymised *audouini* with *scutellatus*, and divided *A. scutellatus* into two subspecies *A. s. hardyi* and *A. s. scutellatus*. Considering the high morphological variability within *A. scutellatus* and *A. dumerili*, Arnold (1983) doubted the validity of *A. dumerili*, and suggested a different approach: the lumping of *A. dumerili*, *A. inornatus* and *A. scutellatus* into *A. scutellatus scutellatus*. Besides this, he agreed with the recognition of *A. longipes,* and *A. aureus,* and the synonymy of *audouini* with *scutellatus*.

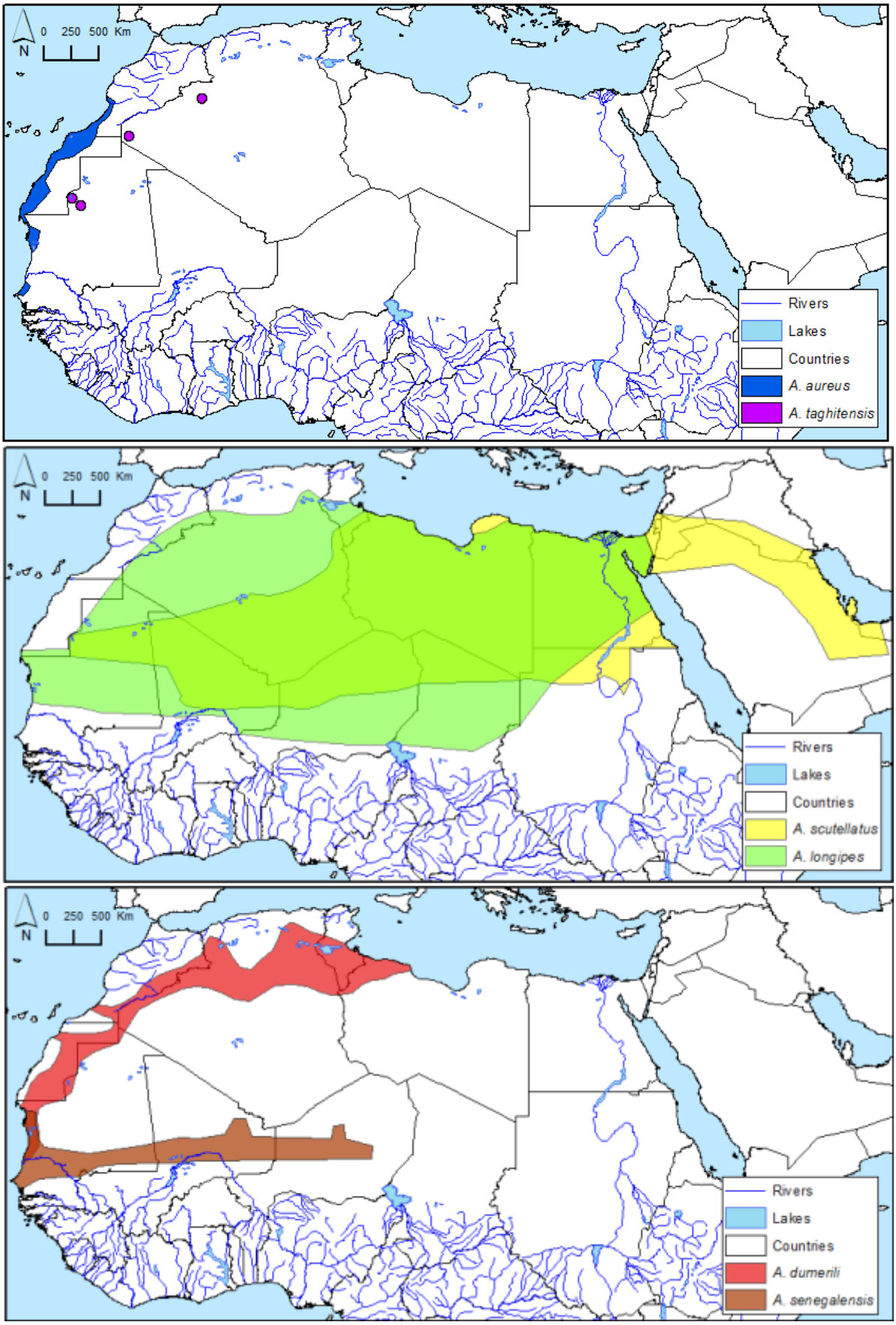

Fig. 5 – Known distribution in North Africa of the species included in the *Acanthodactylus scutellatus* group. Adapted from Crochet et al. (2003).

Overall, some authors agreed on the recognition of particular taxa, but were unable to reach a consensus about others, especially about what to call to populations of a particular geographical area. One of the major reasons for the lack of agreement is related to the number of species for the populations named *A. scutellatus* by Arnold, and their names. And despite the apparent agreement on the existence of a group called *A. longipes,* there was some controversy about its validity, with populations apparently difficult to distinguish from *A. dumerili* and *A. scutellatus* using morphological data (Mellado & Olmedo 1990). This disagreement is well illustrated by the detection of *A. longipes* in Egypt by Baha El Din (1994), classified before as *A. scutellatus*. This author later suggested splitting it into two species, describing the eastern Egyptian populations as a new species, *A. aegyptius* (Baha El Din 2007). Finally, Geniez and Foucart (1995) claimed finding a new species for the complex in Algeria, described as *A. taghitensis*. While still poorly known, its presence was already detected in several localities, including in non-coastal areas of Mauritania. This group appears closely-related to *A. aureus*, and to occur in allopatry with this species (Geniez & Lluch 2004).

More recently, the observation of *A. scutellatus audouini* in northern Mauritania added *A. scutellatus* to the list of species known to occur in this country, which is the richest in what concerns the diversity of species of the *scutellatus* complex (Geniez & Lluch 2004). Crochet *et al*. (2003), based on large sampling and a robust multivariate morphological analysis, suggested that several forms occur in sympatry in multiple locations of that country, and recognized six biological species for the complex: *A. aureus*, *A. dumerili*, *A. longipes*, *A. scutellatus*, *A. senegalensis,* and *A. taghitensis* (Fig. 5). The species *A. aureus* exhibits a set of morphologically original traits, such as the number of supralabial scales and their position regarding the suboculars, appearing to be the most distinctive form within the *scutellatus* group (Crochet *et al*. 2003). In the partial mitochondrial phylogeny of the genus, *A. aureus* was supported as a different species from *A. scutellatus*, and considerable differentiation was suggested between *A. scutellatus* and *A. longipes* (Harris & Arnold 2000).

The distributions in this complex are essentially allopatric at a small scale, with one species being replaced by another as the habitat changes. In the areas where *A. dumerili* and *A. senegalensis* coexist, *A. dumerili* occupies the coastal white dunes, which tend to be avoided by *A. senegalensis,* which prefers the red continental dunes (Crochet *et al*. 2003). While *A. aureus* occurs mostly along the Atlantic coast on littoral dunes, among other sandy grounds, *A. taghitensis* is found farther from the coast and seems to prefer areas interchanging rocky grounds with partially sandy ones. Although most of their distribution overlaps, *A. longipes* occurs typically in large dune massifs and *A. scutellatus* in the flatter areas (Crochet *et al*. 2003; Trape *et al* 2012).

The life-traits of the *scutellatus* species group, such as the high population density, fast sexual maturation, diet, and short life-cycle (Duvdevani and Borut, 1974; Cissé *et al*. 1977; Cissé and Karns 1978; Mellado & Olmedo 1990; Schleich 1996), can potentially promote rapid changes in demography and size of the distributional area. They are prone to quick cycles of expansions and contractions, causing complex patterns of gene flow, and thus may form complex hybrid zones. However, there is still too scarce knowledge about this matter. Arnold (1983) and Salvador (1982) argued about two species occurring in the coastal region of Mauritania, respectively: *A. aureus* and *A. dumerili*, and *A. aureus* and *A. scutellatus*. Intermediate specimens were claimed to exist between *A. dumerili dumerili* and *A. dumerili exiguus* of Salvador. This was later refuted by Crochet *et al*. (2003), whose results showed that the traits are not truly intermediate, and argued that since *A. d. exiguus* is sympatric with and morphologically different from *A. d. dumerili sensu* Salvador, different names should be applied. The form with intermediate specimens retained the name of *A. dumerili*, while the other was renamed *A. senegalensis*.

In Mauritania, where multiple of the described species coexist in sympatry, individuals with intermediate morphology were observed. They could merely reflect high morphological diversity within these taxa, but it is also possible that these individuals are hybrids. Molecular studies are essential to distinguish between the two hypotheses and provide a correct insight (Crochet *et al*. 2003). After decades of taxonomic disagreements, controversial synonymies and unclear species boundaries, the unreliability of conclusions solely based on species distributions and morphology is undeniable. Therefore a molecular phylogeny for the species group is needed. Furthermore, the assessment of gene flow in areas of sympatry would be critical for a better understanding of the species boundaries, and elucidate the cases of putative hybridization.

1.4. Molecular markers and evolutionary studies

Genetic approaches offer significant insights for evolutionary studies, which prosper from the remarkable progress on molecular markers (Wan *et al*. 2004). Every marker should be chosen objectively based on the questions one need to answer (Schlotterer 2004), taking into account the potential biological implications of each marker and the techniques to obtain them. Markers differ in the frequency and type of errors they exhibit in the analyses, statistical independence, information content and dominance relationships (Vignal *et al*. 2002). Marker development is generally needed for non-model organisms, with the cost biasing the marker choice in most studies (Schlotterer 2004).

DNA sequencing grants complete information about the focused region (Schlotterer 2004). Phylogenetic and phylogeographic studies relied for decades mostly on mitochondrial DNA (mtDNA); easy amplification, maternal inheritance and absence of recombination led to its popularity to answer evolutionary questions (Ballard & Whitlock 2004). The mtDNA evolves about 5–10 times faster than single-copy nuclear DNA (scnDNA), but still offers access to a distant range, on the order of potentially millions of years (Wan *et al*. 2004). But since many processes act on the genome, sequences from different regions may exhibit distinct genealogical histories. Precise and robust analyses require the combination of the information from multiple loci (Selkoe & Toonen 2006). Nuclear and organellar markers differ strongly in the way they evolve, in addition to the differences in inheritance, which may be reflected in discordant or at least different evolutionary histories (Sunnucks 2000). Disagreements between organellar and nuclear markers can also be due to sampling error, introgression and incomplete lineage sorting (Ballard & Whitlock 2004). The fact that different markers underlie distinct mutation rates can be an advantage, because they provide information from distinct timescales (Crandall *et al*. 2000). The scnDNA shows the slowest evolutionary rate. Both mtDNA and scnDNA encompass historical genetic variation, being suitable to resolve taxonomic uncertainties (Wan *et al*. 2004).

Microsatellites, also known as short tandem repeats (STR), are iterations of 1-6 nucleotides motifs, with a length varying usually between five and 40 repeat units (Selkoe & Toonen 2006). They are found in every organism, typically at high frequencies, and are a great portion of the noncoding DNA. As STRs present high mutation rates, with an average of $5 \cdot 10^{4}$ per locus per generation, they are highly polymorphic, and therefore ideal to studying processes that act on ecological time scales (Schlotterer 2004; Selkoe & Toonen 2006). Variable Number of Tandem Repeats (VNTRs) markers such as microsatellites are indicated for population genetic analyses and one of the best option to infer modern genetic patterns (Wan *et al*. 2004). Inferences from historical to recent events can be achieved when combining data from different categories of molecular markers (Crandall *et al*. 2000).

1.5. Objectives

This study aims to understand the phylogenetic relationships and evolutionary history of the *Acanthodactylus scutellatus* species group in North Africa, and to assess gene flow in a contact zone in the western part of the Sahara desert. Genetic information from multiple time scales was obtained through the combined use of different molecular markers to address two main issues:

1) Phylogenetic analyses allowed determining the main historical lineages for the species complex, and inferring their phylogenetic relationships. More specifically, this aim aims to answer the following questions:
i) Which are the main evolutionary lineages and how are they related?
ii) Are these lineages consistent with the current systematics of the species group?
iii) Is there geographical structure in the genetic diversity?
iv) Is there cryptic diversity?

2) Population analyses allowed estimating the current genetic structure for the species group and test for putative admixture between species. This step addressed the estimation of gene flow across a contact zone between contemporary lineages for the species group in a region of Mauritania. The questions that emerge are:
i) Is there gene flow between main lineages in the contact zone?
ii) If there is gene flow, how can it be characterized? A description would be attempted in terms of its direction, periodicity and magnitude.

The results of the study are expected to increase the understanding about the species boundaries, relationships, history and diversity in the *Acanthodactylus scutellatus* group, in addition to contributing to the knowledge about evolutionary processes shaping the diversity in the Sahara-Sahel.

2. Methods

2.1. Study area and samples

The phylogenetic analyses were conducted in North Africa, covering almost all the range of *Acanthodactylus scutellatus* species complex. Samples were available from Algeria, Egypt, Libya, Mauritania, Morocco, Niger, Senegal, Sudan and Tunisia; and additional samples from Israel and Lebanon were also included (Fig. 6). The population genetics study was conducted on coastal Mauritania, including the PN Banc d'Arguin (PNBA), in Mauritania (Fig. 7). The area includes a contact zone between four of the recognized species (*A. aureus, A. dumerili, A. longipes* and *A.senegalensis*), where morphologically intermediate individuals have been observed (Crochet *et al*. 2003). A total of 859 georeferenced tissue samples from *Acanthodactylus* species were ready available for this study. A total of 803 were collected by CIBIO researchers of the BIODESERTS group during field missions in North Africa between 2003 and 2014 (Annex A2). A total of 56 were acquired from museum collections (Moshen Kalboussi collection, N.H.M. of Creete, Said Larbes collection, University of Barcelona, ZFMK Bonn Collection). Additionally, 16 sequences were granted by Karin Tamar and Salvador Carranza (CSIC-UPF) – see section A4 from Annexes. From the total pool of samples, 485 were selected to be sequenced, in a way that maximized the geographical coverage of parental populations of taxa across North

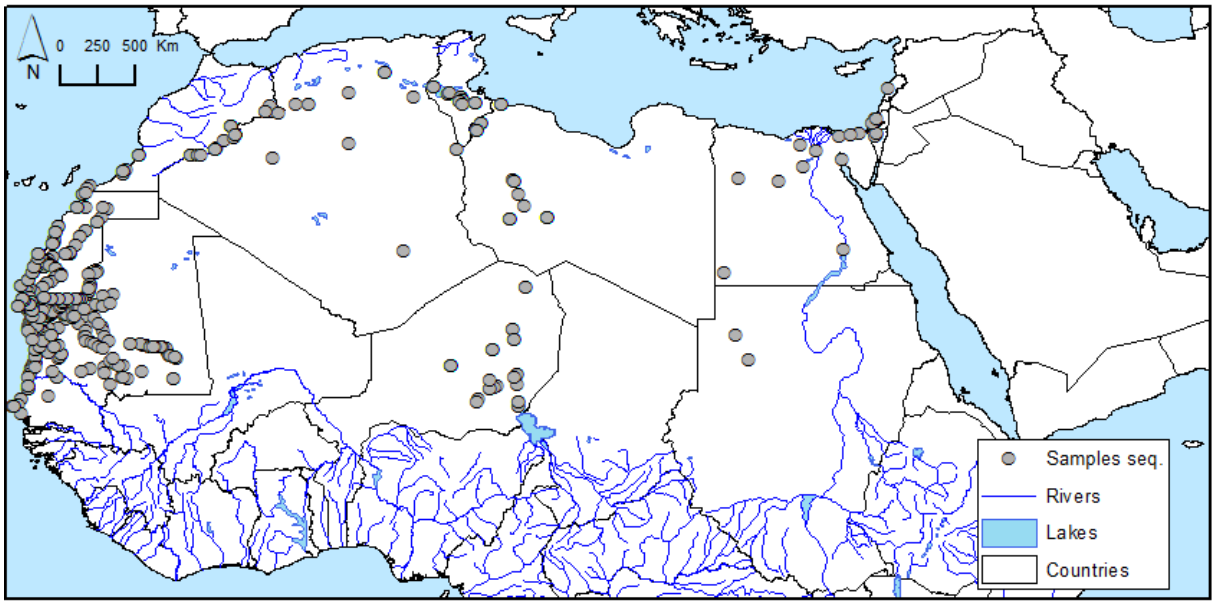

Fig. 6 - Distribution of the sequenced samples of the *Acanthodactylus scutellatus* species complex

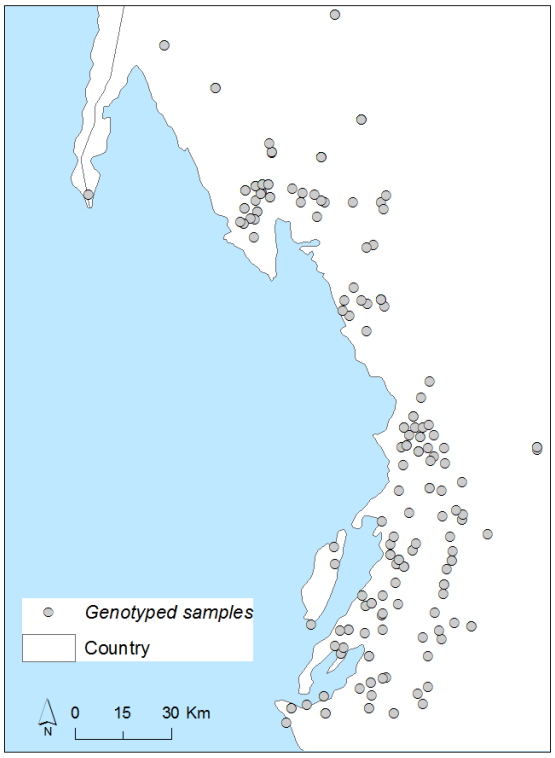

Fig. 7 - Distribution of genotyped individuals in the contact zone in Mauritania

Africa, including at least one sample from each locality across North Africa (Fig. 6), except for the contact zone in Mauritania, for which 210 samples were selected to be sequenced and genotyped (Fig. 7).

A Global Positioning System (GPS) was used to record the location of the samples collected in the field, on the WGS84 datum. Their representation was performed via the Geographical Information System ESRI ® ArcMap 10.0. Information about sample codes, species, countries and localities are in the Annex section A2.

2.2. Laboratory procedures

2.2.1. DNA extraction

Every sample consisted of tail tip tissue. Total genomic DNA extractions were performed using QIAGEN's EasySpin Kit, following an adapted protocol for tissue samples (available in Annex A1).

The success of DNA extractions was evaluated by electrophoresis on 0.8% agarose gels stained with Gel Red, in TBE 0.5x buffer, for about 10 to 15 minutes at 300V. Further visualization through UV radiation was achieved in a Biorad Universal Hood II Quantity One 4.4.0. Low quality and quantity DNA extractions suffered no dilutions for their use in the

subsequent processes. In the case of high success in the extractions, samples were diluted with ultrapure water in an appropriate level. Extractions were kept at -20ºC.

