



# Diversity, distribution and conservation of reptiles in the West Sahara-Sahel

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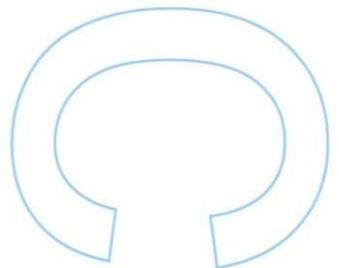
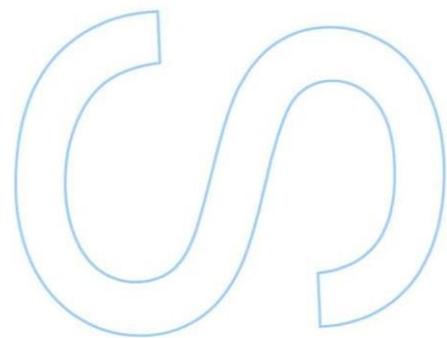
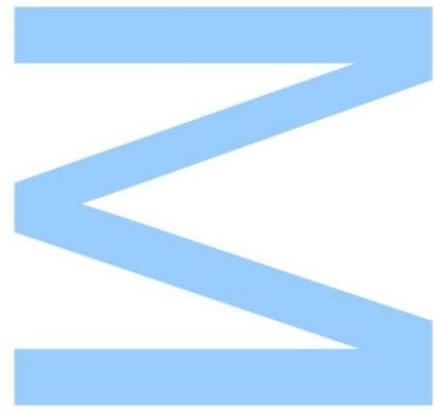
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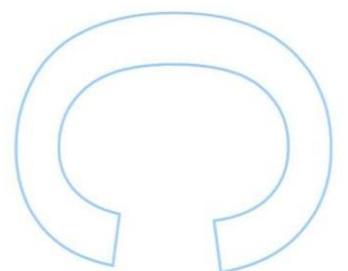
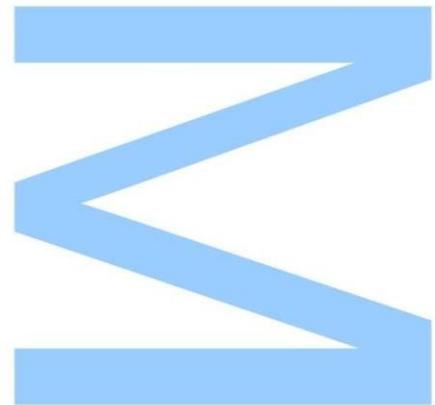




Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

Porto, \_\_\_\_\_ / \_\_\_\_\_ / \_\_\_\_\_



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## Abstract

Although biodiversity plays a key role in maintaining ecosystem function and persistence, there is global biodiversity loss at an alarming rate. Overall knowledge about biodiversity in deserts and arid regions is still very poor in comparison to other biomes. Deserts hold 25% of terrestrial vertebrate's species and may provide important findings about adaptations to extreme environments. The region of West Sahara-Sahel (WSS) in Africa has an increased biodiversity, due to the location in a transition zone between the Palaearctic and Afro-tropic biogeographic realms. Preliminary molecular studies detected cryptic diversity in some reptile species, suggesting that the diversity of the group is still poorly known in the region. In this study, molecular and spatial tools were combined to unravel reptile diversity in the WSS. The aim was to answer the following questions: 1) How many reptile phylogenetic units occur in the WSS? 2) Which is the distribution of the reptile phylogenetic units? 3) Where are the areas with accumulating diversity of reptile phylogenetic units located? 4) Is the current network of protected areas covering the regions which accumulate the highest diversity? A COI barcode was used to build a barcoding reference library for the WSS reptiles based on a total of 755 samples from 109 taxa. Phylogenetic analyses were conducted on 542 sequences to identify phylogenetic units. Delimitation approaches were used to identify the phylogenetic units occurring in the reptiles from WSS and detect cryptic diversity. Geographical Information Systems were used to map the distribution of phylogenetic units, the richness of lineages, and to quantify gaps in the current network of WSS protected areas for reptile diversity.

A DNA barcoding library representing more than 80% of the described reptile diversity of the WSS was assembled, including a barcode for *Agama impalearis* (new species recorded). Four new putative cryptic species were identified in the WSS (and another five outside), and 93 mitochondrial lineages were retrieved. Four mountain endemic species were found. Reptile richness was concentrated in mountains, especially in Assaba Mountain. The regions concentrating the highest reptile diversity were not represented in the current protected areas.

DNA barcoding library obtained in this study provides a valuable tool for identifying and assessing the diversity of WSS reptiles. Biodiversity distribution is spatially structured and mountains display an important biological role as refugium and as local biodiversity hotspots. The implementation of protected areas in mountains should be taken under advice to conserve reptile diversity.

## **Keywords**

Barcoding, COI, Cryptic diversity, GIS, Lineages Richness, Putative species, Species Richness

## Resumo

Ainda que a biodiversidade desempenhe um papel vital na manutenção da função e persistência de um ecossistema, encontra-se uma perda global de biodiversidade a um ritmo alucinante. O conhecimento geral sobre a biodiversidade nos desertos e regiões áridas é, ainda, muito pobre por comparação com outros biomas. Os desertos alojam 25% dos vertebrados terrestres e, desta forma, podem proporcionar importantes achados sobre adaptações a ambientes extremos. A região do Sahara-Sahel Ocidental (SSO) em África tem uma biodiversidade alargada, devido à sua localização numa zona de transição entre as regiões biogeográficas do Paleoártico e Afro-tropical. Estudos moleculares preliminares detetaram diversidade críptica em algumas espécies de répteis, sugerindo que a diversidade do grupo ainda é pouco estudada na região.

