Combining ecological niche modeling and phylogeographic analyses to address climatic stability and persistence in four *Tarentola* species across the West Sahara

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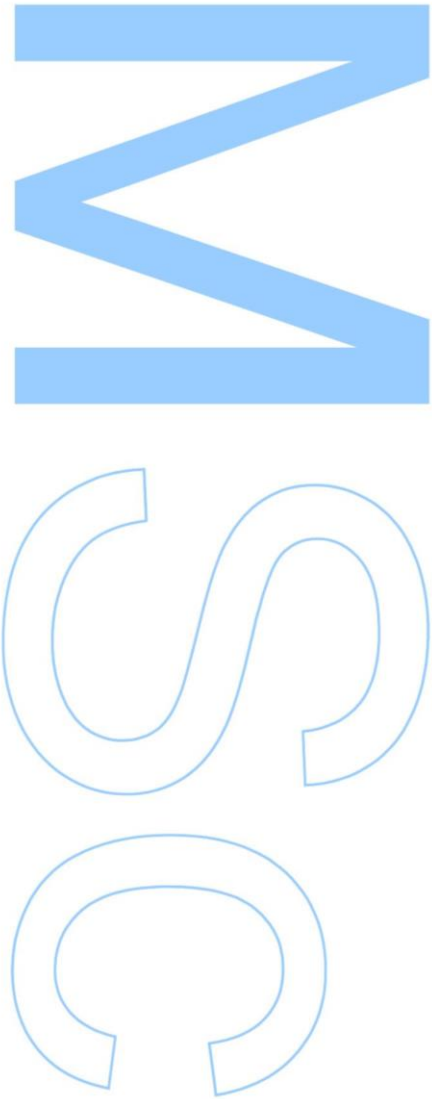

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Resumo

O clima do passado influenciou os padrões de distribuição da biodiversidade. No entanto falta conhecimento em partes remotas do mundo, como o Oeste de Africa, onde os padrões actuais de biodiversidade terão sido influenciados por oscilações entre períodos húmidos e secos. Nesta região, o deserto do Sahara actua como uma barreira para espécies não adaptadas a condições áridas, mas no passado pensa-se que terão existido vários corredores durante os períodos húmidos. Alguns podem até ter persistido ao longo do tempo, como o o Sahara Atlântico. Este estudo usou quatro espécies do género *Tarentola* (*T. annularis*, *T. chazaliae*, *T. hoggarensis* and *T. parvicarinata*), e uma combinação de modelos baseados em nichos ecológicos e análises filogeográficas para inferir a estabilidade climática da região. Um total de 140 amostras foras sequenciadas para um fragmento de 12S com 388pb. Os modelos ecológicos foram construídos com o Maxent. Os resultados genéticos mostram uma concordância com a identificação morfológica das espécies, e um elevado nível de subestruturação geográfica em *T. hoggarensis* e *T. parvicarinata*. *Tarentola annularis* não apresenta sinais de diferenciação ao longo de maioria da sua distribuição, enquanto *T. chazaliae* tem diversidade genética mas esta não se encontra geograficamente estruturada. Os modelos ecológicos revelam áreas estáveis para todas as espécies em regiões mais costeiras, com a excepão de *T. parvicarinata*, cujas áreas estáveis foram as montanhas da Mauritânia. Para *T. chazaliae* a área estável identificada foi um pequeno fragmento na fronteira entre Marrocos e o Sahara Ocidental. Embora não tenham sido completamemte concordantes, os resultados genéticos e ecológicos complementam-se e fornecem uma visualização mais completa dos processos evoluticos no Sahara-Sahel.

Palavras-chave: *Tarentola*, clima passado, modelos baseados em nichos ecológicos, abordagem integrativa, Norte de África, Sahara, Sahel, corredor ecológico, áreas climaticamente estáveis.

Abstract

Past climatic changes influenced the patterns of biodiversity distribution. Research is lacking for remote parts of the world, such as West Africa, where current biodiversity patterns are likely to have been influenced by oscillations between wet and dry climatic periods. In the region, the Sahara desert acts as a barrier to species not adapted to arid conditions, but in the past many corridors are thought to have existed during wet periods. Some may even have persisted through time, as the Atlantic Sahara. This study used four species of the genus *Tarentola* (*T. annularis*, *T. chazaliae*, *T. hoggarensis* and *T. parvicarinata*), and a combination of ecological niche-based models and phylogeographic analyses to infer the climatic stability of the region. A total of 140 samples were sequenced for a 12S fragment of 388bp. ENMs were constructed using Maxent. The genetic results show concordance with the morphological species identification, and a high level of geographic substructuration in *T. hoggarens*is and *T. parvicarina*ta*. Tarentol*a *annularis* shows no signs of differentiation throughout most of its range, while *T. chazali*ae has genetic diversity but it is not geographically structured. ENMs reveal stable areas for all species in more coastal regions with the exception o*f T. parvicarina*ta, which had stable areas in the Mauritanian mountains. For *T. chazali*ae the stable area identified is a small coastal patch in the border between Morocco and Western Sahara. Despite not being completely concordant, genetic and ecological results complement each other and provide a more complete visualization of evolutionary processes in the Sahara-Sahel.

Keywords: *Tarentola*, past climate, ecological niche-based models, integrative approach, North Africa, Sahara, Sahel, ecological corridor, climatically stable areas.

Index

Figure Index

Table Index

1. Introduction

1.1. Climate

Climate comprises all the aspects of the hydrosphere-atmosphere-land system, with all their complex interactions and feedbacks, in a limited time scale (Quante, 2010). Since the origin of life about 3.5 billion years ago, Earth's climate has oscillated due to both external (changes in solar radiation) and internal factors (changes in orbit, tectonic plates and volcanoes) as well as life itself (e.g. through photosynthesis, organisms altered the composition of the atmosphere). As life continually evolved, it became more involved in climatic cycles, such as water and carbon, changing the energy budget of the Earth, and ultimately impacting life itself (Quante, 2010). These climatic changes over time led to diversification processes, while spatial variability, in conjunction with landscape features, shaped species distributions (Slatkin, 1987; Brown and Lomolino, 1996).

1.1.1. Climate variation in space and time

Climatic conditions greatly vary all over the world (e.g. temperature; Figure 1) and this is primarily the result of the interaction between solar radiation (which is higher between the tropics), atmospheric circulation and oceanic currents (Rickelfs, 2008).

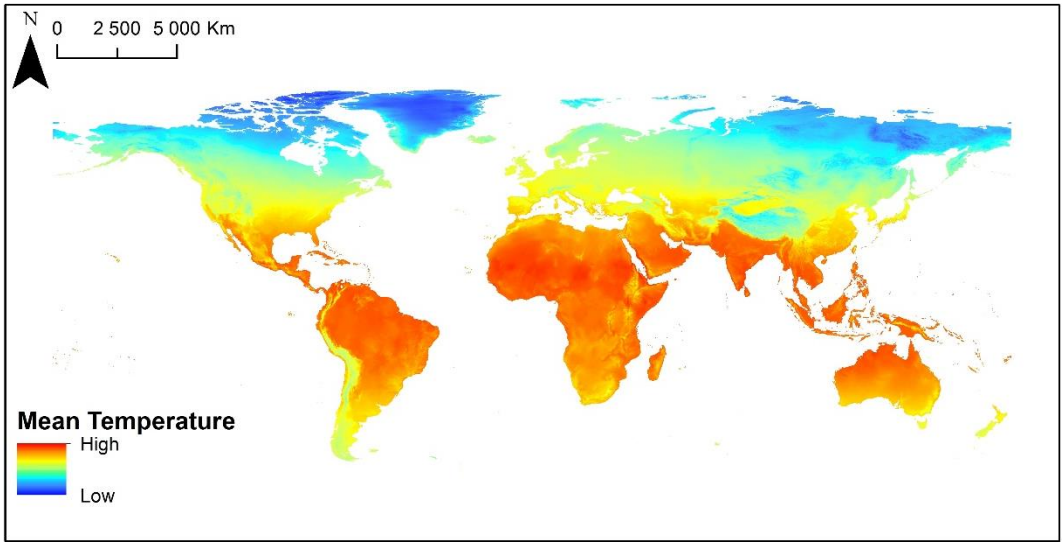

Figure 1: Worldwide mean annual temperature (from WorldClim; www.worldclim.org)

Topographic and geographic features can also affect climate at a local or regional scale. For instance, mountains usually present higher variation in temperature and more precipitation levels than surrounding lowlands, while coastal areas tend to have less variation in temperature than the interior, as well as more precipitation (Rickelfs, 2008).

Through time, climate has also changed considerably. For instance, during the Cretaceous, Earth's temperature was higher than at the present (Quante, 2010). At the end of this epoch, however, a decline in the concentration of CO_2 in the atmosphere led to a cooling of the planet (Quante, 2010). The Arctic ice cap started to grow, marking the beginning of the Pleistocene ca. 2.4 Mya (Damuth, 1975; Hewitt, 2000). The Pleistocene period is characterized by strong oscillations in climate, alternating between glacial periods, where the ice sheets spread through most of northern landmasses (i.e. North America, North of Europe and Siberia), and warmer interglacial periods, where the ice cap was restricted to northernmost latitudes (i.e. Antarctica and sometimes Greenland) (Quante, 2010). Tropical and temperate regions were compressed towards the equator during glacial periods and an increased aridity lead to a reduction of tropical forests and expansion of deserts (Hewitt, 2000, 2004). The oceans, covering about two-thirds of the planet's surface, had a strong influence on these oscillations (Quante, 2010) and were also affected by them, as with the spread of the glaciers, sea level was considerably reduced (Hewitt, 2000). These oscillations have been shown to have occurred in cycles of 100, 41 and 21 thousand years that result from the complex interaction of changes in the Earth's orbit, tilt and axial wobble, and affected the amount of energy received from the sun. In turn, a big part of the energy received is transported by the oceanic currents, allowing for fast and global climate changes (Zagwijn, 1992; Hewitt, 2000).The transition between the Last Glacial Maximum and the Holocene period about 11,700 years ago (Walker *et al.,* 2009) was dramatic. Ice caps started to retreat rapidly, and a large volume of water flooded the land and formed many lakes, as well as created continental islands and reshaped the coastal line (Quante, 2010). Around the Middle Holocene (about 6,000 years ago), solar radiation was 8% higher and lower than today in the peak of summer and winter respectively in the northern hemisphere. This caused and increased ocean evaporation, resulting in higher continental precipitation (Quante, 2010).

Information on past climate comes from many sources. Ice cores can give detailed climatic information over the last 400.000 years, though most are only informative for the last 125.000 years. Through these ice cores, annual analyses of gases, isotopes and acidity can be performed (Seierstad *et al.,* 2014). Sediment analyses can also give information on distant climate, though in a more indirect way and

with less resolution. It is also more restricted to humid habitats, as sedimentation is less likely to occur in dry habitats (Kröpellin *et al.,* 2008). Pollen records also provide invaluable indirect evidence, as most can be identified at the species level, and thus, detailed habitat reconstructions of past times can be performed (Whitmore et al., 2005; e.g. Russia, Tarasov *et al.,* 2005; Central Africa, Jolly *et al.,* 1998; South Africa, Palazzesi and Barreda 2012). Fossils can also be informative, but their scarcity and difficulty of identification of some fragments can limit their usefulness (Williams *et al.,* 1998; Hewitt, 2000).

1.1.2. Current patterns of biodiversity

Biodiversity is not homogeneously distributed across the Earth (Brown and Lomolino, 1996). Tropical regions harbor the highest levels of biodiversity in most of the taxa, while Polar Regions the lowest (Gaston, 2000). Such latitudinal gradient occurs both at a regional and continental scales (Hillebrand, 2004). Topographical gradients are also frequent and usually mountain ranges harbor higher diversity than flat areas (Simpson, 1964). Islands are also regions of great scientific interest, as due to their isolation they usually present a unique species assemble (MacArthur and Wilson, 2015).

Current distributional patterns of biodiversity can be mostly attributed to climatic oscillations that occurred in the Late Pleistocene (Carnaval *et al.,* 2009). In general, during the unfavorable periods of these climatic oscillations, most species went extinct or their ranges became reduced in large parts of their distributional area. But some were able to survive in specific climatic suitable areas (i.e. refugia), subsequently expanding during favorable conditions, while others were able to disperse to new locations (Hewitt, 2000). Additionally, some species were able to persist in their previous range by adapting to the new climatic conditions through niche shifts (Hoffmann and Sgrò, 2011). However, landscape features, ocean currents and latitude regionally modulated the global patterns of climate. Futhermore, each species reacted differently to climate changes owing to their own ecological requirements and life-history traits (Hewitt, 2000). Species with different affinities responded differently to the same climate changes (Hewitt, 1999), although some were able to adapt to the new conditions (Hoffmann and Sgrò, 2011). In temperate regions, warm adapted species experienced range contraction during cold periods, expanding again during warmer periods (e.g. species with Mediterranean affinity; Hewitt, 2004), while cold or mountain adapted species expanded their ranges

during cold periods and contracted in warm ones (e.g. species with Euro-Siberian affinity; Hewitt, 2004). A similar process likely happened with tropical species, albeit these expansions and contractions occurred in a lesser geographic extent (Hewitt, 2004). During humid periods, usually associated with cooler climate, xeric species likely became isolated and diversified, expanding during dry periods (e.g. *Chalcides*, Carranza *et al.,* 2008; Brito *et al.,* 2014). Contrarily, mesic species expanded during humid periods, contracting and becoming isolated when climate became dryer (e.g. *Taterillus,* Dobigny *et al.,* 2005; Hewitt, 2000, 2001). The effects of climatic oscillations on biodiversity patterns have been extensively addressed in many regions of the world (e.g. in Europe and North America; see Murphy and Weiss, 1992; Taberlet *et al.,* 1998; Hewitt, 2000, 2001; Weiss and Ferrand, 2007; Sommer and Zachos, 2009), but research is lacking for remote regions (Brito *et al.,* 2014).

1.2. The Sahara-Sahel

North Africa is a region of great biogeographic interest. The great diversity of habitats, heterogeneous landscapes and complex geologic and climatic histories all contribute to the biodiversity uniqueness of this region (Le Houérou, 1997; Comes 2004; Sayre *et al.,* 2013).

The Sahara desert and the adjacent arid region Sahel cover most of North Africa and correspond to two of the biggest ecoregions of the continent (about 11,230,000 km^2; Olson *et al.,* 2001). These areas present a high diversity of topographic features, as well as a heterogeneous climate resulting from spatial variability in both temperature and precipitation. The transition between the Paleartic and Afrotropical biogeographic realms correspond to the limit between the Sahara and the Sahel (Olson *et al.,* 2001). This leads to great latitudinal variation in species distributions and high local biodiversity (Dumont *et al.,* 1982; Le Houérou, 1992; Brito *et al.,* 2016).

Species inhabiting these regions present unique adaptive features to cope with the severe environmental conditions, such as scarce and unpredictable food and water, and extreme temperatures and solar radiation (see Brito *et al.,* 2014). Species ranges are usually under strong climatic control, and many species have patchy distributions. The evolutionary processes that led to the adaptation of organisms to such extreme environments also led to high rates of endemism (Ward, 2009).

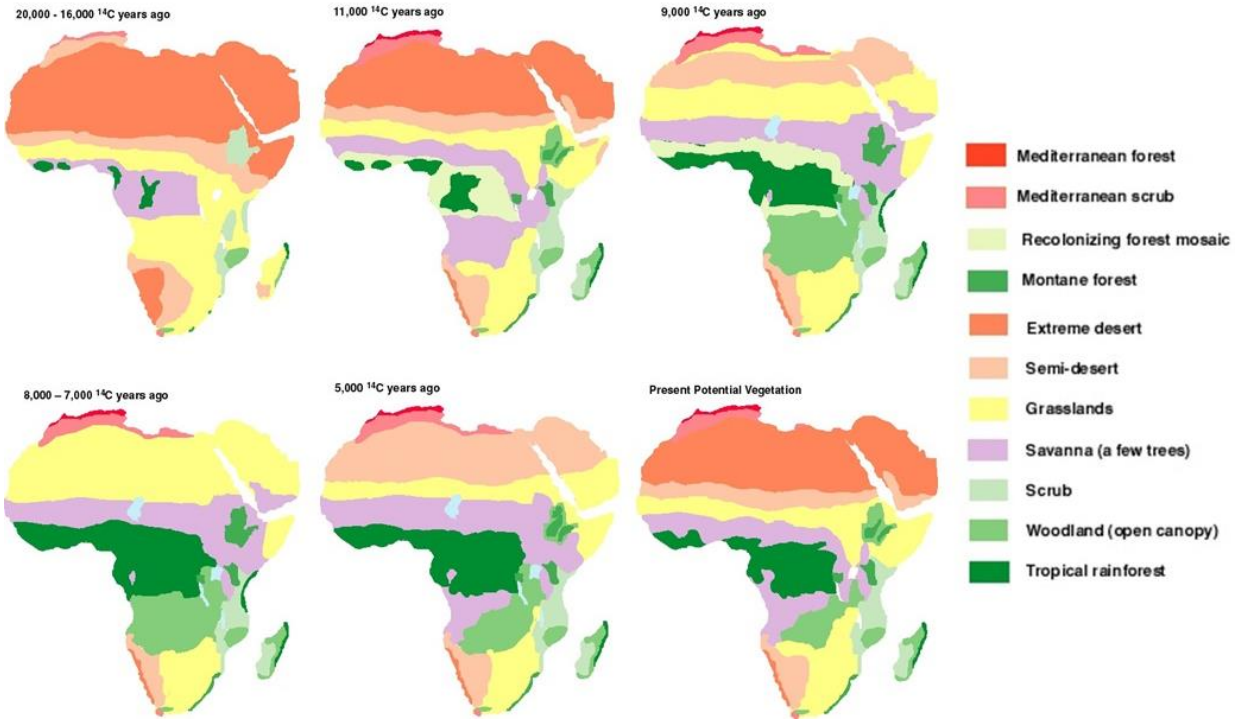

Figure 2: Latitudinal shifts in major habitats during climatic oscillations occurred in Africa since the Last Glacial Maximum until present. Adapted from Adams and Faure 2004.

The Sahara was not always a desert. Desertification is estimated to have begun about 7 million years ago in Chad (Schuster *et al.,* 2006; Zhang *et al.,* 2014), or between 6 and 2.5 million years ago in western areas (Swezey, 2009). Since its formation, this region has experienced, and is still experiencing, strong climatic fluctuations resulting from feedbacks between precipitation and vegetation cover (Wang *et al.,* 2008; Clausen, 2009). Since the Pliocene (5.3 Mya), the Sahara-Sahel went through several dry-wet cycles, at least eight to ten in the past 125,000 years (Le Houérou, 1997). These shifts greatly modified geomorphic processes, which was subsequently accompanied by changes in fauna and flora. During the humid periods, afro-tropical habitats expanded northwards (Figure 2), probably followed by an expansion of afro-tropical species such as fishes (e.g. *Barbus macrops*, *Clarias anguillaris* Trape, 2009), amphibians (e.g. *Hoplobatrachus occipitalis*; *Amietophrynus xeros* Tellería, 2009) and reptiles (e.g. *Echis leucogaster*; *Crocodylus suchus* Trape and Mane, 2006; Brito *et al.,* 2011a). The Sahara-Sahel was covered by a dense river network with multiple basins and large lakes (Drake *et al.,* 2011). These wetlands were covered by extensive vegetation (Gasse, 2000; Kröpelin *et al.,* 2008). The last wet period occurred between the Late Pleistocene (14,500 ya) and the Middle Holocene, ending between 6,000-5,000 years ago, when aridity began to increase significantly, with mesic communities disappearing and lake levels

decreasing (Foley *et al.,* 2003; Holmes, 2008). Current patterns of biodiversity in the Sahara-Sahel resulted from these climatic and land-cover oscillations (see Brito *et al.,* 2014).

1.2.1. Evolution in Sahara-Sahel

Despite recent studies in the region, evolutionary processes in the Sahara-Sahel remain largely unknown. Phylogeographic studies are revealing that diversification and speciation in the Sahara-Sahel are most likely related to spatial and temporal variation of the desert extent (Brito *et al.,* 2014). While the southern limit of the Sahara moved significantly during the Quaternary (1-6Mya), the northern limit appears to have retained approximately the same position (Le Houérou, 1997). The onset of the Sahara itself is likely to have created vicariance in a North-South axis, affecting diversification processes in many species. For example, the separation of two Macroscelidae mammals has been linked to the formation of the Sahara, with no apparent secondary contact ever since (Douady *et al.,* 2003). Carranza *et al.,* 2008 have also linked the origin and divergence of skinks and the age of the Sahara.