2.2.2. Amplification and sequencing of mtDNA and scnDNA

Among successfully used genes for phylogeny estimation in the genus *Acanthodactylus*, two mitochondrial (12S and Cytochrome-b) and one single copy nuclear (C-mos) gene were chosen to be used for this study. For primers and conditions see the section A6 in the Annexes. Polymerase Chain Reactions (PCRs) were performed in 10µl reaction volumes comprising 5µl of MyTaq (MyTaq™ Mix, Bioline), 3µl of pure water, 0.5µl of both reverse and forward primers, and 1µl of template DNA. All PCRs were executed using a negative control in order to check for contaminations. All PCRs were performed in a Biometra T-professional Thermocycler or a C1000 thermal cycler.

After the amplification, the PCR products were accessed by electrophoresis on 2% agarose gels stained with Gel Red, using a mass DNA ladder (NZYDNA Ladder V), with further conditions being identical to the ones described for the extraction electrophoreses.

All PCR products were cleaned before sequencing, using ExoSap. The purification and sequencing process was executed by a commercial company (Macrogen Inc., Netherlands) using the same primers used in the amplification process.

Sequences were edited and aligned in Geneious Pro 4.8.5 using the Muscle alignment tool, applying the default settings. The final alignments consisted of 756 total bp for the mtDNA (373 bp for 12S and 383 bp for Cyt-b), and 513 bp for the scnDNA (C-mos).

2.2.3. Optimization and microsatellite genotyping

Microsatellite selection

A microsatellite library for the *Acanthodactylus scutellatus* group was developed through high-throughput genomic sequencing technology at GenoScreen (http://www.genoscreen.fr/). The genomic library was constructed from 12 specimens of *A. aureus*, collected along the distribution area. The goal was to design and optimize microsatellite markers that would allow cross-species amplification for the study group. An initial set of 50 markers was chosen from the database considering criteria such as the quality ranking (primers expressed as “best” were preferred), repeat motif (balanced motifs were favoured), as well as estimated size and melting temperatures, in a way that would allow to create five groups of markers for multiplexing (10 markers per multiplex). Markers with larger repeat numbers (usually more variable) and larger motifs (tri and

tetranucleotides) were preferred; dinucleotides were avoided because they can be more problematic during the scoring process (they are prone to more stuttering). Multiplexes were designed by grouping in the same multiplex markers with comparable melting temperatures of the reverse primers. Marker choice and assignment to multiplexes was done so that minimum intervals of about 50-70 bp existed between loci labelled with the same dye colour, within a multiplex. AUTODIMER (Vallone & Butler 2004) was run to screen for intramolecular hairpins structures and primer dimers within multiplexes, to avoid high complementarity that would interfere with the amplification success in the multiplexes. These precautions and some others listed below are necessary for applying efficiently a multiplexing approach - amplifying in the same PCR reaction more than one pair of primers (Markoulatos *et al*., 2002).

Microsatellite optimization

Seven samples were selected, representative of the diversity of the sample pool in terms of morphology and geography (including individuals assigned morphologically to distinct species, and from different localities), in order to proceed to laboratory tests for the microsatellite optimization. Using the same samples across this phase allowed a robust evaluation of the adjustments done to primer concentrations or to the PCR programs. The choice of extension and annealing conditions in the programs attempted a balance between high amplification success of the target loci, and yielding low amount of unspecific product. A touchdown PCR approach was applied to cover the range of annealing temperatures in the multiplexes.

Uniplex PCRs were performed to exclude multiple low-quality markers in early phases of the testing process, and for a better understanding of marker's individual amplification success, preferred conditions, and profile. A negative control was used in every reaction, and the reaction volumes of approximately 10µl included: 5µl of Master Mix (Taq PCR Master Mix, QIAGEN), 2.5µl of pure water, 0.5µl of primer forward, 0.5µl of primer reverse, 0.5µl of fluorescent tail, and 1µl of template DNA.

Primer concentrations began as equimolar as possible, and successive tests were done in order to make the necessary adjustments to concentrations and temperatures during PCRs. Primer concentrations within a multiplex were optimized to be adequate for the amplification success of each loci, and for the strength of their assigned fluorescent dye (NED and PET are weaker than FAM and VIC), while trying to keep them as balanced as possible. Concentration needs to be increased in some loci due to preferential amplification of others; loci amplifying very easily had their concentration lowered. A trade-off was also

attempted between concentration and primer-dimer formation; raising primer concentration much above template concentration results in more abundant primer dimer (Markoulatos *et al*. 2002). PCR temperatures were also raised or lowered to obtain the best amplification success for each multiplex. The needed modifications were made after consecutively evaluating the performance in the test samples, until a good overall amplification and genotyping success became possible for the loci of each multiplex. During this process, other candidate loci ended up being discarded and some were shifted to other multiplexes, or had their label changed to improve efficiency. The main reasons for exclusion were weak amplification, unsuitability for cross-species amplification, lack of polymorphism, and unreadable profile. If a marker had to be excluded from its multiplex, but would fit in another and amplify well enough with its respective conditions, the marker was shifted to that other multiplex. Such shifts allowed avoiding the exclusion of functional loci and beneficiated performance.

Genotyping

Multiplex organization for the post-optimization set of 30 markers, primers concentrations and amplification conditions are summarized in the Annexes (A7 and A8). The following description of the genotyping methods regards all multiplex PCRs performed, including the ones performed during the optimization phase.

A total of 18 *A. aureus*, 115 *A. dumerili* A, and 77 *A. longipes* were genotyped. All PCRs were performed in approximately 10µl reaction volumes, including: 5µl of Master Mix, 3µl of pure water, 1µl of primer mix, and 1µl of template DNA. Amplification was performed in Biorad T100 Thermal Cyclers, and a negative control was used in each PCR. The amplification success was assessed with the same procedure explained previously for mtDNA and scnDNA. PCR products were diluted if necessary according to the observed quality in the agarose gels. They were later separated by capillary electrophoresis on an automatic sequencer ABI3130xl Genetic Analyzer (AB Applied Biosystems), using 1µl of amplification product for 10µl of formamide+ 75-400 (-250) LIZ NEW size standard.

Microsatellite reading and scoring was performed in GENEMAPPER v4.0 (Applied Biosystems). Sizing bin windows were manually created, by comparison to the allelic ladder. Automated scoring was combined with manual checking, in order to mitigate the amount of errors. Suspicious genotypes were declared as missing data.

2.3. Data analyses

2.3.1. Phylogenetic analysis

Three outgroup taxa were included in these analyses. One sample of *Acanthodactylus maculatus* and one of *A. erythrurus* were sequenced for each marker while sequence data from *Acanthodactylus boskianus* for the selected markers were obtained from GeneBank (see section A3 of Annexes for the accession numbers).

Data was organized to produce two concatenated datasets, one containing both mitochondrial genes, and the other with the concatenation of the three sequenced genes (12s, cytb and C-mos). The 466 total sequences were collapsed into haplotypes using the online software FABOX (Villesen 2007) before the assessment the phylogenetic inferences, creating datasets of 321 sequences.

The best-fitting model of nucleotide substitutions for each mtDNA and nDNA fragments was determined with jMODELTEST v.2.1.5 (Guindon & Gascuel 2003; Darriba *et al*. 2012), using the Akaike Information Criterion (AIC). The models indicated as best were TPM2uf+I+G for 12S and C-mos, and TrN+I+G for Cyt-b.

Bayesian analysis was run in BEAST v.1.7.5 (Drummond *et al*. 2012) with unlinked substitution models for each gene. Strict and relaxed clocks, and a coalescence constant size and Yule models were used as tree priors. Three independent MCMC runs of 100 million generations were implemented, sampling every 10,000 generations and 20% of the trees were discarded as burn-in. The convergence of chains and ESSs for all parameters was verified using TRACER v.1.6. (Effective sample sizes (ESSs) higher than 300 for all parameters). Log and tree files of the three independent runs were combined using the software LOGCOMBINER v.1.7.5, and then the subsequent maximum clade credibility summary tree with posterior probabilities for each node was obtained using TREEANNOTATOR V.1.7.5. The resulting tree was visualized and edited with FigTree v.1.4.1 (http://tree.bio.ed.ac.uk/software/figtree).

The software TCS v.1.2.1 (Clement *et al*. 2000) was used to create the mitochondrial haplotype network. Sequences with a significant number of undefined states (the ones for which at least one of the genes was lacking in the concatenated alignment) were excluded. As a result, the network was calculated with a total of 414 sequences. The network was edited manually. Calculations of sequence divergence were performed in MEGA v.6 (Tamura *et al*. 2013) based on Kimura-2 parameter model.

Samples were preliminarily assigned to the putative species based on morphology. Later, given the results obtained in these phylogenetic analyses, the assignment was

compared to the revealed clades. Based on this comparison, and considering information about type localities, the clades were assigned to species.

2.3.2. Population genetic analysis

Samples and loci presenting at least 40% of missing data were discarded from the analysis. The final dataset included 18 samples from *A. aureus*, 114 from *A. dumerili* A, and 76 from *A. longipes*. MICROCHECKER v.2.2.3 (Oosterhout *et al*. 2004) was used for the identification of potential genotyping errors, using the Bonferroni correction to infer confidence intervals. Tests of departures from Hardy-Weinberg Equilibrium (HWE) and assessment of Linkage Disequilibrium (LD) were computed in ARLEQUIN v.3.11 (Excoffier and Lischer 2010). The sampling was continuous across the contact zone and there were no isolated sampling locations neither known geographical populations to consider. Defining populations based on genetic clusters within species was also not possible since no strong signal of structure existed within each species in the contact zone. Therefore, lineages were divided in artificial populations defined based on the samples distribution. Three loci presented significant deviations and potential null alleles (Ac11, Ac31 and Ac32). It is not clear to whitch extent the null alleles and heterozygote deficiency observed in these result from intrinsic marker properties or are related to population structuring and inadequate division of samples in populations. With this in mind, in order to test the potential influence of these problematic loci in the results, the software STRUCTURE v.2.3.3 (Pritchard *et al*. 2000; Falush *et al*. 2003) was run with and without those loci. Given the mostly similar STRUCTURE results, which were the main analyses to be performed with the microsatellites, the loci were maintained in the final dataset.

The Bayesian Clustering approach implemented in the software package STRUCTURE was used to infer population structure and putative admixture between species. The admixture model was used, assuming correlated allele frequencies, for a number of clusters (K) = 1-6. Five repetitions of MCMC run of 800 000 iterations following a burn-in of 200 000 steps were performed. STRUCTURE HARVESTER v.0.6.94 (Earl & VonHoldt 2011) was used to determine the probability of each (K) and select the most likely number of clusters, based on the mean values of likelihood [L(K)] and statistic Delta K (Evanno method; Evanno *et al*. 2005). A Principal Coordinate Analysis (PCA) was performed in GENEALEX v.6.5 (Peakall & Smouse, 2012), using individual-by-individual genetic

distances. The same software allowed calculating pairwise F-statistics between the identified clusters.

3. Results

3.1. Laboratory overview

The amplification success includes 433 samples for Cyt-b, 452 for 12S and 441 for C-mos. Samples from museums had overall lower quality than samples collected in the field.

Regarding the microsatellites, more markers passed the screening process than the ones included in the final dataset. The markers that passed the major filtering process were organized in the four multiplexes described in appendix A8, however, only those that could be amplified for all species present in the contact zone were included in the dataset for the population analyses (which contained 15 microsatellites after discarding the markers with more than 40% missing data).

3.2. Phylogeny of the *A. scutellatus* complex

The phylogeny inference revealed six main clades, which are designated in Fig. 8 (mtDNA tree) and Fig. 9 (combined mtDNA and nuDNA tree) as 1) *Acanthodactylus taghitensis* (purple); 2) *A. aureus* (blue); 3) *A. longipes* (green); 4) *A. scutellatus* (yellow); 5) *A. dumerili* B (dark red); 6) *A. dumerili* A (red). The taxonomy of each clade was inferred considering the putative assignments of the clades to described species given the morphology (see section A2 to consult the morphological and genetic assignments of each sample included in the trees datasets) and partially based on the inclusion of type locality specimens. Most of the diversity was found in North-West Africa, in particular in Morocco, with samples from five of the six lineages (Fig. 10). There were no major discrepancies between the samples distribution and the known range. Only *A. scutellatus* and *A. longipes* were found in the easternmost regions, such as Libya and Egypt. *A. aureus*, *A. dumerili* and *A. longipes* are lineages distributed also in more southern regions, until the Sahel, while *A. aureus* occured only along the Atlantic coast. The other two lineages were only found inland and the clade *A. longipes* exhibits the widest range.

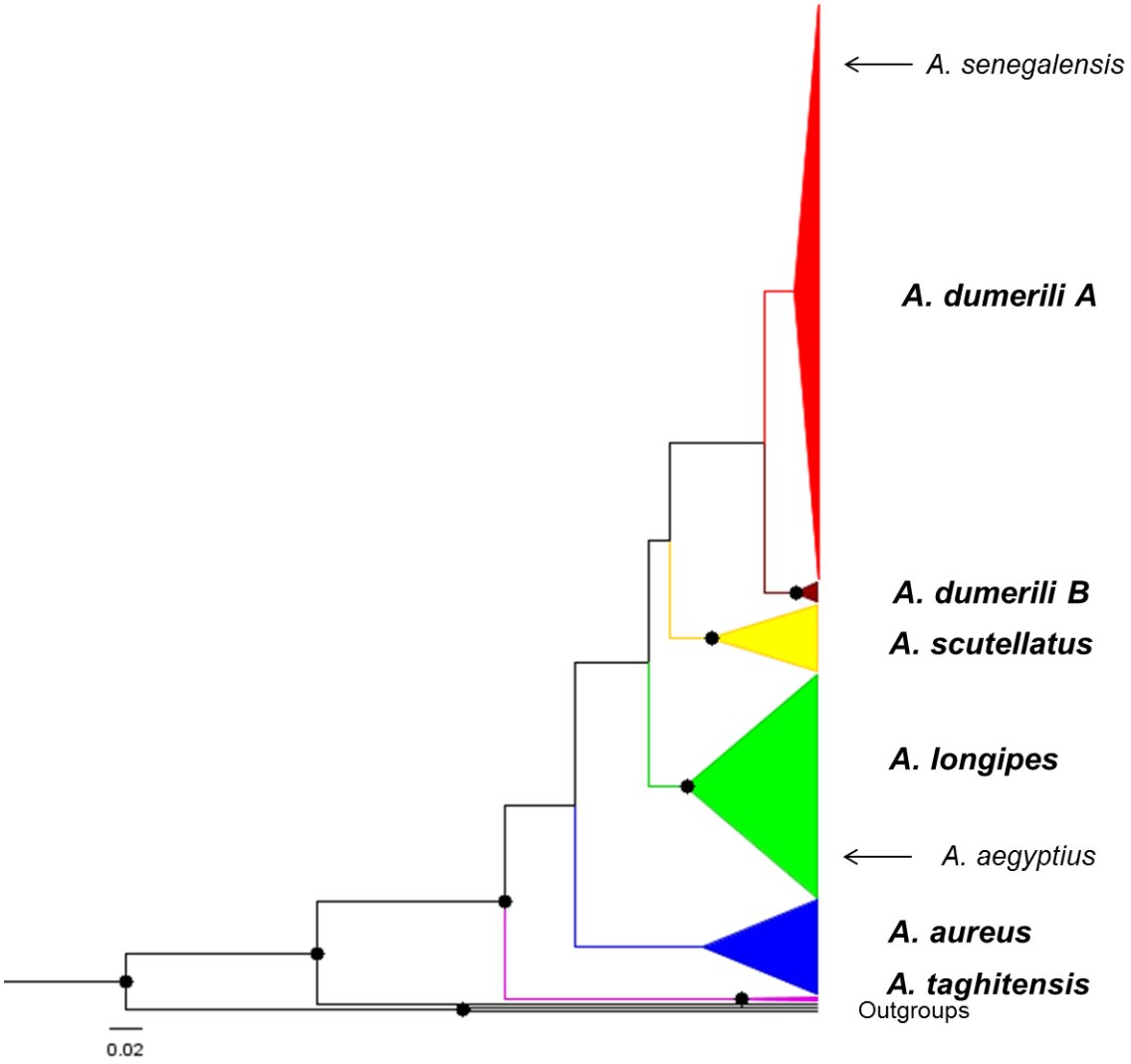

Fig. 8 - Bayesian mitochondrial phylogenetic tree of the *Acanthodactylus scutellatus* species complex based on a concatenated dataset of 756 bp, containing the 12S and Cyt-b genes. *A. erythrurus, A. maculatus* and *A. boskianus* were used as outgroups. The nodes with a Bayesian Posterior Probability (BPP) over 95% are highlighted with a black dot. The scale bar indicates 2% sequence divergence. Each clade/lineage referred in the main text is highlighted with a different colour: 1) *A. taghitensis* (purple); 2) *A. aureus* (blue); 3) *A. longipes* (green); 4) *A. scutellatus* (yellow) 5) *A. dumerili* B (dark red); 6) *A. dumerili* A (red). Arrows indicate the location in the tree of the samples assigned to *A. aegyptius* and *A. senegalensis*.

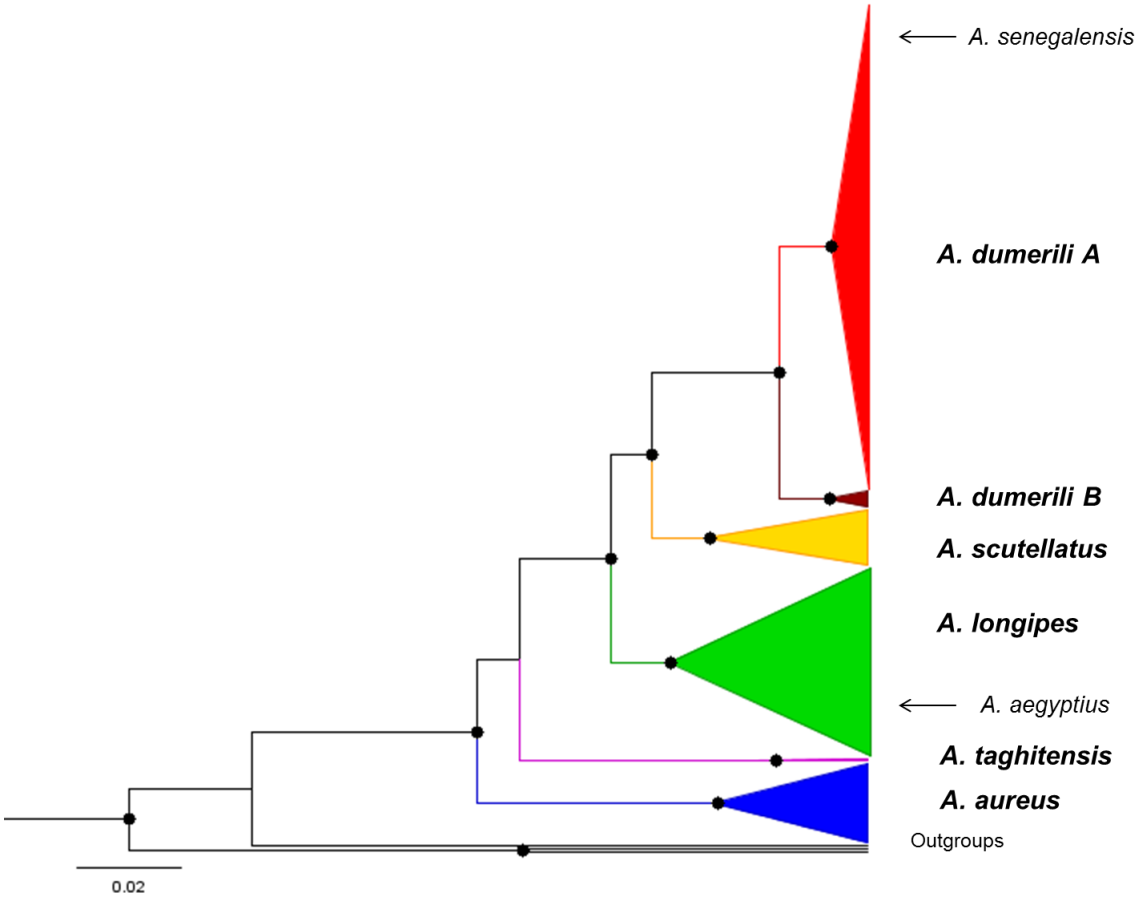

Fig. 9 - Bayesian total phylogenetic tree of the *Acanthodactylus scutellatus* species complex based on a concatenated dataset of 1269 bp, containing the 12S, Cyt-b and C-mos genes. *A. erythrurus, A. maculatus* and *A. boskianus* were used as outgroups. The nodes with a Bayesian Posterior Probability (BPP) over 95% are highlighted with a black dot. The scale bar indicates 2% sequence divergence. Each clade/lineage referred in the main text is highlighted with a different colour: 1) *A. aureus* (blue); 2) *A. taghitensis* (purple); 3) *A. longipes* (green); 4) *A. scutellatus* (yellow) 5) *A. dumerili* B (dark red); 6) *A. dumerili* A (red). Arrows indicate the location in the tree of the samples assigned to *A. aegyptius* and *A. senegalensis*.