Nesta tese, as ferramentas espaciais e moleculares foram combinadas de forma a revelar a diversidade reptil no SSO. A intenção prendia-se com a resposta às seguintes questões: 1) Quantas unidades filogenéticas reptis surgem no SSO? 2) Qual é a distribuição de unidades filogenéticas reptis? 3) Onde se encontram as áreas de unidades filogenéticas reptis com diversidade acumulada? 4) Será que a atual rede de áreas protegidas cobrem as regiões que acumulam a maior diversidade? Foi usado um *COI barcode* para construir uma biblioteca de referência de *barcoding* para os répteis de SSO, partindo de um total de 755 amostras de 109 taxa. As análises filogenéticas foram realizadas em 542 sequências para a identificação de unidades filogenéticas. Foram usados métodos de delimitação para identificar as unidades filogenéticas presentes nos répteis de OSS e para detetar a diversidade críptica. Utilizaram-se Sistemas de Informação Geográfica para mapear a distribuição das unidades filogenéticas, a riqueza das linhagens e para quantificar as lacunas na atual rede de áreas protegidas da OSS de diversidade reptil.

Uma biblioteca de *barcoding* de ADN representando mais de 80% da diversidade reptil descrita no OSS foi reunida, incluindo o *barcode* para a *Agama impalearis* (nova espécie registada). Quatro novas potenciais espécies crípticas foram identificadas na OSS (conjuntamente com outras cinco localizadas fora da região mencionada), e foram recolhidas 93 linhagens mitocondriais. Foram encontradas quatro espécies endémicas das montanhas. A riqueza reptil estava concentrada nas montanhas, particularmente na Montanha Assaba. As regiões que concentravam a maior diversidade reptil não se encontram representadas nas atuais áreas protegidas.

A biblioteca de *barcoding* de ADN obtida neste estudo proporciona uma ferramenta valiosa para a identificação e avaliação da diversidade dos répteis do OSS. A distribuição da biodiversidade está estruturada espacialmente e as montanhas desempenham um

importante papel biológico de refúgio e como hotspots de biodiversidade local. A implementação de áreas protegidas nas montanhas devia ser tida em conta, com vista à conservação da diversidade reptil.

## **Palavras-Chave**

Barcoding, COI, Diversidade críptica, SIG, Riqueza de linhagens, Espécies potenciais, Riqueza de espécies

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## Abbreviation index

COI - cytochrome c oxidase I

GIS - Geographical information systems

mtDNA - Mitochondrial DNA

p.p – Posterior probability

R<sup>2</sup>- R-squared

UTM - Universal Transverse Mercator

WSS - West Sahara Sahel

# 1. Introduction

## 1.1 Global biodiversity crisis

Biodiversity has an important role in controlling ecosystem function and stability, providing several indirect essential services obtained from the natural ecosystems together with tremendous direct economic benefits (Singh, 2002). Still, the world is facing the first mass extinction since the dinosaurs (Sheehan *et al.*, 1991; Ceballos *et al.*, 2015). We are witnessing a loss of biodiversity at an alarming rate, with the current extinction rates exceeding what was expected from the fossil record (Barnosky *et al.*, 2011). While past extinctions happened during a period of million years, the current mass extinction will probably take place in a much shorter span of time of about 200 years (Singh, 2002). Humans play a major role in this crisis, since the current anthropogenic activities lead to habitat loss and fragmentation, overfishing, overhunting, introducing invasive species, spreading diseases, polluting, and causing climate change (Tittensor *et al.*, 2014; Barnosky *et al.*, 2011; Hoffmann *et al.*, 2010). In response to the global crisis, recent efforts have been made at the highest international levels to stop the decline of biodiversity and ecosystem services (Krishna Krishnamurthy and Francis, 2012), such as the Convention on Biological Diversity's 2020 Strategic Plan for Biodiversity (Joppa *et al.*, 2016). Recently, a set of Sustainable Development Goals (SDG) were adopted by several countries as part of the 2030 Agenda for Sustainable Development (UN General Assembly, 2015). Among the 17 defined SDGs, the "Life on land" aims at halting biodiversity loss. Still, it has been argued that no significant improvements in halting biodiversity loss will be achieved by 2020, comparing to 2010 (Tittensor *et al.*, 2014).

The available knowledge about overall global biodiversity is still very limited (Whittaker *et al.*, 2005). Even with 250 years of taxonomic studies, much of the biodiversity is still to be described or to be discovered (Krishna Krishnamurthy and Francis, 2012). Since the majority of species have not been formally described yet, the quantification of biodiversity loss is likely very underestimated (Dirzo and Raven, 2003; Joppa *et al.*, 2011). In addition, given that estimates of biodiversity loss do not consider population extinctions or community changes, then biodiversity loss estimates are further undervalued (Mendenhall *et al.*, 2012).

Global biodiversity conservation requires the preservation of the variability between individuals, species and ecosystems (Jensen, 1990). One way to do it is by looking at species genetic diversity, since intra-specific diversity allow obtaining insights into how they evolved (Mooers, 2007) and into the potential for future evolutionary change (Solbrig, 1991).

Given that the frequencies of unique or rare haplotypes possibly result from natural selection, if ecological or environmental factors are driving the genetic patterns, then they can putatively indicate local adaptation events (Nielsen *et al.*, 2017). Thus, genetic diversity is considered as an important conservation trait since high levels of diversity can increase individual fitness and population resilience (Hughes *et al.*, 2008). Additionally, there is also evidence for a probable correlation between genetic diversity and species richness (Wright *et al.*, 2015; Selkoe *et al.*, 2016), which together likely improve ecosystem function and resilience (Reusch *et al.*, 2005; Bernhardt and Leslie, 2013). Thus, preserving genetic variation is critical to mitigate the potential impacts of climate change on biodiversity (Smith *et al.*, 1993).