The occurrence of several cycles of wet-dry periods also had a profound impact on diversification processes of biota in the Sahara-Sahel region. These oscillations are estimated to have occurred in cycles of 100,000-20,000 years (Le Houérou, 1997), greatly shifting the range of savannah and desert environments and constrained the distribution and genetic structure of many species (Brito *et al.,* 2014). The many cycles of expansions and contractions resulted in contrasting patterns. Unsuitable climatic periods would have led to divergence due to absence of gene flow between refugia (e.g. Guillaumet *et al.,* 2008) while dispersal and gene flow would occur along geographical corridors during suitable climatic periods (e.g. Gaubert *et al.,* 2012). For instance, Dobigny *et al.,* 2005 showed that during the last million years, the West African *Taterillus* gerbils were likely restricted to refugia during dry periods and expanded in wet periods while being constrained by rivers, which probably led to parapatric species distribution and genetic evidence of bottlenecks. Similarly, the arid-adapted *Stenodactylus* geckos, which are present throughout Arabia and North Africa, exhibit high genetic diversity, with geological events and climatic instability as the main probable drivers of their divergence (Metallinou *et al.,* 2012). Nonetheless, despite these strong variations, mountains likely acted as refugia for many species since allowed vertical shifts of environmental

conditions and thus, population persistence through time (Messerli and Winiger, 1992; Gonçalves *et al.,* 2012, Velo-Antón *et al.,* 2014).

The Sahara-Sahel is an excellent model for studying the effects of extreme climate oscillations on biodiversity dynamics. However, such studies are still scarce, especially for species truly adapted to its arid conditions. Studies on patterns and processes in the region have largely been neglected and the role of landscape and climate remains poorly understood (Brito *et al.,* 2014).

1.2.2. Trans-Saharan biodiversity corridors

One pattern that emerges is that the Sahara desert effectively acts as a barrier to many species that are not fully adapted to arid conditions. However, there is plenty of evidence that many non-Saharan species persist in refugia throughout the desert (see Brito *et al.,* 2014). This suggests the existence of biodiversity corridors (narrow strips of habitat that connects two or more suitable patches; Rickelfs, 2008) across the Sahara (Figure 3), at least during wet periods. Brito *et al.,* 2014 reported that many of these isolated populations exist in restricted habitats within oases and mountains that are currently surrounded by sandy and rocky areas. But in the past, these areas were probably connected by savannah-like habitats during humid periods (Gasse, 2000; Kröpelin *et al.,* 2008), therefore forming a network of biodiversity corridors in a North-South axis (Dumont, 1982; Drake *et al.,* 2011). Most of these corridors disappeared during dry periods, as they did during the present, but others may have persisted even during dry periods. The Atlantic and Red Sea coasts, as well as the Nile River (see Figure 3) stand out as the most probable ones (Brito *et al.,* 2014). The Nile River has been regarded as a long and narrow oasis that prevailed at least since the last glaciation (Krings *et al.,* 1999). The Atlantic and Red Sea coasts are influenced by the sea proximity, and thus have a milder climate (Brito *et al.,* 2009; Brito *et al.,* 2011b). There have been some studies addressing the Nile River and the Red Sea coast as biogeographic corridors, mainly for the dispersal of hominins (e.g. Drake *et al.,* 2011). However, no study addressed the climatic stability of the Atlantic Sahara as a corridor over time, linking such stability to the genetic structure of species.

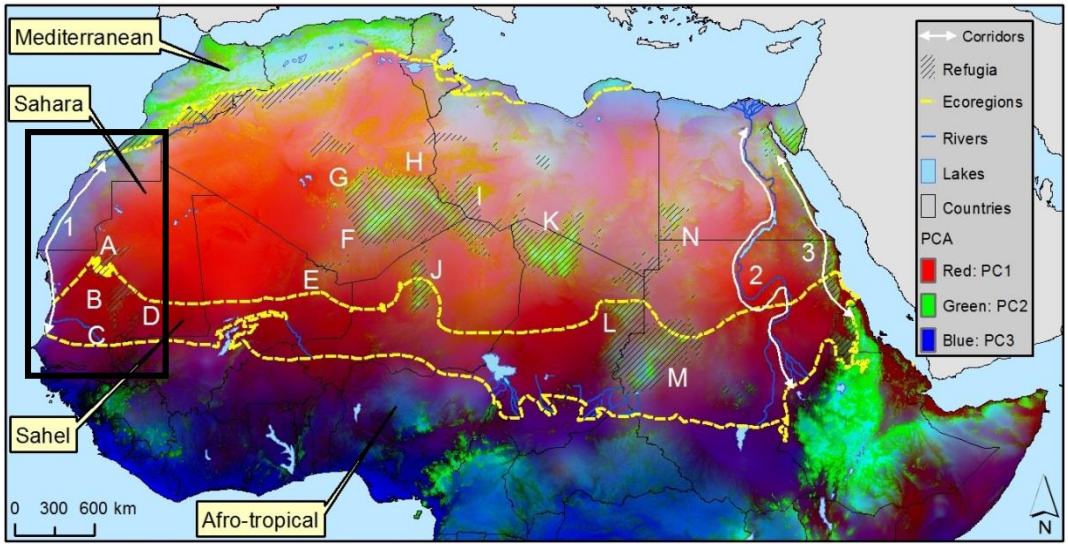

Figure 3: Environmental variability in North Africa derived by Spatial Principal Component Analysis (SPCA). The West Sahara region comprised within the black rectangle corresponds to the proposed corridor. Adapted from Brito *et al.,* 2014.

1.2.3. The West Sahara region

The West Sahara is a variable region in terms of climatic and habitat factors. At this respect, three units can be currently identified (Figure 4):

1. The Atlantic Coastal Desert, consisting of a narrow strip of land along the Mauritanian and Western Saharan coast, extending just 40 km inland, which has being previously identified as a WWF ecoregion (Olson *et al.,* 2001). It comprises the westernmost part of the Sahara and is characterized by low altitude (maximum of 200 m) and sandy or gravy substrate (Olson *et al.,* 2001). The climate is hot and dry, with low and sporadic rainfall, but mists from the Atlantic are common, bringing humidity when condensing (Olson *et al.,* 2001). Desertification in this region is thought to have begun more recently than in other areas (Swezey, 2009). Nevertheless, it was also subject to several dry-wet cycles, albeit less intense than in more inland areas due to the influence of the sea. One important landscape feature, however, is that during the Last Glacial Maximum sea levels decreased, and thus terrestrial habitat increased.

2. Inland regions to the Atlantic Coastal Desert, suffer less influence from the sea, and are thus characterized by increasing warmer temperatures and low precipitation.

Annual rainfall is usually between 100 and 200 mm, increasing from north to south, but droughts lasting several years are not uncommon. It also has low altitude and the substrate is mostly composed of sand. Some more arid resistant plant species of Sahelian origin can be found in this region, such as *Acacia* trees (Olson *et al.,* 2001).

3. Inland rocky areas, such as the mountains of Gueltat Zemour and Adrar Soutuf in Western Sahara, and the Mauritanian mountains of Adrar Atar, Tagant and Assaba. These mountains have moderate altitude, ranging from 300 to 900 meters, and the substrate is mostly rocky. They also present a unique climate, with an increase of humidity comparing to the surrounding areas, especially in the southern mountains. This

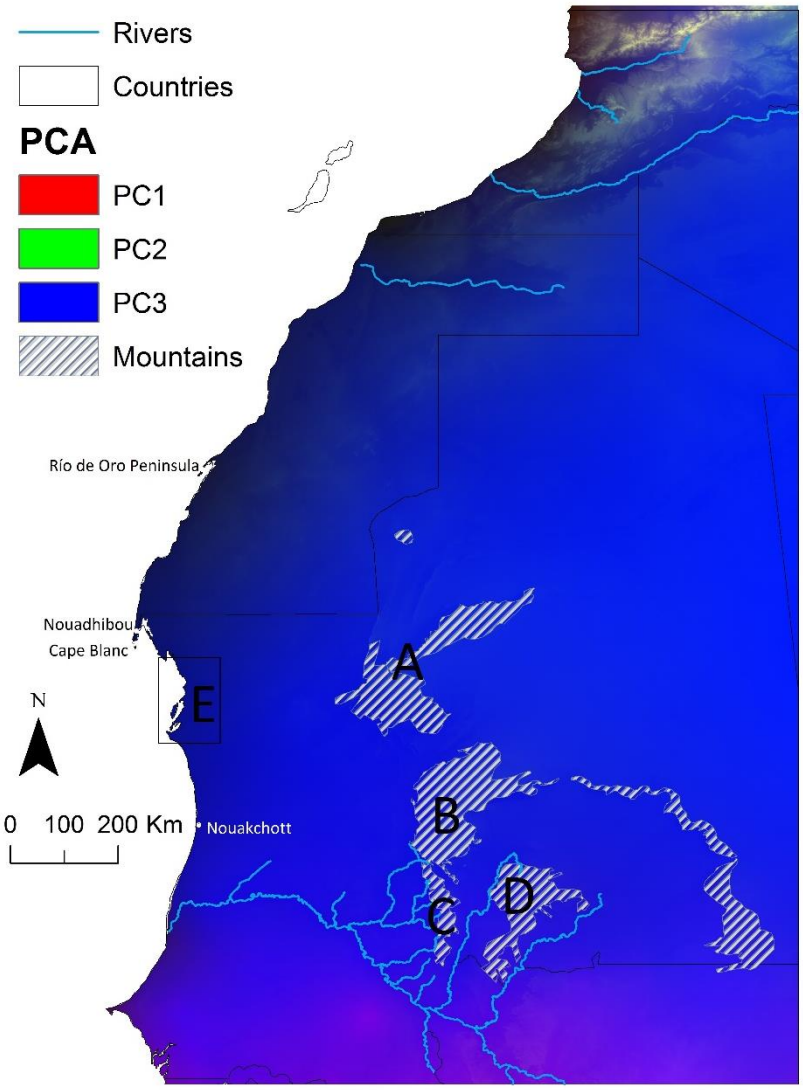

Figure 4: Environmental variability in West Sahara derived by Spatial Component Analysis (SPCA). Main rivers and mountains are represented; A: Adrar-Atar; B: Tagant; C: Assaba; D; Affolé; E: Parc National Banc du d'Arguin.

allows the persistence of relict populations of both Mediterranean and Sahelian taxa in the middle of the desert (Olson *et al.,* 2001).

In the West Sahara, climate has importantly varied through the Late Pleistocene (Figure 5). Generally, in the Last Inter Glacial (LIG, 120 kya), maximum temperature and annual precipitation were higher than in the present, especially in more inland regions. During the Last Glacial Maximum (LGM, 21 kya), maximum temperature and annual precipitation decreased. In the Middle Holocene (MidHol, 6 kya), climate became slightly warmer and wetter. Maximum temperature in the Atlantic Coastal Desert remained reasonably constant, being slightly lower during the LGM. However, annual precipitation varied greatly, being moderately high during the LIG and decreasing towards the present. More inland areas suffered greater oscillations, especially in precipitation. Maximum temperature was higher during the LIG, decreasing towards the LGM and remaining approximately constant until the present. Annual precipitation was significantly higher during the LIG, greatly decreasing towards the LGM. In the Middle Holocene, annual precipitation increased, but then decreased again towards the present. In mountainous areas, similarly to coastal regions, climate was more constant, and generally maintaining a cooler and wetter climate than the surrounding areas. During the LIG climate was warmer, with temperature decreasing towards the Middle Holocene, slightly increasing in the present. Annual precipitation remained more or less constant, only being slightly higher in the Middle Holocene.

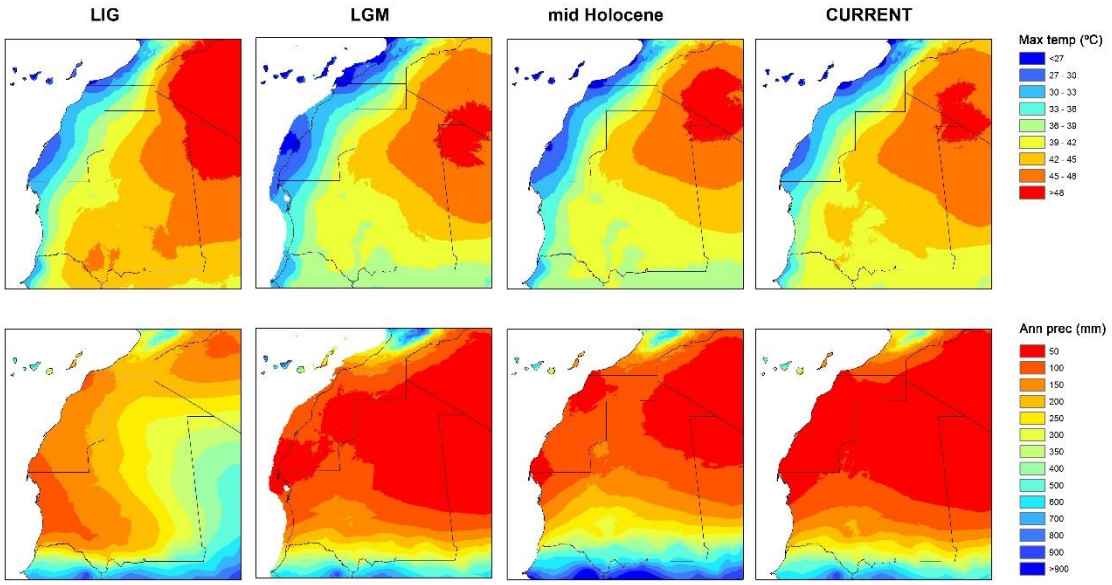

Figure 5: Variation of maximum temperature (Max Temp) and annual precipitation (Ann Prec) in the West Sahara through time. For the LGM only one GLM is represented, corresponding to CCSM (Community Climate System Model) scenario. Climatic variables were retrieved from WorldClim (www.worldclim.org).

The effect of climatic variability in the West Sahara biodiversity remains poorly understood. Many species with continuous distribution in either north or south of the West Sahara desert present isolated populations on the other side and some even have relict populations in suitable patches in the mountains thus, suggesting this region as a corridor (Brito *et al.,* 2014). When distributional patterns of taxa inhabiting the West Sahara are analysed, four main patterns of species distributions can be differentiated:

1) Species showing a disjoint distribution

Some species can be found both north and south of the West Sahara, with no connection in between. This is the case of some Afrotropical species such as the puff adder *Bitis arieta*ns, which inhabits a range of semi-arid open habitats extending throughout sub-Saharan Africa, and north of the Sahara in isolated populations in southwest Morocco. This species presents a distinct West African clade, with indivisuals from both north and south of the Sahara grouped together (Trape and Mane, 2006; Barlow *et al*., 2013).

2) Species endemic to the coastal region

Some species are endemic to the Atlantic coastal desert, having a restricted distribution near the Atlantic coast and only expanding a few kilometers inland. For instance, the small lizard *Acanthodactylus aureus* exists only along the Atlantic Sahara (Crochet *et al.,* 2003; Trape *et al.,* 2012), but recent inferences on its genetic variability indicated high levels of structuration along it (Lopes, 2014).

3) Species with almost continuous distributions from one realm to another

Some species are typically Mediterranean and expand to the Sahara and sometimes Sahel, while others are major sub-Saharan but can be found in Saharan mountains. The Mediterranean snake *Psammophis schokari* exemplifies the first case as it is distributed across the Mediterranean in North Africa and penetrates along the western Sahara coast, reaching Mauritania (Trape and Mane, 2006; Rato *et al.,* 2007). Exemplifying the second case, the North African *Agama boulengeri* lizard which is Sahelian, but colonized the Sahara, presumably during wet periods, diversifying during dry ones; currently, presenting north-south genetic sub-structure (Trape *et al.,* 2012; Gonçalves *et al.,* 2012). *Crocodylus suchus* is also another example (see Trape *et al.,* 2012), thought to have colonized the Mauritanian mountains from southern ranges during

wet periods, with subsequent isolation when climate became drier (Velo-Antón *et al.,* 2014).

4) Species with widespread distributions in arid conditions

Species adapted to arid conditions typically have a widespread distribution throughout the Sahara (Brito *et al.,* 2014). These species present adaptations to cope with extreme climate, and thus are expected to have reacted in an opposite manner than non-Saharan species. For instance, the arid adapted *Cerastes* vipers are widespread throughout the Sahara (Trape and Mane, 2006), including one species adapted to rocky and another to sandy areas (Brito *et al.,* 2011b). Similarly, *Acanthodactylus dumerili* (including the synonymous *A. senegalensis*; Lopes, 2014) is also widespread, though it avoids the central areas of the Sahara (Trape *et al.,* 2012). While no genetic studies exist for the viper species*, A. dumerili* was found to have two lineages, with one distributed throughout all the southern range and another apparently restricted to Morocco (Lopes, 2014).

These four patterns of species distribution described here are apparently related to the distribution of climatic variability. For instance, the coastal desert corresponds to an area of milder temperatures than the interior regions (Olson *et al.,* 2001). This particular climate, derived from the proximity of the sea, likely restricted endemic species to the coastal desert. When comparing current climatic patterns in the region with predictions of past climate, both temperature and precipitation are more variable in inland areas, while remaining more constant near the coast (Fig. 4). Similarly to other African mountains (Messerli and Winiger, 1992), Saharan mountains also present lower temperatures than the surrounding low altitude areas, as well as higher precipitation (Olson *et al.,* 2001). Mountains likely acted as climatic refugia favoring the persistence of species not adapted to arid conditions during dry periods as the current. For example, Vale *et al.,* 2015 showed that Mauritanian gueltas, despite their small size, are important micro-hotspots of biodiversity. All of these inferences raise the question of whether climatic stability in the different regions of the West Sahara resulted in the persistence of ecological corridors through which species could cross the Sahara even during unfavorable periods.

1.3. Species model: *Tarentola*

Reptiles constitute good models to infer the role of climatic variability on biogeographical patterns. Being ectothermic, reptiles are dependent on environmental temperature, presenting several behavioral, physiological and anatomical traits that allow them to regulate their internal temperature with low energy costs (Shine, 2005). As a result, reptiles were highly susceptible to the climatic oscillations of the Pleistocene, and their low dispersal capacity hindered their ability to track suitable habitat (Araújo and Pearson, 2005).

In this thesis, I used geckos of the genus *Tarentola* to infer the role of climatic oscillations as drivers of their genetic structure. The genus *Tarentola* (Fam. Phyllodactylidae) comprises 21 species, distributed across North Africa, coastal regions of the Mediterranean Sea, Macaronesia and the West Indies (Cuba and Bahamas; Rato *et al.,* 2012; TRD, 2016). All species present low inland dispersal capacity and are morphologically similar, with a large head, short plump body, slender limbs and short tail. They form a monophyletic clade, being phylogenetically close to *Pachydactylus* and *Hemidactylus* (Carranza *et al.,* 2002). The genus is formed by two main clades, one containing species as *Tarentola annularis*, *T. chazaliae* and *T. ephippiata*, and the other the likes of *T. mauritanica* and *T. deserti*, *T. boehemei* and *T. fascicularis* (Rato *et al.,* 2012). The origin of the genus goes back to the Miocene with the split between the two main clades occurring in this period (Rato *et al.,* 2012). Furthermore, high genetic divergence has been found within *T. mauritanica*, suggesting several cryptic species (Rato *et al.,* 2015). However, no study addressed in detail the genetic structure within the other *Tarentola* species.

Four *Tarentola* species inhabit the West Sahara (Schleich *et al.,* 1996, Trape *et al.,* 2012):

1) Tarentola annularis (Geofrey-St-Hilaire, 1827) is a relatively large species, with a maximum snout-vent length (SVL) of 14 cm, and can be identified by the presence of four white spots in the shoulder region arranged in a square (Figure 6A). This species has a widespread distribution from the Western coast to Somalia and Egypt, and can be found in rocky desert plains along the Sahel and some parts of the Sahara (Figure 6A; Trape *et al.,* 2012).