The target species constitute monophyletic clades. Both the mitochondrial tree (Fig. 8) and the tree including the nuclear gene (Fig. 9) recovered the same main clades, and the same assignment of samples to these clades. The mt-scnDNA tree yielded supported clades, with small differences in the Bayesian posterior probability values (BPP). The most important difference in topology regarded the position of *A. taghitensis* and *A. aureus*. In the mitochondrial tree, *A. taghitensis* appears as the most basal taxon, followed by a nearly well-supported clade grouping *A. aureus* and the remaining lineages. The mt-scnDNA tree shows *A. aureus* as the most basal clade, followed by a poorly supported clade grouping *A. taghitensis* and the remaining lineages, preventing robust inferences about its exact position based on this tree. Independently of the tree considered, the results indicated *A. aureus* and *A. taghitensis* as the basal clades, but the unclear support prevents saying with confidence which of them is more basal. *Acanthodactylus longipes* and *A. scutellatus* are

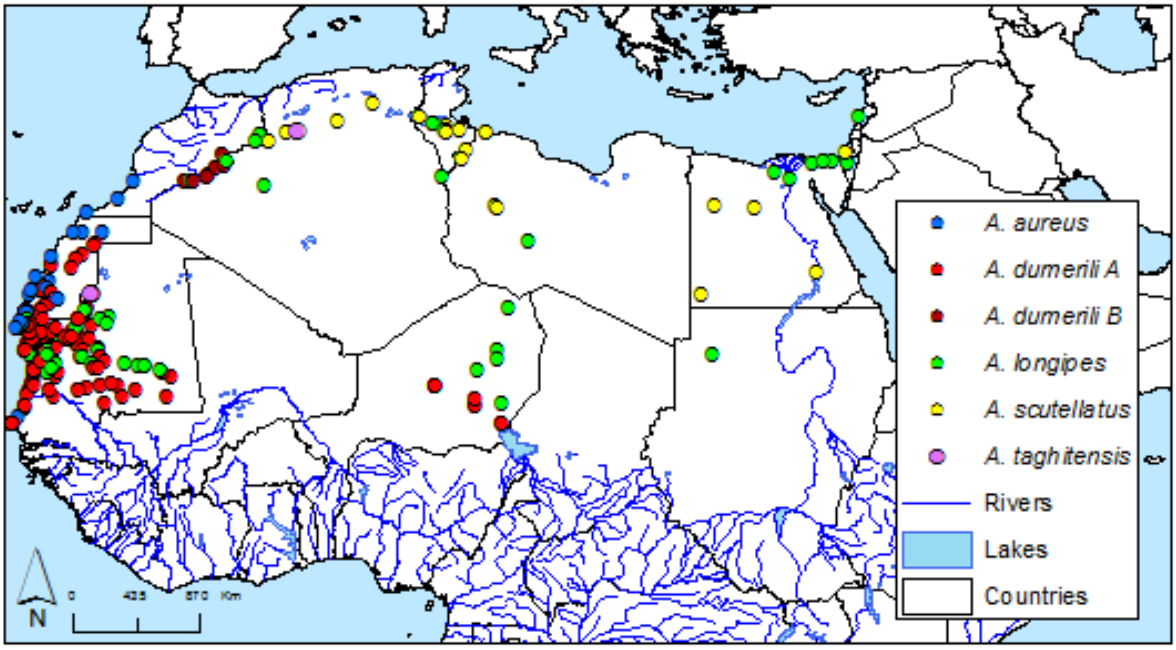

Fig. 10 - Distribution of lineages of the *Acanthodactylus scutellatus* species complex in North Africa, based on the phylogenetic results. Each clade/lineage referred in the main text is highlighted with a different colour: 1) *A. taghitensis* (purple); 2) *A. aureus* (blue); 3) *A. longipes* (green); 4) *A. scutellatus* (yellow) 5) *A. dumerili* B (dark red); 6) *A. dumerili* A (red).

supported in both trees, but only the mt-nuDNA tree shows support for their relationship; they appear in that tree as closely related, although not sister taxa. *A. dumerili* splits into two well supported sublineages: *A. dumerili* A and *A. dumerili* B, which are the clades that emerged more recently. *A. dumerili* B, apparently restricted to Morocco, is an undescribed lineage revealed among what was considered *A. dumerili* or *A. senegalensis*.

The previously suggested distinctness of *A. taghitensis* and *A. aureus* was supported by the phylogenetic results. *A. taghitensis* and *A. aureus* appeared in the trees as monophyletic, each of them exhibiting high divergence from the other main lineages. Based on the assignments done to the samples used, *A. aegyptius* was not revealed here as genetically different from *A. longipes,* exhibiting a small divergence from the other sublineages of this large supported clade. *A. dumerili* and *A. senegalensis* are not genetically differentiable in the trees. The samples from the type localities of *A. dumerili* and *A. senegalensis* are contained within *A. dumerili* A, with low divergence and lack of support regarding their separation within this lineage. Additionally, the genetic distances calculated between the main clades exhibited in most cases high values, confirming their distinctiveness (Table 1). *A. taghitensis* was the more distinct, with values ranging from 13.6-15.2%. The lower genetic divergence occurred between *A. dumerili* A and *A. dumerili* B, with 4.5%.

In what concerns allopatry of the main clades, *A. dumerili* A occurs in southern Morocco, while *A. dumerili* B can be found in northern areas, along the border between Morocco and Algeria. The remaining patterns are related to genetic structure within clades

and are better illustrated by the haplotype network (Fig. 11). The inferred mtDNA network shows a neat separation of the same main lineages obtained in the trees, with an evident geographical pattern in the genetic variation (see Annex A9 to consult the assignment of samples to haplotypes and haplogroups). No haplotypes are shared between the main clades, neither between very distant geographic regions within each clade (Fig. 12). A total of 172 haplotypes were recovered. Within the main lineages, the major haplogroups tended to be allopatric. Such pattern is very evident within *A. aureus*, *A. scutellatus*, and *A. longipes*. For *A. aureus*, one of the haplogroups (haplogroup E) include samples from Southern Morocco and Northern Mauritania, without haplotypes shared between Morocco and Mauritania, and genetic structure was present among the 10 Moroccan haplotypes. Haplogroups A, B and C are distributed in this order from North to South Morocco along the coast, while haplogroup D and the haplotype 6 can be found inland, in the central region of the country. The networks showed *A. dumerili* A as the most diverse lineage (102 haplotypes), contrasting with *A. longipes*, for which only 33 haplotypes were found despite this group having the widest distribution. Haplogroups in *A. longipes* (F, G, H and I) are clearly allopatric; four of the isolated haplotypes can be found in allopatry in Morocco, and the fifth in Libya. The pattern of allopatry is also visible in *A. scutellatus*. No great structuration was observed within *A. dumerili* B (haplogroup M), but such is expected since only four haplotypes restricted to a very small geographical area represented this lineage. Within *A. dumerili* A, the haplogroup N contains most haplotypes, being distributed from southern Morocco, a large portion of Mauritania, and Northern Senegal, until Niger, with the major separation regarding the haplotypes from Niger. Another group of haplotypes from Mauritania was found in *A. dumerili* A (haplogroup O), showing geographical overlap with haplogroup N (Fig. 12). The haplotypes representing in the network the type localities of *A. dumerili* (haplotype 117) and *A. senegalensis* (haplotypes 130 and 131) present some separation, but are contained within the same haplogroup (N). Finally, it was not possible to make inferences for *A. taghitensis* (haplotype 1) since from the few localities where the species is known, only one is covered in the haplotypes available in the network dataset (the other locality included in our sampling was not included in the network due to missing data in one of the genes).

Table 1 - Mean genetic distances (uncorrected p-distances) between the six evolutionary clades of the *A. scutellatus* group revealed in the phylogenetic analyses, based on a mitochondrial dataset (12S and Cyt-b) of 756 bp. Standard error estimates are shown above the diagonal.

	Taxon	1	2	3	4	5	6
1	*A. taghitensis*		0.013	0.014	0.014	0.013	0.014
2	*A. aureus*	0.136		0.013	0.012	0.013	0.013
3	*A. longipes*	0.148	0.141		0.010	0.011	0.010
4	*A. scutellatus*	0.152	0.131	0.101		0.009	0.010
5	*A. dumerili* B	0.141	0.130	0.111	0.089		0.007
6	*A. dumerili* A	0.144	0.111	0.107	0.094	0.045	

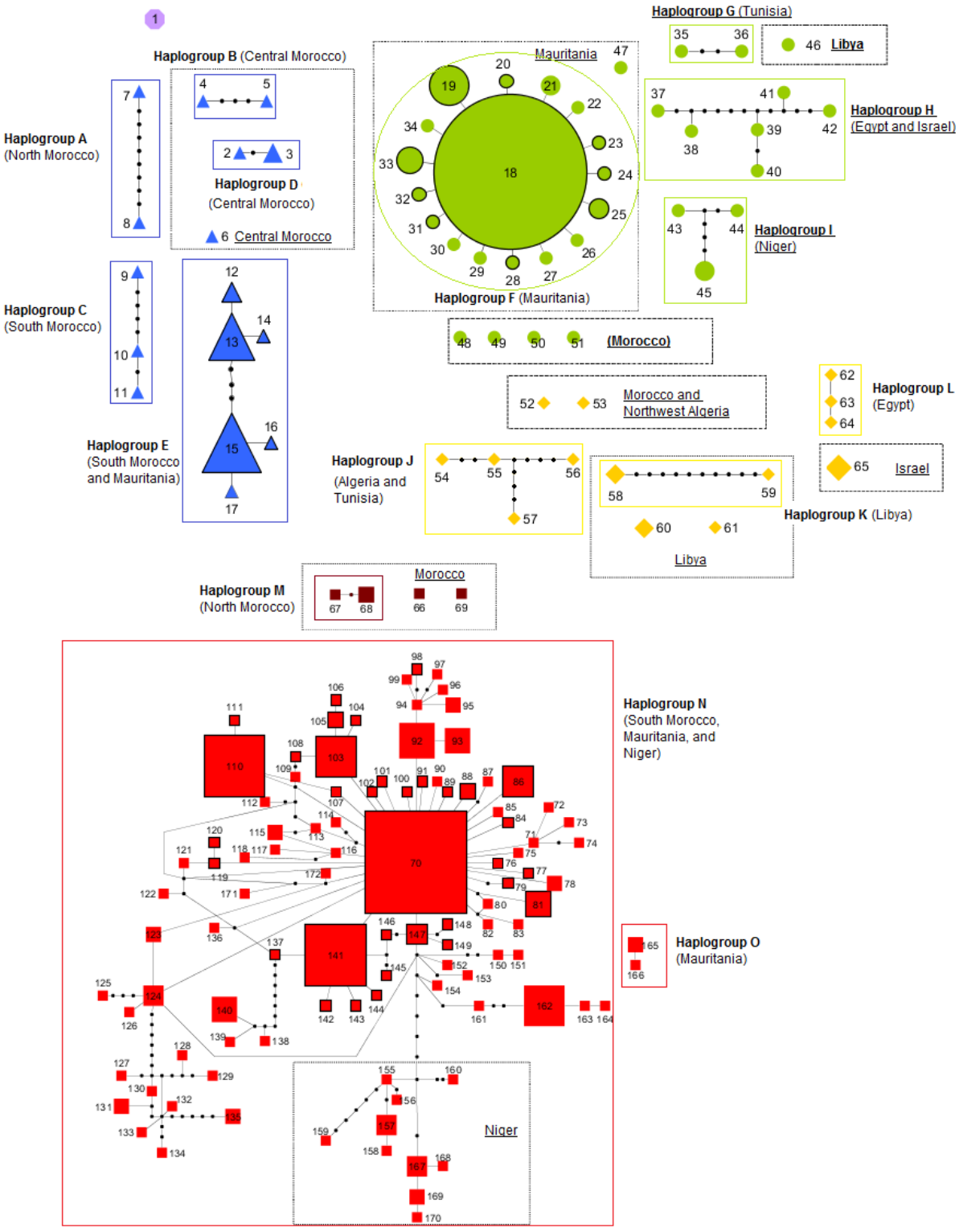

Fig. 11 - TCS mitochondrial haplotype network of the *Acanthodactylus scutellatus* species complex, assuming a 95% parsimony threshold, based on a concatenated mitochondrial dataset of 756 bp (12S and Cyt-b genes) and 414 sequences. Each symbol represents a different haplotype. Symbol sizes are roughly proportional to haplotype frequency. Small black circles dividing single lines are inferred haplotypes. Haplotypes from the contact zone are highlighted with a black border. Each clade/lineage referred in the main text is highlighted with a different colour: 1) *A. taghitensis* (purple); 2) *A. aureus* (blue); 3) *A. longipes* (green); 4) *A. scutellatus* (yellow) 5) *A. dumerili* B (dark red); 6) *A. dumerili* A (red).

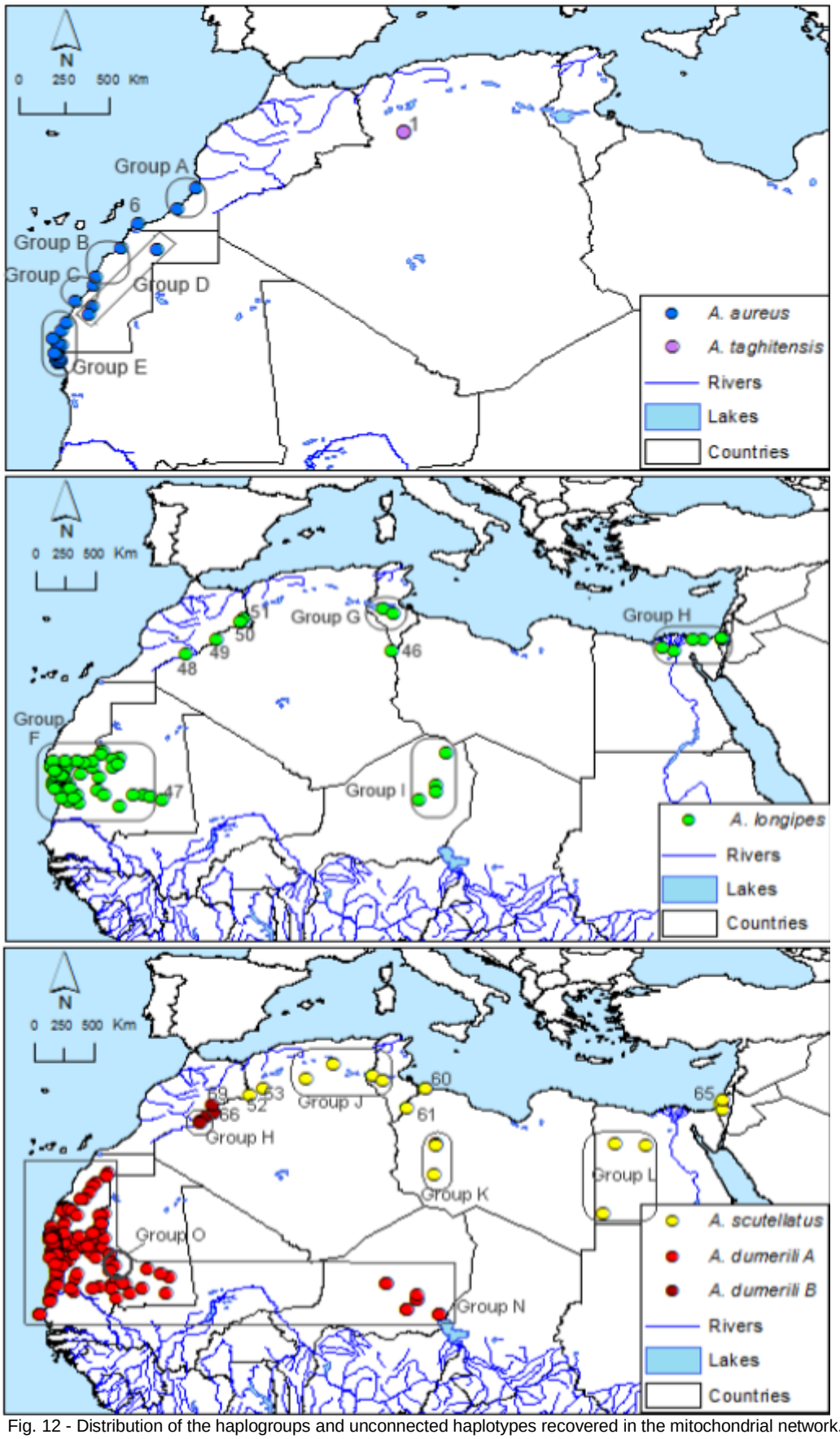

Fig. 12 - Distribution of the haplogroups and unconnected haplotypes recovered in the mitochondrial network. Group's names and numbers of ungrouped haplotypes are represented.

3.3. Population analyses

The population analyses were conducted for the three main lineages obtained in the phylogenetic analyses that are present in the contact zone under study: 1) *A. dumerili* A; 2) *A. longipes,* and 3) *A. aureus* (Fig. 13). The optimal number of clusters indicated by Delta K and L (K) methods was K=3 (Fig. 14 A). As shown in the STRUCTURE bar plot (Fig. 14 B), the detected genetic clusters were completely concordant with the lineages and the individual assignments recovered in the phylogenetic analyses, using mitochondrial and nuclear genes. The separation between each cluster is clear, with an average proportion of membership of Q_1=99.4% for *A. aureus* (blue), Q_2=97.1% for *A. dumerili* A (red), and Q_3=94.0% for *A. longipes* (green). Individual proportions of membership can be consulted in Annex A5. One individual belonging to *A. dumerili* A (sample 6463) must be highlighted, since it exhibited 71.3% of assignment to its respective cluster and 28.3% to *A. aureus*. However, the 90% probability interval for this individual is very large, ranging from 0.53 to 0.90 in the assignment to *A. dumerili* A, and from about 0.10 to 0.46 in the assignment to *A. aureus*. The PCA (Fig. 15) supports the STRUCTURE result, showing a clear separation of the same three clusters, in this case without suspicious individuals.