The available knowledge about the global, regional, and local distributions for most of the known species is mostly incomplete, a problem named as Wallacean shortfall by Lomolino (2004). Regional knowledge biases occur for the well sampled areas of North America, Australia and Western Europe while other areas, such as Africa and other politically unstable regions, are still poorly studied (Meyer *et al.*, 2016). Biases also occur in certain types of biomes, such as tropical forests that are normally perceived as potential biodiversity hotspots, which in turn stimulates more scientific attention and data collection (e.g. Liu *et al.*, 2015; Barlow *et al.*, 2016; Ocampo-Peñuela *et al.*, 2016). On the contrary, remote regions of restricted accessibility, often subjected to civil conflict and war, are less well sampled and remain poorly known (Brito *et al.*, 2009; Strange *et al.*, 2007). This is especially evident in deserts and arid regions, where little conservation investment and action have been placed in comparison to tropical biomes (Davies *et al.*, 2012; Durant *et al.*, 2012). About 17% of the world's land mass is covered by deserts and arid regions and despite having low primary productivity, deserts harbour 25% of terrestrial vertebrates species and together with xeric shrublands are in the first three richest biomes for terrestrial vertebrate species (Hassan, Scholes and Ash, 2005; Mace *et al.*, 2005). An enormous potential is thus hidden within desert biodiversity; its study can lead to important findings in physiological and genetic traits related with resistance to extreme temperatures and water stress contexts (Durant *et al.*, 2014).

## 1.2 The West Sahara-Sahel ecoregions of Africa

The Sahara desert, together with the neighbouring arid Sahel, is the largest warm desert in the world and both represent two main ecoregions of the African continent, covering about 11,230,000 km<sup>2</sup> (Dinerstein *et al.*, 2017).

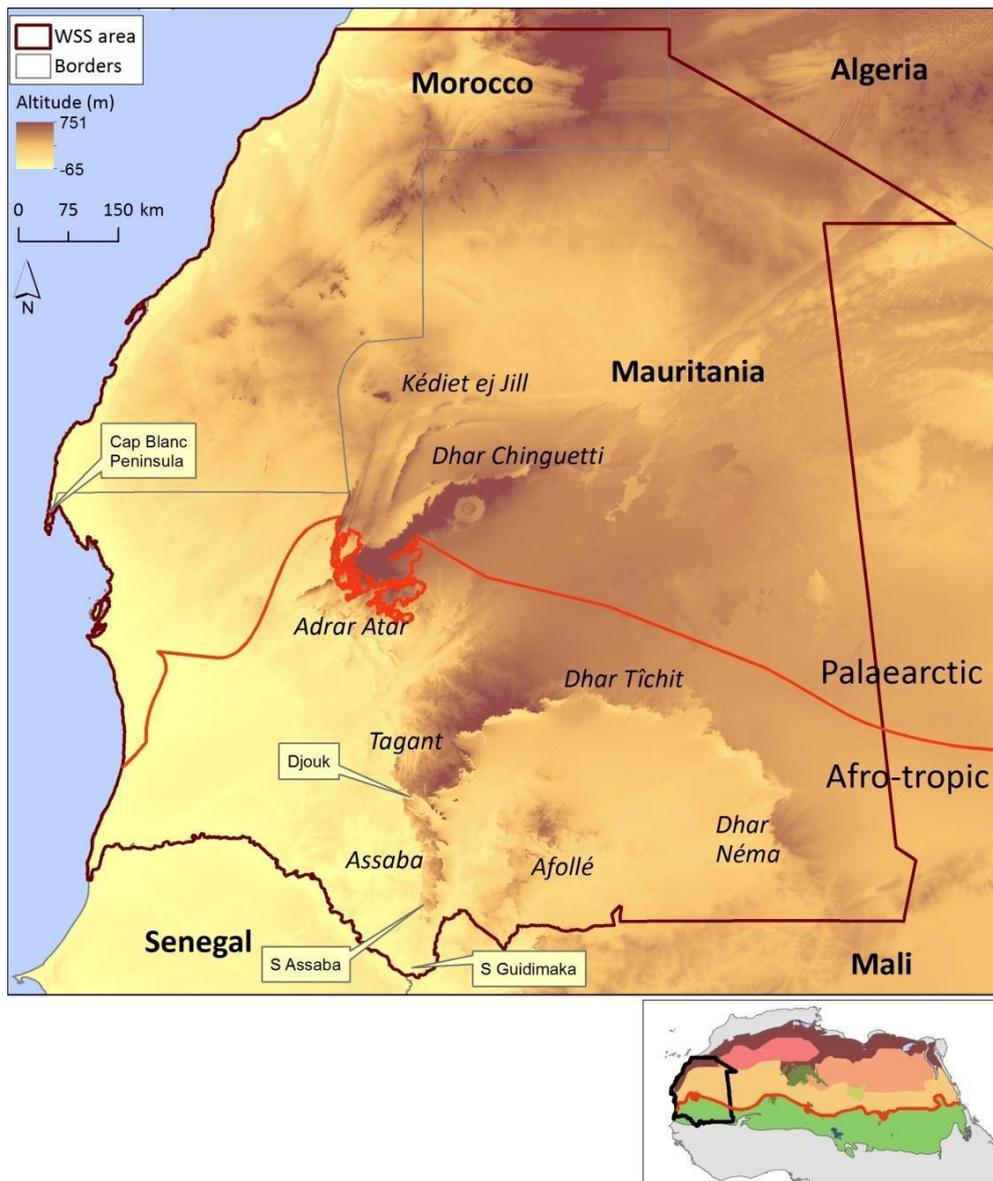


Figure 1 – Location of the West Sahara-Sahel (WSS) and distribution of ecoregions (coloured polygons; adapted from Dinerstein *et al.*, 2017) in North Africa (small inset). Location of major mountain massifs and transition line between Palaeartic and Afro-tropic regions in the WSS. Location of particular localities mentioned in the text are depicted by text boxes.

The West Sahara-Sahel (WSS), covering Mauritania and southern Morocco, exhibits high diversity of topographic features, from salt pans below sea level to mountain plateaux, and heterogeneous climates due to the substantial variability in temperature and precipitation (Brito *et al.*, 2014). The WSS comprises the transition zone between the Palaeartic and Afro-tropic biogeographical realms, and thus constitutes a biogeographic crossroad where endemic species are found beside range-limit populations of species of Palaeartic and Afro-tropic affinity (Brito *et al.*, 2014; Le Houerou, 1997). Cyclic climatic oscillations since at least from 6 to 2.5 Mya have affected the region, when the Sahara desert appeared in western areas (Schuster *et al.*, 2006). The last wet period occurred in the mid-Holocene and ended around 6 to 5000 years ago, when extreme increase in aridity caused the disappearance of mesic vegetation communities and collapse of most water-bodies (Foley *et al.*, 2003;

Holmes, 2008).