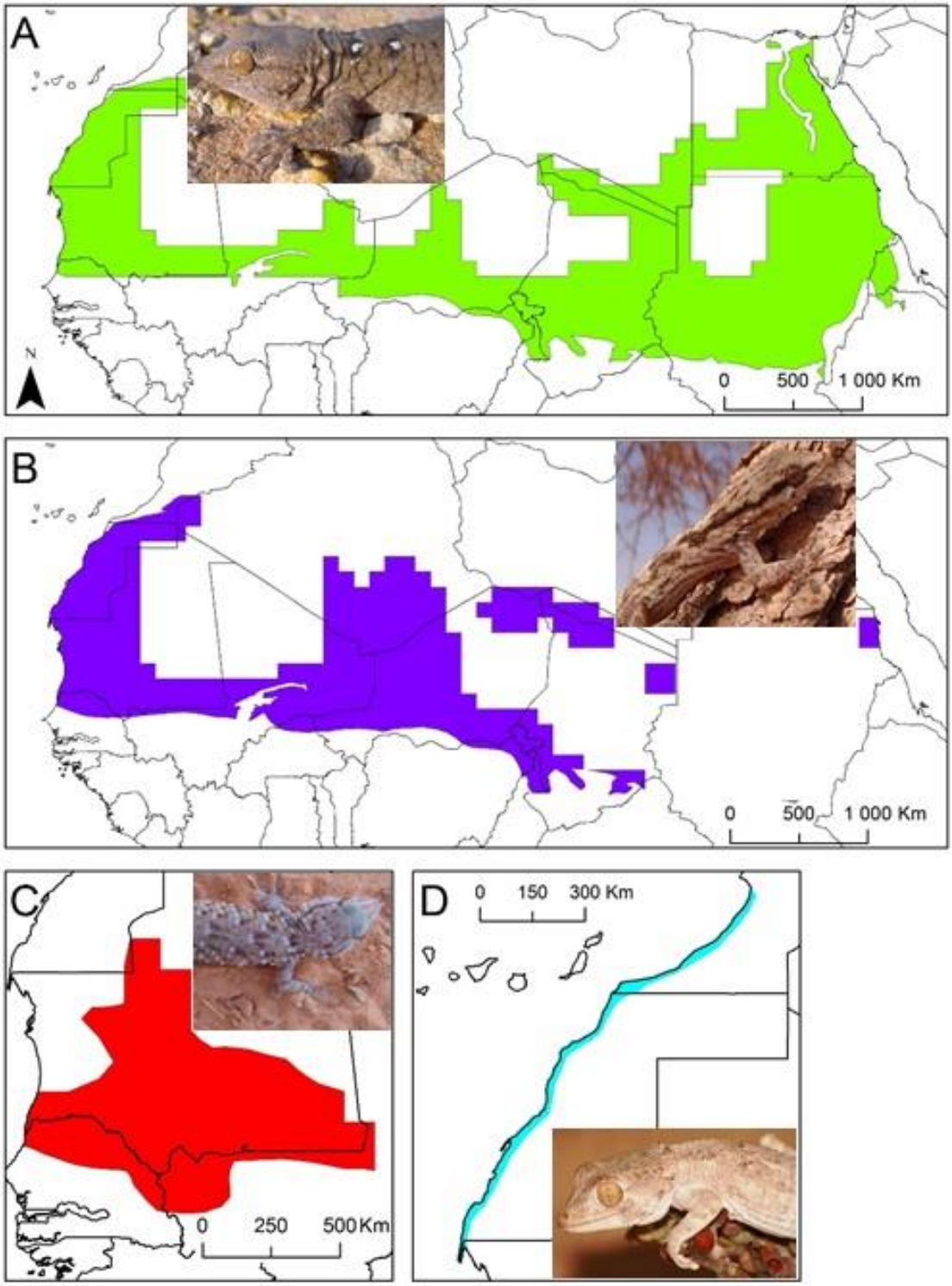

Figure 6: Known distributional ranges of the study species in North Africa, with photography of a representative individual: A) *T. annularis*, B) *T. hoggarensis*, C) *T. parvicarinata* and D) *T. chazaliae*. Adapted from Bons and Geniez, 1996 and Trape et al 2012

2) Tarentola hoggarensis (Werner, 1937) is relatively small, with a maximum SVL of 8.2 cm and characterized by four transversal dark bands in the dorsum (Figure 6B). It inhabits arid areas of Western Sahel and part of the West Sahara (Figure 6B), where *Acacia* trees are more or less abundant. It was previously considered as a subspecies of *T. ephippiata,* but it was more recently elevated to species level (Trape *et al.,* 2012). Another former subspecies of *T. ephippiata* recently elevated to species level is *T. senegambiae*. This species is larger, with a maximum SVL of 13 cm, and is restricted to Senegal, Gambia and Guinea-Bissau. It is typically arboreal, though it can be found in buildings and walls (Trape *et al.,* 2012). However, molecular studies are lacking to confirm the validity of these species and to determine their relation with *T. ephippiata* and *T. hoggarensis*.

3) Tarentola parvicarinata (Joger, 1980) has a maximum SVL of 9.7 cm, a lighter coloration and large tubercles in the tail (Figure 6C; Trape *et al.,* 2012). No molecular study has included this species, and thus its phylogenetic relationship with the remaining *Tarentola* species remains unknown. This species is found almost excusively in mountain regions with rocky substrate, namely in Mauritania and Mali (Figure 6C; Trape *et al.,* 2012).

4) Tarentola chazaliae (Mocquard, 1895) is smaller, with a maximum SVL of 7.4 cm, and a disproportionately large head (Figure 6D). It is sometimes called helmeted gecko because it presents a row of conical occipital tubercles that resemble a helmet. Previously considered to be part of the genus *Geckonia*, but an integrative study with morphological and molecular markers revealed that it is in fact a *Tarentola* (Carranza *et al.,* 2002). This species is endemic to the Atlantic Sahara, inhabiting cooler and more humid habitats than the other species, and extending their distribution just a few kilometers inland (Figure 6D). It prefers sandy and rocky soils with large deposits of vegetation (Trape *et al.,* 2012).

By apparently displaying distinct ecological/habitat requirements, these four *Tarentola* species offer an interesting biological system to investigate the effect of Pleistocene climatic oscillations on their genetic structure across the West Sahara. The four species are expected to follow one of the continuous distributional patterns previously described: (i) *T. annularis* would follow the pattern of the widespread arid-adapted species like *A. dumerilli* and thus, genetic structuration should be low; (ii) *T.*

chazaliae is expected to follow the endemic distribution pattern, similar to *A. aureus* and thus, genetic structure should be high; and (iii) *T. hoggarensis* and (iv) *T. parvicarinata* would display an almost continuous distribution pattern, being both Sahelian species penetrating northwards but adapted to different habitats (i.e. *Acacia* trees the former, mountainous areas the latter). These species should exhibit similarities with *A. boulengeri* or *C. suchus*, for instance, and thus genetic structuration would be dependent on specific habitat characteristics.

1.4. Genetic and ecological approaches

Combining phylogeographic and Ecological Niche-based Modelling to study patterns of biodiversity has seen recent attention, as both approaches complement each other well (Kozak *et al.,* 2008; Alvarado-Serrano and Knowles, 2014).

Phylogeography is the integration of several genetic data sources in a geographic context in order to understand the relationship between geographic features and species distributions (Hickerson *et al.,* 2010). The choice of molecular marker is central to phylogeography, and different markers are used to help answering different questions. Each molecular marker has different rates of evolution and can be subjected to different selective pressures. Thus, working with mitochondrial (mt) or nuclear DNA (nDNA) might yield different results (e.g. Wan *et al.,* 2004). The choice of marker will depend on the question being addressed. For example, microsatellites are suitable for studying recent dynamics such as at a population level due to their fast evolutionary rate (Goldstein and Schlotterer, 1999), while coding nDNA is more conservative, being more indicated to infer relations among distant taxa, as genera or families (Zhang and Hewit, 2003). MtDNA is less conservative, thus being better suited to infer both interspecific and intraspecific relationships (Wan *et al.,* 2004). However, mtDNA is maternally inherited, reflecting only the matrilineal evolutionary history. The effective population size of mtDNA is also only a fourth of nuclear markers, resulting in a faster lineage sorting and allele extinction rates, leading to a potential oversimplification of phylogenetic relations and underestimation of genetic diversity (Zhang and Hewitt, 2003). Within the same class of markers (e.g. mtDNA), some variation exists as far as mutation rate is concerned, with different markers being suitable to address different time scales (Wan *et al.,* 2004). MtDNA is the most commonly used tool for phylogeographic analyses.

Genetic data has some shortcomings when trying to build phylogegraphic hypothesis. For example, phylogeographic approaches are limited when addressing "where" biogeographic events occurred. Genetic data can indicate population expansion or contraction, but provides limited information on where those populations occurred when they were large or small (Peterson, 2009). This question, however, can be independently inferred with Ecological Niche-based Models (ENMs). ENMs correspond to a series of methods that aim to determine the ecological niche of a species (fundamental vs. realized; see Sillero, 2011) and its potential distribution (Peterson, 2011). Although hybrid approaches exists, ENMs can be divided into correlative and mechanistic approaches. Correlative approaches aim to determine the realized ecological niche of a species (i.e. the Grinnellian niche, *sensu* Soberón, 2007) by associating its distribution with the environmental conditions of where the species occurs. On the other hand, mechanistic approaches try to spatially map different aspects of the fundamental ecological niche of species, linking functional traits with the environmental conditions that species experiment along their ranges (Wiens *et al.,* 2009; Alvarado-Serrano and Knowles, 2014). Mechanistic approaches, however, require detailed information on the physiological tolerances of the organisms, for instance, which remain unknown for most species. Thus, most studies use correlative approaches as data on species presence (or absence to a lesser extent) is widely available (Elith *et al.,* 2010; Alvarado-Serrano and Knowles, 2014).

ENMs are not exempt from assumptions and uncertainties either. For instance, when building the models, it is impossible to measure all the variables that potentially influence the niche of a species (i.e. the Fundamental niche) and thus there is always the risk of a species niche being determined by unmeasured variables (Wiens *et al.,* 2009). Another assumption is that species are in equilibrium with the environment, that is, the suitable habitat is fully occupied (Soberón and Peterson, 2005). However, a species may not be present in a suitable patch of habitat if a recent disturbance eradicated it from that area or if a patch only became available recently and the species was not yet able to colonize it (Wiens *et al.,* 2009). On the other hand, some individuals might be found in a patch no longer suitable for the species due to having high longevity (such as with some plant species; Foden *et al.,* 2007). To assume equilibrium with the environment, it is also necessary to assume that individuals are capable of dispersing to suitable locations (Pearson and Dawson, 2003). However, species with low dispersal rates may not be able to track the shifting suitable habitat and must either adapt to the new conditions, or survive in refugia (e.g. Velo-Antón *et al.,* 2015). ENMs also assume

that each species react to the environment independently, with their niches not being influenced by biotic interactions (Wiens *et al.,* 2009). This has been shown not to be true (Araújo and Luoto, 2007), as by definition, a species realized niche is restricted by the interactions with other species (Soberón, 2007) but remains an overwhelming challenge for most studies (Wiens *et al.,* 2009; Kissling *et al.,* 2012). ENMs also assume that the niche of a species is conserved, that is, it is an immutable characteristic of a species, thus remaining constant through space and time. This assumption justifies the possibility of extrapolating models to different time periods (Wiens and Graham, 2005). Finally, sampling the complete climatic niche where a species is able to thrive is considered one of the main factors affecting the reliability of SDM predictions. To measure the true adaptive potential of a species, it is necessary to sample the entire climatic niche of said, minimizing the uncertainties associated when projecting to a different time period (Thuiller, 2004; Wisz *et al*., 2008; Nogués-Bravo, 2009; Barbet-Massin *et al*., 2010; Martínez-Freiría *et al.,* 2016).

Some uncertainties must also be taken into consideration. Some violate the assumptions, but others have different sources. For instance, climatic conditions for the past cannot be directly measured, and must be derived. Different methods take into consideration different parameters, and predict different conditions. Furthermore, there are several algorithms used to construct ENMs, which deal with the assumptions differently, and can lead to different predictions. The quality of the data used can also have and important impact on the ENMs. The records of species occurrence need to be reliable, as well as the data on the environmental conditions. Finally, the scale at which the ENMs are constructed, need to be concordant with the scale at which the environmental variables affect the niche of the species. For example, climatic variables can define the niche of a species at a coarse scale, but at a finer scale, other factors, such as species interaction or land cover can have a bigger impact. Acknowledging the assumptions and uncertainties of ENMs is essential to correctly interpret ENMs (Wiens *et al.,* 2009; Alvarado-Serrano and Knowles, 2014; Martínez-Freiría *et al.,* 2016).

Phylogeographic and ENM tools complement each other well. ENMs can be used to interpret the patterns of genetic variations by visually comparing the geographic distribution of the genetic patterns with the projected distribution of a species (Alvarado-Serrano and Knowles, 2014). For example, Martínez-Freiría *et al.,* 2015 identified northwest Iberia as climatic refugia for *Vipera seoanei* based on mtDNA genetic pattern, which was corroborated by an ENM approach that identified the same area as suitable, both in the present and in the past. Habitat suitability can also be converted into probable

migration routes that can be compared to genetic distances (McRae, 2006). On the contrary, habitat suitability can be used to identify environmental barriers and compare with genetic breaks (e.g. Velo-Antón *et al*., 2015). When predicting species distributions for several time periods, it is possible to identify regions of environmental stability where species could have persisted over time. Carnaval *et al.,* 2009 identified stable areas of tropical forests along the Brazilian coast for the past 21,000 years and compared them with genetic data for three frog species. As predicted, the populations from the stable areas presented a genetic signature that was different from the populations of unstable areas.

In this thesis I will take advantage of the combination of phylogeographic and ENM approaches to address evolutionary scenarios in West Sahara to try to understand whether this region served as a stable ecological corridor through time.

1.5. Objectives

This study aims to test the effect of climatic oscillations in the West Sahara by addressing the genetic structure of four species of the genus *Tarentola* with different ecological affinites. In order to fulfil this objective, two approaches will be used:

1 – Ecological Niche-based Modelling in order to understand how the study species respond to current and past climatic conditions in the Atlantic Sahara. This approach aims to answer the following questions: i) What are the climatic variables that most determine the distribution of the study species? ii) How suitable the West Sahara is for each species over time?

2 – Phylogeographic analysis to analyze the genetic structure of each study species. More specifically, this aims to answer two questions: i) Are the species genetically structured across the West Sahara? And if they are ii) how are the different lineages geographically distributed?

Overall, the genetic patterns and ENMs obtained for each species are compared within *Tarentola* and also with other reptile studies from the same geographic area in order to better understand the role of climate in shaping genetic structure in the West Sahara.

2. Methods

2.1. Study area

The study area covers the West Sahara region, spreading from Senegal to southern Morocco (13.5ºN to 35.5ºN) and thus including the Atlantic Sahara ecoregion, and the adjacent inland region and mountain ranges of West Sahara and Mauritania until 6ºW (see Figures 4 and 7).

The Fishnet tool from the Data Management Toolbox in ArcGIS (ESRI, 2006) was used to define the study area in a fashion that would include all the observations used and a 100 km buffer around them. A shapefile of the contour of the African countries was used to better visualize the spatial distribution of both the inputs (samples and climatic variables; see below) and the outputs.

2.2. Sampling

For Ecological Niche-based Models (ENM), 284 observations of *T. annularis*, 108 of *T. chazaliae*, 164 of *T. hoggarensis* and 339 of *T. parvicarinata* at 1 x 1 km resolution (WGS 1980 datum) were taken into account. Observations were gathered from fieldwork carried by BIODESERTS and collaborators (231 of *T. annularis*, 19 of *T. chazaliae*, 147 of *T. hoggarensis* and 339 of *T. parvicarinata* at at GPS precision), and from already published national atlases (e.g. Bons and Geniez, 1996; 53 of *T. annularis*, 89 of *T. chazaliae* and 16 of *T. hoggarensis*). In each species dataset, levels of spatial clustering were assessed with the Nearest Neighbor Index (NNI) from the Spatial Analyst extension in ArcGIS (ESRI, 2006), and a random removal of localities from clusters of each species occurrence was done to decrease it (e.g. Brito *et al*., 2011b; Martínez-Freiría *et al*., 2015). Finally, to perform ENMs, a low clustered distribution was obtained for each species (z-score=-1.642, NNI=0.894 for *T. annularis* with 62 presences; z-score=-1.627, NNI =0.854 for *T. chazaliae* with 34 presences; z-score=-1.646, NNI =0.883 for *T. hoggarensis* with 51 presences; z-score=-1.597, NNI =0.921 for *T. parvicarinata* with 112 presences; Table 1).

Table 1: Sample sizes for building ENMs for each species. Total: total number of observations; Training: number of observations used to construct the model; Validation: number of observations used to test the models.

Species	Total	Training	Validation
T. annularis	62	50	12
T. chazaliae	34	28	6
T. hoggarensis	51	41	10
T. parvicarinata	112	90	22
Total	259	209	50

For molecular analyses, tissue samples were available from South-Western Morocco, Western Sahara, Mauritania, Senegal and Mali (Figure 7). Samples were collected during fieldwork missions carried by the BIODESERTS group from 2004 to 2014. Each sample locality was recorded with a Global Positioning System (GPS) on WGS84 datum. From a total of 183 samples, 38 of *T. annularis*, 12 of *T. chazaliae*, 48 of *T. hoggarensis* and 72 of *T. parvicarinata* were selected to be sequenced in a way that would maximize the geographical coverage of the distribution of each species. The remaining 13 samples included other species, *T. senegambiae* (1 sample), *T. boehmei* (3 samples), *T. deserti* (2 samples), *T. mauritanica* (2 samples) and *Tarentola* sp (5 samples) were also sequenced and used as outgroups for genetic analyses (Figure 7).

2.3. Climatic variables

For current conditions, 19 bioclimatic variables were downloaded from WorldClim (http://www.worldclim.org/) at 30 arc seconds (~1 x 1 km) resolution (Hijmans *et al.,* 2005). All variables were imported to ArcMap and cut to the study area using the Extract by Mask tool from the Spatial Analyst extension (ESRI, 2006). Spatial correlation among variables was assessed with the Band Collection Statistics tool from the Spatial Analyst extension and variables with high correlation ($R > 0.70$) were discarded. Three sets of variables were defined, all with low correlation. The variables in each set were visualized in ArcMap to check for spatial artifacts and inconsistencies (e.g. Kidd and Ritchie, 2000). The set chosen included six variables, three related to temperature and three to precipitation (Table 2), and thought to be biologically important for the study species (Rato *et al.,* 2014).

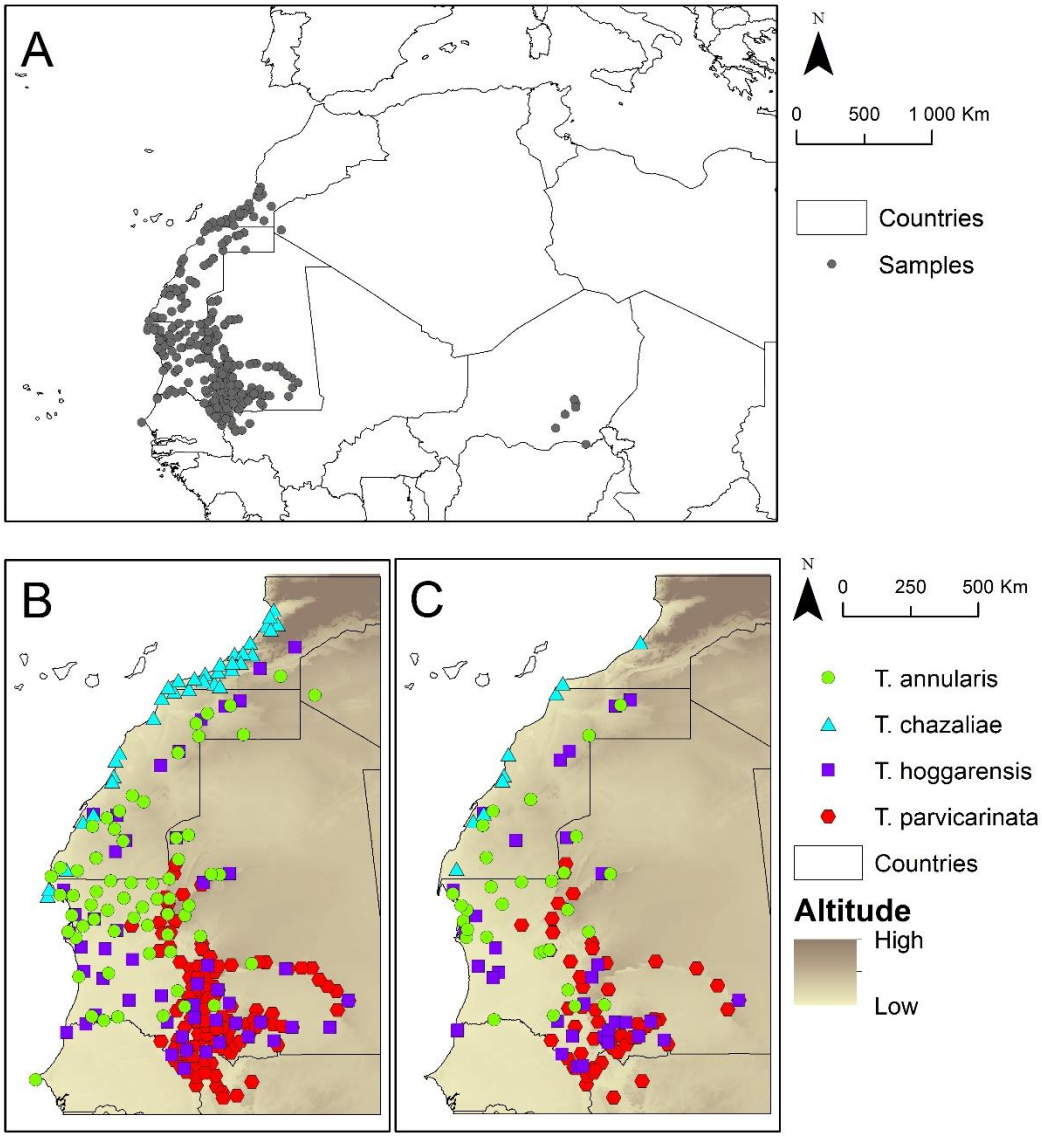

Figure 7: Spatial distribution of the distributional records and samples used in this thesis. A) Total number of samples and records used for both the ecological and molecular approach. B) Distributional records per species used to build the ENMs. C) Tissue samples used for the molecular analyses, per species. Six samples of *T. hoggarensis* from Niger (see A) were also included in molecular analyses.