The neat separation of the groups was also noticeable when considering the pairwise F_{ST} values (Table 2), which, following the author's guidelines (Wright 1978), indicate great genetic differentiation.

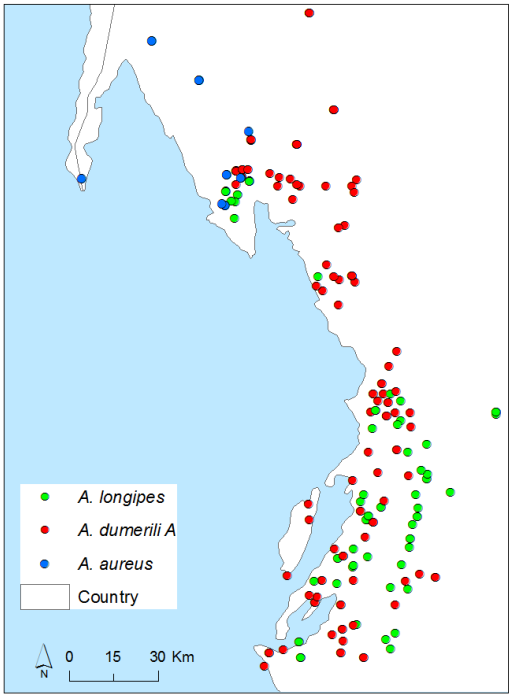

Fig. 13 - Distribution of samples according to the STRUCTURE results. Each cluster is highlighted with a different colour: 1) *A. aureus* (blue); 2) *A. longipes* (green); 3) *A. dumerili* A (red).

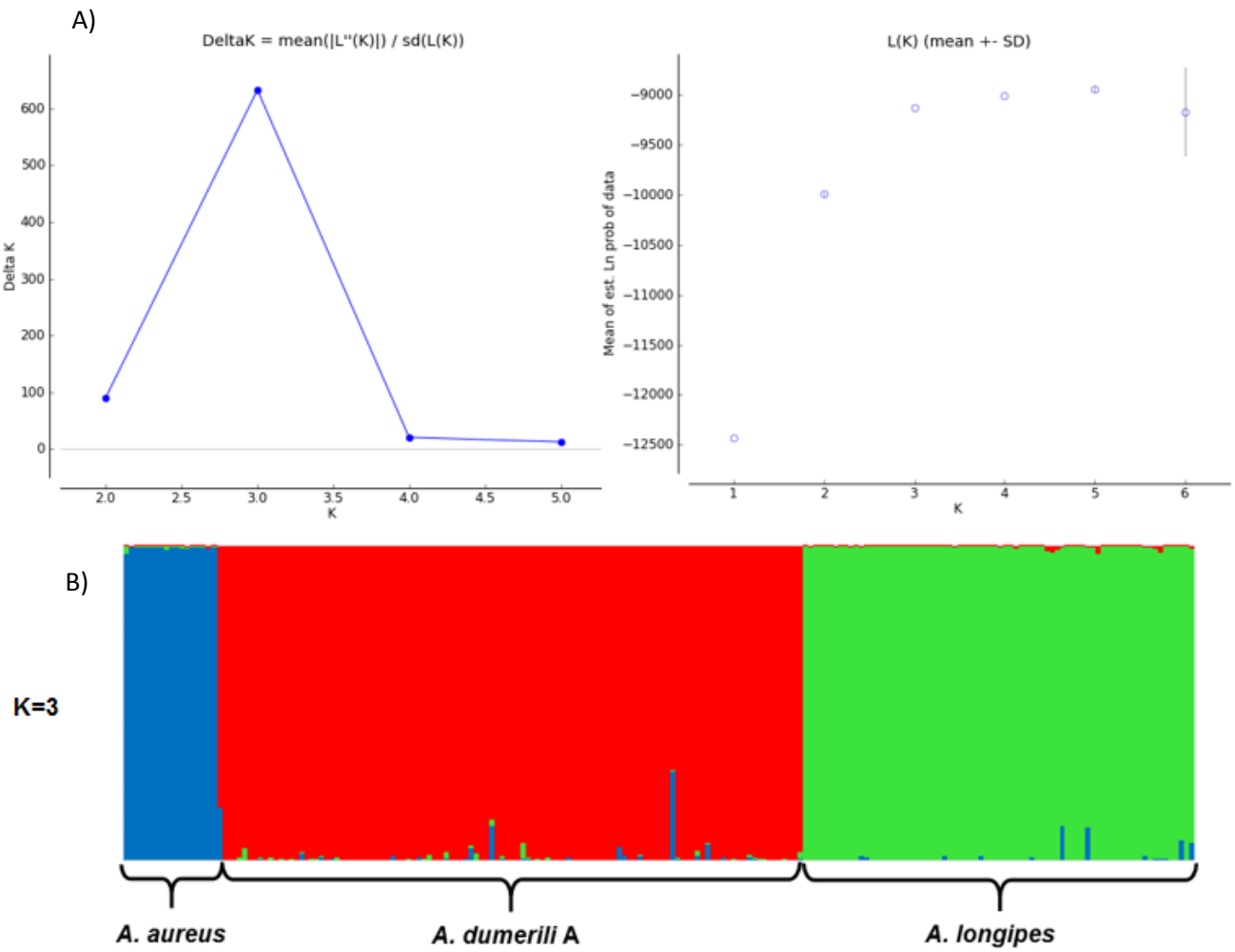

Fig. 14 – A) STRUCTURE HARVESTER graphic outputs of Delta K and Mean L(K), and B) STRUCTURE bar plot showing the assignment of 208 genotyped individuals of the contact zone for the optimal number of clusters (K=3). The analysis was based on 15 microsatellite loci. Each cluster is represented by a different colour: 1) *A. aureus* in blue; 2) *A. dumerili* A in red; 3) *A. longipes* in green. Each vertical bar represents an individual.

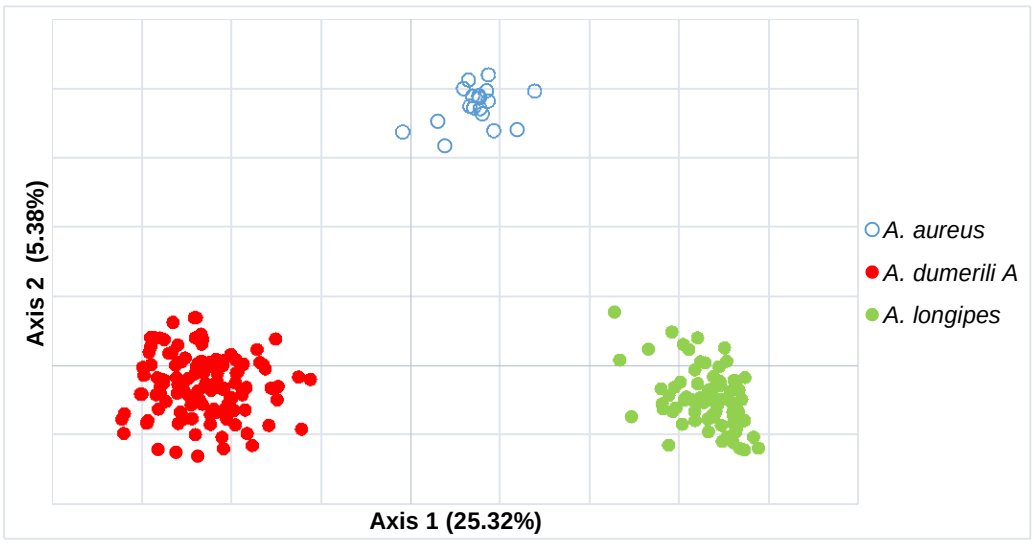

Fig. 15 - PCA output graph based on individual-by-individual genetic distances, for 208 genotyped samples of the contact zone and dataset of 15 microsatellite loci. Each point represents an individual, and each lineage is represented by a different colour: 1) *A. aureus* in blue; 2) *A. dumerili* A in red; 3) *A. longipes* in green.

Table 2: Pairwise Fst values of the three identified genetic clusters of the contact zone

Cluster 1	**Cluster 2**	**F_{ST}**
A. aureus	*A. dumerili* A	0.275
A. aureus	*A. longipes*	0.248
A. dumerili A	*A. longipes*	0.326

4. Discussion

4.4.1. Phylogenetic relationships and taxonomic implications

The inferred phylogeny recovers as main clades some of the currently described species but do not support others, showing the systematics of the *Acanthodactylus scutellatus* group needs revision. There were frequent inconsistencies between the main clades found and the putative assignments of the specimens they contain. The trees and networks illustrate well most of the conflicts in the literature, in particular the difficulty in defining *A. scutellatus*, and the distinct views over the identities of *A. dumerili*, *A. scutellatus* and *A. longipes* (Salvador 1982; Arnold 1983; Mellado & Olmedo 1990; Crochet *et al*. 2003).

The Bayesian trees show that *A. aureus* and *A. taghitensis* are evolutionarily close, but not sister taxa. The lack of a fully resolved mt-nuDNA phylogenetic tree impedes an accurate assessment of the phylogenetic position of *A. taghitensis* and *A. aureus*, which might be related to incomplete lineage sorting processes. Both *A. taghitensis* and *A. aureus* appear as the two most basal lineages among the extant clades of the *A. scutellatus* complex. Moreover, these two species are the only cases where the genetic results agree completely with the previous morphological studies (Salvador 1982; Arnold 1983; Mellado & Olmedo 1990; Geniez & Foucart 1995; Crochet *et al*. 2003). Considering that *A. aureus* and *A. taghitensis* are monophyletic, well supported, and both morphologically and genetically distinct, their species status is corroborated. There were no doubts in the preliminary species assignments based on morphology for all *A. taghitensis,* and most *A. aureus*. The only *A. aureus* samples assigned with less certainty, including one individual that appeared to be intermediate with *A. taghitensis,* belong to the haplogroup D (Fig. 11). Contrary to all the other *A. aureus* haplotypes, which occur on the Atlantic coast, the haplotypes contained in this haplogroup are the only ones found farthest from the coast in Morocco. Given the possibly peculiar morphology and exceptional distribution of the individuals contained in this haplogroup, further research would be recommended to try to assess the extent of its distinctiveness.

Acanthodactylus longipes and *A. scutellatus* are genetically distinct (10.1% of sequence divergence; Table 1) and constitute two valid species, but genetic lineages found in this work and phenotypic assignment during fieldwork do not perfectly match in these two species. A well supported clade designated as *A. longipes* shows a clearly distinct evolutionary lineage, but also includes samples that were putatively assigned to more than one of the recognized species. Most samples were assigned to *A. longipes* without uncertainty, however, some other individuals were also assigned to *A. scutellatus*. There were doubts in the identification of what is morphologically considered *A. scutellatus*.

These discrepancies seem to be caused by the unreliability of external morphology for species identification, rather than by paraphyly of the species as they are currently described. A few of the misidentified samples from the contact zone were confirmed to be misidentifications with the microsatellites (supporting their genetic assignment based on the trees). Molecular data should be always obtained in future studies concerning populations of these species, in order to proceed to an objective and correct identification of the specimens. Additionally, the molecular results of this study do not support *A. aegyptius* as a different species (Baha El Din 2007). The specimens identified under this name were not evolutionarily differentiated from *A. longipes*. Despite such lack of divergence, the suggested morphological and ecological distinctiveness for the populations named *A. aegyptius* can indicate processes of local adaptation, maybe related to the vegetation cover (Baha El Din 2007).

Acanthodactylus dumerili and *A. senegalensis* are in the need of taxonomic revision, since there is no significant divergence between them to consider them as valid species. This lack of significant differentiation seems to justify the difficulties in the assignment based on morphological characters. For instance, a small number of samples within *A. dumerili* A were assigned to *A. longipes*. *Acanthodactylus dumerili* and *A. senegalensis* are not recovered as evolutionary differentiated in the trees. And although some differentiation can be observed in the network between the haplotypes from the type localities of these two recognized species, that differentiation is low, since they are included in the same haplogroup. Additional data based on a nuclear network would allow confirming these patterns. Besides the taxonomic implication of this discrepancy between morphology and molecular data, there are consequences regarding other matters. In regions where *A. dumerili* and *A. senegalensis* where claimed to co-exist, diversity is reduced by one species, as these two are lumped together. Moreover, the observation of individuals with intermediate morphology and consequent suspects of hybridization (Crochet *et al*. 2003) are a result of *A. dumerili* and *A. senegalensis* in fact not being distinct, merely reflecting high morphological diversity. The distinct morphologies are real, but they occur at the population level and not between different evolutionary lineages. Evidently, this lumping has also an effect on what becomes the distribution of the species. The consequences for the contact zone focused in this study are that instead of the four distinct evolutionary lineages initially expected to occur there, only three remained, and the need to check for hybridization between *A. dumerili* and *A. senegalensis* ceases to exist. Also, further research is needed concerning the systematics of the other "*A. dumerili*" found (*A. dumerili* B). Considering the distance between *A. dumerili* A and B (4.5%) and their allopatric distribution, they may be considered different subspecies, but a nuclear network is needed to help with these

taxonomic inferences. Further and integrative research is required for this clade to be properly described.

The network was useful to show the high coherence between the distribution of diversity and geographical features. The only haplogroups found in sympatry were the two haplogroups from *A. dumerili A* (N and O). One important aspect to consider regarding the size of the haplotypes is that our sampling was biased towards Mauritania, and the genetic diversity outside Mauritania might have been underestimated. Haplogroup N, the more diverse, includes mostly samples from Mauritania. Within this haplogroup, the observable separation of the haplotypes from Niger is coherent with their large geographical distance from the remaining haplotypes. Large geographical distance between different haplogroups is also observable (within *A. longipes and A. scutellatus*). The same cannot be said about *A. aureus* or *A. dumerili* B since they have much smaller distributions, but despite smaller distances between haplotypes, these are nevertheless structured according to geography.

Considering the high diversity observed in North-West Africa, the origin of the *A. scutellatus* species complex has probably occurred in this region. The mountain chains in this region may have played a role by acting as refugia in periods of climatic instability and facilitating isolation (e.g., Barata *et al*. 2012). The clades *A. dumerili* A and *A. dumerili* B seem to reflect a North-South vicariance, apparently separated by the valley of the As Saquia al-Hamra (southern Morocco). This landscape feature may be presently acting as a major geographical barrier between these forms. However, further fieldwork is needed to confirm the pattern of allopatry observed in this study.

4.4.2. Sampling gaps

Despite the work done in this study, there is a lack of knowledge about the recovered evolutionary lineages, mostly in what concerns diversity and distributional range. There may be an overestimation of the species distribution in the currently available distribution maps (Fig. 5). These reflect the extent of species occurrence, which may not correspond to the effective area of occupancy. Distinguishing between these two aspects of the distributions in the species under study is highly relevant. Countries such as Mali, Algeria, Libya, Chad and Niger are strikingly undersampled, and should be priority targets for future field surveys and increased sampling effort.

The sequences obtained for *A. taghitensis* were insufficient to make further inferences on the distribution of its genetic diversity. More complete molecular data is needed, especially from other localities where it is known to occur but samples were not available. To understand the evolutionary history of this group, it would be important to

understand more in detail the distribution of *A. taghitensis*, which seems to be underestimated. Eastern parts of Western Sahara have been suggested also as priority to look for the species (Geniez & Lluch 2004). Likewise, further work is needed to define more in detail the range of *A. dumerili* B.

Sampling would be very important in the area where *A. scutellatus audouini* was claimed to occur in Mauritania (Geniez & Lluch 2004). The filling of such sampling gaps and molecular identification of specimens can help confirming the presence of the species in certain areas, potentially unravel cryptic or unknown lineages, and provide a better understanding of the distribution of lineages. The samples assigned in this study to *A. scutellatus* cover a much smaller geographic area that what is seen as the known distribution, despite the total sampling covering other areas where it could occur. The discrepancy can be explained by the lack of sampling in other regions, or it could indicate a more restricted distribution for the group. Further studies are needed to clarify the subject.

4.3. Hybridization assessment

Surprisingly, the results present a scenario without gene flow. There is one individual with a suspiciously lower than average proportion of membership, but given the large confidence interval, it cannot be pointed out as hybrid. The difficulty in the assignment for this individual could be due to an effect of missing data, although information is lacking for only three loci of the 15, and the same amount of missing data for other specimens did not yield problems in their assignments. This absence of gene flow means that the interpretation in the field of some individuals as possible hybrids results from the already discussed unreliability of external morphological characters for discriminating between those species. This result highlights the importance of using molecular methods when dealing with the lizards of the *A. scutellatus* species complex.

The absence of gene flow in sympatric conditions has important consequences regarding systematics. Our results suggest that the three lineages found in the target contact zone can be considered “good species” also according to the biological species concept (Butlin *et al*. 2012). They maintain their genetic distinctiveness even in sympatric areas where gene flow is expected to occur, if nothing prevented it. This result was maintained across the large sample numbers and the large geographical area covered, and was confirmed with different types of molecular markers. Future assessments with nuclear networks should be performed for additional support. Sequencing results did not suggest historical hybridization events between the considered units, and the contemporary scale reached with microsatellites supports the lack of gene flow among the three species.

The lack of gene flow in the contact zone raises questions about the mechanisms involved to prevent it. A first possibility implies the presence of reproductive barriers between species due to pre- or post-zygotic isolating mechanisms (Slatkin 1987; Sobel *et al*. 2010; Butlin, R. *et al*. 2012; Abbott *et al*. 2013). There could be pre-mating barriers causing assortative mating (Sobel *et al*. 2010), such as recognition of conspecific males or courtship behaviour, although such behaviour appears absent in the case of *Acanthodactylus* species (Harris & Arnold 2000). Incompatibility in genitalia would serve also as pre-zygotic barrier (Masly 2011), but since there is the possibility of homoplasy, study of genitalia of the *Acanthodactylus* species should be analysed in detail to test this hypothesis. Hemipenial structures were already indicated as complex in *Acanthodactylus* and having maybe the potential to promote very fast evolution (Arnold 1983). However, there are no studies focusing the hemipenial differences within the *A. scutellatus* species group in particular. Gametic incompatibilities would prevent zygote formation, but not mating (Butlin *al*. 2012). Alternatively, the gene flow barrier can be a post-zygotic one; in this case, interspecific individuals would be able to mate, but hybrids would be unviable or have very low fitness (Sobel *et al*. 2010). Hybrid sterility is highly improbable as only mechanism, because hybrids should be detected. The unviable hybrid could occur in the embryonic stage (Lu & Bernatchez 1998). Eventually, if given low hybrid fitness in the parental habitats (Seehausen 2004), the individuals could be born but have such high mortality that they would not reach a juvenile or adult stage. Finally, lack of hybridization might be related with habitat selection. Ecological segregation between lineages has been suggested (Crochet *et al*., 2003) and ecological models in coastal Mauritania suggested very distinct relationships between lineage presence and environmental variation (Sow *et al*. 2014). Strong habitat selection would act as an environmental barrier (Shurtliff *et al*. 2013; Tarroso *et al*. 2014). Ecological niche modelling would be an appropriate tool to study the patterns of habitat selection. Overall, the range of possible explanations for the absence of gene flow is large, and the true scenario probably implies a combination of reproductive isolation and habitat selection.