Biodiversity distribution in the Sahara-Sahel appears to be linked to environmental changes (Brito *et al.*, 2016). The dynamics of climate oscillation in the region and the arid periods may have induced allopatric diversification and speciation events in taxa restricted to mountains or aquatic environments for long periods (Brito *et al.*, 2014). Areas with more precipitation and subsequent more primary productivity are found to have higher species richness, but the central Sahara accumulates most of the threatened and endemic species (Brito *et al.*, 2016). The WSS mountains constitute isolated suitable areas for a wide array of species, acting as biodiversity refugia and playing an important role in the diversification patterns observed across the region (Brito *et al.*, 2014). In particular, water-bodies in mountains are particularly important places, as they disproportionately accumulate biodiversity representation considering their very small size (Brito *et al.*, 2014). For instance, about 78 % of Mauritanian endemics and 32% of the considered vertebrates were present in 69 analysed water-bodies, and the Assaba mountain gathered the most diverse water-bodies (Vale *et al.*, 2015). The last 10 years of taxonomic research brought new insights regarding the vertebrates occurring in the WSS, showing that there is still urgent research to be made to uncover the hidden cryptic biodiversity (reviewed by Brito *et al.*, 2014). Molecular studies have been demonstrating that what was once recognised as a widespread species, in fact they contain multiple evolutionary lineages, sometimes cryptic, with restricted and fragmented distributions (Brito *et al.*, 2014). These findings are completely altering what we knew about the biodiversity patterns. Emphasising that the current knowledge on biodiversity is incomplete (Brito *et al.*, 2016). Thus, updated information regarding intra-specific diversity across the Sahara Sahel is mostly needed to accurately map biodiversity distribution patterns and to lay the basis for the understanding of evolutionary and landscape processes associated with biodiversity distribution.

Human activities are on the rise across the Sahara-Sahel and human population has almost doubled in the region from 1990 to 2010 (OECD, 2014; Brito *et al.*, 2016). Although the region still displays some of the lowest levels of urbanization in the world, the rate at which is happening is startling (OECD, 2014). Desert ecosystems are predicted with strong and fast climate changes (Loarie *et al.*, 2009), which taken together with the major undergoing habitat changes calls for urgent identification of biodiversity hotspots where to allocate conservation efforts.

### **1.2.1 The case of reptiles in the West Sahara-Sahel**

The reptiles of the WSS provide a case-study for addressing biodiversity distribution in remote regions. Presently, 77 reptile species have been identified in the WSS (Geniez *et al.*,

2000; Padial, 2006; Trape and Mané, 2006; Trape *et al.*, 2012), from Mediterranean, North-Saharan, Saharan, Sahelian, Tropical and Macaronesian origin (Geniez *et al.*, 2000) (Table 1). Preliminary studies based in molecular markers have detected cryptic diversity in some species, such as in *Agama* and *Uromastyx* lizards or *Stenodactylus* geckos (Gonçalves *et al.*, 2012; Harris, Vaconcelos, and Brito, 2007; Metallinou *et al.*, 2012), suggesting that a significant amount of diversity with taxa remains undescribed (Brito *et al.*, 2014). Thus, a complete assessment of the group's diversity is lacking and the distribution of such diversity is mostly unknown. Such information is urgently needed given that many suitable habitats for reptiles are endangered due to intense wood harvesting and increasing agro-pastoral use (Padial, 2006). Regional red-list categorised 14% of the reptiles of Morocco as threatened (Pleguezuelos *et al.*, 2010), but such data are unavailable for Mauritania. The identification of cryptic diversity combined with the definition of phylogenetic units using genetic tools would certainly contribute for the conservation planning of regional reptile diversity (Hawlitschek *et al.*, 2016).

Table 1 – List of reptile families and species known to occur in the West Sahara Sahel (adapted from Geniez *et al.*, 2000; Padial, 2006; Trape and Mané, 2006; Trape *et al.*, 2012). \* indicates species for which no sample was available (see section 2. Methods below).

Family	Species	Family	Species
Agamidae	<i>Agama agama</i>	Lacertidae	<i>Mesalina guttulata</i>
	<i>Agama boensis</i>		<i>Mesalina olivieri</i>
	<i>Agama boueti</i>		<i>Mesalina pasteuri</i>
	<i>Agama boulengeri</i>		<i>Mesalina rubropunctata</i>
	<i>Trapelus boehmei</i>		<i>Mesalina sp. nov.</i>
	<i>Uromastyx dispar</i>		
Atractaspididae	<i>Atractaspis microlepidota</i>	Lamprophiidae	<i>Boaedon fuliginosus</i>
	<i>Atractaspis micropholis</i> *		<i>Dromophis praeornatus</i> *
Boidae	<i>Gongylophis muelleri</i>		<i>Rhagerhis moilensis</i>
Chamaeleonidae	<i>Chamaeleo africanus</i>	Leptotyphlopidae	<i>Rhamphiophis oxyrhynchus</i>
	<i>Chamaeleo senegalensis</i> *		<i>Myriopholis algeriensis</i>
Colubridae	<i>Bamanophis dorri</i>	Pelomedusidae	<i>Myriopholis boueti</i> *
	<i>Dasypeltis sahelensis</i>		<i>Pelomedusa olivacea</i> *
	<i>Hemorrhois algirus</i>	Phyllodactylidae	<i>Pelusios adansonii</i> *
	<i>Lytorhynchus diadema</i>		<i>Ptyodactylus rivapadiali</i>
	<i>Psammophis cf. rukwae</i>		<i>Tarentola annularis</i>
	<i>Psammophis elegans</i>		<i>Tarentola chazaliae</i>
	<i>Psammophis schokari</i>		<i>Tarentola ehippiata</i>
	<i>Spalerosophis diadema</i>		<i>Tarentola hoggarensis</i>
<i>Telescopus tripolitanus</i>	<i>Tarentola parvicarinata</i>		
		<i>Tarentola senegambiae</i>	
Crocodylidae	<i>Crocodylus suchus</i>	Pythonidae	<i>Python sebae</i>
Elapidae	<i>Elapsoidea trapei</i> *	Scincidae	<i>Chalcides delislei</i>
	<i>Naja nigricollis</i>		<i>Chalcides sphenopsiformis</i>
Eublepharidae	<i>Hemitheconyx caudicinctus</i> *		<i>Scincopus fasciatus</i>
Gekkonidae	<i>Hemidactylus angulatus</i>		<i>Scincus albifasciatus</i>
	<i>Stenodactylus mauritanicus</i>		<i>Trachylepis perrotetii</i>