Table 2: Climatic variables used for building the Ecological Niche-based Models of *Tarentola*, with units and range of variation for current conditions.

Variable	Units	Range
Temperature Seasonality	ºC	1092-8005
Min Temperature of Coldest Month	ºC	-14.4 - 18.9
Mean Temperature of Warmest Quarter	ºC	11.0 - 36.9
Annual Precipitation	mm	8 - 954
Precipitation of Driest Quarter	mm	0 - 91
Precipitation of Coldest Quarter	mm	0 - 662

For past climatic conditions, the same six variables were downloaded from WorldClim for three time periods: Last Interglacial (LIG; ~120,000–140,000 years BP; Otto-Bliesner *et al.,* 2006), Last Glacial Maximum (LGM; ~21,000 years BP; Paleoclimate Modelling Intercomparison Project Phase II) and Middle Holocene (MidHol; ~6,000 years BP; Coupled Model Intercomparison Project #5. LIG variables were at 30 arc seconds resolution and accounted for one Global Circulation Model (GCM), NCAR-CCSM (Community Climate System Model; Otto-Bliesner *et al.,* 2006). LGM variables were at 2.5 arc minutes (~5 x 5 km) resolution and corresponded to three GCMs: CCSM4 (Community Climate System Model, ver. 4, Collins *et al*., 2006), MIROC-ESM (Model for Interdisciplinary Research on Climate, ver. 3.2, Hasumi and Emori, 2004) and MPI-ESM-P (Max Planck Institute). These GCMs differ in temperature and precipitation, and in the West Sahara CCSM predicts the warmest climate, while MPI-ESM-P predicts the coolest, and MIROC predicts the wettest climate, while MPI-ESM-P predicts the driest. For the Middle Holocene, one GCM was retrieved: CCSM4 All variables were imported to ArcMap and cut to the study area using the Extract by Mask tool from the Spatial Analyst extension (ESRI, 2006). Finally, variables, both from present and for past conditions were exported from ArcMap to ASCII format in order to be used for the modelling analyses.

2.4. Lab Procedures

2.4.1. DNA Extraction

Samples consisted of tail tip tissue and were extracted with the EasySpin Genomic DNA Tissue kit (Citomed) following an adapted protocol (Annex A1). The quality and quantity of the DNA extracted was evaluated with an electrophoresis in a 0,8% Agarose gel stained with Gel Red in TBE 0,5x for 10-15 minutes at 300V. A Biorad Universal Hood II Quantity One 4.4.0 was used to visualize the gels exposed to UV radiation. According to this evaluation DNA extractions were proportionally diluted with ultrapure water to prevent possible inhibitions in the PCR reactions.

2.4.2. Marker selection

Several mtDNA markers were tested in order to obtain reliable information for phylogenetic/phylogeographic reconstructions. First, the cytochrome oxidase I (COI; Castaneda and de Queiroz, 2011) was initially chosen as it is a commonly used marker in phylogeographic studies due to its fast evolutionary rate (Knowlton and Weigt, 1998). However, after obtaining and aligning the first sequences, they presented a pattern typical of nuclear genes, with double peaks in many positions of the chromatogram. Thus, it was concluded that the selected primers were probably amplifying a nuclear pseudogene, and the marker was discarded. Another gene commonly used in phylogeographic studies was then selected: cytochrome-b (cyt-b; Kocher *et al.,* 1989), and the same process of amplification and sequencing was performed. However, after aligning the first sequenced samples and building a preliminary Neighbour-Joining Tree, there were some inconsistencies between morphology and the tree obtained. The most glaring one is that *T. chazaliae*, which is morphologically quite distinct from the remaining species, did not form a unique clade, but was rather spread through the other clades, with one individual falling among *T. annularis* and the remaining within *T. hoggarensis*. This pattern could be due to a real evolutionary process, or could be as simple as a contamination. In order to distinguish between the two, another gene was necessary, and thus 12S (Kocher *et al.,* 1989) was finally selected, which had already been successfully used in previous works with the genus *Tarentola* (e.g. Rato *et al.,* 2012). As

this strange pattern was not found with 12S, contamination is unlikely, although more research will be needed to understand the unexpected pattern obtained in cyt-b.

2.4.3. Amplification and sequencing

Polymerase Chain Reactions (PCRs) were performed in a total volume of 10µl containing 5µl of MyTaq™ HS Mix (BioLine), 3µl of ddH_2O, 0.5µl of each primer (forward and reverse) and 1µl of template DNA. The PCR conditions are as follows: an initial denaturation at 95ºC for 10 minutes, followed by 35 cycles of denaturation at 95ºC for 30s, annealing at 52ºC for 30s and extension at 72ºC for 30s, and a final extension at 72ºC for 10 minutes. Reamplification of samples that yielded too low amounts of product was performed, using the same PCR conditions. A negative control was used in every reaction to check for contaminations. All PCRs were performed in a Biometra T100 thermocycler.

All PCR products were assessed by electrophoresis in 2% Agarose gels stained with Gel Red and with a mass DNA ladder (NZIDNA ladder V).

Part of the PCR products were purified and sequenced by a commercial company (Macrogen Inc, Netherlands). The remaining PCR products were purified using ExoSAP-IT® PCR clean-up Kit (GE Healthcare). The sequencing followed the BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) protocol. The sequencing products were cleaned in sephadex and then rehydrated with ddH_2O. Finally, the cleaned sequencing products were read by capillary electrophoresis in a ABI3130xl Genetic Analyzer (AB Applied Biosystems).

2.5. Data analyses

2.5.1. Ecological Niche-based Models

A Maximum Entropy approach was used to build ENMs (Phillips *et al.,* 2006). For this modeling technique, only presence data is required and has been shown that consistently performs well comparing with other methods (Elith *et al.,* 2006), especially when the sample size is low (Hernandez *et al.,* 2006; Wisz *et al.,* 2008). Both the

presence records and the six climatic variables were imported to Maxent ver. 3.3.3 (Phillips *et al.,* 2006). For each species, a total of 20 replicates were run with a random seed and 80-20% of the data was used for training-testing, respectively. In each replicate, the observations were chosen by bootstrapping, which allows sampling with replacement. These settings ensure that in each replicate a different and random subset of the data is used to train and test the models. Models were run with auto-features and the fit of each individual model was evaluated with the Area Under the Curve (AUC) of the receiver-operating characteristic (ROC) plot. Individual replicates were then projected to the different past climatic scenarios.

Individual models were averaged and standard deviation of presence probabilities used as a measure of prediction uncertainty (Buisson *et al.,* 2010). Mean models reclassified in ArcGIS with the Reclassify tool of the Spatial Analyst extension into binomial suitable/unsuitable maps. The threshold used was the minimum training presence (MTP; Liu *et al.,* 2013), which ensures that all presence records fall within the suitable area. All presence records were used to calculate this value on ArcGIS using the Extract Values to Table tool of the Geostatistical Analyst Toolbox. The reclassified models for each species were added with the Raster Calculator from the Spatial Analyst toolbox to build the stable suitable areas through time.

Variable importance for explaining the distribution of *Tarentola* geckos was determined by the average percent contribution to the models. This was done by both building models with only one of the variables, and models with all but that variable. Response curves of univariate models were visually examined to determine the preferred climatic conditions of each species (Phillips *et al.,* 2006).

2.5.2. Phylogenetic analyses

Sequences were edited and aligned in Geneious Pro 4.8.5 (Drummond *et al.,* 2009) using the Geneious alignment tool with default parameters. The alignment was then checked by eye to correct small misalignments and ambiguous positions. Every position that was not possible to determine the correct base, was considered as missing data (an N was edited into the alignment). The final alignment consisted of 388bp.

In order to estimate the root of the tree, two outgroups were used: one sequence of *Hemidactylus turcicus* and one of *Pachydactylus turneri*, both downloaded from GenBank. These two genera were chosen for being phylogenetically close to *Tarentola*

(Carranza *et al.,* 2002). A total of 58 more samples were also downloaded from GenBank (see Annex A3 for accession numbers), comprising *T. annularis* (three sequences), *T. chazaliae* (four sequences), *T. hoggarensis* (four sequences), *T. boehemei* (15 sequences), *T. desertii* (22 sequences), *T. fascicularis* (two sequences), *T. mauritanica* (six sequences) and *T. neglecta* (two samples). All sequences of the four study species that were available were downloaded (no sequences were available for *T. parvicarinata*). For *T. mauritanica*, the six sequences represent all the African clades found by Rato *et al.,* 2012. As the majority of the GenBank sequences were shorter than the ones obtained in the lab, the tips were filled with missing data in order to maintain the genetic information obtained for most of the sequences.

The 210 aligned sequences were collapsed into haplotypes with the online tool FABOX (Villesen, 2007) before further analyses, resulting in 94 unique haplotypes. In order to find the best-fitting model of nucleotide substitutions, JMODELTEST v.2.1.4 (Darriba *et al.,* 2012) was used, and Bayesian Information Criterion (BIC) scores calculated to select the appropriate model (TrN + I + G).

Bayesian Analyses were performed in BEAST v.1.8.2 (Drummond *et al.,* 2012). A lognormal relaxed clock was used, Yule process chosen as tree prior and ucld.mean Normal (initial value: 0.00827, mean: 0.00827, Stdev: 0.00162; according to Rato *et al.,* 2012). Three independent Markov Chains of Monte Carlo (MCMC) runs of 80 million generations were performed, sampling at every 8,000 generations. The stationarity and convergence of the runs were evaluated in Tracer v.1.5 (Rambaut and Drummond, 2007), and values of Effective Sample Size (ESS) higher than 300 for all parameters were found. For building the final tree, the .log and .tree files of the three independent runs were combined with LogCombiner v.1.8.2 and summarized in TreeAnnotator v.1.8.2 (Drummond *et al.,* 2012) with maximum clade credibility. FigTree v.1.3.1 (Rambaut, 2009) was used to visualize and edit the resulting tree.

2.5.3. Genetic structure

TCS v.1.21 was used to create haplotype networks with default parameters and a 95% threshold. To run this software, shorter sequences cannot be filled with missing data (Joly *et al.,* 2007). As the sequences downloaded from GenBank were ~80bp shorter than the ones obtained in the lab, several mutating positions would be lost by cutting the sequences short. In order to evaluate the importance of this loss, two datasets

were built for each of the four study species considered: one including all sequences available, but with the sequences being shorter, and another one including only the longest sequences. The resulting networks were edited manually.

To build the trees for the individual species, the data sets containing only the sequences from the study area were collapsed into haplotypes in FABOX (*T.* annularis N = 6; *T* chazaliae N = 9; *T. hoggarensis* N = 13; *T. parvicarinata* N = 21), the best-fitting model of nucleotide substitution calculated in jMODELTEST v.2.1.4 (F81 for *T. annularis*, K80 for *T. chazaliae*, HKY + I for *T. hoggarensis* and TrN + I for *T. parvicarinata*), and a Bayesian tree built in BEAST v.1.8.2. A lognormal relaxed clock was used, and a Coalescent: Bayesian Skyline tree prior chosen. Three independent MCMC runs of 50 million generations and sampling every 5000 generations were performed, and the outputs treated as described above.

2.5.4. Genetic distances

Taking into account the results of the phylogenetic tree and networks, sequences were divided into groups corresponding to lineages within species in MEGA v.6 (Tamura *et al.,* 2013) and genetic distances between groups calculated with Kimura-2 parameter model and 1000 bootstrap. Uncorrected pairwise distances between the lineages of each species were also calculated with the same parameters.

3. Results

3.1. General Overview

For molecular analyses, samples from 29 *T. annularis*, 9 *T. chazaliae*, 40 *T. hoggarensis*, 62 *T. parvicarinata*, 3 *T. boehmei*, 2 *T. deserti*, 2 *T. mauritanica* and 3 *Tarentola* sp. were successfully sequenced, corresponding to 82% of samples. Sequences aligned consisted of 388 bp. Despite the short length of obtained sequences for 12S, the resulted genetic information allowed to evaluate the intraspecific patterns of genetic structure in the studied region. Ecological Niche-based Models were successfully built for each species, all of them showing high performance. They allowed the identification of the climatic niche of the species and to map it through time.

3.2. Species/lineages identification

The phylogenetic tree revealed seven major clades (Figure 8): *Tarentola boehmei* (light grey), *Tarentola mauritanica* (light grey), *Tarentola fascicularis/deserti* (light grey) *Tarentola parvicarinata* (red), *Tarentola chazaliae* (blue), *Tarentola annularis* (green) and *Tarentola hoggarensis* (purple). All study species are represented with different colors to facilitate the interpretation throughout the text.

Most species also present intraspecific substructure. The taxonomy of each clade was determined by the putative assignment of the individuals to a species by their morphology, and corroborated with the inclusion of previously validated sequences of each species available in GenBank, with the exception of *T. parvicarinata*. For this species, individuals from the type locality were included.

The target species form a well-supported clade, sister to the also well supported clade containing the remaining, non-study species. *T. boehemei* is divided in two well supported sub-clades. The *T. mauritanica* clade forms five well supported clades, each representing a lineage or species found by Rato *et al.,* 2012 (not shown); *T. neglecta* falls within the *T. deserti* and *T. fascicularis* clade.

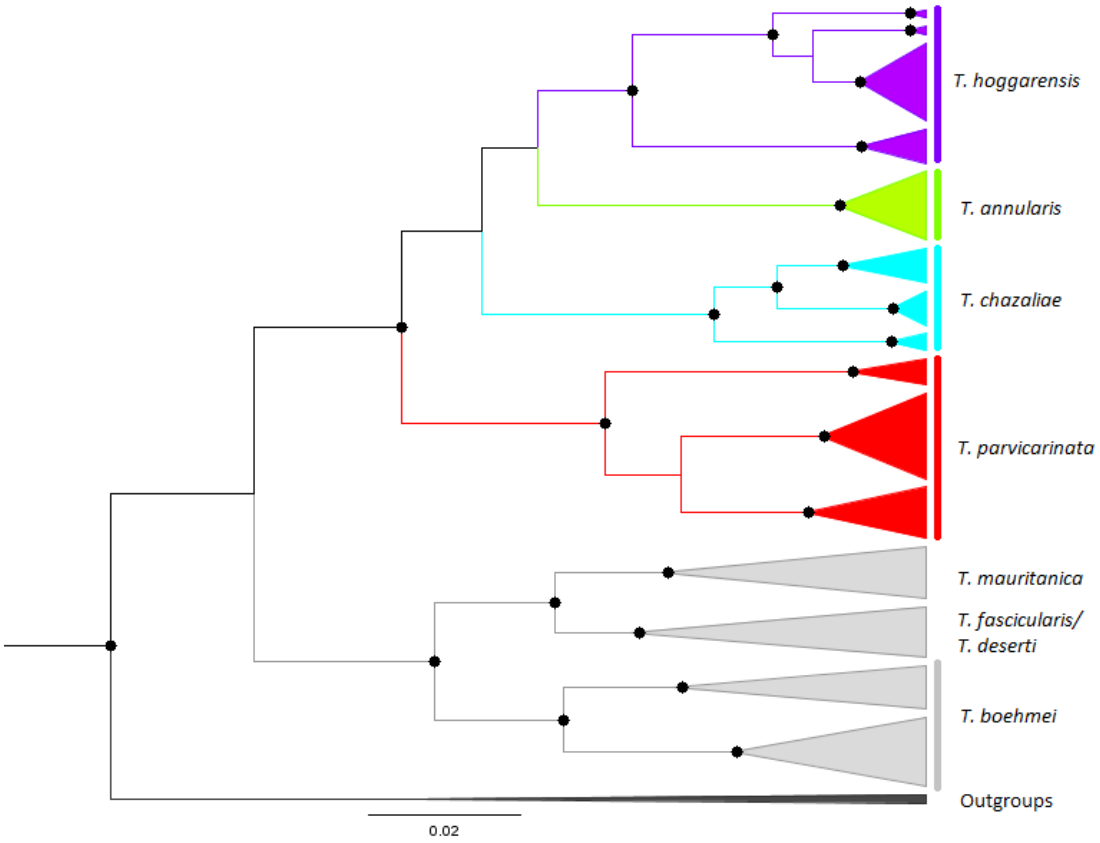

Figure 8: Bayesian phylogenetic tree of the *Tarentola* species based on 388 bp of the 12S gene. *Pachydactylus turneri* and *Hemidactylus turcicus* were used as outgroups. The nodes with a Bayesian Posterior Probability (BPP) over 95% are highlighted with black dots. The scale bar indicates 2% sequence divergence. Each species referred in the main text is highlighted with a different colour: 1) *T. parvicarinata* in red; 2) *T. chazaliae* in light blue; 3) *T. annularis* in green; 4) T. *hoggarensis* in purple. Outgroups are represented in dark grey and non-study *Tarentola* species in light gray.

The phylogenetic relationships of the study species are not resolved, though a common ancestor is supported. The clade of *T. parvicarinata* presents three well supported clades, although the relation between them is not supported. The clade of *T. chazaliae* also presents three supported clades. *Tarentola annularis* presents no substructure. *Tarentola hoggarensis* presents four main sublineages, with one particularly divergent from the others.

The haplotype networks (Figures 9B to 12B) recovers, for each study species, the same lineages shown in the phylogenetic tree.

Tarentola annularis shows very few haplotypes separated by few mutational steps (Figure 9B). There is one large haplotype containing most samples (N = 23) widespread across all the study area, and four other small haplotypes differing in one mutational step with no particular geographic arrangement.

Tarentola chazaliae presents three distinct haplogroups (Figure 10B). There is a large haplogroup encompassing samples throughout all the known distribution of the species, including both the northern- and southernmost samples. The other two haplogroups are geographically contained within the large one, with one ranging from Sebkha Imlilil to Chtoukane and the other from Chtoukane to Tarfaya.

Tarentola hoggarensis is divided into four allopatric haplogroups (Figure 11B). One of the haplogroups is not connected to the remaining ones, and corresponds to the more divergent lineage in the tree. This lineage is restricted to the south of Mauritania, close to the Senegal River. Another haplogroup consists of a main haplotype (N = 15) and a few single haplotypes differing in one mutational step. These individuals are distributed throughout Mauritania and Western Sahara. There are, however, two groups of haplotypes that differ from this main group by six and seven mutational steps. One of these groups contains all the samples from Niger with the exception of one sample, and the other includes the remaining sample from Niger and the easternmost sample from Mauritania.