5. Concluding remarks and future research

This study is the first to assess gene flow at a contemporary scale in dry-adapted Saharan herpetofauna. It is also one of the few to date that checked for hybridization with molecular tools in a contact zone of the Sahara-Sahel, and the first to do so in a contact zone of *Acanthodactylus* species. Further work is needed to fight the taxonomic and geographical biases of studies reaching modern patterns in the Sahara-Sahel.

This study provides for the first time microsatellite markers developed for the *Acanthodactylus* genus. The developed and optimized microsatellites allow future studies on population structure, gene flow, and demographic patterns in this group.

Sampling gaps need to be filled, particularly to better investigate the biogeographic history of the genus and to unveil phylogeographic patterns of each species. Sampling some geographical areas will require great effort, but deeper understanding about the biodiversity of the focused organisms, and associated history and processes, will not be achieved unless this effort is taken. Although useful insights were obtained, the knowledge for now is insufficient for a complete appreciation of the biodiversity and evolution of the species group.

Ecological and ecophysiological studies need to be combined with genetic data to obtain deeper understanding of habitat selection patterns. In the future, ecological modelling can bring understanding about the patterns and evolution of the *scutellatus* species group. Morphological studies focusing hemipenial morphology among the genetic lineages of this species group will be essential to study the potential role of incompatibilities in genitalia acting as reproductive barrier.

The present study contributed to an improved knowledge about the dry-adapted *Acanthodactylus scutellatus* species group and evolutionary processes shaping the diversity in the Sahara-Sahel. Hopefully, future research will shed more light on the diversity and evolutionary history of this group of organisms.

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Annexes

A1)

Adapted EasySpin protocol of the Genomic DNA Microplate Tissue Kit # SP-DT

1. Cut up 30mg tissue and place in Deep Well collection Plate.
2. Add 300µl of ACL solution (Animal Cell Lysis solution) to Deep Well Collection Plate, and 20 µl Proteinase K, then seal.
3. Incubate at 55ºC until the tissue is completely lyzed (usually 1-3 hours). Occasionally vortex. Incubation in shaking water bath can reduce lysis time.
4. Cool to room temperature. Vortex for 20 seconds and centrifuge 14000 rpm for 5 minutes.
5. Pipette 300µl of supernatant into an EasySpin 96-Well plate (if pellet not visible, repeat previous step) and add 300µl of AB solution. Seal, mix by occasionally inverting plate and keep for 2 minutes.
6. Centrifuge at 4000 rpm for 2 minutes with a rotor for microtiter plates. Discard the flow-through.
7. Add 500µl Wash solution to each well of 96-Well Plate and spin at 8000 rpm for 1 minute.
8. Discard flow-through and place EasySpin 96-Well Plate back to the same Deep Well collection plate.
9. Add 500µl Wash solution to each well of the EasySpin 96-Well Plate, spin at 8000 rpm for 1 minute. Discard the flow-through and spin once more at 14000 rpm for 5 minutes to remove residual amount of Wash solution.
10. Transfer the EasySpin 96-Well Plate to a 96-Well storage plate. Add 30-50µl Elution Buffer to the EasySpin 96-Well Plate; incubate at 50º for 10 minutes.
11. Centrifuge at 14000 rpm for 5 minutes.
12. Measure DNA quantity by UV absortion at A260 (1.0 OD unit is equivalent of 50 µg). Assess genomic DNA quality by an analytical agarose gel.

A2)

List of sequenced samples used in the mt and mt-nuDNA concatenated datasets (from the pool of initially selected samples, only the samples that amplified for at least one molecular maker are included here), putative species assignment based on morphology, the local, country and clades they belong to based on the phylogenetic results.

Analysis code	Putative species	Local	Country	Clade
1273	*A. aureus*	Tarfaya	Morocco	*A. aureus*
1474	*A. erythrurus*	Parc National Theniet el Had: Kaf Sahchine	Algeria	*A. erythrurus*
1476	*A. maculatus*	Dar Chioukh - 40 km Nord Est Djelfa : Ain Rouss	Algeria	*A. maculatus*
1637	*A. dumerili/senegalensis*	Zmeila el Beida	Mauritania	*A. dumerili* A
1795	*A. longipes*	Grâret Timinit	Mauritania	*A. longipes*
1881	*A. dumerili/senegalensis*	Terjît	Mauritania	*A. dumerili* A
1926	*A. dumerili/senegalensis*	Aoueloul crater	Mauritania	*A. dumerili* A
1933	*A. sp.*	Oued Oûl Moûssa	Mauritania	*A. dumerili* A
1934	*A. sp.*	Oued Oûl Moûssa	Mauritania	*A. dumerili* A
1935	*A. sp.*	Oued Oûl Moûssa	Mauritania	*A. dumerili* A
1940	*A. longipes*	Léhélim dunes	Mauritania	*A. longipes*
1945	*A. dumerili/senegalensis*	Eydh Kerim	Mauritania	*A. dumerili* A
1978	*A. sp.*	Rachid, 25km SE of	Mauritania	*A. dumerili* A
2068	*A. dumerili/senegalensis*	Tâmchekket, 10km SW of	Mauritania	*A. dumerili* A
2198	*A. sp.*	Gleib Mari Tarecha	Mauritania	*A. dumerili* A
2270	*A. dumerili/senegalensis*	Oumm Mgheirît	Mauritania	*A. dumerili* A
2731	*A. dumerili/senegalensis*	PN Banc d'Arguin, 5km N of Oûdei el Ghassâl	Mauritania	*A. dumerili* A
2734	*A. longipes*	PN Banc d'Arguin, Bguent	Mauritania	*A. longipes*
2736	*A. longipes*	PN Banc d'Arguin, Bguent	Mauritania	*A. longipes*
2743	*A. dumerili/senegalensis*	PN Banc d'Arguin, NE of El Mounâne	Mauritania	*A. dumerili* A
2745	*A. longipes*	PN Banc d'Arguin, 5km E of El Mounâne	Mauritania	*A. longipes*
2746	*A. longipes*	PN Banc d'Arguin, Oued Nouafferd	Mauritania	*A. longipes*
2750	*A. dumerili/senegalensis*	PN Banc d'Arguin, Dló Matai	Mauritania	*A. dumerili* A
2757	*A. dumerili/senegalensis*	PN Banc d'Arguin, dunes NW of N-Tâbiyât	Mauritania	*A. dumerili* A
2758	*A. dumerili/senegalensis*	PN Banc d'Arguin, floodplain SE of Tachekché	Mauritania	*A. dumerili* A
2763	*A. dumerili/senegalensis*	PN Banc d'Arguin, N of Baie d'Arguin	Mauritania	*A. dumerili* A
2766	*A. sp.*	PN Banc d'Arguin, Kerekchet et Teintâne	Mauritania	*A. aureus*
2768	*A. dumerili/senegalensis*	PN Banc d'Arguin, Kerekchet et Teintâne	Mauritania	*A. dumerili* A
2769	*A. dumerili/senegalensis*	PN Banc d'Arguin, Kerekchet et Teintâne	Mauritania	*A. dumerili* A
2777	*A. dumerili/senegalensis*	PN Banc d'Arguin, 5km NW of El Kerekchi well	Mauritania	*A. dumerili* A
2779	*A. dumerili/senegalensis*	PN Banc d'Arguin, 5km NE of El Kerekchi well	Mauritania	*A. dumerili* A
2831	*A. longipes*	Azeffâl dunes, S of Chami	Mauritania	*A. longipes*
2836	*A. dumerili/senegalensis*	Tîgjafât, 5km S of	Mauritania	*A. dumerili* A
2841	*A. longipes*	Akchâr dunes, N of Akjoujt	Mauritania	*A. longipes*

2844	*A. dumerili/senegalensis*	Tiberguent well	Mauritania	*A. dumerili* A
2848	*A. dumerili/senegalensis*	Ain Oumm Rekba	Mauritania	*A. dumerili* A
2851	*A. dumerili/senegalensis*	Oum Khzâma	Mauritania	*A. dumerili* A
2856	*A. dumerili/senegalensis*	Touâma	Mauritania	*A. dumerili* A
2858	*A. dumerili/senegalensis*	Guellâbet 'Alla	Mauritania	*A. dumerili* A
2861	*A. dumerili/senegalensis*	El Mokhanza	Mauritania	*A. dumerili* A
2866	*A. longipes*	Ech Chig	Mauritania	*A. longipes*
2877	*A. dumerili/senegalensis*	Oued Jraif	Mauritania	*A. dumerili* A
2894	*A. longipes*	El Beyyed	Mauritania	*A. longipes*
2933	*A. dumerili/senegalensis*	Jâlet Atîl	Mauritania	*A. dumerili* A
2947	*A. dumerili/senegalensis*	Lekoueirîyât	Mauritania	*A. dumerili* A
2951	*A. dumerili/senegalensis*	Tâmkarkart	Mauritania	*A. dumerili* A
2955	*A. longipes*	Tâmkarkart, extreme SE of	Mauritania	*A. longipes*
2980	*A. longipes*	Tamassoumit, oued 20km E of	Mauritania	*A. longipes*
2992	*A. dumerili/senegalensis*	Inikchâne	Mauritania	*A. dumerili* A
3008	*A. dumerili/senegalensis*	Iguevane II, 20km NW of	Mauritania	*A. dumerili* A
3260	*A. dumerili/senegalensis*	Tarhad Rhiz N'Dégué	Mauritania	*A. dumerili* A
3270	*A. dumerili/senegalensis*	Tarf Mendjoura, dunes on basis of plateaux	Mauritania	*A. dumerili* A
3288	*A. dumerili/senegalensis*	Boumedeit, towards Bou Bleï'Îne	Mauritania	*A. dumerili* A
3314	*A. dumerili/senegalensis*	Oued Guérou	Mauritania	*A. dumerili* A
3458	*A. dumerili/senegalensis*	Gueltet Thor, 10km S of	Mauritania	*A. dumerili* A
3473	*A. dumerili/senegalensis*	Poleti	Mauritania	*A. dumerili* A
3487	*A. dumerili/senegalensis*	El Ferghailiya well, 10km SW of	Mauritania	*A. dumerili* A
3493	*A. dumerili/senegalensis*	Tidemmallîne well, 5km NW of	Mauritania	*A. dumerili* A
3497	*A. longipes*	El Mabroûk well, 3km NE of	Mauritania	*A. longipes*
3503	*A. longipes*	Mednet el Fras	Mauritania	*A. longipes*
3521	*A. longipes*	Bir Boû Khbeira wells, 8km SW of	Mauritania	*A. longipes*
3526	*A. longipes*	PN Banc d'Arguin, Anagoum puit, 2km W of	Mauritania	*A. longipes*
3535	*A. longipes*	PN Banc d'Arguin, Tâfrajoût	Mauritania	*A. longipes*
3537	*A. dumerili/senegalensis*	PN Banc d'Arguin, Tâfrajoût	Mauritania	*A. dumerili* A
3542	*A. longipes*	PN Banc d'Arguin, Oûdei el Ghassâl	Mauritania	*A. longipes*
3553	*A. dumerili/senegalensis*	PN Banc d'Arguin, Tifegtene, 2km SW of	Mauritania	*A. dumerili* A
3557	*A. longipes*	PN Banc d'Arguin, Tifegtene, 2km SW of	Mauritania	*A. longipes*
3558	*A. dumerili/senegalensis*	PN Banc d'Arguin, Nouâmghâr, 15km NE of	Mauritania	*A. dumerili* A
3561	*A. longipes*	PN Banc d'Arguin, Nouâmghâr, 15km E of	Mauritania	*A. longipes*
3562	*A. longipes*	PN Banc d'Arguin, Nouâmghâr, 15km E of	Mauritania	*A. longipes*
3563	*A. longipes*	PN Banc d'Arguin, Nouâmghâr, 15km E of	Mauritania	*A. longipes*
3572	*A. longipes*	PN Banc d'Arguin, Aleib et Talah, 7km W of	Mauritania	*A. longipes*
3575	*A. dumerili/senegalensis*	PN Banc d'Arguin, Aleib et Talah, 12km SW of	Mauritania	*A. dumerili* A
3581	*A. dumerili/senegalensis*	PN Banc d'Arguin, Aghoueinît	Mauritania	*A. dumerili* A
3582	*A. dumerili/senegalensis*	PN Banc d'Arguin, Aghoueinît	Mauritania	*A. dumerili* A
3585	*A. dumerili/senegalensis*	PN Banc d'Arguin, Aghoueinît	Mauritania	*A. dumerili* A

3594	*A. dumerili/senegalensis*	PN Banc d'Arguin, Tidra island	Mauritania	*A. dumerili* A
3599	*A. dumerili/senegalensis*	PN Banc d'Arguin, Teichott, 2km N of	Mauritania	*A. dumerili* A
3607	*A. longipes*	PN Banc d'Arguin, Iouîk, 16km SE of	Mauritania	*A. longipes*
3608	*A. dumerili/senegalensis*	PN Banc d'Arguin, Iouîk, crossroad to	Mauritania	*A. dumerili* A
3615	*A. dumerili/senegalensis*	PN Banc d'Arguin, Râs Tafarît, 16km E of	Mauritania	*A. dumerili* A
3618	*A. dumerili/senegalensis*	PN Banc d'Arguin, Adeim el Marrâr	Mauritania	*A. dumerili* A
3622	*A. dumerili/senegalensis*	PN Banc d'Arguin, Grâret Zra	Mauritania	*A. dumerili* A
4282	*A. dumerili/senegalensis*	Saint-Louis Peninsula	Senegal	*A. dumerili* A
4283	*A. dumerili/senegalensis*	Saint-Louis Peninsula	Senegal	*A. dumerili* A
4284	*A. dumerili/senegalensis*	Dakar	Senegal	*A. dumerili* A
4285	*A. dumerili/senegalensis*	Dakar	Senegal	*A. dumerili* A
4286	*A. dumerili/senegalensis*	Dakar	Senegal	*A. dumerili* A
4441	*A. dumerili/senegalensis*	Oumm el Kcheb	Mauritania	*A. dumerili* A
4448	*A. dumerili/senegalensis*	Aftoût es Sâhel	Mauritania	*A. dumerili* A
4459	*A. dumerili/senegalensis*	Aftoût es Sâhel	Mauritania	*A. dumerili* A
4470	*A. dumerili/senegalensis*	Argoui Tichilit	Mauritania	*A. dumerili* A
4477	*A. dumerili/senegalensis*	Chott Boul, 13km S of	Mauritania	*A. dumerili* A
5002	*A. dumerili/senegalensis*	Nouakchott, 140km NE of	Mauritania	*A. dumerili* A
5004	*A. dumerili/senegalensis*	Akjoujt, 40km SW of	Mauritania	*A. dumerili* A
5019	*A. longipes*	Bennichchâb, 15km NW of	Mauritania	*A. longipes*
5025	*A. longipes*	Agoueilîl el Hâmi	Mauritania	*A. longipes*
5029	*A. dumerili/senegalensis*	Khatt en Negued	Mauritania	*A. dumerili* A
5037	*A. longipes*	Sebkhet Te-n-Dghâmcha	Mauritania	*A. longipes*
5040	*A. longipes*	PN Banc d'Arguin, Goûd Anagoum, 2km NW of	Mauritania	*A. longipes*
5041	*A. longipes*	PN Banc d'Arguin, Goûd Anagoum, 5km NW of	Mauritania	*A. longipes*
5045	*A. longipes*	PN Banc d'Arguin, Goûd Anagoum, 6km NW of	Mauritania	*A. longipes*
5046	*A. dumerili/senegalensis*	PN Banc d'Arguin, Afreighilât 1	Mauritania	*A. dumerili* A
5047	*A. longipes*	PN Banc d'Arguin, Afreighilât 2	Mauritania	*A. longipes*
5049	*A. longipes*	PN Banc d'Arguin, Oûdei el Ghassâl, 1km S of	Mauritania	*A. longipes*
5052	*A. dumerili/senegalensis*	PN Banc d'Arguin, Oûdei el Ghassâl, 1km N of	Mauritania	*A. dumerili* A
5054	*A. longipes*	PN Banc d'Arguin, Aleib et Talah 1	Mauritania	*A. longipes*
5062	*A. longipes*	PN Banc d'Arguin, Mednet ed Dâya 1	Mauritania	*A. longipes*
5069	*A. longipes*	PN Banc d'Arguin, Mednet ed Dâya 3	Mauritania	*A. longipes*
5070	*A. longipes*	PN Banc d'Arguin, Mednet ed Dâya 3	Mauritania	*A. longipes*
5073	*A. longipes*	PN Banc d'Arguin, Azeffâl 1	Mauritania	*A. longipes*
5075	*A. longipes*	PN Banc d'Arguin, Azeffâl 2	Mauritania	*A. longipes*
5076	*A. longipes*	PN Banc d'Arguin, Azeffâl 2	Mauritania	*A. longipes*
5080	*A. longipes*	PN Banc d'Arguin, Azeffâl 3	Mauritania	*A. longipes*
5087	*A. longipes*	PN Banc d'Arguin, Azeffâl, Elb en Nouçç 1	Mauritania	*A. longipes*
5093	*A. longipes*	PN Banc d'Arguin, Azeffâl, Elb en Nouçç, 1km N of	Mauritania	*A. longipes*
5094	*A. longipes*	PN Banc d'Arguin, Azeffâl, Elb en Nouçç, 1km N of	Mauritania	*A. longipes*