	<i>Stenodactylus petrii</i>		<i>Trachylepis quinquetaeniata</i>
	<i>Stenodactylus sthenodactylus</i>	Sphaerodactylidae	<i>Pristurus adrarensis</i>
	<i>Tropicolotes algericus</i>	Testudinidae	<i>Centrochelys sulcata</i>
	<i>Tropicolotes tripolitanus</i>	Trionychidae	<i>Cyclanorbis senegalensis</i> *
Lacertidae	<i>Acanthodactylus aureus</i>		<i>Trionyx triunguis</i> *
	<i>Acanthodactylus boskianus</i>	Typhlopidae	<i>Indotyphlops braminus</i>
	<i>Acanthodactylus busacki</i>	Varanidae	<i>Varanus exanthematicus</i>
	<i>Acanthodactylus dumerillii</i>		<i>Varanus griseus</i>
	<i>Acanthodactylus longipes</i>		<i>Varanus niloticus</i>
	<i>Acanthodactylus scutellatus</i>	Viperidae	<i>Bitis arietans</i>
	<i>Acanthodactylus taghitensis</i>		<i>Cerastes cerastes</i>
	<i>Latastia longicaudata</i>		<i>Cerastes vipera</i>
			<i>Echis pyramidum</i>

### 1.3 DNA Barcoding as a tool for biodiversity assessment

DNA barcoding can aid to bridge the gap of knowledge in poorly studied areas, providing means to understand local species diversity and evaluate intra-specific variability (Krishna Krishnamurthy and Francis, 2012). The technique uses molecular markers to amplify short and highly variable DNA sequences (Hebert *et al.*, 2003), which allow effective species identification through comparison of similarities of sequenced barcodes with a reference database (Hebert and Gregory, 2005). The Consortium for the Barcode of Life ("CBOL", 2004) suggested that this approach should meet certain operational criteria to assure informative taxonomical identification: 1) a single gene of roughly 600 base pairs, cytochrome c oxidase I (COI) in the 5' end of mitochondrial DNA (mtDNA) is sequenced and used as a barcode; 2) the same barcode, same region of the same gene, is used universally in order to develop standardised protocols; and lastly 3) the obtained sequences are then analysed with distance based approaches to identify specimens and hence their taxon (Savolainen *et al.*, 2005; Rubinoff, 2006; Krishnamurthy and Francis, 2012).

DNA barcoding has shown to provide invaluable source of information for forensic studies (Carvalho *et al.*, 2015; Rolo *et al.*, 2013), biodiversity inventories (Telfer *et al.*, 2015; Walther *et al.*, 2013), quantifications of phylogenetic diversity (Smith, Hallwachs, and Janzen, 2014), population monitoring and demographic studies (Craft *et al.*, 2010; Kunprom, Sopaladawan, and Pramual, 2015; Alfonsi *et al.*, 2013), tracking illegally trade species (Yan *et al.*, 2013; Welton *et al.*, 2013; Zhang *et al.*, 2015), detection of rare or secretive animals (Schnell *et al.*, 2012), and invasive species identification (Xu *et al.*, 2016). Barcoding has provided an amazing input to taxonomic research by aiding in the identification of species and in the discovery of new, sometimes cryptic, ones (Hebert *et al.*, 2004; Vieites *et al.*, 2009; Padial *et al.*, 2010; Murphy *et al.*, 2013).

The divergences found in COI allow the discrimination of closely allied species, so COI can be used as an effective tool in species identification (Hebert *et al.*, 2003). Divergences reflect

both the high rates of sequence change in COI and the constraints on intraspecific mitochondrial DNA divergence, the latter are in part due to selective sweeps mediated via interaction with the nuclear genome (Hebert *et al.*, 2003). Additionally, the higher divergences exhibited in some 'species' does not compromise the use of COI marker for their identification. Instead, it is quite the opposite, since COI may allow the delimitation of regional lineages within species (Hebert *et al.*, 2003). Barcoding is now commonly accomplished using operational taxonomic units (e.g. Jones *et al.*, 2011; Pentinsaari *et al.*, 2017)). Blaxter *et al.* (2005) defined 'molecular operational taxonomic unit' has a cluster of individuals identified based on sequence similarity. This has revolutionized the way how units worthy of monitoring and conservations efforts are selected (Adamowicz, 2015).

Although COI is a valuable marker, it should be used with caution when relying only on this barcode for species discovery or identification. Given that COI is maternally inherited and that distinct evolutionary processes may act differently on either genders (Shaw, 2002; Trewick, 2008; Will and Rubinoff, 2004), nuclear pseudogenes of mitochondrial origin (numts) are common in main eukaryotes' clades (Krishnamurthy and Francis, 2012) and mitochondrial introgression has been detected (Cong *et al.*, 2017). As such, Bergsten *et al.*, (2012) argued that the best approach for achieving higher identification rates is to do it at small geographic scales. As expected, intraspecific diversity increases with geographical sampling scale due to phylogeographic structure and isolation by distance phenomena (Figure 2). Bergsten *et al.*, (2012) also noticed that with increasing geographic sampling scale there is a decrease in interspecific divergence, since more closely related, allopatrically distributed, species occur over larger areas, and there is an increase in the amount of non-monophyletic species. Given that spatial scale is relevant when using identification and delimitations approaches that rely on species monophyly, national and regional barcoding initiatives are best for maximal identification precision (Bergsten *et al.*, 2012).