Tarentola parvicarinata is divided into three allopatric haplogroups that correspond to the three lineages recovered by the tree (Figure 12B). The northern haplogroup presents two main haplotypes separated by four mutational steps, each one with some haplotypes differing only in one mutational step. These two groups are also allopatric, with the smaller one restricted to the northern mountains of Adrar-Atar and the other in Tagant and Assaba. The central haplogroup is restricted to Tagant and also shows some geographic structure, albeit not much due to the restricted range. The southern haplogroup presents a main haplotype distributed throughout the entire range of this lineage in Assaba and Affolé, another smaller haplotype restricted to the west and two small haplotypes in the periphery of the larger one, all differing from the main one by one mutational step. There are also two individual haplotypes that differ by four and seven steps from the main haplotype and the samples are located in Mali, separated from the main cluster of individuals.

3.3. Ecological Niche-based Models

3.3.1. ENMs evaluation

In the four species, ENMs presented high AUC values, in both training and testing, with low standard deviation (Table 3).

3.3.2. Environmental factors related to species distribution

Ecological models identified the most important environmental variables related to the current distribution of each species (Table 3). Overall, temperature seasonality was found to be important for all species and annual precipitation was also important for all species with the exception of *T. chazaliae*. For *T. annularis* the most important variable was the precipitation of the coldest quarter; for *T. chazaliae* and *T. parvicarinata* it was the mean temperature of the warmest quarter; for *T. hoggarensis* it was the temperature seasonality (Table 3).

Table 3: Number of observations to train and test models for each species, average and standard deviation (SD) of training and test AUC derived from the 20 replicates, minimum training presence logistic threshold (MTP), and average percent contribution (with standard deviation) of each variable in the models. Most important variables are marked (*).

	T. annularis	*T. chazaliae*	*T. hoggarensis*	*T. parvicarinata*
# Training-test	50 - 12	28 - 6	41 - 10	90 - 22
Training AUC (SD)	0.86 (0.02)	0.99 (0.00)	0.85 (0.02)	0.94 (0.00)
Test AUC (SD)	0.83 (0.05)	0.98 (0.007)	0.81 (0.04)	0.91 (0.02)
MTP	0.111	0.170	0.145	0.107
Temperature Seasonality	25.87* (8.25)	15.27* (4.54)	53.24* (10.47)	13.40* (4.00)
Minimum Temperature of Coldest Month	6.74 (5.74)	0.52 (0.52)	2.28 (2.58)	17.59* (3.77)
Mean Temperature of Warmest Quarter	12.90 (11.17)	64.74* (9.31)	4.75 (5.29)	39.50* (3.87)
Annual Precipitation	25.40* (9.22)	4.63 (2.48)	31.47* (7.78)	26.07* (6.69)
Precipitation of Driest Quarter	2.40 (2.53)	3.35 (2.98)	2.29 (2.34)	2.77 (1.88)
Precipitation of Coldest Quarter	26.69* (14.73)	11.49 (7.74)	5.97 (6.02)	0.67 (0.60)

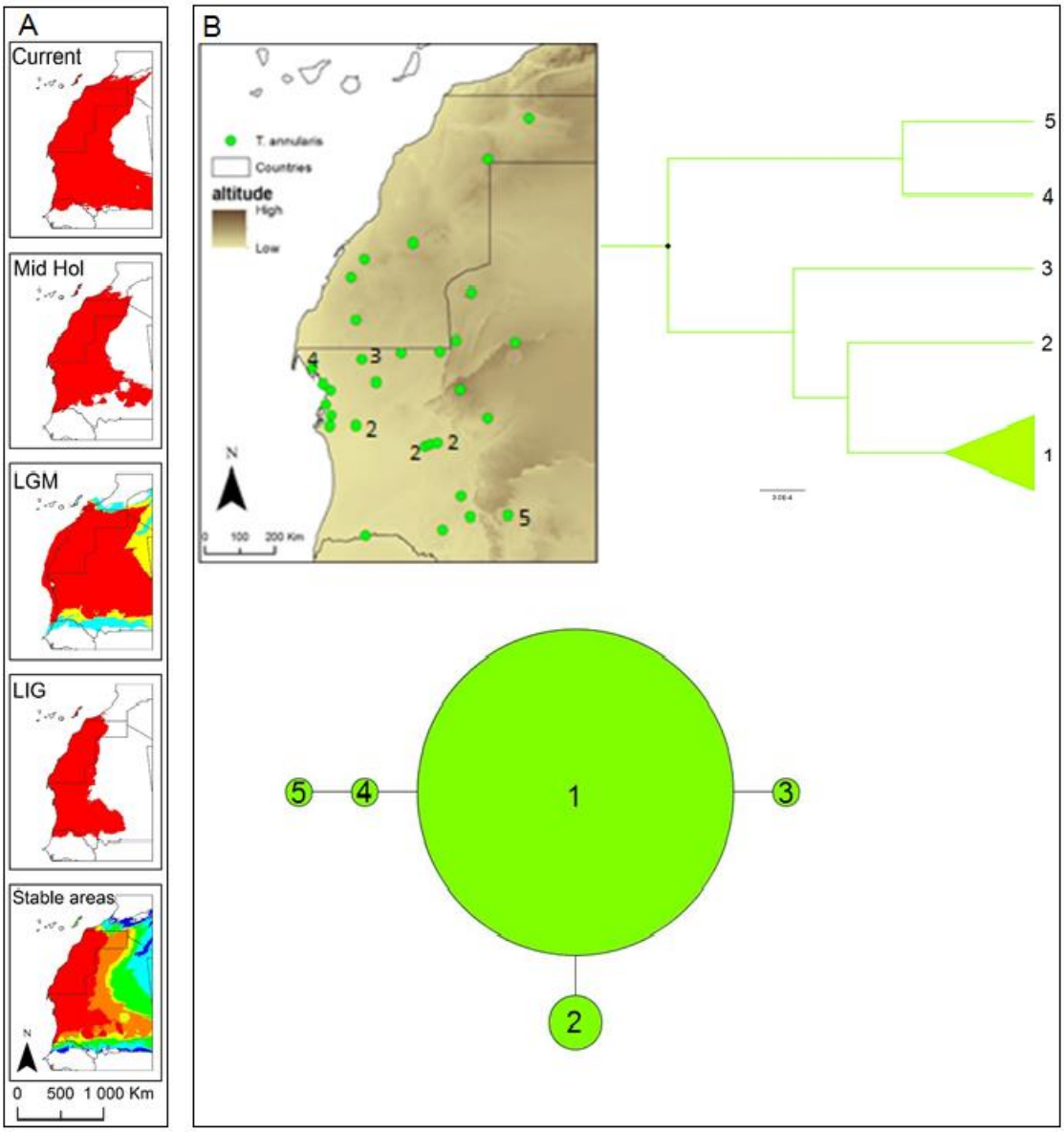

Figure 9: Individual analyses for *Tarentola annularis*. A) Suitable area predicted by Maxent for current and past conditions. For the LGM, the red color corresponds to areas predicted by the three scenarios, yellow to predictions of two, and blue to predictions of only one. Bottom map corresponds to the stable areas. Warmer colors indicate more higher number of periods of for climate suitabilityle climate. B) Phylogeography of *T. annularis*. The TCS mitochondrial network assumes a 95% parsimony threshold. Each circle corresponds to one haplotype and their size is approximately proportional to the number of shared individuals of that haplotype. The Bayesian tree is reduced to haplotypes. Supported nodes are shown with a black dot. The map shows the geographic distribution of the haplotypes in the study area. Numbers next to each dot correspond to the number of the haplotype; haplotype 1 was not represented in order not to overload the image, i.e. all dots with no number correspond to haplotype 1.

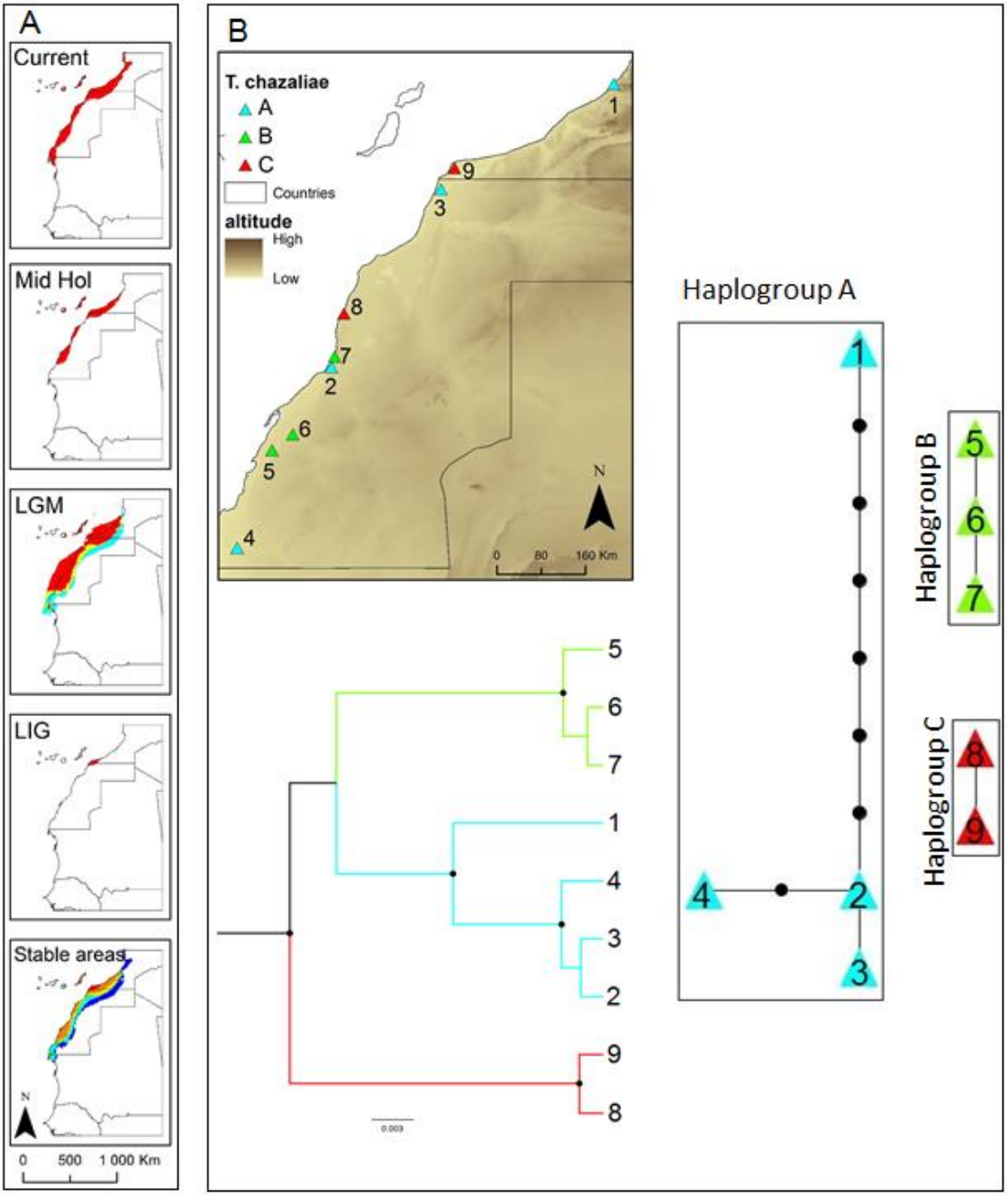

Figure 10: Individual analyses for *Tarentola chazaliae*. A) Suitable area predicted by Maxent for current and past conditions. For the LGM, the red color corresponds to areas predicted by the three scenarios, yellow to predictions of two, and blue to predictions of only one. Bottom map corresponds to the stable areas. Warmer colors indicate more periods of suitable climate. B) Phylogeography of *T. chazaliae.* The TCS mitochondrial network assumes a 95% parsimony threshold. Each triangle corresponds to one haplotype and their size is approximately proportional to the number of shared individuals of that haplotype. Different colors correspond to different haplogroups. The Bayesian tree is reduced to haplotypes. Suppoerted nodes are represented by a black dot. The map shows the geographic distribution of the haplotypes in the study area. Numbers next to each dot correspond to the number of the haplotype. Colors correspond to different haplogroups and are the same as in the network.

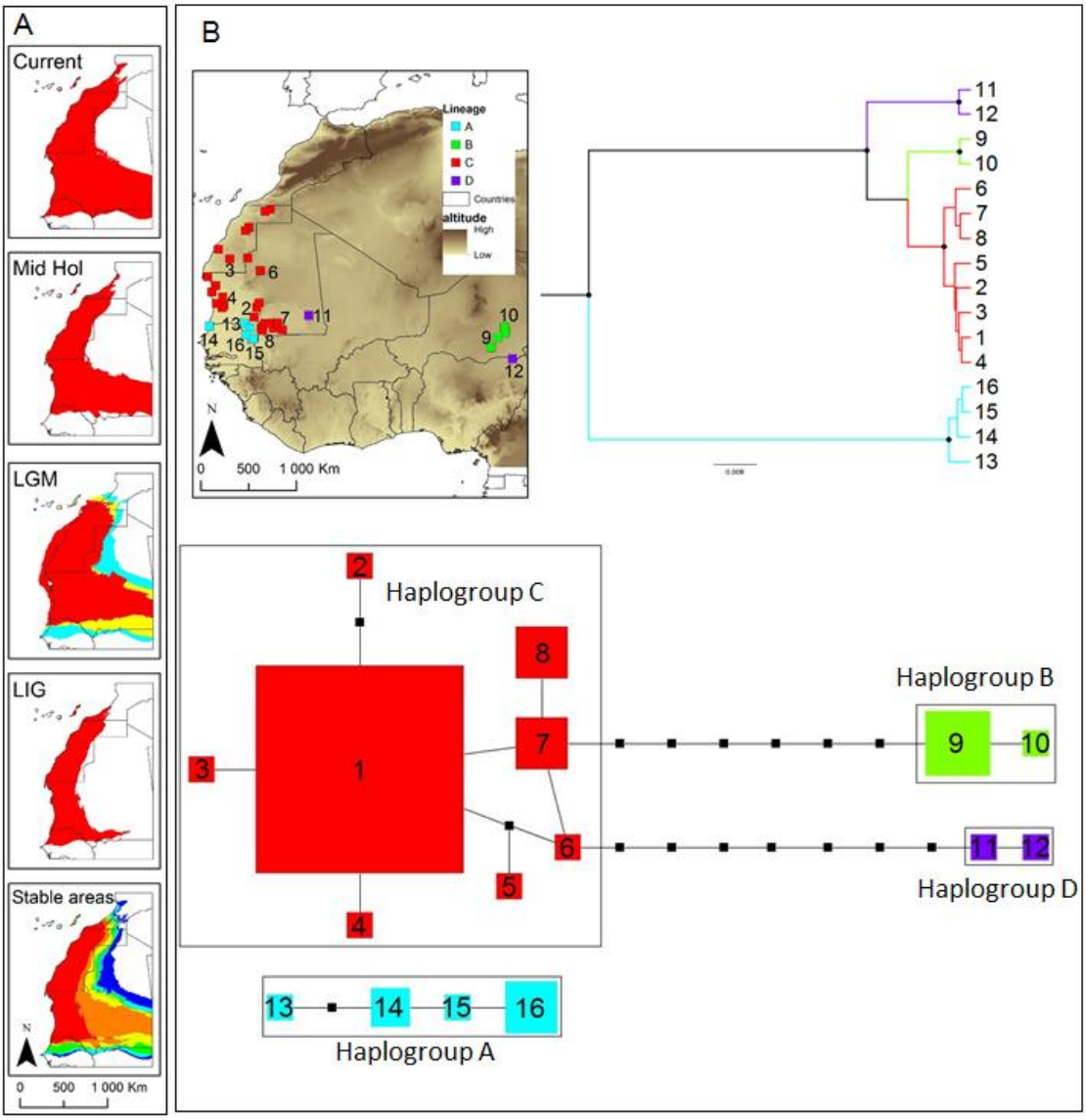

Figure 11: Individual analyses for *Tarentola hoggarensis*. A) Suitable area predicted by Maxent for current and past conditions. For the LGM, the red color corresponds to areas predicted by the three scenarios, yellow to predictions of two, and blue to predictions of only one. Bottom map corresponds to the stable areas. Warmer colors indicate more periods of suitable climate. B) Phylogeography of *T. hoggarensis.* The TCS mitochondrial network assumes a 95% parsimony threshold. Each square corresponds to one haplotype and their size is approximately proportional to the number of shared individuals of that haplotype. The Bayesian tree is reduced to haplotypes. Supported nodes are represented by a black dot. The map shows the geographic distribution of the haplotypes in the study area. Numbers next to each dot correspond to the number of the haplotype; haplotype 1 was not represented in order not to overload the image, i.e. all dots with no number correspond to haplotype 1.

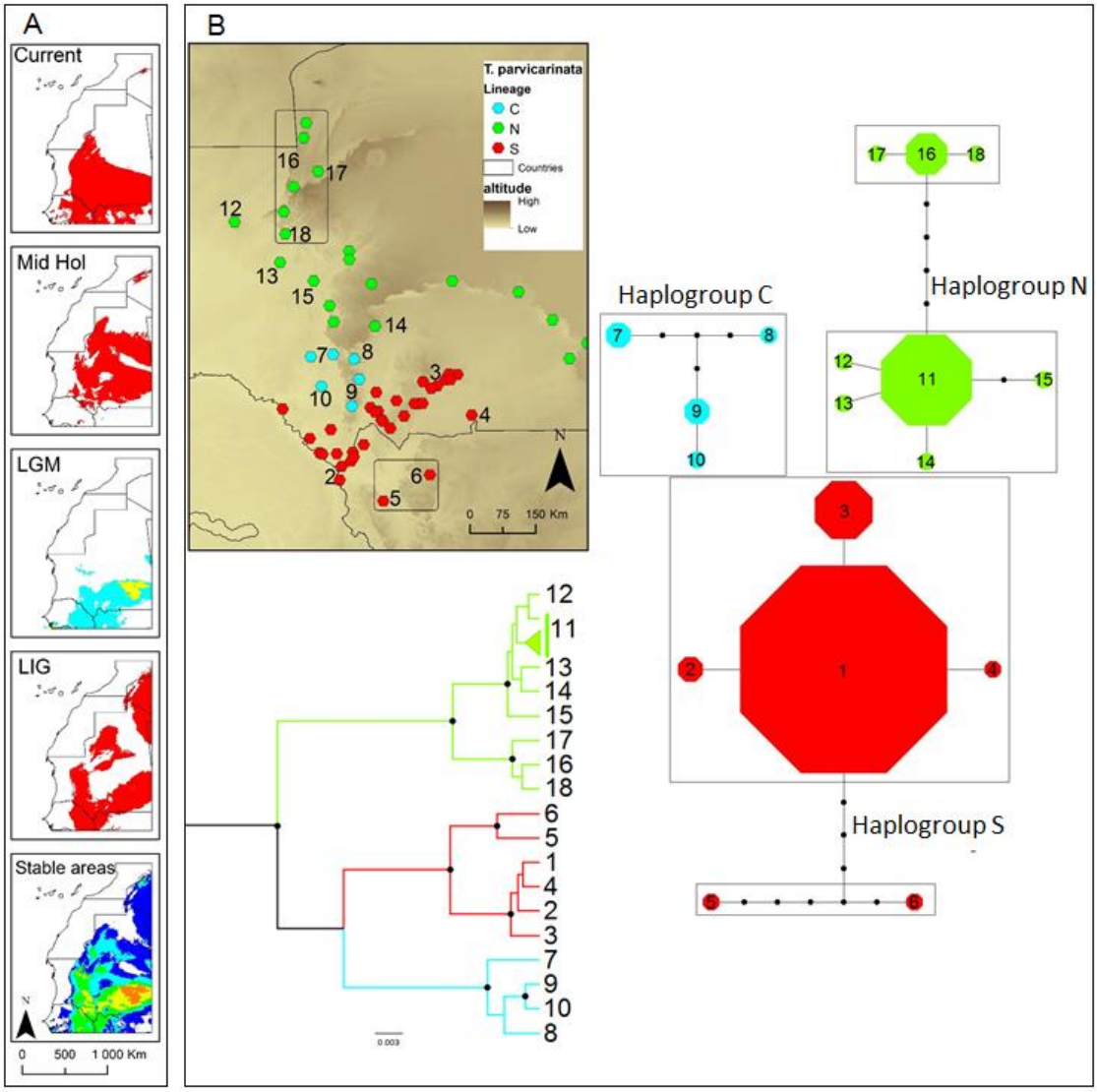

Figure 12: Individual analyses for *Tarentola parvicarinata*. A) Suitable area predicted by Maxent for current and past conditions. For the LGM, the yellow corresponds to areas predicted by two scenarios (no suitable area was predicted for MIROC) and blue to predictions of only one. Bottom map corresponds to the stable areas. Warmer colors indicate more periods of suitable climate. B) Phylogeography of *T. parvicarinata*. The TCS mitochondrial network assumes a 95% parsimony threshold. Each hexagon corresponds to one haplotype and their size is approximately proportional to the number of shared individuals of that haplotype. The Bayesian tree is reduced to haplotypes. Supported nodes are represented with a black dot. The map shows the geographic distribution of the haplotypes in the study area. Number next to each dot correspond to the number of the haplotype; haplotype 1 and 11 were not represented in order not to overload the image, i.e. all red dots with no number correspond to haplotype 1 and all green dots with no number correspond to haplotype 11.