5098	*A. longipes*	PN Banc d'Arguin, Azeffâl, Elb en Nouçç, 2km N of	Mauritania	*A. longipes*
5099	*A. longipes*	PN Banc d'Arguin, Azeffâl, Elb en Nouçç, 3km N of	Mauritania	*A. longipes*
5103	*A. dumerili/senegalensis*	PN Banc d'Arguin, Oûl el Qâdi	Mauritania	*A. dumerili* A
5106	*A. longipes*	PN Banc d'Arguin, Taguilâlet Jreik 1	Mauritania	*A. longipes*
5110	*A. longipes*	PN Banc d'Arguin, Gherd Ayyer	Mauritania	*A. longipes*
5111	*A. dumerili/senegalensis*	PN Banc d'Arguin, Agreigrât, 1km E of	Mauritania	*A. dumerili* A
5119	*A. longipes*	PN Banc d'Arguin, Aguilâl, 1km W of	Mauritania	*A. longipes*
5120	*A. longipes*	PN Banc d'Arguin, Aguilâl, 1km W of	Mauritania	*A. dumerili* A
5126	*A. dumerili/senegalensis*	PN Banc d'Arguin, Aguilâl 1	Mauritania	*A. dumerili* A
5135	*A. dumerili/senegalensis*	PN Banc d'Arguin, Aguilâl 4	Mauritania	*A. dumerili* A
5137	*A. longipes*	PN Banc d'Arguin, Oued Nouafferd 1	Mauritania	*A. longipes*
5139	*A. dumerili/senegalensis*	PN Banc d'Arguin, Oued Nouafferd 3	Mauritania	*A. dumerili* A
5153	*A. dumerili/senegalensis*	PN Banc d'Arguin, Ouein Zidîne	Mauritania	*A. dumerili* A
5158	*A. dumerili/senegalensis*	PN Banc d'Arguin, Amgheououas es Sâhli	Mauritania	*A. dumerili* A
5160	*A. dumerili/senegalensis*	PN Banc d'Arguin, Amgheououas es Sâhli	Mauritania	*A. dumerili* A
5162	*A. dumerili/senegalensis*	PN Banc d'Arguin, Amgheououas es Sâhli, 3km NW of	Mauritania	*A. dumerili* A
5163	*A. longipes*	PN Banc d'Arguin, Kerekchet et Teintâne, E side 1	Mauritania	*A. longipes*
5164	*A. longipes*	PN Banc d'Arguin, Kerekchet et Teintâne, E side 1	Mauritania	*A. longipes*
5167	*A. longipes*	PN Banc d'Arguin, Kerekchet et Teintâne, E side 2	Mauritania	*A. longipes*
5168	*A. longipes*	PN Banc d'Arguin, Kerekchet et Teintâne, central 1	Mauritania	*A. longipes*
5171	*A. aureus*	PN Banc d'Arguin, Kerekchet et Teintâne, central 2	Mauritania	*A. aureus*
5172	*A. aureus*	PN Banc d'Arguin, Kerekchet et Teintâne, central 2	Mauritania	*A. aureus*
5173	*A. aureus*	PN Banc d'Arguin, Kerekchet et Teintâne, W side 1	Mauritania	*A. aureus*
5176	*A. aureus*	PN Banc d'Arguin, Kerekchet et Teintâne, W side 3	Mauritania	*A. aureus*
5177	*A. longipes*	PN Banc d'Arguin, Kerekchet et Teintâne, W side 3	Mauritania	*A. longipes*
5181	*A. dumerili/senegalensis*	PN Banc d'Arguin, Kerekchet et Teintâne, central 3	Mauritania	*A. dumerili* A
5192	*A. longipes*	Goût	Mauritania	*A. longipes*
5201	*A. dumerili/senegalensis*	Tijirît 2	Mauritania	*A. dumerili* A
5213	*A. dumerili/senegalensis*	Sebkhet Nich	Mauritania	*A. dumerili* A
5221	*A. dumerili/senegalensis*	Ichkrâne el Khader	Mauritania	*A. dumerili* A
5234	*A. dumerili/senegalensis*	Boû el Gra', 2km W of	Mauritania	*A. dumerili* A
5249	*A. dumerili/senegalensis*	Gleibât Ajayât, 4km NW of	Mauritania	*A. dumerili* A
5258	*A. longipes*	Çtel Ogmâne	Mauritania	*A. longipes*
5267	*A. dumerili/senegalensis*	Çtel Ogmâne, 12km SW of	Mauritania	*A. dumerili* A
5274	*A. longipes*	Ntalfa, 6km SE of	Mauritania	*A. longipes*
5275	*A. dumerili/senegalensis*	Imkebdene, N extreme	Mauritania	*A. dumerili* A
5293	*A. sp.*	PN Banc d'Arguin, Ouein Zidîne	Mauritania	*A. dumerili* A

5294	*A. dumerili/senegalensis*	PN Banc d'Arguin, El Kerekchi, 5km W of	Mauritania	*A. dumerili* A
5298	*A. dumerili/senegalensis*	PN Banc d'Arguin, El Kerekchi, 8km NE of	Mauritania	*A. dumerili* A
5771	*A. dumerili/senegalensis*	Châr, valley	Mauritania	*A. dumerili* A
5789	*A. dumerili/senegalensis*	Châr, 10km N of	Mauritania	*A. dumerili* A
5790	*A. dumerili/senegalensis*	Châr, 10km N of	Mauritania	*A. dumerili* A
5800	*A. dumerili/senegalensis*	Açâbet Châr	Mauritania	*A. dumerili* A
5804	*A. dumerili/senegalensis*	Tâjâlet el Gara	Mauritania	*A. dumerili* A
5805	*A. longipes*	Tâjâlet el Gara	Mauritania	*A. longipes*
5820	*A. dumerili/senegalensis*	Zouérat, 17km NE of	Mauritania	*A. dumerili* A
5823	*A. taghitensis*	Zouérat, 11km NE of	Mauritania	*A. taghitensis*
5827	*A. dumerili/senegalensis*	Guelb Atomai	Mauritania	*A. dumerili* A
5833	*A. dumerili/senegalensis*	Tâjâlet el Gara	Mauritania	*A. dumerili* A
5834	*A. dumerili/senegalensis*	Tâjâlet el Gara	Mauritania	*A. dumerili* A
5846	*A. longipes*	Guelb el Hâlma	Mauritania	*A. longipes*
5883	*A. longipes*	Aderg	Mauritania	*A. longipes*
5919	*A. dumerili/senegalensis*	Nouakchott, 100km NE of	Mauritania	*A. dumerili* A
5923	*A. dumerili/senegalensis*	PNBA: Tidra island, northern half	Mauritania	*A. dumerili* A
5924	*A. dumerili/senegalensis*	PNBA: Tidra island, northern half	Mauritania	*A. dumerili* A
5995	*A. dumerili/senegalensis*	PNBA: Nouâmghâr, 2km E of	Mauritania	*A. dumerili* A
5996	*A. longipes*	PNBA: Nouâmghâr, 2km E of	Mauritania	*A. longipes*
5997	*A. dumerili/senegalensis*	PNBA: Nouâmghâr, 2km E of	Mauritania	*A. dumerili* A
6076	*A. longipes*	Nega pass	Mauritania	*A. longipes*
6275	*A. dumerili/senegalensis*	PNBA: Agneitîr	Mauritania	*A. dumerili* A
6277	*A. dumerili/senegalensis*	PNBA: Agneitîr	Mauritania	*A. dumerili* A
6281	*A. dumerili/senegalensis*	PNBA: Agneitîr	Mauritania	*A. dumerili* A
6282	*A. dumerili/senegalensis*	PNBA: Agneitîr	Mauritania	*A. dumerili* A
6285	*A. dumerili/senegalensis*	PNBA: Agneitîr	Mauritania	*A. dumerili* A
6288	*A. dumerili/senegalensis*	PNBA: Tâfrâjoût, 2km W of	Mauritania	*A. dumerili* A
6289	*A. dumerili/senegalensis*	PNBA: Tâfrâjoût, 2km W of	Mauritania	*A. dumerili* A
6297	*A. dumerili/senegalensis*	PNBA: Tâfrâjoût, 4km W of	Mauritania	*A. dumerili* A
6298	*A. longipes*	PNBA: Chrîfîya	Mauritania	*A. longipes*
6299	*A. dumerili/senegalensis*	PNBA: Chrîfîya	Mauritania	*A. dumerili* A
6301	*A. longipes*	PNBA: Sebkhet Râs el Mâ	Mauritania	*A. longipes*
6302	*A. longipes*	PNBA: Toueigueret, 1km SW of	Mauritania	*A. longipes*
6303	*A. longipes*	PNBA: Toueigueret, 1km SW of	Mauritania	*A. dumerili* A
6306	*A. longipes*	PNBA: Toueigueret, 2km SW of	Mauritania	*A. longipes*
6307	*A. dumerili/senegalensis*	PNBA: Tîla, 4km NW of	Mauritania	*A. dumerili* A
6308	*A. longipes*	PNBA: Tîla	Mauritania	*A. longipes*
6315	*A. dumerili/senegalensis*	PNBA: Kôra, 1km NW of	Mauritania	*A. dumerili* A
6317	*A. longipes*	PNBA: Kôra	Mauritania	*A. longipes*
6318	*A. longipes*	PNBA: Kôra	Mauritania	*A. longipes*
6319	*A. longipes*	PNBA: Ackenjeîl	Mauritania	*A. longipes*
6320	*A. longipes*	PNBA: Ackenjeîl	Mauritania	*A. longipes*
6321	*A. longipes*	PNBA: Ackenjeîl	Mauritania	*A. dumerili* A
6322	*A. longipes*	PNBA: Ackenjeîl, 2km NE of	Mauritania	*A. longipes*

6325	*A. dumerili/senegalensis*	PNBA: Ackenjeîl, 4km NE of	Mauritania	*A. dumerili* A
6329	*A. dumerili/senegalensis*	PNBA: Mdeinet Taouadouaddît	Mauritania	*A. dumerili* A
6330	*A. dumerili/senegalensis*	PNBA: Mdeinet Taouadouaddît	Mauritania	*A. dumerili* A
6331	*A. dumerili/senegalensis*	PNBA: Mdeinet Taouadouaddît	Mauritania	*A. dumerili* A
6339	*A. longipes*	PNBA: Elb en Nouçç, extreme S	Mauritania	*A. longipes*
6340	*A. longipes*	PNBA: Elb en Nouçç, extreme S	Mauritania	*A. longipes*
6347	*A. dumerili/senegalensis*	PNBA: Grâret Agoueifa	Mauritania	*A. dumerili* A
6348	*A. longipes*	PNBA: Grâret Agoueifa	Mauritania	*A. longipes*
6349	*A. longipes*	PNBA: Grâret Agoueifa	Mauritania	*A. longipes*
6352	*A. longipes*	PNBA: Taguîlâlet Jreik	Mauritania	*A. longipes*
6353	*A. longipes*	PNBA: Taguîlâlet Jreik	Mauritania	*A. dumerili* A
6356	*A. longipes*	PNBA: Taguîlâlet Jreik, 1km W of	Mauritania	*A. longipes*
6360	*A. dumerili/senegalensis*	PNBA: Taguîlâlet Jreik, 2km W of	Mauritania	*A. dumerili* A
6363	*A. dumerili/senegalensis*	PNBA: Taguîlâlet Jreik, 2km W of	Mauritania	*A. dumerili* A
6364	*A. dumerili/senegalensis*	PNBA: Taguîlâlet Jreik, 2km W of	Mauritania	*A. dumerili* A
6369	*A. longipes*	PNBA: Adeim el Marrâr, 4km W of	Mauritania	*A. longipes*
6374	*A. longipes*	PNBA: Oued Nouafferd, 2km S of	Mauritania	*A. longipes*
6375	*A. dumerili/senegalensis*	PNBA: Oued Nouafferd, 2km S of	Mauritania	*A. dumerili* A
6376	*A. dumerili/senegalensis*	PNBA: Oued Nouafferd, 2km S of	Mauritania	*A. dumerili* A
6377	*A. dumerili/senegalensis*	PNBA: Oued Nouafferd	Mauritania	*A. dumerili* A
6378	*A. dumerili/senegalensis*	PNBA: Oued Nouafferd	Mauritania	*A. dumerili* A
6383	*A. longipes*	PNBA: Aguilâl	Mauritania	*A. longipes*
6384	*A. dumerili/senegalensis*	PNBA: Aguilâl	Mauritania	*A. dumerili* A
6386	*A. longipes*	PNBA: Aguilâl	Mauritania	*A. longipes*
6390	*A. dumerili/senegalensis*	PNBA: Dlo' Matai	Mauritania	*A. dumerili* A
6391	*A. dumerili/senegalensis*	PNBA: Dlo' Matai	Mauritania	*A. dumerili* A
6394	*A. dumerili/senegalensis*	PNBA: Dlo' Matai	Mauritania	*A. dumerili* A
6405	*A. dumerili/senegalensis*	PNBA: Jerf el Oûstâni, 4km E of	Mauritania	*A. dumerili* A
6410	*A. dumerili/senegalensis*	PNBA: Îmgoûtene, 2km E of	Mauritania	*A. dumerili* A
6411	*A. dumerili/senegalensis*	PNBA: Îmgoûtene, 3km NE of	Mauritania	*A. dumerili* A
6414	*A. longipes*	PNBA: Îmgoûtene, 5km NE of	Mauritania	*A. longipes*
6415	*A. dumerili/senegalensis*	PNBA: Îmgoûtene, 7km NE of	Mauritania	*A. dumerili* A
6416	*A. dumerili/senegalensis*	PNBA: Oued ech Chibka	Mauritania	*A. dumerili* A
6421	*A. dumerili/senegalensis*	PNBA: Ouein Zidîne	Mauritania	*A. dumerili* A
6426	*A. dumerili/senegalensis*	PNBA: Sebkhet Dbâdeb et Teintâne, 8km SE of	Mauritania	*A. dumerili* A
6431	*A. dumerili/senegalensis*	PNBA: Sebkhet Dbâdeb et Teintâne, 4km E of	Mauritania	*A. dumerili* A
6433	*A. dumerili/senegalensis*	PNBA: Sebkhet Dbâdeb et Teintâne, 2km E of	Mauritania	*A. dumerili* A
6435	*A. aureus*	PNBA: Sebkhet Dbâdeb et Teintâne, W margin	Mauritania	*A. aureus*
6436	*A. longipes*	PNBA: Sebkhet Dbâdeb et Teintâne, W margin	Mauritania	*A. longipes*
6438	*A. longipes*	PNBA: Kerekchet et Teintâne, extreme S	Mauritania	*A. longipes*
6445	*A. dumerili/senegalensis*	PNBA: Kerekchet et Teintâne, Western face	Mauritania	*A. dumerili* A
6446	*A. aureus*	PNBA: Kerekchet et Teintâne, Western face	Mauritania	*A. aureus*
6448	*A. aureus*	PNBA: Kerekchet et Teintâne, Western face	Mauritania	*A. aureus*

6449	*A. aureus*	PNBA: Kerekchet et Teintâne, central	Mauritania	*A. aureus*
6450	*A. dumerili/senegalensis*	PNBA: Kerekchet et Teintâne, central	Mauritania	*A. dumerili* A
6451	*A. longipes*	PNBA: Kerekchet et Teintâne, central	Mauritania	*A. longipes*
6452	*A. longipes*	PNBA: Kerekchet et Teintâne, central	Mauritania	*A. longipes*
6453	*A. dumerili/senegalensis*	PNBA: Kerekchet et Teintâne, central	Mauritania	*A. dumerili* A
6456	*A. longipes*	PNBA: Kerekchet et Teintâne, central	Mauritania	*A. dumerili* A
6457	*A. dumerili/senegalensis*	PNBA: Kerekchet et Teintâne, central	Mauritania	*A. dumerili* A
6458	*A. aureus*	PNBA: Kerekchet et Teintâne, central	Mauritania	*A. aureus*
6460	*A. dumerili/senegalensis*	PNBA: Kerekchet et Teintâne, central	Mauritania	*A. dumerili* A
6461	*A. dumerili/senegalensis*	PNBA: Kerekchet et Teintâne, central	Mauritania	*A. dumerili* A
6462	*A. dumerili/senegalensis*	PNBA: Kerekchet et Teintâne, central	Mauritania	*A. dumerili* A
6463	*A. dumerili/senegalensis*	PNBA: Kerekchet et Teintâne, central	Mauritania	*A. dumerili* A
6468	*A. dumerili/senegalensis*	PNBA: Kerekchet et Teintâne, central	Mauritania	*A. dumerili* A
6469	*A. dumerili/senegalensis*	PNBA: Kerekchet et Teintâne, central	Mauritania	*A. dumerili* A
6470	*A. dumerili/senegalensis*	Kerekchet et Teintâne, extreme N	Mauritania	*A. dumerili* A
6473	*A. dumerili/senegalensis*	Kerekchet et Teintâne, extreme N	Mauritania	*A. dumerili* A
6474	*A. dumerili/senegalensis*	Kerekchet et Teintâne, extreme N	Mauritania	*A. dumerili* A
6477	*A. aureus*	Kerekchet et Teintâne, extreme N	Mauritania	*A. aureus*
6660	*A. dumerili/senegalensis*	Nguigmi, 60km N of	Niger	*A. dumerili* A
6661	*A. dumerili/senegalensis*	Nguigmi, 60km N of	Niger	*A. dumerili* A
6664	*A. longipes*	Ngourti, 30km S of	Niger	*A. dumerili* A
6667	*A. dumerili/senegalensis*	Ngourti, 80km N of	Niger	*A. longipes*
6725	*A. dumerili/senegalensis*	Termit, Madaranga	Niger	*A. dumerili* A
6726	*A. dumerili/senegalensis*	Termit, Madaranga	Niger	*A. dumerili* A
6747	*A. dumerili/senegalensis*	Termit, Nourou Nga	Niger	*A. dumerili* A
6764	*A. dumerili/senegalensis*	Tasker, 8km N of	Niger	*A. dumerili* A
6769	*A. dumerili/senegalensis*	Tasker, 8km S of	Niger	*A. dumerili* A
7245	*A. aureus/taghitensis*	Laasallien	Morocco	*A. aureus*
7254	*A. dumerili/senegalensis*	Tiglalatin, W of	Morocco	*A. dumerili* A
7255	*A. dumerili/senegalensis*	Legcheinat	Morocco	*A. dumerili* A
7272	*A. dumerili/senegalensis*	Oued Awletiss, SE of	Morocco	*A. dumerili* A
7276	*A. dumerili/senegalensis*	Oued Zbayra	Morocco	*A. dumerili* A
7282	*A. dumerili/senegalensis*	Oued Clayina	Morocco	*A. dumerili* A
7293	*A. dumerili/senegalensis*	Oued Tindkine	Morocco	*A. dumerili* A
7301	*A. dumerili/senegalensis*	Raim Ould Ad-Dlile	Morocco	*A. dumerili* A
7308	*A. aureus*	Raglamhoun	Morocco	*A. aureus*
7325	*A. dumerili/senegalensis*	Khanfrat Al Faj	Morocco	*A. dumerili* A
7334	*A. dumerili/senegalensis*	Hfor An-Naçrani, W of	Morocco	*A. dumerili* A
7339	*A. aureus*	Oued Hawl	Morocco	*A. aureus*
7347	*A. dumerili/senegalensis*	Oued Hawl	Morocco	*A. dumerili* A
8252	*A. dumerili/senegalensis*	Amerdoul Bou Tazoult	Morocco	*A. dumerili* B