Until recently, COI was scarcely used for barcoding reptiles. This was mainly due to the methodological challenges arising from the high variability of DNA sequences making difficult the binding of primers (Murphy *et al.*, 2013). With the development of new primers (Nagy *et al.*, 2012), the use of COI for barcoding became widespread in the last years (e.g. Vences *et al.*, 2012; Hawlitschek *et al.*, 2013; Vasconcelos *et al.*, 2016), and supported the establishment of the global initiative *Cold Code* that aims at barcoding all herpetofauna (Murphy *et al.*, 2013).

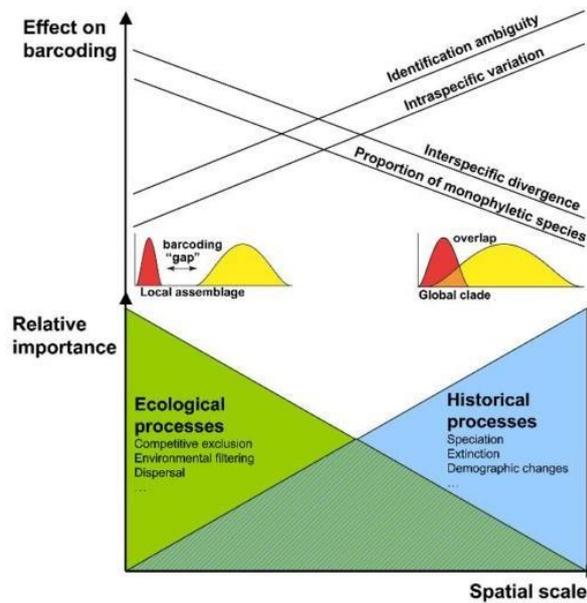


Figure 2. Schematic representation of the relative importance of processes as spatial (and temporal) scale increases and the effect on DNA barcoding criterion (adapted from Bergsten *et al.*, 2012). The linear slopes are simplifications and the nature of the scale effects can be non-continuous and chaotic across different domains of scale (see Wiens, 1989). The small red and yellow graphs depict the presence or absence of barcoding gap, i.e., the overlap between inter- and intra-specific genetic distances (adapted from Meyer and Paulay, 2005).

Overall, barcoding studies enhance the understanding of global species diversity. For a better understanding of biodiversity distribution patterns, this approach should be used in conjunction with spatial tools, such as Geographical Information Systems (GIS). This combination culminates into spatially explicit maps of genetic information with valuable phylogeographic information to inform effective conservation planning (Krishna Krishnamurthy and Francis, 2012). GIS provide valuable tools to investigate geographic related processes for conservation planning, for example mapping regions concentrating high species richness and optimised reserve design solutions (Brito *et al.*, 2016), the modelled distribution of poorly known species (Papeş and Gaubert, 2007), or the predictions of range shifts induced by climate-change (Thuiller *et al.*, 2006).

## 1.4 Objectives

This work aims at increasing the available knowledge on the diversity, distribution and conservation of reptiles in West Sahara-Sahel. In detail, it is expected to answer the following questions:

- 1) How many reptile phylogenetic units occur in the West Sahara-Sahel?
- 2) Which is the distribution of reptile phylogenetic units?
- 3) Where are the areas accumulating diversity of reptile phylogenetic units located?
- 4) Is the current network of protected areas covering the regions accumulating the highest diversity?

Molecular and spatial tools will be used in an integrative approach to derive the reptile phylogenetic units' distribution allowing identifying richness areas and priority areas for the conservation of reptile biodiversity in WSS. Considering the huge information gaps on local species richness and individual species' ranges at all taxonomic levels and that biodiversity mapping in the WSS is still poor (Brito *et al.*, 2014), it is expected to improve the current knowledge at these levels for local reptile fauna by providing data on reptiles' phylogenetic diversity and their respective current biogeographical patterns. The creation of a barcoding reference library for the WSS reptiles will also contribute for the 'Cold Code' global initiative that aims to barcoding global herpetofauna (Murphy *et al.*, 2013).

## 2. Methods

### 2.1 Study area

The study area is located in the West Sahara-Sahel (WSS) and comprises Mauritania and southern Morocco (Figure 3). With a total area of 1,024,538 km<sup>2</sup>, the WSS includes vast arid areas with a series of scattered scarps-like mountains (scarps separating sandstone plateaus) facing south-west. Mountain rock pools, locally known as gueltas, are located at the base of some of the escarpments. There are eight mountains and nine ecoregions (Dinerstein *et al.*, 2017) within the WSS with distinct distributions and areas (Table 2).