The profiles of the response curves for the most important variables shared by species exhibited different pattern for each species i.e. each species reacts differently to the same variables (Figure 13). *T. annularis* occurs in areas with low annual precipitation and has a narrow range of precipitation throughout which it occurs. On the other hand, *T. parvicarinata* and *T.* hoggarensis occur in slightly more humid areas (though still with

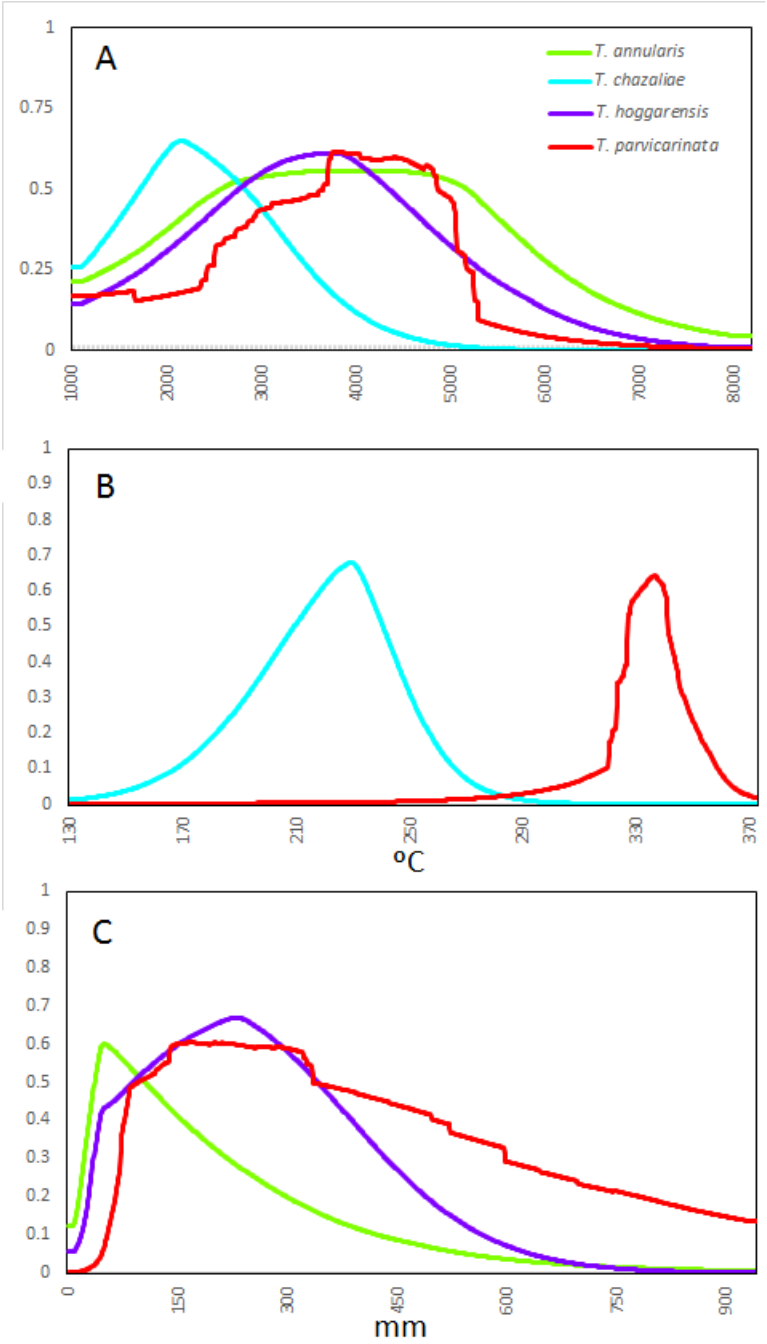

Figure 13: Response curves for the bioclimatic variables most related to the distribution of *Tarentola*. Curves represent the average probability of occurrence derived from 20 model replicates along the bioclimatic gradients. A) Temperature seasonality; B) Mean Temperature of the Warmest Quarter; C) Annual precipitation.

low precipitation) and throughout a broader range of precipitation. The mean temperature of the warmest quarter also shows distinct patterns in these species. *T. chazaliae* prefers cooler temperatures of around 23ºC, while *T. parvicarinata* prefers higher temperatures (around 34ºC). As for temperature seasonality, *T. chazaliae* occurs in areas with low values, that is, areas where the temperature is relatively stable, while the remaining species prefer more intermediate values (Figure 13).

3.3.3. Predicted suitable areas for current conditions

The models obtained successfully identified suitable areas for the four species, mostly fitting the distribution of the distinct datasets. High probability of occurrence of *T. annularis* and *T. hoggarensis* was found across all of Western Sahara and Mauritania, with the exception of the northwestern part of the study area. The models of *T. chazaliae* identified areas only in the Atlantic coast, extending just a few kilometers inland. *T. parvicarinata* only has suitable cells in the southern mountains of Mauritania and a small patch in the border between Morocco and Algeria, in the Northeast of the study area (Annex A5).

MTP thresholds (see Table 3) were applied to each species average probabilistic model (and past projections), producing presence/absence predictions for current conditions which mostly resemble probabilistic models (see Figures 9A-12A).

3.3.4. Past suitable areas and stable areas

Ecological models identified suitable areas in the past for the four species. Overall, during the LIG all species presented a more restricted distribution, reaching their maximum in the LGM (in all three scenarios). In the Middle Holocene the distribution was an intermediate extension between the LIG and LGM, though slightly more restricted that in the present (Figures 9A to 12A).

Projections from ENMs for *T. annularis* predict a smaller range, more restricted to coastal areas during the LIG comparing to the predicted area for the present. For the LGM, the three alternative scenarios predict approximately the same area, but with small differences among them. LGM CCSM predicts suitable areas more to the south when

compared with the other two, with reduced suitable areas in northeastern Mauritania. LGM Melgmbi is the scenario that predicts less suitable areas in southern Mauritania, while predicting more suitable areas in the eastern part of the study area. LGM MIROC shows a more intermediate pattern, predicting few suitable areas in southern Mauritania and high suitability in the northeastern. Projections for the Middle Holocene predict a more restricted suitable area than for the present, with a patchy distribution in the southeastern limit. Climatically stable areas were mainly restricted by the LIG projections and are found mostly in the western half of Mauritania and Western Sahara.

For *T. chazaliae* the ENM projections for LIG predicted two small suitable areas, one restricted to southwestern Morocco and the other very patchy in Río de Oro peninsula. Predictions for LGM scenarios greatly differ from each other. LGM CCSM predicts a narrower area near the coast, with a more accentuated narrowing in northwestern Western Sahara and a patchy distribution in the southern limit. LGM Melgmbi, on the other hand, predicts a wider area, extending more to the north, but highly restricted in the southern limit. LGM MIROC is an intermediate among the other two projections, predicting a narrower distribution than melgmbi, but wider than CCSM. It also predicts a wider suitable area in the south of the study area. For the Middle Holocene the predictions show two suitable areas, one in southern Morocco and other in coastal Western Sahara. These two suitable areas are separated from each other by an extension of about 70 km. Stable climatic areas occur near the coast in the border between Morocco and Western Sahara and are mainly restricted by predictions from LIG.

For *T. hoggarensis* the ENM projections predict a smaller range during the LIG, mainly restricted to coastal areas, expanding inland in the south along the Senegal River. The three alternative scenarios for the LGM show different predictions. LGM CCSM predicts a similar area to the present with only the northern limit occurring slightly more to the south and expanding to the coastline. LGM Melgmbi predicts less suitable area in the south, but more in the eastern parts, while the northern limit is similar to CCSM. LGM MIROC predicts a more restricted suitable area, with much less suitable areas in the south and northwest of Mauritania, as well as southern Morocco. For the Middle Holocene, the predictions are similar to the present albeit more restricted in the northern, southern and western limits. Stable climatic areas are mainly restricted by the predictions for the LIG, found mostly in the coast and extending between 200 and 300 km inland.

In *T. parvicarinata* ENM projections for the LIG predict a very patchy distribution throughout almost all the study area, with the exception of Western Sahara. There are

three major regions of suitability, one comprising the major mountain complexes of Mauritania with the exception of Tagant, other in central Mauritania and another in the northeastern limit of the study area, comprising southwestern Algeria and northeastern Mauritania. The alternative scenarios for the LGM resulted in very different outputs, with projections to LGM Melgmbi showing no suitability. LGM CCSM predicts a small, patchy distribution in Mauritania, mainly in Adrar-Atar and Affolé. LGM MIROC predicts a continuous distribution in the south of Mauritania and Senegal. For the Middle Holocene the models predict a continuous suitable area in central Mauritania, encompassing all the major mountains and a small patch in the northeast of the study area. When considering all alternative scenarios for the LGM, climatically stable areas cannot be found; however, if only CCSM and MIROC are considered, stable areas are found in southeastern Mauritania.

3.4. Genetic distances

The genetic distances calculated between the main lineages of *Tarentola chazaliae* show values of about 0.045 for all lineages.

The more divergent lineage of *Tarentola hoggarensis*, which also corresponds to separated haplotype, presents a genetic distance of 0.10 when compared to the remaining lineages. Within the remaining lineages of *T. hoggarensis* distances are around 0.03 among them.

Tarentola parvicarinata also shows values of about 0.045 between the three distinct lineages. The North lineage has a distance of 0.048 to the south lineage, and 0.05 to the central one. The central and south lineages show a genetic distance of 0.04 (Table 4).

Table 4: Genetic distances (diagonal left) between the lineages of each study species with standard deviation (diagonal right). Numbers in red represent the genetic distances between the lineages of each species. TA stands for *T. annularis*, TCA, TCB and TCC for lineages A. B and C of *T. chazaliae*, THA, THB, THC and THD for lineages A, B C and D of *T. hoggarensis*, and TPS, TPC and TPN for lineages S, C and N of *T. parvicarinata*.

	T. annularis	*T. chazaliae*			*T. hoggarensis*				*T. parvicarinata*		
	TA	TCA	TCC	TCB	THA	THC	THD	THB	TPS	TPC	TPN
TA		0.024	0.022	0.027	0.021	0.022	0.022	0.022	0.018	0.016	0.016
TCA	0.126		0.011	0.011	0.028	0.028	0.026	0.028	0.023	0.023	0.021
TCC	0.110	0.042		0.012	0.030	0.029	0.029	0.030	0.024	0.021	0.022
TCB	0.139	0.044	0.048		0.030	0.030	0.029	0.030	0.025	0.025	0.025
THA	0.111	0.158	0.163	0.166		0.022	0.021	0.021	0.028	0.025	0.024
THC	0.113	0.157	0.158	0.169	0.114		0.009	0.008	0.023	0.023	0.023
THD	0.113	0.138	0.159	0.162	0.107	0.030		0.011	0.021	0.023	0.022
THB	0.111	0.157	0.165	0.168	0.106	0.023	0.037		0.023	0.023	0.022
TPS	0.088	0.122	0.125	0.133	0.160	0.123	0.113	0.130		0.010	0.012
TPC	0.076	0.124	0.107	0.139	0.141	0.129	0.128	0.133	0.040		0.012
TPN	0.075	0.113	0.112	0.132	0.134	0.128	0.124	0.126	0.048	0.051	

4. Discussion

This work assessed the effect of past climatic oscillations in the distribution and genetic diversity of four *Tarentola* species, namely *T. annularis*, *T. chazaliae*, *T. hoggarensis* and *T. parvicarinata*, in the West Sahara-Sahel. ENMs were used to infer biogeographical scenarios for species persistence according to their realized climatic niches and to compare them to phylogeographic patterns derived from mitochondrial data. Despite using only one slow evolving mitochondrial gene, this study recovered an unexpected and remarkably high intraspecific diversity and genetic structure for some species (see Figures 9B to 12B), which is mostly concordant to paleoclimatic reconstructions. The combination of both approaches is novel for the region, as well as the genetic assessment for some species (e.g. *T. parvicarinata)*.

4.1. Validity of the study species and environmental factors related to their occurrence

Phylogenetic inferences revealed interesting patterns for the relationships between *Tarentola* species (Figure 8). Although no statistical support exists, the four study species form a monophyletic clade sister to the clade containin*g T. mauritanica, T. deserti, T. fascicularis, T. bohemei* an*d T. neglecta*, as was recovered by Rat*o et al*., 2012. These two sister clades are thought to have diverged during the Miocene, about 15.38 Mya (Rato *et al.,* 2012). The most divergent lineag*e* withi*n Tarentola hoggarensi*s presents two lineages with more than 10% divergence. This raises the hypothesis that this lineage is in fact a distinct species, *T. senegambia*e. Formerly a subspecies of *T. ephippiat*a, *T. senegambia*e was recently elevated to species level by Trape *et al.*, 2012. The distinction was mostly based on body size and geographic distribution but there are no molecular results to support this claim. However, it was not possible to sequence the only *T. senegambia*e sample available for this study, and the lack of overlap between the described species range and the locations where this lineage was found does not allow to confirm the validity of this species.

In general, the four species showed distinct environmental correlates which suggest different ecological affinities. *Tarentola annularis* is the most arid-adapted of the species studied, and that is captured in the ENMs. The most important variables for this

species are temperature seasonality, where it occurs in a wide range, and low precipitation, which are typical from desert conditions (Ward, 2009). Furthermore, ENM analyses performed elsewhere (Sow *et al.,* 2014) also recovered annual precipitation as an important variable restricting the occurrence of *T. annularis*. Sow *et al.,* 2014 also used habitat variables to construct the ecological models and recovered distance to sandy areas and seasonal rivers as important variables. The arid-adapted *A. dumerili* was modelled in the same study, with the same variables being identified as important.

Tarentola chazaliae is mainly restricted by low mean temperature of the warmest quarter and temperature seasonality, which is also to be expected from a species adapted to a coastal area, where climate is milder and more constant (Ward, 2009). Similar results were found by Sow *et al.,* 2014, with low annual temperature restricting the occurrence of *T chazaliae* and for other coastal species, like *A. aureus*, low annual temperature being important variables. For *A. aureus*, however, annual precipitation was also recovered as an important variable by Sow *et al.,* 2014, which was not found to be as important for *T. chazaliae* and might be related to its wider distribution and climatic niche (Trapé *et al.,* 2012). The same study also recovered distance to sandy areas as an important habitat variable for this species.

Tarentola hoggarensis is mostly restricted by temperature seasonality and annual precipitation, with intermediate values for both variables. Being a Sahelian species, it should be expected that it is adapted to less extreme environmental conditions (Brito *et al.,* 2014), due to the milder climatic conditions, and that is what was recovered. Sow *et al.,* 2014 recovered annual precipitation as an important variable for this species (considered to be *T. ephippiata* in that study) which is consistent with our findings.

Tarentola parvicarinata was found to be restricted by high mean temperature of the warmest quarter and a wide range of seasonal temperature, and low to medium annual precipitation. Other Mauritanian mountain species, such as *Agama boulengeri* were shown to be mostly restricted by environmental variables, namely distance to *gueltas*, and not so much by climatic variables (Vale *et al.,* 2012). Nevertheless, annual precipitation was the climatic variable identified as the most important in this study. Despite not being tested, species environmental correlates and response curves suggest some degree of niche divergence. The exception was *T. annulari*s and *T. hoggarensi*s which inhabit similar climatic zones in the study area and response curves for the most important environmental correlates present similar profiles (albeit *T. annulari*s can be found in a wider range; Fig. 7). Habitat selection might be playing an important role acting as segregation factor between these two species (Rato *et al., 2015*), as the first species occurs in rock outcrops and the later in *Acacia* trees. A recent work found evidence of

both niche divergence and conservatism within *Tarentola mauritan*ica species complex (R*ato et al.,* 2015). That is, some species within the complex evolved while conserving their niche, while others diverged.

4.2. Response to climatic oscillations

Pleistocene climatic oscillations in the Sahara resulted in drastic changes in habitat types and vegetation cover (Le Houérou, 1997) which led to large shifts in species ranges (Kröpelin *et al*., 2008). This appears to have happened with our species although the lack of habitat variables prevents further conclusions from being drawn.

Tarentola annularis is a widespread and climatically tolerant species in the present, and accordingly, presented a widespread distribution through time (Figure 9A). Its distribution was more restricted to coastal areas during the LIG and more widespread during the LGM. However, the low genetic diversity and lack of geographic structure appear to suggest slow range shifts (Hewitt, 1999). On the other hand, the populations from the study area are in the margin of the species distribution, and thus the low genetic diversity could be a result of a recent colonization from the east. However, there is a lack of genetic divergence between the populations from this area and the individuals from Egypt (Annex A6), which could either be indicative of a recent expansion from another region, or reflect the poor sampling of the Egyptian populations. Also, during the LIG, there is no suitable area for this species in the southeast of Mauritania, i.e. *T. annularis* appears to have been isolated in more coastal regions during this period. During the LIG, precipitation was higher in East Mauritania, which could explain the absence of suitable habitat for an arid adapted species with a preference for low precipitation regions (Figure 13C) such as *T. annularis*. Other arid adapted species, such as *Cerastes cerastes* and *C. vipera,* present a similar response curve to annual precipitation, and a similar predicted suitable area for the present, especially *C. vipera* (Brito *et al.,* 2011b). *Stenodactylus sthenodactylus* and *S. mauritanicus* also present a wide distribution across the Sahara, but Metallinou *et al.,* 2012 recovered two lineages in each species, one comprising the eastern populations and another comprising the western. However, we lack samples from the remaining range of *T. annularis* to determine whether this species is homogeneous throughout its range or presents structuration.

Tarentola chazaliae, was found to be continuously restricted to coastal areas (Figure 10A), but the predicted suitable area greatly varied through time. This large

variation in suitable area is reflected in the lack of geographic structuration in the genetic structure of this species. The reason behind this lack of geographic structuration is unknown. Perhaps the lower sea level during the LGM allowed the spread of a major lineage through most of the suitable habitat, while other environmental conditions prevented other lineages from dispersing. Another possible explanation is that this species has high dispersal capacity and all lineages were able to spread through the current species range after being restricted during the LGM. *Acanthodactylus aureus,* has recently been shown to not only be genetically structured, as is *T. chazaliae*, but the lineages are geographically structured (Lopes, 2014). Contrary to *T. chazaliae*, *A. aureus* has a wider distribution, being found in more inland regions (Trapé *et al.,* 2012). This results in a potentially wider niche and higher tolerance to climatic conditions, as recovered by Sow *et al.,* 2014. *T. chazaliae* on the other hand is less tolerant which probably led to only one very restricted putative refugia during the LIG. However, the reduced sample size of our study greatly limits the conclusions that can be drawn.

Tarentola hoggarensis, also recovered a widespread distribution through time, except during the LIG (Figure 11A). As with *T. annularis*, the increase in precipitation in East Mauritania during this period could be responsible for this pattern. Contrary to *T. annularis*, *T. hoggarensis* presents a high level of genetic differentiation, which is geographically structured. The southernmost samples present a high genetic distance from the remaining individual, possibly constituting another species. The remaining three lineages present an East-West structuration. Many other species are known to have colonized the western regions of the Sahara from the East (e.g. Agama, Gonçalves *et al.,* 2012; *Stenodactylus*, Metallinou *et al.,* 2012; *Vulpes*, Leite *et al.,* 2015) during favourable periods, becoming isolated and diverging during unfavourable ones. This could also have occurred with *T. hoggarensis*. *Ptyodactylus ragazzii* has a similar distribution and has been shown to present genetic structuration in an East-West axis (Froufe *et al.,* 2013). However, the existence of a lineage that is present in both Mauritania and Niger (despite the low sample size) could be indicative of the presence of a corridor in the Sahel, not sampled in the current study. ENMs appear to support this hypothesis, as there is suitable habitat in southern Mauritania reaching the eastern limit of the study area except during the LIG. More sampling and a wider area for modelling are needed to properly address this hypothesis.