8254	*A. dumerili/senegalensis*	Talmaidert	Morocco	*A. dumerili* B
8255	*A. dumerili/senegalensis*	Taouz, W of	Morocco	*A. dumerili* B
8402	*A. dumerili/senegalensis*	Boukra, 40km NW of	Morocco	*A. aureus*
8990	*A. dumerili/senegalensis*	Chott Tigri, 12km W of	Morocco	*A. longipes*
8992	*A. dumerili/senegalensis*	Oued Es Safsaf, dunes above dam	Morocco	*A. scutellatus*
8998	*A. dumerili/senegalensis*	Bouarfa, 20km of; towards Mengoub (N10)	Morocco	*A. longipes*
9019	*A. dumerili/senegalensis*	Smara, 21km W of; Laayoune-Smara (N5)	Morocco	*A. aureus*
9108	*A. aureus*	Chtoukane	Morocco	*A. aureus*
9135	*A. aureus*	Atf	Morocco	*A. aureus*
9139	*A. aureus*	Oum Al Fissan	Morocco	*A. aureus*
9141	*A. dumerili/senegalensis*	Graret Ouchfegt	Morocco	*A. dumerili* A
9142	*A. dumerili/senegalensis*	Graret Ouchfegt	Morocco	*A. dumerili* A
9143	*A. dumerili/senegalensis*	Graret Ouchfegt	Morocco	*A. dumerili* A
9144	*A. dumerili/senegalensis*	Graret Ouchfegt	Morocco	*A. dumerili* A
9145	*A. aureus*	Graret Ouchfegt	Morocco	*A. aureus*
9148	*A. dumerili/senegalensis*	Zmoul at Teha	Morocco	*A. dumerili* A
9153	*A. dumerili/senegalensis*	Oued Aj Jenna	Morocco	*A. dumerili* A
9168	*A. dumerili/senegalensis*	Lahraycha	Morocco	*A. dumerili* A
9171	*A. dumerili/senegalensis*	Adrar Souttouf	Morocco	*A. dumerili* A
9179	*A. dumerili/senegalensis*	Koudyat Laghnam	Morocco	*A. dumerili* A
9184	*A. dumerili/senegalensis*	Galb Ska'dwiyat	Morocco	*A. dumerili* A
9191	*A. dumerili/senegalensis*	Oued El Melah	Morocco	*A. dumerili* A
9196	*A. dumerili/senegalensis*	Oued Archane	Morocco	*A. dumerili* A
9198	*A. aureus*	Ghrad Al 'Angra	Morocco	*A. aureus*
9205	*A. aureus*	Krikchet	Morocco	*A. aureus*
9648	*A. longipes*	Nebyet el Dris el Abiad	Mauritania	*A. dumerili* A
9687	*A. dumerili/senegalensis*	Tarf Tagouraret	Mauritania	*A. dumerili* A
9704	*A. dumerili/senegalensis*	Kerkour	Mauritania	*A. dumerili* A
9711	*A. longipes*	Tinigart	Mauritania	*A. longipes*
9774	*A. dumerili/senegalensis*	El Rhimiya	Mauritania	*A. dumerili* A
9775	*A. dumerili/senegalensis*	El Rhimiya	Mauritania	*A. dumerili* A
9790	*A. longipes*	Tîchît, 5km NW of	Mauritania	*A. longipes*
9807	*A. longipes*	Foum Ajâr	Mauritania	*A. longipes*
9910	*A. dumerili/senegalensis*	Akoueijât el Hemâr	Mauritania	*A. dumerili* A
9911	*A. dumerili/senegalensis*	Akoueijât el Hemâr	Mauritania	*A. dumerili* A
9912	*A. longipes*	Chami, NW of	Mauritania	*A. longipes*
9913	*A. longipes*	Chami, NW of	Mauritania	*A. longipes*
9914	*A. longipes*	Chami, NW of	Mauritania	*A. longipes*
9915	*A. longipes*	Chami, NW of	Mauritania	*A. longipes*
9917	*A. longipes*	Chami, NW of	Mauritania	*A. longipes*
9999	*A. aureus*	Derraman	Morocco	*A. aureus*
A004	*A. longipes*	Guelb Nouatil	Mauritania	*A. longipes*
A011	*A. aureus/taghitensis*	Fort Guerguerat, 70km N of	Morocco	*A. aureus*
A086	*A. dumerili/senegalensis*	Tozeur, 7km W of	Tunisia	*A. scutellatus*
A092	*A. dumerili/senegalensis*	Douz, 11km NW of	Tunisia	*A. scutellatus*

A094	*A. longipes*	Douz, 11km NW of	Tunisia	*A. longipes*
A103	*A. dumerili/senegalensis*	Pipeline track, 40km N of Ksar Ghilane	Tunisia	*A. scutellatus*
A110	*A. dumerili/senegalensis*	Pipeline track, 22km NE of Ksar Ghilane	Tunisia	*A. scutellatus*
A116	*A. longipes*	Ksar Ghilane at the Roman fort	Tunisia	*A. longipes*
A120	*A. dumerili/senegalensis*	Pipeline track, 20km SE of Ksar Ghilane	Tunisia	*A. scutellatus*
A122	*A. dumerili/senegalensis*	Tataouine, 17km NE of	Tunisia	*A. scutellatus*
A133	*A. sp.*	Jadi Resort; 7km E of Zuara	Libya	*A. scutellatus*
A134	*A. sp.*	Jadi Resort; 7km E of Zuara	Libya	*A. scutellatus*
A137	*A. dumerili/senegalensis*	Nalut, 10km S of	Libya	*A. scutellatus*
A140	*A. dumerili/senegalensis*	Nalut, 72km S of	Libya	*A. scutellatus*
A147	*A. longipes*	Ghadames, 7km NW of	Libya	*A. longipes*
A154	*A. dumerili/senegalensis*	Idri, 105km N of	Libya	*A. scutellatus*
A155	*A. dumerili/senegalensis*	Idri, 95km N of	Libya	*A. scutellatus*
A162	*A. dumerili/senegalensis*	Messak Mallet; 95km W of Tesawa	Libya	*A. scutellatus*
A170	*A. longipes*	Crossroad to Al Katrun; 100km E of Murzuq	Libya	*A. longipes*
A180	*A. longipes*	Ténéré: Madama	Niger	*A. longipes*
A182	*A. longipes*	Ténéré: Dirkou, 40km N of	Niger	*A. longipes*
A184	*A. longipes*	Ténéré: Bilma, 15km N of	Niger	*A. longipes*
A187	*A. longipes*	Ténéré: Fachi	Niger	*A. longipes*
A191	*A. dumerili/senegalensis*	Ténéré: Tazolé well	Niger	*A. dumerili* A
A192	*A. dumerili/senegalensis*	Ténéré: Tazolé well	Niger	*A. dumerili* A
A194	*A. dumerili/senegalensis*	Ténéré: Tazolé well	Niger	*A. dumerili* A
A195	*A. dumerili/senegalensis*	Ténéré: Tazolé well	Niger	*A. dumerili* A
A196	*A. dumerili/senegalensis*	Ténéré: Tazolé well	Niger	*A. dumerili* A
A197	*A. dumerili/senegalensis*	Ténéré: Tazolé well	Niger	*A. dumerili* A
A198	*A. dumerili/senegalensis*	Ténéré: Tazolé well	Niger	*A. dumerili* A
A212	*A. dumerili/senegalensis*	S.Louis, 200km of	Senegal	*A. dumerili* A
A215	*A. dumerili/senegalensis*	S.Louis peninsula	Senegal	*A. dumerili* A
A219	*A. dumerili/senegalensis*	PN Langue Barbarie: piste S.Louis-Louga	Senegal	*A. dumerili* A
A226	*A. dumerili/senegalensis*	Nouakchott, 80km S of	Mauritania	*A. dumerili* A
A238	*A. dumerili/senegalensis*	Nouakchott, 30km S of	Mauritania	*A. dumerili* A
A242	*A. dumerili/senegalensis*	Nouakchott, 30km S of	Mauritania	*A. dumerili* A
A243	*A. dumerili/senegalensis*	Nouakchott, 30km S of	Mauritania	*A. dumerili* A
A245	*A. dumerili/senegalensis*	Nouakchott, 10km N of	Mauritania	*A. dumerili* A
A248	*A. dumerili/senegalensis*	Nouakchott, 10km N of	Mauritania	*A. dumerili* A
A252	*A. dumerili/senegalensis*	Nouakchott, 12km N of	Mauritania	*A. dumerili* A
A253	*A. dumerili/senegalensis*	Nouakchott, 12km N of	Mauritania	*A. dumerili* A
A254	*A. dumerili/senegalensis*	Nouakchott, 12km N of	Mauritania	*A. dumerili* A
A262	*A. dumerili/senegalensis*	Nouakchott, 15km N of	Mauritania	*A. dumerili* A
A264	*A. dumerili/senegalensis*	Nouakchott, 15km N of	Mauritania	*A. dumerili* A
A266	*A. dumerili/senegalensis*	Nouakchott, 15km N of	Mauritania	*A. dumerili* A
A267	*A. dumerili/senegalensis*	Nouakchott, 15km N of	Mauritania	*A. dumerili* A
A268	*A. dumerili/senegalensis*	Nouakchott, 15km N of	Mauritania	*A. dumerili* A
A269	*A. dumerili/senegalensis*	Nouakchott, 10km N of	Mauritania	*A. dumerili* A

A270	*A. dumerili/senegalensis*	Nouakchott, 10km N of	Mauritania	*A. dumerili* A
A271	*A. longipes*	Akchâr dunes; 115km N of Nouakchott	Mauritania	*A. longipes*
A273	*A. longipes*	Nouakchott, 20km NE of	Mauritania	*A. longipes*
A275	*A. dumerili/senegalensis*	Nouakchott, 70km NE of	Mauritania	*A. dumerili* A
A276	*A. longipes*	Nouakchott, 70km NE of	Mauritania	*A. dumerili* A
A281	*A. longipes*	Akjoujt, 100km SW of	Mauritania	*A. longipes*
A287	*A. dumerili/senegalensis*	Atar, 110km SW of	Mauritania	*A. dumerili* A
A288	*A. dumerili/senegalensis*	Atar, 90km SW of	Mauritania	*A. dumerili* A
A290	*A. dumerili/senegalensis*	Choûm, 40km W of	Mauritania	*A. dumerili* A
A293	*A. dumerili/senegalensis*	Choûm, 40km W of	Mauritania	*A. dumerili* A
A296	*A. longipes*	Choûm, 50km W of	Mauritania	*A. longipes*
A311	*A. longipes*	Choûm, 100km W of	Mauritania	*A. longipes*
A318	*A. longipes*	Choum, 140km W of	Mauritania	*A. longipes*
A328	*A. dumerili/senegalensis*	Nouâdhibou, 200km E of	Mauritania	*A. dumerili* A
A332	*A. longipes*	Nouâdhibou, 200km E of	Mauritania	*A. longipes*
A334	*A. longipes*	Nouâdhibou, 90km E of	Mauritania	*A. longipes*
A335	*A. dumerili/senegalensis*	Nouâdhibou, 90km E of	Mauritania	*A. dumerili* A
A336	*A. dumerili/senegalensis*	Nouâdhibou, 90km E of	Mauritania	*A. dumerili* A
A337	*A. dumerili/senegalensis*	Nouâdhibou, 160km S of	Mauritania	*A. dumerili* A
A338	*A. dumerili/senegalensis*	Nouâdhibou, 160km S of	Mauritania	*A. dumerili* A
A339	*A. dumerili/senegalensis*	Nouâdhibou, 160km S of	Mauritania	*A. dumerili* A
A340	*A. dumerili/senegalensis*	Nouâdhibou, 160km S of	Mauritania	*A. dumerili* A
A341	*A. dumerili/senegalensis*	Nouâdhibou, 160km S of	Mauritania	*A. dumerili* A
A342	*A. dumerili/senegalensis*	Nouâdhibou, 160km S of	Mauritania	*A. dumerili* A
A343	*A. longipes*	Nouâdhibou, 160km S of	Mauritania	*A. dumerili* A
A344	*A. longipes*	PN Banc d'Arguin, Bir el Gareb, 15km S of	Mauritania	*A. longipes*
A345	*A. longipes*	PN Banc d'Arguin, Bir el Gareb, 15km S of	Mauritania	*A. longipes*
A346	*A. longipes*	PN Banc d'Arguin, Bir el Gareb, 15km S of	Mauritania	*A. longipes*
A347	*A. longipes*	PN Banc d'Arguin, Bir el Gareb, 15km S of	Mauritania	*A. longipes*
A348	*A. dumerili/senegalensis*	PN Banc d'Arguin, Bir el Gareb, 15km S of	Mauritania	*A. dumerili* A
A349	*A. dumerili/senegalensis*	PN Banc d'Arguin, Bir el Gareb, 15km S of	Mauritania	*A. dumerili* A
A350	*A. dumerili/senegalensis*	Nouâdhibou, 110km S of	Mauritania	*A. dumerili* A
A351	*A. dumerili/senegalensis*	Nouâdhibou, 110km S of	Mauritania	*A. dumerili* A
A352	*A. dumerili/senegalensis*	Nouâdhibou, 110km S of	Mauritania	*A. dumerili* A
A353	*A. dumerili/senegalensis*	Nouâdhibou, 110km S of	Mauritania	*A. dumerili* A
A354	*A. dumerili/senegalensis*	Nouâdhibou, 110km S of	Mauritania	*A. dumerili* A
A355	*A. dumerili/senegalensis*	Nouâdhibou, 110km S of	Mauritania	*A. dumerili* A
A356	*A. dumerili/senegalensis*	Nouâdhibou, 110km S of	Mauritania	*A. dumerili* A
A357	*A. dumerili/senegalensis*	Nouâdhibou, 110km S of	Mauritania	*A. dumerili* A
A359	*A. aureus*	Nouâdhibou, 70km S of	Mauritania	*A. aureus*
A360	*A. aureus*	Nouâdhibou, 70km S of	Mauritania	*A. aureus*
A362	*A. aureus*	Nouâdhibou, 70km S of	Mauritania	*A. aureus*
A363	*A. aureus*	Nouâdhibou, 70km S of	Mauritania	*A. aureus*

A366	*A. aureus*	Nouâdhibou, 40km S of	Mauritania	*A. aureus*
A367	*A. aureus*	Nouâdhibou, 40km S of	Mauritania	*A. aureus*
A369	*A. aureus*	Nouâdhibou, 40km S of	Mauritania	*A. aureus*
A370	*A. aureus*	Cape Blanc, 4km N of	Mauritania	*A. aureus*
A376	*A. aureus*	Fort Guerguerat, 70km N of	Morocco	*A. aureus*
A382	*A. aureus*	Dakhla, 240km S of crossroad for	Morocco	*A. aureus*
A386	*A. aureus*	Dakhla, 190km S of crossroad for	Morocco	*A. aureus*
A392	*A. aureus*	Dakhla, 140km S of crossroad for	Morocco	*A. aureus*
A395	*A. aureus*	Dakhla, 20km N of	Morocco	*A. aureus*
A401	*A. aureus*	Dakhla, 100km N of crossroad for	Morocco	*A. aureus*
A410	*A. aureus*	Boujdour, 110km S of	Morocco	*A. aureus*
A416	*A. aureus*	Laayoune, 70km S of	Morocco	*A. aureus*
A435	*A. aureus*	25km S of Aoreora	Morocco	*A. aureus*
A456	*A. dumerili/senegalensis*	40km E of Foum Zguid	Morocco	*A. dumerili* B
A462	*A. longipes*	Erg Mhazil - 80km E of Foum Zguid	Morocco	*A. longipes*
A468	*A. dumerili/senegalensis*	112km E of Foum Zguid	Morocco	*A. dumerili* B
A469	*A. dumerili/senegalensis*	60km E of Tagounite	Morocco	*A. dumerili* B
A517	*A. dumerili/senegalensis*	12km N of Erfoud	Morocco	*A. dumerili* B
A526	*A. longipes*	Erg Chebbi	Morocco	*A. longipes*
A556	*A. aureus*	Bou Soun	Morocco	*A. aureus*
A765	*A. aureus*	M'boro-sur-mer	Senegal	*A. aureus*
A766	*A. longipes*	Guerzim	Algeria	*A. longipes*
A768	*A. dumerili/senegalensis*	Bou Trekfine	Algeria	*A. scutellatus*
A769	*A. dumerili/senegalensis*	Near the exit for Abfahrt Ain Ourka	Algeria	*A. scutellatus*
A773	*A. dumerili/senegalensis*	Tiguent, S of	Mauritania	*A. dumerili* A
A776	*A. dumerili/senegalensis*	Niakoul Rap	Senegal	*A. dumerili* A
A778	*A. dumerili/senegalensis*	Cambérène	Senegal	*A. dumerili* A
A781	*A. scutellatus*	Beirut	Lebanon	*A. longipes*
A784	*A. scutellatus*	Darfur: Merga (Nukheila)	Sudan	*A. longipes*
A787	*A. scutellatus*	Gilf Kebir	Egypt	*A. scutellatus*
A788	*A. scutellatus*	10km NW of Cairo	Egypt	*A. longipes*
A790	*A. scutellatus*	Wadi Natroun	Egypt	*A. longipes*
A791	*A. scutellatus*	Al Arish	Egypt	*A. longipes*
A792	*A. scutellatus*	Bahariya oasis	Egypt	*A. scutellatus*
A796	*A. longipes*	Herrour ridge	Mauritania	*A. longipes*
A797	*A. longipes*	Agouadir, 5km SW of	Mauritania	*A. longipes*
A798	*A. longipes*	Idîni, 75km NE of	Mauritania	*A. longipes*
A800	*A. dumerili/senegalensis*	Nouâmghâr, 3km S of	Mauritania	*A. dumerili* A
A817	*A. dumerili/senegalensis*	23km W Foughala	Algeria	*A. scutellatus*
A825	*A. dumerili/senegalensis*	Nouakchott, N of	Mauritania	*A. dumerili* A
A826	*A. dumerili/senegalensis*	Rkiz lake, E of	Mauritania	*A. dumerili* A
A827	*A. dumerili/senegalensis*	Kankossa, N of	Mauritania	*A. dumerili* A

A3)

List of sequences obtained from GenBank

Taxon	Accession Number	Gene	Country	Reference
A. boskianus	KJ567672.1	12S	Egypt	Unpublished
A. boskianus	KJ567768.1	Cyt-b	Egypt	Unpublished
A. boskianus	KJ547966.1	C-mos	Egypt	Unpublished

A4)

List of sequences obtained from Karin Tamar and Salvador Carranza (CSIC-UPF), putative species assignment based on morphology, local, country and clade to which the sample belongs according to the phylogenetic results.

Code	Putative species	Local	Country	Clade
E7021323	*A. taghitensis*	El-Abiodh Sidi Cheikh	Algeria	*A. taghitensis*
E7021324	*A. taghitensis*	El-Abiodh Sidi Cheikh	Algeria	*A. taghitensis*
EGY 02/2012 - 20	*A. s. audouini*	El Khattara, W bank, Aswan	Egypt	*A. scutellatus*
HUJ - 23473	*A. s. scutellatus*	Nizzanim sands	Israel	*A. scutellatus*
IBES3533	*A. s. audouini*	11172 - Sitra Oasis	Egypt	*A. scutellatus*
SPM002352(42)	*A. aegyptius*	Zerenuk, N. Sinai (Zaraniq)	Egypt	*A. longipes*
SPM002912	*A. aegyptius*	N Sinai - 17 km NW to Bir Qatia	Egypt	*A. longipes*
TAU - R.16331	*A. aegyptius*	Ashelim	Israel	*A. longipes*
TAU - R.16389	*A. s. scutellatus*	Bir Mashash sands	Israel	*A. scutellatus*
TAU - R.16400	*A. aegyptius*	Bir Mashash sands	Israel	*A. longipes*
TAU - R.16410	*A. s. scutellatus*	Holon sands	Israel	*A. scutellatus*

A5)

List of genotyped samples from the contact zone, cluster to which each sample was assigned and proportion of membership.