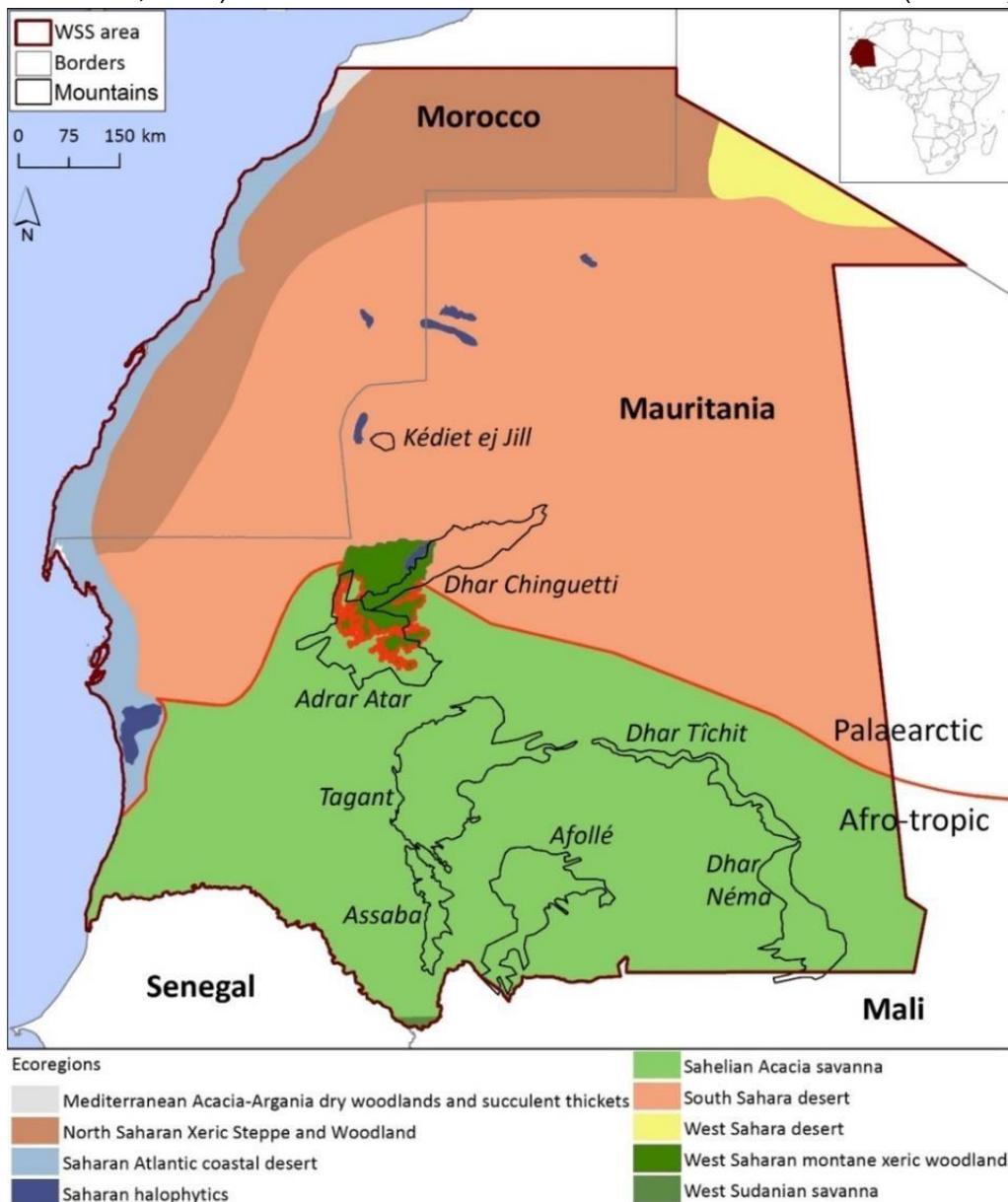


Figure 3– Distribution of mountains and ecoregions (adapted from Dinerstein *et al.*, 2017) within the West Sahara-Sahel, and limit between the Palaeartic and Afro-tropic biogeographical realms.

Table 2. Mountains and Ecoregions found in the WSS and their respective area (km<sup>2</sup>) and percentage of coverage.

Region	Area (km <sup>2</sup> )	%
<b>Mountain</b>		
Adrar Atar	11037	1.08
Afollé	10836	1.06
Assaba	3643	0.36
Dhar Chinguetti	7646	0.75
Dhar Néma	8103	0.79
Dhar Tichit	3863	0.38
Kédiét ej Jill	469	0.05
Tagant	18923	1.85
<b>Ecoregion</b>		
Mediterranean Acacia-Argania dry woodlands and succulent thickets	1554	0.15
North Saharan Xeric Steppe and Woodland	128796	12.57
Saharan Atlantic coastal desert	31004	3.03
Saharan halophytics	4599	0.45
Sahelian Acacia savanna	310270	30.28
South Sahara desert	463828	45.27
West Sahara desert	13119	1.28
West Saharan montane xeric woodlands	6278	0.61
West Sudanian savanna	569	0.06

## 2.2 Sampling

A total of 755 samples were available for this study (Figure 4). Samples were collected by researchers and collaborators of BIODESERTS research group during 34 field expeditions to North Africa (<http://biodeserts.cibio.up.pt/expeditions>). Samples were assigned to previously existing taxonomical units based on external morphological characters and following standard identification keys (Geniez *et al.*, 2004; Trape and Mané, 2006; Trape, Trape, and Chirio, 2012)

Geographic coordinates of all samples were collected in the field with a Global Positioning System (GPS) on the WGS-1984 datum. Metadata of all samples were inserted in a georeferenced database and a Geographical Information System (ArcGIS) was used for display of distribution data. Tissue samples were preserved in tubes with 100% ethanol to guarantee DNA integrity.