Tarentola parvicarinata clearly presents three distinct allopatric lineages. The same pattern has previously been identified on other species such as *Agama boulengeri* (Gonçalves *et al.,* 2012) and *Crocodylus suchus* (Velo-Antón *et al.,* 2014) which might also result from climatic oscillations. ENMs for past conditions, however, are unable to

shed much light on the exact events, as the predicted suitable areas do not clearly show isolation between the mountain ranges. Furthermore, the distinct scenarios for the LGM are very different, with one inclusively predicting no suitable area for this species. *Tarentola parvicarinata* prefers rocky substrates (Trape *et al.,* 2012), which could explain why the lineages remained isolated in different mountains even during humid periods. The lack of habitat information when building the ENMs could be adding an important bias to the models. Genetic distances of around 4% seem to support this long isolation. The suitable patch in the northwest of the study area was almost connected to southern suitable areas during the LIG, raising the hypothesis that the species could be present there.

4.3. Stability in West Sahara

When comparing the stable areas for all species the pattern that emerges is that during past periods all species had their distributions more restricted to coastal areas. The only exception is *T. parvicarinata*, which is not present in the coastal region. The stable areas are mostly restricted by the LIG distribution, again with the exception of *T. parvicarinata*. By overlapping the stable areas for all species (Figure 14), there is no stable area common for all species. There is, however, a small area stable for three species, *T. annularis*, *T. chazaliae* and *T. hoggarensis*. This area is mostly restricted by the stable area for *T. chazaliae*. There is also a large overlap between the stable areas of *T. annularis* and *T. hoggarensis* restricted to the coast, but extending some kilometres inland. Further inland, the area is only stable for one species. This shows that closer to the coast the climate has been more stable through time. However, for *T. chazaliae* the stable area is very limited. This appears to indicate that the corridor persisted through time for some species, but not for all. Nevertheless, this corridor appears to have been restricted to coastal areas, with its width depending on the ecological characteristics of each species.

4.4. Concordance between ecological and molecular approaches

Integrating ENMs and phylogeography often yields incomplete concordance in ecological and genetic signals (e.g. Beatty and Provan 2013; Igea *et al.,* 2013), but in some cases both approaches completely agree (e.g. Moussalli *et al.,* 2009*;* Martínez-

Freiría *et al*., 2015). This study has mixed results in relation to such concordance. Discrepancies between ecological and genetic signals could result from several factors (see Alvarado-Serrano *et al.,* 2014), such as incomplete sampling of the species niche, lack of incorporation of adequate predictors in the analyses or erroneous selection of the ecological units to model. Also, ecological niche-based reconstructions of past ranges are subject of many uncertainties, particurlaly when models are projected to novel climates or different areas (Wiens *et al.,* 2009; Alvarado-Serrano *et al.,* 2014).

Sampling the complete niche where a species is able to thrive can greatly modify ENM outputs (Wiens *et al*., 2009; Martínez-Freiría *et al.,* 2016). In order to capture the

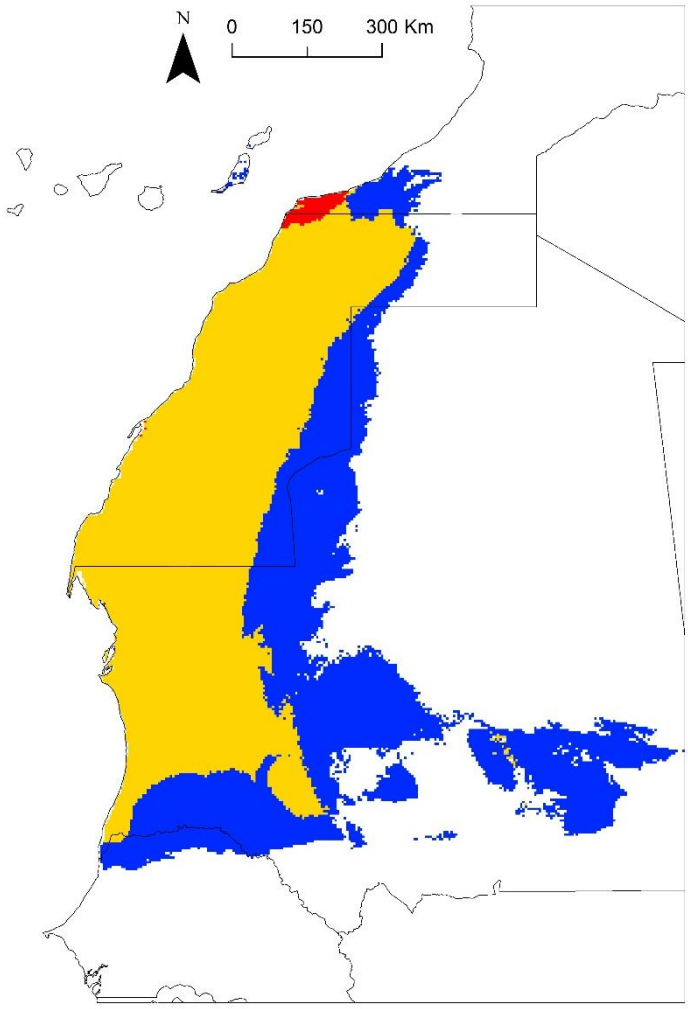

Figure 14: Sum of stable areas for all species. Red corresponds to the overlap of the stable areas for three species (*T. annularis*, *T. chazaliae* and *T. hoggarensis*), yellow corresponds to the overlap of the stable areas of two species and blue corresponds to areas that are stable for only one species.

entire ecological range of a species, all the distributional range must be comprised (Alvarado-Serrano *et al.,* 2014). In this work, only the complete distributional range of *T. chazalia*e was included. For the remaining three species partial distributional ranges

were used, preventing a thorough analysis at intraspecific level, as well as the capture of the entire range of environmental conditions in which each species can occur.This may have led to an underestimation of the ecological niche, and thus of their potential distribution in the past. On the other hand, widespread species may present specific local adaptations, especially at the range margins, that cannot be captured when building global models. This in turn can lead to less accurate predictions and an overestimation of a species ecological niche at the range margins when modelling their entire range (Vale *et al.,* 2014). *T. annularis*, being a climatically tolerant species, appears to not have many local adaptations, and thus models built for the entire or restricted range are not expected to differ much. However, *T. hoggarensis* is less tolerant, and colonized West Africa from the more humid Sahel, and thus is expected to present local adaptations to more arid conditions. As a result, building ecological models for the entire range would portentially not capture these adaptations, resulting in an overestimation of the suitable area. For *T. parvicarinata* almost the entire range was included when building the ecological models. Accordingly, almost all the environmental conditions where the species can occur was considered. However, failiure to include the entire range could have led to an underestimation of the suitable area.

Habitat variables can have a great impact on the distributional ranges of species showing low vagility, such as most reptiles, or specialized species that only occur in a limited set of variables (Sow *et al.,* 2014). However, it is not possible to infer their past distribution. As a result, when building ENMs for past conditions, only climatic variables can be used, which may not capture the entire range of species distribution (Wiens *et al.,* 2009; Alvarado-Serrano *et al.,* 2014). The four study species have distinct habitat preferences, which was not taken into consideration when building the ENMs. For instance, despite the models predicting a similar suitable area for *T. annularis* and *T. hoggarensis*, and these species being found in sympatry at a large spatial scale, *T. annularis* prefers sandy habitat, while *T. hoggarensis* can only be found in *Acacia* trees (Trape *et al.,* 2012). Using landscape features to build the models might yield different results, but they could not be projected to the past. *Tarentola parvicarinata* can only be found in mountainous rocky areas, but the models predicted suitable areas in the past that are currently covered by sand. Being in fact rocky and suitable in the past or covered in sand like in the present, would have different implications for this species distribution and dispersal. Species interaction can restrict the distribution of a species to a fraction of their fundamental niche, but are difficult to reliably measure and incorporate in ENMs (Pearson and Dawson, 2003). This may also result in a bias where an area is identified

as suitable, but the species is not present there. Thus, using only climatic variables to build ecological models could also lead to an overestimation of suitable areas. New techniques, such as remote sensing, could help solving this problem in the future (e.g. Deblauwe *et al.,* 2016).

Projecting the ecological models to the past can also affect the identification of suitable areas. Past climatic conditions may fall outside of the physiological tolerance of a species, which can potentially inflate the predicted suitable area of a species (Alvarado-Serrano *et al.,* 2014). Maxent tries to deal with this through clamping. In the present study high clamping values fall outside of the predicted suitable area of the species, and thus where the species potentially occurred climate remained within the tolerance values of the species.

Despite using a generally slow evolving gene, 12S (Wan *et al.,* 2004), this work recovered high levels of genetic divergence even at intraspecific levels, which potentially result in lack of niche conservatism between lineages. That is, the models are being built for entities higher than species, when in fact the lineages could have different ecological requirements. The inclusion of all this environmental variability potentially led to an overestimation of past suitable areas, preventing the identification of possible isolated patches that matched the molecular results. By modelling each lineage independently it would be possible to circumvent this issue. However, a more extensive sampling and the use of more molecular markers would be necessary to clearly define the lineages. Furthermore, *Tarentol*a history dates back to the Miocene (Rato *et al.*, 2012), and thus the lineages recovered in this study could have originated before the LIG. This discrepancy between the ecological and molecular time scales could also result in a lack of concordance in the results. Using faster evolving markers, such as other mitochondrial genes, microsatellites or NGS approaches could provide greater insights to the more recent phylogeographic processes in these species (Velo-Antón *et al.*, 2013). However, slow-evolving molecular markers could also be useful to track the allopatric distribution of ancient lineages through time. Assuming a 0.6% genetic divergence used in previous studies (Carranza *et al.,* 2000; Rato *et al.,* 2012), the lineages of *T. chazaliae* diverged between 7 and 8 Mya, the lineages of *T. parvicarinata* 6.7-8.5 Mya, and for *T. hoggarensis*, the most divergent lineage separated from the other 17.7-19 Mya, while among the remaining lineages, divergence is estimated to have occurred 3.8-5.3 Mya. However, Rato *et al.,* 2012 calculate that the two main *Tarentola* clades diverged 15.38 Mya, contradicting the calculations for *T. hoggarensis*. This could be the result of the 0.6% genetic divergence having been calculated with both 12S and cyt-b for *Gallotia* (Carranza *et al.,* 2000). These authors argue that both genera are heterothermic and

relatively closely related, and thus using data from one genera to calibrate the other is appropriate. Our results appear to contradict this, but nonetheless it is evident that all *Tarentola* lineages diverged some million years ago. Comparing with other works, most clades of *T. mauritanica* recovered by Rato *et al.,* 2010 show a genetic distance between 2.8 and 5.4%, with one presenting 9%. Rato *et al.,* 2016 recovered lineages with a genetic distance between 2.7 and 7.7%. These clades were also recovered and well supported by Rato *et al.*, 2012, and recognized as full independent lineages that should be further accessed to determine if they are different species. Although the relation between the lineages recovered by Rato *et al.,* 2012 differs from the one recovered by Rato *et al.,* 2016, the authors still advise a taxonomic revision. Similar values of genetic distance in our study species raises the question of whether the lineages recovered correspond to higher taxonomic entities, especially the most divergent lineage within *T. hoggarensis* that should be object of independent ecological model assessments. However, as only one gene was used, the results obtained may not correspond to the true history of the species, and more unlinked genes are needed to infer the biogeographic history of *Tarentola* in North Africa.

5. Concluding remarks and future prospects

This study exemplifies how ecological and molecular tools can be integrated to infer the response of species to past climatic oscillations, even when not fully agreeing on the recovered signal.

There is a remarkable lack of knowledge on genetic information for North African *Tarentola*. Phylogeographic studies with *Tarentola* species have been mostly focused on the *T. mauritanica* clade, overlooking the species studied here. Besides the work of Carranza *et al.,* 2002, Rato *et al.,* 2012, Rato *et al.,* 2015 and Rato *et al.,* 2016, which all focused the *T. mauritanica* species complex, few sequences of *Tarentola* were available for the targeted species. This limits the comparisons and inferences that can be made for this species. For example, it remains unknown if the lack of divergence between Egyptian and Mauritanian *T. annularis* is real or results are biased due to the inadequate sampling. Furthermore, the notable divergence recovered by the genetic marker used was unexpected, and more markers (both mitochondrial and nuclear) are needed to clarify the evolutionary history of these species, and to coherently delimitate evolutionary and taxonomic units (e.g. in *T. hoggarensis*). Comprehensive ENM studies, increasing the sampling across the entire range of these species and the use of both habitat and climatic variables would allow better inferring biogeographical scenarios for the *Tarentola* genus, and each particular species.

The present studied allowed to shed some light on how variation in climatic conditions over time has affected biodiversity patterns in the West Sahara-Sahel, as well as confirming the existence of a stable and semi-permeable corridor in the region. These results will also help clarify biogeographic patterns in other species, as many present similar distributions and tolerances to the climatic conditions in the region (e.g. *A. aureus*, Lopes, 2014; *S. sthenodactylus*, Metallinou *et al.,* 2012; *C. cerastes*, Brito *et al.,* 2011b).

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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 flowthrough.

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 this study with the putatuve species assignment based on morphology, local, country, clade from the phylogenetic tree, lineage within the tree (when applicable), and haplotype number from Figures 9B to 12B (Haplotype Network 1) and Annex A6 (Haplotype Network 2).

Analysis code	Species	Local	Country	Lineage	Haplotype Network 1	Haplotype Network 2
1617	*T. annularis*	Amchigdef, 2km S of	Mauritania	A	1	1
1627	*T. annularis*	Bedmeijât	Mauritania	A	1	1
1916	*T. annularis*	Lemmoizîne	Mauritania	A	1	1
1936	*T. annularis*	Oued Oûl Moûssa	Mauritania	A	1	1
2749	*T. annularis*	PN Banc d'Arguin, Dló Matai	Mauritania	A	1	1
2895	*T. annularis*	El Beyyed	Mauritania	A	1	1
3471	*T. annularis*	Oued Saouat	Mauritania	A	1	1
3610	*T. annularis*	PN Banc d'Arguin, Iouîk, 10km E of	Mauritania	A	1	1
4560	*T. annularis*	Fanaye Niakouar, 5km E of	Mauritania	A	1	1
4932	*T. annularis*	Oumm 'Aoueili, 5km S of	Mauritania	A	1	1
5039	*T. annularis*	PN Banc d'Arguin, Goûd Anagoum, 2km NW of	Mauritania	A	1	1
5056	*T. annularis*	PN Banc d'Arguin, Aleib et Talah 2	Mauritania	A	1	1
5222	*T. annularis*	Bir Îgueni, 2km SW of	Mauritania	A	1	1
5257	*T. annularis*	Çtel Ogmâne	Mauritania	A	3	
5768	*T. annularis*	Châr, valley	Mauritania	A	1	1
5816	*T. annularis*	Zouérat	Mauritania	A	1	1
6007	*T. annularis*	Letfatar, 35km SW of	Mauritania	A	1	1
6408	*T. annularis*	PNBA: Jerf el Oûstâni, 4km NE of	Mauritania	A	1	1
6465	*T. annularis*	PNBA: Kerekchet et Teintâne, central	Mauritania	A	4	
7232	*T. annularis*	Hassi Hawza	Western Sahara	A	1	1
7252	*T. annularis*	Tiglalatin, W of	Western Sahara	A	1	1
7296	*T. annularis*	Oued Tindkine	Western Sahara	A	1	1
7318	*T. annularis*	Tayart Labwir, SE of	Western Sahara	A	1	1
7337	*T. annularis*	Oued Hawl	Western Sahara	A	1	1
7874	*T. annularis*	Nouameline	Mauritania	A	5	

9183	*T. annularis*	Gleybat Al Maçrane	Western Sahara	A	1	1
11392	*T. annularis*	Bou Naga, SW of	Mauritania	A	2	2
11406	*T. annularis*	Bou Naga, SW of	Mauritania	A	2	2
SPM002386	*T. annularis*	W de El Cairo, road to Baharyia	Egypt	A		
	T. annularis		Egypt	A		
	T. annularis		captive speciemen	A		
7164	*T. boehmei*	Oued Zouwa	Morocco			
7193	*T. boehmei*	Jebel Ouarkziz	Morocco			
10263	*T. boehmei*	Ait Sawh, SE of	Morocco			
DB1604	*T. boehmei*	Foum Zguid	Morocco			
DB876	*T. boehmei*	Sidi Mohand OuSourou, Mirleft	Morocco			
DB11024	*T. boehmei*	Near Oed Iriri	Morocco			
DB9000	*T. boehmei*	0.5 Km N. of Bou-Azzer	Morocco			
DB859	*T. boehmei*	Abattekh	Morocco			
DB880	*T. boehmei*	Abattekh	Morocco			
DB399	*T. boehmei*	2km S of Zawyat Sidi Blal	Morocco			
DB241	*T. boehmei*	Akka Ighane	Morocco			
DB785	*T. boehmei*	Imi El Had	Morocco			
DB878	*T. boehmei*	Idufkir, Sidi Ifni	Morocco			
DB1411	*T. boehmei*	Gorges near Guelmin	Morocco			
DB795	*T. boehmei*	Tioulit	Morocco			
DB242	*T. boehmei*	Akka Ighane	Morocco			
DB262	*T. boehmei*	Aït-Bekkou	Morocco			
DB1864	*T. boehmei*	Aouinet Torkoz	Morocco			
SC64	*T. boehmei*	Akka Ighane	Morocco			
561	*T. chazaliae*	Laayoune, 40km N of (in litoral dune field)	Western Sahara	A	3	3
5721	*T. chazaliae*	Graret An-Nwayeb	Western Sahara	C	8	7
7351	*T. chazaliae*	Sebkha Imlilil, E of	Western Sahara	B	5	5
9092	*T. chazaliae*	Tarfaya, 20km S of	Morocco	C	9	7
9109	*T. chazaliae*	Chtoukane	Western Sahara	B	7	6
9116	*T. chazaliae*	Oued Lakra, S of	Western Sahara	A	2	3
9201	*T. chazaliae*	Ghrad Al 'Angra	Western Sahara	A	4	4
9921	*T. chazaliae*	Atf	Western Sahara	B	6	5
10603	*T. chazaliae*	Sidi Warziq, S of	Morocco	A	1	1
DB863	*T. chazaliae*	Abattekh	Morocco	B		5
DB884	*T. chazaliae*	Tafnidilt, Tan-Tan	Morocco	A		2
Geckoni 2	*T. chazaliae*	Tan-Tan Plage	Morocco	B		5

Geckoni 1	*T. chazaliae*	Laâyoune	Morocco	C		8
8343	*T. deserti*	Bou'arfa, SW of	Morocco			
10236	*T. deserti*	Ait Sawh, SE of	Morocco			
DB473	*T. deserti*	Guemar, El Oued	Algeria			
DB1262	*T. deserti*	Road between Messaad and Ain Kheneg ed Defia	Algeria			
DB326	*T. deserti*	Merzouga	Morocco			
DB8998	*T. deserti*	7 Km South of Rizane	Morocco			
DB3278	*T. deserti*		Morocco			
DB3124	*T. deserti*		Morocco			
DB480	*T. deserti*	Taibet, Touggourt	Algeria			
DB467	*T. deserti*	Guemar, El Oued	Algeria			
DB325	*T. deserti*	Merzouga (house)	Morocco			
DB363	*T. deserti*	Road between Messaad and Ain Kheneg ed Defia	Algeria			
DB9006	*T. deserti*	Rissani	Morocco			
DB481	*T. deserti*	El Goléa, El Goléa (Sahara)	Algeria			
DB9005	*T. deserti*	Rissani	Morocco			
DB9015	*T. deserti*	7 Km South of Rizane	Morocco			
DB9011	*T. deserti*	Erfoud area	Morocco			
DB488	*T. deserti*	El Ateuf, Ghardaia (Sahara)	Algeria			
DB327	*T. deserti*	Merzouga	Morocco			
DB9007	*T. deserti*	Rissani	Morocco			
DB9016	*T. deserti*	7 Km South of Rizane	Morocco			
SC62	*T. deserti*	Erfoud	Morocco			
DB3178	*T. deserti*		Morocco			
3468	*T. hoggarensis*	Moit	Mauritania	A	13	
3520	*T. hoggarensis*	Bir Boû Khbeira wells, 8km SW of	Mauritania	C	1	1
3541	*T. hoggarensis*	PN Banc d'Arguin, Tâjgourît	Mauritania	C	1	1
4626	*T. hoggarensis*	Mbout, 10km N of	Mauritania	A	16	
4993	*T. hoggarensis*	Nouakchott, 45km NE of	Mauritania	C	1	1
5003	*T. hoggarensis*	Nouakchott, 140km NE of	Mauritania	C	1	1
5828	*T. hoggarensis*	Guelb Atomai	Mauritania	C	1	1
5901	*T. hoggarensis*	Tarf Tazazmout	Mauritania	C	5	
6032	*T. hoggarensis*	Tin Waadine, guelta	Mauritania	C	1	1
6080	*T. hoggarensis*	Bougâri, tâmoûrt	Mauritania	C	7	
6110	*T. hoggarensis*	Aouînet Nanâga	Mauritania	C	2	
6475	*T. hoggarensis*	Kerekchet et Teintâne, extreme N	Mauritania	C	1	1
7220	*T. hoggarensis*	Hassi Aglat An Nakhla	Western Sahara	C	1	1