Code	Cluster	Proportion of membership
2766	*A. aureus*	0.974
5171	*A. aureus*	0.996
5172	*A. aureus*	0.997
5173	*A. aureus*	0.997
5176	*A. aureus*	0.997
6435	*A. aureus*	0.997
6446	*A. aureus*	0.997
6448	*A. aureus*	0.997
6449	*A. aureus*	0.987
6458	*A. aureus*	0.996
6477	*A. aureus*	0.997
A359	*A. aureus*	0.992
A360	*A. aureus*	0.992
A362	*A. aureus*	0.996
A363	*A. aureus*	0.997
A367	*A. aureus*	0.997
A369	*A. aureus*	0.991
A370	*A. aureus*	0.996
2731	*A. dumerili* A	0.831
2743	*A. dumerili* A	0.998
2750	*A. dumerili* A	0.997
2757	*A. dumerili* A	0.997
2758	*A. dumerili* A	0.99
2763	*A. dumerili* A	0.961
2768	*A. dumerili* A	0.997
2769	*A. dumerili* A	0.997
2777	*A. dumerili* A	0.988
2779	*A. dumerili* A	0.997
3537	*A. dumerili* A	0.991
3553	*A. dumerili* A	0.997
3558	*A. dumerili* A	0.994
3575	*A. dumerili* A	0.997
3581	*A. dumerili* A	0.995
3582	*A. dumerili* A	0.997
3585	*A. dumerili* A	0.973
3594	*A. dumerili* A	0.997
3608	*A. dumerili* A	0.993
3615	*A. dumerili* A	0.996
3618	*A. dumerili* A	0.987
3622	*A. dumerili* A	0.997

5052	*A. dumerili* A	0.991
5103	*A. dumerili* A	0.997
5111	*A. dumerili* A	0.996
5120	*A. dumerili* A	0.998
5126	*A. dumerili* A	0.997
5135	*A. dumerili* A	0.997
5139	*A. dumerili* A	0.996
5153	*A. dumerili* A	0.997
5158	*A. dumerili* A	0.997
5160	*A. dumerili* A	0.997
5162	*A. dumerili* A	0.997
5181	*A. dumerili* A	0.984
5294	*A. dumerili* A	0.998
5298	*A. dumerili* A	0.997
5923	*A. dumerili* A	0.993
5924	*A. dumerili* A	0.997
5995	*A. dumerili* A	0.99
5997	*A. dumerili* A	0.992
6275	*A. dumerili* A	0.983
6277	*A. dumerili* A	0.997
6281	*A. dumerili* A	0.995
6282	*A. dumerili* A	0.976
6285	*A. dumerili* A	0.997
6288	*A. dumerili* A	0.996
6289	*A. dumerili* A	0.997
6297	*A. dumerili* A	0.993
6299	*A. dumerili* A	0.953
6303	*A. dumerili* A	0.98
6307	*A. dumerili* A	0.997
6315	*A. dumerili* A	0.996
6321	*A. dumerili* A	0.869
6325	*A. dumerili* A	0.997
6329	*A. dumerili* A	0.986
6330	*A. dumerili* A	0.996
6331	*A. dumerili* A	0.997
6347	*A. dumerili* A	0.997
6353	*A. dumerili* A	0.944
6360	*A. dumerili* A	0.991
6363	*A. dumerili* A	0.996
6364	*A. dumerili* A	0.995
6375	*A. dumerili* A	0.997
6376	*A. dumerili* A	0.99
6377	*A. dumerili* A	0.997
6378	*A. dumerili* A	0.997
6384	*A. dumerili* A	0.997
6390	*A. dumerili* A	0.989

6391	*A. dumerili* A	0.996
6394	*A. dumerili* A	0.997
6405	*A. dumerili* A	0.997
6410	*A. dumerili* A	0.997
6411	*A. dumerili* A	0.997
6415	*A. dumerili* A	0.997
6416	*A. dumerili* A	0.997
6421	*A. dumerili* A	0.997
6426	*A. dumerili* A	0.997
6431	*A. dumerili* A	0.955
6433	*A. dumerili* A	0.985
6445	*A. dumerili* A	0.997
6450	*A. dumerili* A	0.996
6453	*A. dumerili* A	0.984
6456	*A. dumerili* A	0.998
6457	*A. dumerili* A	0.996
6460	*A. dumerili* A	0.996
6461	*A. dumerili* A	0.997
6462	*A. dumerili* A	0.997
6463	*A. dumerili* A	0.713
6468	*A. dumerili* A	0.992
6469	*A. dumerili* A	0.996
6470	*A. dumerili* A	0.998
6473	*A. dumerili* A	0.997
6474	*A. dumerili* A	0.97
9910	*A. dumerili* A	0.996
9911	*A. dumerili* A	0.943
A335	*A. dumerili* A	0.997
A336	*A. dumerili* A	0.995
A338	*A. dumerili* A	0.989
A339	*A. dumerili* A	0.998
A340	*A. dumerili* A	0.995
A341	*A. dumerili* A	0.997
A342	*A. dumerili* A	0.996
A348	*A. dumerili* A	0.981
A349	*A. dumerili* A	0.99
A350	*A. dumerili* A	0.995
A351	*A. dumerili* A	0.993
A352	*A. dumerili* A	0.997
A353	*A. dumerili* A	0.997
A354	*A. dumerili* A	0.997
A355	*A. dumerili* A	0.995
A356	*A. dumerili* A	0.997
A357	*A. dumerili* A	0.998
A800	*A. dumerili* A	0.974
2734	*A. longipes*	0.995

2736	*A. longipes*	0.995
2739	*A. longipes*	0.997
2740	*A. longipes*	0.997
2742	*A. longipes*	0.996
2745	*A. longipes*	0.997
2746	*A. longipes*	0.993
2831	*A. longipes*	0.997
3535	*A. longipes*	0.998
3542	*A. longipes*	0.993
3557	*A. longipes*	0.997
3561	*A. longipes*	0.982
3562	*A. longipes*	0.99
3563	*A. longipes*	0.997
3572	*A. longipes*	0.996
3607	*A. longipes*	0.997
5040	*A. longipes*	0.997
5041	*A. longipes*	0.995
5045	*A. longipes*	0.996
5049	*A. longipes*	0.996
5054	*A. longipes*	0.997
5062	*A. longipes*	0.997
5069	*A. longipes*	0.998
5070	*A. longipes*	0.996
5073	*A. longipes*	0.998
5075	*A. longipes*	0.997
5076	*A. longipes*	0.997
5080	*A. longipes*	0.986
5087	*A. longipes*	0.996
5093	*A. longipes*	0.993
5094	*A. longipes*	0.997
5098	*A. longipes*	0.998
5099	*A. longipes*	0.997
5106	*A. longipes*	0.996
5110	*A. longipes*	0.984
5119	*A. longipes*	0.997
5137	*A. longipes*	0.998
5163	*A. longipes*	0.998
5164	*A. longipes*	0.992
5167	*A. longipes*	0.997
5168	*A. longipes*	0.998
5177	*A. longipes*	0.989
5996	*A. longipes*	0.996
6298	*A. longipes*	0.997
6301	*A. longipes*	0.988
6302	*A. longipes*	0.996
6306	*A. longipes*	0.997

6317	*A. longipes*	0.982
6318	*A. longipes*	0.978
6319	*A. longipes*	0.984
6320	*A. longipes*	0.885
6322	*A. longipes*	0.994
6339	*A. longipes*	0.997
6340	*A. longipes*	0.997
6348	*A. longipes*	0.997
6349	*A. longipes*	0.893
6352	*A. longipes*	0.995
6356	*A. longipes*	0.971
6369	*A. longipes*	0.997
6374	*A. longipes*	0.997
6383	*A. longipes*	0.997
6386	*A. longipes*	0.997
6414	*A. longipes*	0.997
6436	*A. longipes*	0.997
6438	*A. longipes*	0.997
6451	*A. longipes*	0.998
6452	*A. longipes*	0.981
9913	*A. longipes*	0.994
9914	*A. longipes*	0.986
9915	*A. longipes*	0.974
9917	*A. longipes*	0.992
A334	*A. longipes*	0.998
A344	*A. longipes*	0.998
A345	*A. longipes*	0.932
A346	*A. longipes*	0.997
A347	*A. longipes*	0.935

A6)

Primers and PCR conditions for the sequenced genes of mtDNA and scnDNA

12S primers

12Sa	Forward	AAACTGGGATTAGATACCCCACTAT
12Sb	Reverse	GAGGGTGACGGGCGGTGTGT

Conditions:

Amplification step	Temperature (ºC)	Duration	Number of cycles
Initial denaturation	94ºC	5 minutes	1
Denaturation	94ºC	30 seconds	35
Annealing	56ºC	45 seconds	
Extension	72ºC	1 minute	
Final Extension	72ºC	5 minutes	1

Cyt-b primers

Gludg	Forward	TGACTTGAARAACCAYCGTTG
Cytb2	Reverse	CCCTCAGAATGATATTTGTCCTCA

Conditions:

Amplification step	Temperature (ºC)	Duration	Number of cycles
Initial denaturation	94ºC	5 minutes	1
Denaturation	94ºC	30 seconds	35
Annealing	49ºC	45 seconds	
Extension	72ºC	1:30 minutes	
Final Extension	72ºC	5 minutes	1

C-mos primers

Lsc1-F	Forward	CTCTGGKGGCTTTGGKKCTGTSTACAAGG
Lsc2-R	Reverse	GGTGATGGCAAANGAGTAGATGTCTGC

Conditions:

Amplification step	Temperature (ºC)	Duration	Number of cycles
Initial denaturation	94ºC	5 minutes	1
Denaturation	94ºC	30 seconds	35
Annealing	57ºC	45 seconds	
Extension	72ºC	1:10 minutes	
Final Extension	72ºC	5 minutes	1

A7)

PCR programs for the microsatellite multiplexes

Multiplex 1

Amplification step	Temperature (ºC)	Duration	Number of cycles
Initial denaturation	95º	15min	1
Denaturation	95º	30 seconds	9
Annealing	59º-55º (Touchdown -0.5ºC)	90 seconds	
Extension	72º	30 seconds	
Denaturation	95º	30 seconds	31
Annealing	54º	60 seconds	
Extension	72º	30 seconds	
Final Extension	60º	30 minutes	1

Multiplex 2

Amplification step	Temperature (ºC)	Duration	Number of cycles
Initial denaturation	95º	15 minutes	1
Denaturation	95º	30 seconds	9
Annealing	57º-53º (Touchdown -0.5ºC)	90 seconds	
Extension	72º	30 seconds	
Denaturation	95º	30 seconds	31
Annealing	53º	60 seconds	
Extension	72º	30 seconds	
Final Extension	60º	30 minutes	1

Multiplex 3

Amplification step	Temperature (ºC)	Duration	Number of cycles
Initial denaturation	95º	15 minutes	1
Denaturation	95º	30 seconds	9
Annealing	58º-54º (Touchdown -0.5ºC)	90 seconds	
Extension	72º	30 seconds	
Denaturation	95º	30 seconds	31
Annealing	54º	60 seconds	
Extension	72º	30 seconds	
Final Extension	60º	30 minutes	1

Multiplex4

Amplification step	Temperature (ºC)	Duration	Number of cycles
Initial denaturation	95º	15 minutes	1
Denaturation	95º	30 seconds	9
Annealing	55º-51 (Touchdown -0.5ºC)	90 seconds	
Extension	72º	30 seconds	
Denaturation	95º	30 seconds	31
Annealing	51º	60 seconds	
Extension	72º	30 seconds	
Final Extension	60º	30 minutes	1

A8)

Microssatelite list and marker information

Multiplex	Name	Motif	Size	Volume (µl)	Tail
1	Ac1	atac	242	0.6	FAM
	Ac23	cat	104	0.4	FAM
	Ac5	aac	153	0.6	VIC
	Ac7	gag	113	0.4	NED
	Ac8	ttg	195	0.6	NED
	Ac9	caa	184	0.4	PET
	Ac30	tcta	123	0.6	PET
2	Ac11	aac	291	1.5	FAM
	Ac13	aac	142	0.2	FAM
	Ac14	caa	215	1.5	VIC
	Ac16	agg	90	1	VIC
	Ac21	gga	245	0.8	NED
	Ac17	tacc	159	0.5	NED
	Ac19	aac	205	0.8	PET
	Ac20	gtt	150	2	PET
3	Ac32	ttc	241	0.6	FAM
	Ac33	tgt	126	0.4	FAM
	Ac4	ttc	240	0.4	VIC
	Ac36	tgt	98	0.4	VIC
	Ac37	tcta	158	1.5	NED
	Ac38	gga	90	0.5	NED
	Ac15	caa	142	0.4	PET
4	Ac31	gtt	292	0.6	FAM
	Ac43	caa	96	0.4	FAM
	Ac44	gga	190	0.4	VIC
	Ac45	caa	140	0.4	VIC
	Ac47	aca	163	0.8	NED
	Ac6	ttg	97	0.4	NED
	Ac49	aac	190	0.4	PET
	Ac28	acat	122	0.4	PET

A9)

List of samples used in the mitochondrial network, and their respective haplotype and haplogroup

Code	Haplotype	Haplogroup
1273	6	
1637	70	N
1795	18	F
1881	152	N
1926	154	N
1933	123	N
1934	123	N
1935	126	N
1945	165	O
1978	166	O
2068	140	N
2198	156	N
2270	159	N
2731	144	N
2734	19	F
2736	18	F
2743	103	N
2745	18	F
2746	18	F
2750	86	N
2757	103	N
2758	104	N
2763	70	N
2766	13	E
2768	70	N
2769	119	N
2777	70	N
2779	70	N
2831	18	F
2836	162	N
2841	30	F
2844	162	N
2851	162	N
2856	163	N
2858	164	N
2861	162	N
2866	18	F
2877	125	N
2894	27	F
2933	153	N
2947	161	N

2955	18	F
2980	18	F
3008	165	O
3260	141	N
3270	141	N
3288	139	N
3314	141	N
3458	151	N
3473	150	N
3487	128	N
3493	129	N
3497	18	F
3503	18	F
3521	18	F
3535	18	F
3537	142	N
3542	18	F
3553	76	N
3557	18	F
3558	110	N
3561	18	F
3562	32	F
3563	18	F
3572	18	F
3575	141	N
3581	145	N
3582	146	N
3585	141	N
3594	141	N
3599	141	N
3607	18	F
3608	147	N
3615	70	N
3618	110	N
3622	101	N
4283	117	N
4284	130	N
4285	131	N
4286	131	N
4441	135	N
4448	115	N
4459	115	N
4470	95	N
4477	95	N
5002	82	N
5004	70	N

5019	29	F
5025	18	F
5029	109	N
5037	18	F
5040	18	F
5041	18	F
5045	18	F
5046	70	N
5047	23	F
5052	137	N
5054	33	F
5062	18	F
5069	18	F
5070	18	F
5073	18	F
5075	25	F
5076	25	F
5080	18	F
5087	24	F
5093	18	F
5094	18	F
5098	18	F
5099	18	F
5103	147	N
5106	20	F
5110	18	F
5111	110	N
5119	18	F
5120	141	N
5126	141	N
5135	86	N
5137	18	F
5139	110	N
5153	108	N
5158	70	N
5160	70	N
5162	103	N
5163	18	F
5164	18	F
5167	18	F
5168	18	F
5171	13	E
5172	15	E
5173	12	E
5176	12	E
5177	18	F

5181	70	N
5192	18	F
5201	70	N
5213	78	N
5221	70	N
5234	162	N
5249	162	N
5258	18	F
5267	78	N
5274	18	F
5275	87	N
5293	70	N
5294	70	N
5298	70	N
5771	122	N
5789	90	N
5790	136	N
5800	124	N
5804	124	N
5805	18	F
5820	171	N
5827	70	N
5833	124	N
5834	70	N
5846	18	F
5883	18	F
5919	112	N
5923	141	N
5924	141	N
5995	81	N
5997	81	N
6076	18	F
6275	81	N
6277	79	N
6281	100	N
6282	98	N
6285	81	N
6288	141	N
6289	141	N
6297	141	N
6298	33	F
6299	111	N
6301	19	F
6302	33	F
6303	141	N
6307	143	N

6308	18	F
6315	141	N
6317	19	F
6319	18	F
6321	141	N
6322	19	F
6325	141	N
6329	147	N
6330	110	N
6331	110	N
6339	18	F
6347	110	N
6348	18	F
6349	18	F
6352	18	F
6353	110	N
6356	31	F
6360	110	N
6363	107	N
6364	110	N
6369	18	F
6374	18	F
6375	110	N
6376	110	N
6377	86	N
6378	110	N
6383	18	F
6384	110	N
6386	19	F
6390	103	N
6391	70	N
6394	103	N
6405	103	N
6410	70	N
6411	105	N
6414	18	F
6415	105	N
6416	70	N
6421	84	N
6426	103	N
6431	103	N
6433	70	N
6435	14	E
6436	18	F
6438	18	F
6445	70	N

6446	15	E
6448	15	E
6449	15	E
6450	70	N
6451	18	F
6452	18	F
6453	70	N
6456	89	N
6457	86	N
6458	13	E
6460	70	N
6461	70	N
6462	70	N
6463	91	N
6468	88	N
6469	88	N
6470	77	N
6473	70	N
6474	70	N
6477	15	E
6660	168	N
6661	167	N
6725	169	N
6726	170	N
6747	169	N
6764	167	N
6769	167	N
7245	3	D
7254	172	N
7255	75	N
7272	72	N
7276	73	N
7282	71	N
7293	70	N
7301	70	N
7308	3	D
7325	70	N
7334	70	N
7347	113	N
8252	67	M
8254	68	M
8255	66	
8990	51	
8992	52	
8998	50	
9108	9	C

9141	70	N
9142	70	N
9143	70	N
9144	70	N
9145	2	D
9148	70	N
9153	70	N
9179	70	N
9184	74	N
9196	85	N
9198	17	E
9205	15	E
9648	155	N
9687	158	N
9704	157	N
9711	47	
9775	160	N
9790	34	F
9807	21	F
9910	70	N
9911	110	N
9912	18	F
9913	18	F
9914	18	F
9915	18	F
9917	18	F
A004	21	F
A011	15	E
A086	55	J
A092	56	J
A094	35	G
A116	36	G
A133	60	
A134	60	
A140	61	
A147	46	
A154	58	K
A155	58	K
A162	59	K
A180	43	I
A182	45	I
A184	44	I
A187	45	I
A196	157	N
A198	157	N
A212	134	N

A215	116	N
A219	132	N
A226	135	N
A238	94	N
A242	118	N
A243	99	N
A245	96	N
A248	92	N
A252	93	N
A253	92	N
A254	97	N
A262	93	N
A264	92	N
A266	92	N
A267	92	N
A268	93	N
A269	93	N
A270	92	N
A271	22	F
A273	18	F
A275	80	N
A276	83	N
A281	26	F
A288	162	N
A290	114	N
A293	162	N
A296	18	F
A311	18	F
A318	18	F
A328	121	N
A332	18	F
A334	28	F
A335	70	N
A336	70	N
A337	70	N
A338	102	N
A339	70	N
A340	70	N
A341	70	N
A342	70	N
A344	18	F
A345	18	F
A346	18	F
A347	18	F
A348	149	N
A349	86	N

A350	70	N
A351	70	N
A352	70	N
A353	120	N
A354	70	N
A355	70	N
A356	70	N
A357	106	N
A359	13	E
A360	15	E
A362	13	E
A363	13	E
A366	13	E
A369	16	E
A376	15	E
A382	15	E
A386	11	C
A395	10	C
A410	5	B
A416	4	B
A435	8	A
A462	48	
A469	68	M
A517	69	
A526	49	
A556	7	A
A768	57	J
A769	53	
A773	127	N
A787	63	L
A788	37	H
A790	38	H
A792	62	L
A796	18	F
A797	18	F
A798	18	F
A800	148	N
A817	54	J
A825	92	N
A826	133	N
A827	138	N
E7021323	1	
E7021324	1	
HUJ - 23473	65	
IBES3533	64	L
SPM002352(42)	39	H

SPM002912	40	H
TAU - R.16331	42	H
TAU - R.16389	65	
TAU - R.16400	41	H
TAU - R.16410	65	