7236	*T. hoggarensis*	Hawza, 10km W of	Western Sahara	C	1	1
7271	*T. hoggarensis*	Gtem Larad	Western Sahara	C	1	1
7275	*T. hoggarensis*	Oued Awletiss, SE of	Western Sahara	C	1	1
7413	*T. hoggarensis*	PN Diawling headquarters	Mauritania	A	13	
7574	*T. hoggarensis*	Korokoro	Mauritania	A	15	
9137	*T. hoggarensis*	Atf	Western Sahara	C	1	1
9158	*T. hoggarensis*	Derraman	Western Sahara	C	3	1
9240	*T. hoggarensis*	Sélibabi, 5km SW of	Mauritania	A	16	
9610	*T. hoggarensis*	Moutchikaten	Mauritania	C	7	
9665	*T. hoggarensis*	Oualâta, 5km W of	Mauritania	D	11	
9843	*T. hoggarensis*	Hassi Nyelbo	Mauritania	C	1	1
9916	*T. hoggarensis*	Chami, NW of	Mauritania	C	1	1
10724	*T. hoggarensis*	Toundou Beret	Mauritania	A	14	
10817	*T. hoggarensis*	Ngouye Classified Forest (Senegal river basin)	Mauritania	A	16	
SPM003398	*T. hoggarensis*	25Km W of Smara	Western Sahara	C		
SPM000387	*T. hoggarensis*	Smara	Western Sahara	C		1
Tephi19	*T. hoggarensis*	M'Bour, ORSTOM institute	Senegal	A		
Tephi18	*T. hoggarensis*	M'Bour, ORSTOM institute	Senegal	A		
6642	*T. ephippiata*	Mainé-Soroa, 10km E of	Niger	D	12	
6740	*T. ephippiata*	Termit, Zgaidinga	Niger	B	9	
6753	*T. ephippiata*	Termit, Louli Agadem Nga	Niger	B	10	
6763	*T. ephippiata*	Termit-Kaoboul, 10km NW of	Niger	B	9	
6765	*T. ephippiata*	Tasker, 8km N of	Niger	B	9	
6773	*T. ephippiata*	Kellé, 5km S of	Niger	B	9	
11012	*T. ephippiata*	Tichillit el Beida	Mauritania	C	8	
11031	*T. ephippiata*	Lembeidi	Mauritania	C	8	
11048	*T. ephippiata*	Oued Kmache	Mauritania	C	8	
11283	*T. ephippiata*	Gharghar, S of	Mauritania	C	7	
11309	*T. ephippiata*	Tamourt el Khadra	Mauritania	C	6	
11360	*T. ephippiata*	Oued el Mancour	Mauritania	C	1	1
11381	*T. ephippiata*	Aguilal Fai, SW of	Mauritania	C	4	
DB2159	*T. fascicularis*	Detj oasis	Libya			
DB2156	*T. fascicularis*	Detj oasis	Libya			
10601	*T. mauritanica*	Ar-Khout, N of	Morocco			
10637	*T. mauritanica*	Sidi Akhfennir	Morocco			
DB321	*T. mauritanica*	Essaouira	Morocco			
Tm34	*T. mauritanica*	Al Jadida	Morocco			

Tm141	*T. mauritanica*	Berja/Castala, Almería	Spain		
Tm216	*T. mauritanica*	Conigli islet	Italy		
DB924	*T. mauritanica*	Chouga, Sidi el Bettach	Morocco		
	T. mauritanica				
Ksar_6	*T. neglecta*	Ksar Ghilane	Tunisia		
Ksar_1	*T. neglecta*	Ksar Ghilane	Tunisia		
971	*T. parvicarinata*	Tîntâne, 40km W of (Kediet el Freid)	Mauritania	S	1
1639	*T. parvicarinata*	Atar, Camping Bab Sahara	Mauritania	N	16
1942	*T. parvicarinata*	Touâjîl, 10km N of	Mauritania	N	11
1944	*T. parvicarinata*	Touâjîl, 15km S of	Mauritania	N	11
2191	*T. parvicarinata*	Guelb Samba	Mauritania	S	3
2677	*T. parvicarinata*	El Housseînîya	Mauritania	N	11
2875	*T. parvicarinata*	Ej Jbeilîyât	Mauritania	N	18
2876	*T. parvicarinata*	Ej Jbeilîyât	Mauritania	N	18
2915	*T. parvicarinata*	Ibi el Abiod	Mauritania	N	16
2940	*T. parvicarinata*	Teneddâyet	Mauritania	N	17
2969	*T. parvicarinata*	Tamassoumit	Mauritania	N	15
3004	*T. parvicarinata*	Ain Bâjed	Mauritania	N	11
3459	*T. parvicarinata*	Gueltet Thor, 10km S of	Mauritania	C	7
4659	*T. parvicarinata*	Wali, 10km E of	Mauritania	S	1
4874	*T. parvicarinata*	Gânçai source, 4km S of	Mauritania	C	7
5007	*T. parvicarinata*	Akjoujt, 12km NE of	Mauritania	N	12
5773	*T. parvicarinata*	Châr, valley	Mauritania	N	16
5847	*T. parvicarinata*	Guelb el Hâlma	Mauritania	N	16
6071	*T. parvicarinata*	Nega pass	Mauritania	N	13
6126	*T. parvicarinata*	El Harach, 10km NW of	Mauritania	C	10
6168	*T. parvicarinata*	Artémou mountain	Mauritania	S	1
6187	*T. parvicarinata*	Wompu, 23km SE of (Senegal river basin)	Mauritania	S	1
7623	*T. parvicarinata*	Koumba Ndao, 5km N of	Mauritania	S	1
7738	*T. parvicarinata*	Guelta Meyla	Mauritania	C	9
7816	*T. parvicarinata*	Tarf Tentahara, S slope	Mauritania	C	9
9258	*T. parvicarinata*	Baédiam	Mauritania	S	1
9290	*T. parvicarinata*	Oued Kmache	Mauritania	S	1
9307	*T. parvicarinata*	El Goueissi	Mauritania	S	1
9405	*T. parvicarinata*	Boutounguissi, N of	Mali	S	1
9437	*T. parvicarinata*	Boli Bana river	Mali	S	2
9500	*T. parvicarinata*	Takoutala, W of	Mali	S	5
9555	*T. parvicarinata*	Bindougou, N of	Mali	S	6
9600	*T. parvicarinata*	Gobernie	Mauritania	S	4

9660	*T. parvicarinata*	Tâmoûrt Chkateyl	Mauritania	N	11	
9666	*T. parvicarinata*	Oualâta, 5km W of	Mauritania	N	11	
9695	*T. parvicarinata*	Sgué	Mauritania	N	11	
9729	*T. parvicarinata*	Es Sba	Mauritania	N	11	
9798	*T. parvicarinata*	Zig	Mauritania	N	11	
9840	*T. parvicarinata*	Tidjikja, 8km SW of	Mauritania	N	11	
10780	*T. parvicarinata*	Dindi	Mauritania	S	1	
10846	*T. parvicarinata*	Manael, SE of	Mauritania	S	1	
10892	*T. parvicarinata*	Khabou-Guidimaka, N of	Mauritania	S	2	
10931	*T. parvicarinata*	Bokedinabi, N of	Mauritania	S	1	
10941	*T. parvicarinata*	Bokedinabi, N of	Mauritania	S	1	
10997	*T. parvicarinata*	Kankossa, N of	Mauritania	S	1	
11033	*T. parvicarinata*	Lembeidi	Mauritania	S	1	
11047	*T. parvicarinata*	Oued Kmache	Mauritania	S	1	
11054	*T. parvicarinata*	Oued Lemhara	Mauritania	S	1	
11096	*T. parvicarinata*	Oued Lemhara	Mauritania	S	1	
11147	*T. parvicarinata*	Borie, E of	Mauritania	S	1	
11223	*T. parvicarinata*	Mzalig	Mauritania	S	1	
11251	*T. parvicarinata*	Djafarat	Mauritania	S	1	
11264	*T. parvicarinata*	Djafarat	Mauritania	S	1	
11269	*T. parvicarinata*	Djafarat, W of	Mauritania	S	1	
11282	*T. parvicarinata*	Guelb Cenguetra	Mauritania	S	1	
11299	*T. parvicarinata*	Tamourt el Khcheb	Mauritania	S	3	
11324	*T. parvicarinata*	Dmouch Telli	Mauritania	S	3	
11338	*T. parvicarinata*	Taleb Eley	Mauritania	S	3	
11343	*T. parvicarinata*	Tintane, NE of	Mauritania	S	3	
11348	*T. parvicarinata*	Tintane, SW of	Mauritania	S	3	
11368	*T. parvicarinata*	Taghada el Ouas'a	Mauritania	C	8	
11405	*T. parvicarinata*	Bou Naga, SW of	Mauritania	N	14	
5023	*Tarentola* sp.	Bennichchâb, 15km SW of	Mauritania	A	2	2
6849	*Tarentola* sp.	Chaambi	Tunisia			
10611	*Tarentola* sp.	Toufgounit	Morocco			

A3)

List of sequences obtained from GenBank, with accession number, locality, country and reference

Species		Accession number	Locality	Country	Reference
T. chazaliae	DB863	JQ300722.1	Abattekh	Morocco	Rato et al 2012
T. chazaliae	DB884	Q300542.1	Tafnidilt, Tan-Tan	Morocco	Rato et al 2013
T. chazaliae	Geckoni2	AF363575.2	Tan-Tan Plage	Morocco	Carranza et al 2002
T. chazaliae	Geckoni1	AF363574.2	Laâyoune	Morocco	Carranza et al 2003
T. annularis	SPM002386	JQ300566.1	W de El Cairo, road to Baharyia	Egypt	Rato et al 2013
T. annularis		AF363571.2		Egypt	Carranza et al 2002
T. annularis		DQ275412.1		captive speciemen	Lamb and Bauer 2006
T. deserti	DB473	JQ300766.1	Guemar, El Oued	Algeria	Rato et al 2012
T. deserti	DB1262	JQ300754.1	Road between Messaad and Ain Kheneg ed Defia	Algeria	Rato et al 2012
T. deserti	DB326	JQ300727.1	Merzouga	Morocco	Rato et al 2012
T. deserti	DB8998	JQ300725.1	7 Km South of Rizane	Morocco	Rato et al 2012
T. deserti	DB3278	JQ300723.1		Morocco	Rato et al 2012
T. deserti	DB3124	JQ300718.1		Morocco	Rato et al 2012
T. deserti	DB480	JQ300708.1	Taibet, Touggourt	Algeria	Rato et al 2012
T. deserti	DB467	JQ300679.1	Guemar, El Oued	Algeria	Rato et al 2012
T. deserti	DB325	JQ300658.1	Merzouga (house)	Morocco	Rato et al 2012
T. deserti	DB363	JQ300655.1	Road between Messaad and Ain Kheneg ed Defia	Algeria	Rato et al 2012
T. deserti	DB9006	JQ300647.1	Rissani	Morocco	Rato et al 2012
T. deserti	DB481	JQ300628.1	El Goléa, El Goléa (Sahara)	Algeria	Rato et al 2012
T. deserti	DB9005	JQ300625.1	Rissani	Morocco	Rato et al 2012
T. deserti	DB9015	JQ300615.1	7 Km South of Rizane	Morocco	Rato et al 2012
T. deserti	DB9011	JQ300586.1	Erfoud area	Morocco	Rato et al 2012
T. deserti	DB488	JQ300579.1	El Ateuf, Ghardaia (Sahara)	Algeria	Rato et al 2012
T. deserti	DB327	JQ300564.1	Merzouga	Morocco	Rato et al 2012
T. deserti	DB9007	JQ300562.1	Rissani	Morocco	Rato et al 2012
T. deserti	DB9016	JQ300548.1	7 Km South of Rizane	Morocco	Rato et al 2012
T. deserti	SC62	AF363570.2	Erfoud	Morocco	Rato et al 2012
T. deserti	DB3178	JQ300696.1		Morocco	Rato et al 2012
T. boehmei	DB1604	JQ300748.1	Foum Zguid	Morocco	Rato et al 2012
T. boehmei	DB876	JQ300743.1	Sidi Mohand Ou Sourou, Mirleft	Morocco	Rato et al 2012
T. boehmei	DB11024	JQ300692.1	Near Oed Iriri	Morocco	Rato et al 2012
T. boehmei	DB9000	JQ300668.1	0.5 Km N. of Bou-Azzer	Morocco	Rato et al 2012
T. boehmei	DB859	JQ300656.1	Abattekh	Morocco	Rato et al 2012
T. boehmei	DB880	JQ300652.1	Abattekh	Morocco	Rato et al 2012
T. boehmei	DB399	JQ300651.1	2km S of Zawyat Sidi Blal	Morocco	Rato et al 2012
T. boehmei	DB241	JQ300637.1	Akka Ighane	Morocco	Rato et al 2012
T. boehmei	DB785	JQ300608.1	Imi El Had	Morocco	Rato et al 2012
T. boehmei	DB878	JQ300584.1	Idufkir, Sidi Ifni	Morocco	Rato et al 2012
T. boehmei	DB1411	JQ300582.1	Gorges near Guelmin	Morocco	Rato et al 2012
T. boehmei	DB795	JQ300557.1	Tioulit	Morocco	Rato et al 2012

T. boehmei	DB242	JQ300552.1	Akka Ighane	Morocco	Rato et al 2012
T. boehmei	DB262	JQ300549.1	Aït-Bekkou	Morocco	Rato et al 2012
T. boehmei	DB1864	GU593722.1	Aouinet Torkoz	Morocco	Ceacero et al 2010
T. boehmei	SC64	AF363569.2	Akka Ighane	Morocco	Carranza et al 2002
T. hoggarensis	SPM003398	JQ300746.1	25Km W of Smara	Western Sahara	Rato et al 2012
T. hoggarensis	SPM000387	JQ300681.1	Smara	Western Sahara	Rato et al 2012
T. hoggarensis	Tephi19	AF363573.2	M'Bour, ORSTOM institute	Senegal	Carranza et al 2002
T. hoggarensis	Tephi18	AF363572.2	M'Bour, ORSTOM institute	Senegal	Carranza et al 2002
T. fascicularis	DB2159	JQ300757.1	Detj oasis	Libya	Rato et al 2012
T. fascicularis	DB2156	JQ300646.1	Detj oasis	Libya	Rato et al 2012
T. neglecta	Ksar_6	JQ300712.1	Ksar Ghilane	Tunisia	Rato et al 2012
T. neglecta	Ksar_1	JQ300649.1	Ksar Ghilane	Tunisia	Rato et al 2012
T. mauritanica	DB321	HM014494.1	Essaouira	Morocco	Rato et al 2010
T. mauritanica	Tm34	AY828463.1	Al Jadida	Morocco	Harris et al 2004
T. mauritanica	Tm141	FJ156030.1	Berja/Castala, Almería	Spain	Perera and Harris 2008
T. mauritanica	Tm216	FJ609242.1	Conigli islet	Italy	Harris et al 2009
T. mauritanica	DB924	JQ300631.1	Chouga, Sidi el Bettach	Morocco	Rato et al 2012
T. mauritanica		JQ425045.1			Rato et al 2013

A4)

Primers and PCR conditions for the sequenced mitochondrial gene

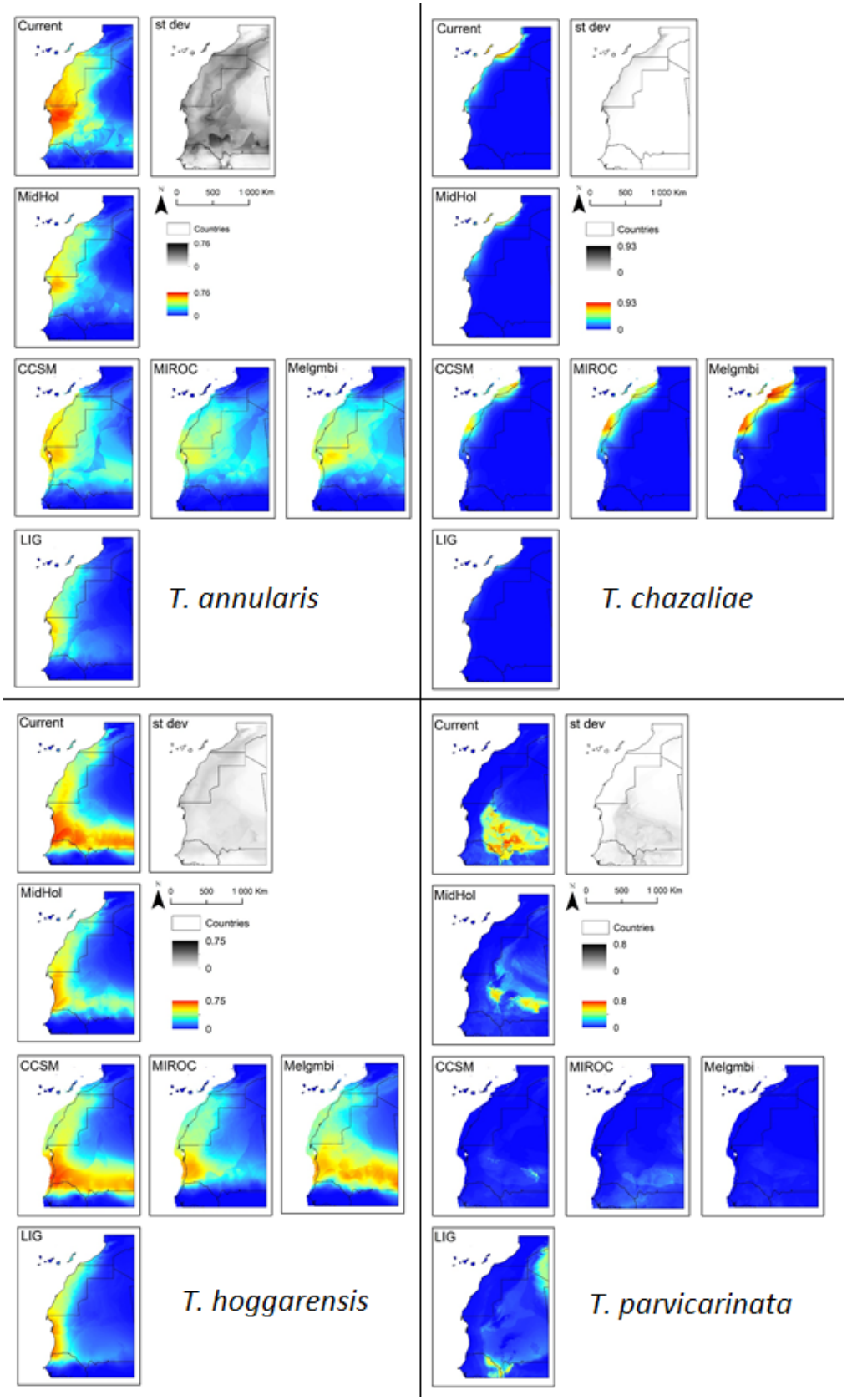
12S primers

L1091	Forward	AAAAAGCTTCAAACTGGGATTAGATACCCCACTAT
H1478	Reverse	TGACTGCAGAGGGTGACGGGCGGTGTGT

PCR conditions

Amplification Step	Temperature (ºC)	Duration	Number of cycles
Initial Denaturation	95	10 minutes	1
Denaturation	95	30 seconds	35
Annealing	52	30 seconds	
Extension	72	30 seconds	
Final Extension	72	10 minutes	1

A5) Probabilistic models for the four study species for the present and the past, and standard deviation.



A6)

Haplotype networks with shorter sequence length and including more individuals.