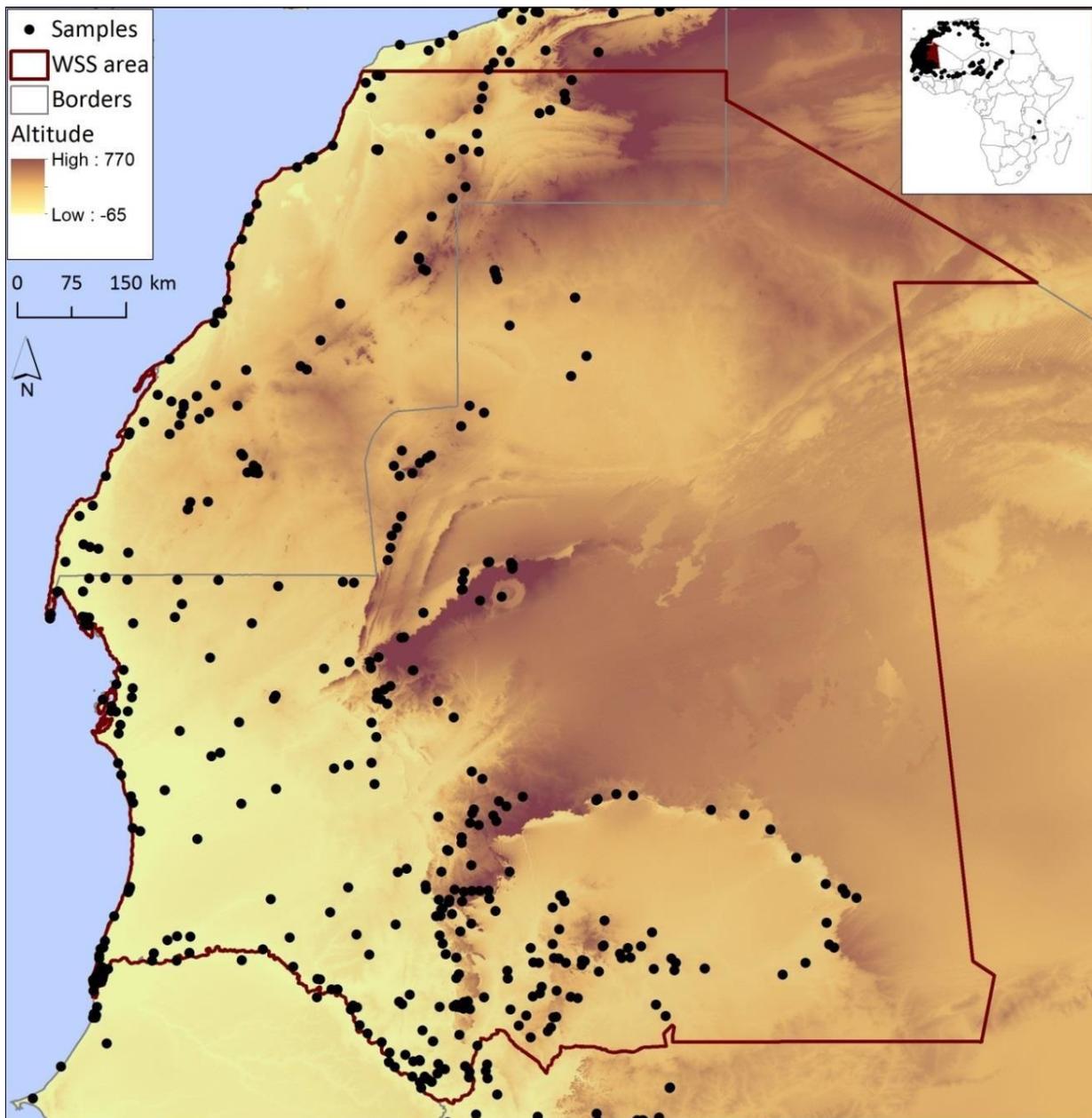


Figure 4. Distribution of 755 samples available for this study in Africa (small inset) and within the West Sahara-Sahel region.

The 755 samples included 695 samples from 109 reptile taxa distributed by 19 families and 60 samples from taxa with uncertain diagnosis at the species level (Table 3). The dataset included 69 species known to occur in the WSS, corresponding to 90% of the diversity described in the WSS region (Table 1). The dataset also included 50 samples from 41 outgroup taxa and 196 samples come from areas outside the WSS.

Samples were selected for analyses in order to: 1) cover the described taxonomic diversity of reptiles in the WSS (Table 2); and 2) cover the known geographic distribution of each taxon within the WSS.

Table 3 List of samples available for the current study for each species. Species identified according to external morphological characters. Uncertain id. indicates samples with uncertain morphological diagnosis. \* indicates outgroup taxon of taxa occurring in the West Sahara-Sahel.

Family	Species	N	Family	Species	N	
Agamidae	<i>Agama agama</i>	11	Lacertidae	<i>Acanthodactylus maculatus*</i>	1	
	<i>Agama boensis</i>	3		<i>Acanthodactylus margaritae*</i>	1	
	<i>Agama boueti</i>	11		<i>Acanthodactylus scutellatus</i>	3	
	<i>Agama boulengeri</i>	12		<i>Acanthodactylus taghitensis</i>	3	
	<i>Agama impalearis*</i>	2		<i>Latastia longicaudata</i>	6	
	<i>Agama paragama*</i>	1		<i>Mesalina guttulata</i>	7	
	<i>Agama tassiliensis*</i>	1		<i>Mesalina olivieri</i>	12	
	<i>Trapelus boehmei</i>	17		<i>Mesalina pasteuri</i>	8	
	<i>Trapelus mutabilis*</i>	1		<i>Mesalina rubropunctata</i>	8	
	<i>Uromastyx acanthinura*</i>	1		<i>Mesalina sp. nov.</i>	12	
	<i>Uromastyx dispar</i>	13		<i>Mesalina symoni*</i>	1	
	<i>Uromastyx geyri*</i>	1		Uncertain id.	17	
	<i>Uromastyx nigriventris</i>	3		Lamprophiidae	<i>Boaedon fuliginosus</i>	4
	Uncertain id.	4			<i>Boaedon lineatus*</i>	1
Atractaspididae	<i>Atractaspis microlepidota</i>	3	<i>Rhagerhis moilensis</i>		15	
	Uncertain id.	2	<i>Rhamphiophis oxyrhynchus*</i>	1		
Boidae	<i>Gongylophis colubrinus*</i>	1	Uncertain id.	1		
	<i>Gongylophis jaculus*</i>	1	Leptotyphlopidae	<i>Myriopholis algeriensis</i>	2	
	<i>Gongylophis muelleri</i>	5		Phyllodactylidae	<i>Ptyodactylus oudrii*</i>	4
Chamaeleonidae	<i>Chamaeleo africanus</i>	3	<i>Ptyodactylus ragazzi*</i>		3	
	<i>Chamaeleo chamaeleon*</i>	2	<i>Ptyodactylus rivapadiali</i>		21	
	<i>Chamaeleo gracilis*</i>	1	<i>Ptyodactylus togoensis</i>		15	
Colubridae	<i>Bamanophis dorri</i>	7	<i>Tarentola annularis</i>		14	
	<i>Crotaphopeltis hotamboeia*</i>	1	<i>Tarentola boehmei*</i>		1	
	<i>Dasypeltis sahelensis</i>	2	<i>Tarentola chazaliae</i>		12	
	<i>Dasypeltis scabra*</i>	2	<i>Tarentola deserti*</i>		2	
	<i>Hemorrhois algirus</i>	8	<i>Tarentola ehippiata</i>		2	
	<i>Hemorrhois hippocrepis*</i>	1	<i>Tarentola hoggarensis</i>		22	
	<i>Lytorhynchus diadema</i>	5	<i>Tarentola mauritanica*</i>		1	
	<i>Psammophis aegyptius*</i>	2	<i>Tarentola parvicarinata</i>		15	
	<i>Psammophis cf. rukwae</i>	7	<i>Tarentola senegambiae</i>		16	
	<i>Psammophis elegans</i>	5	Uncertain id.		9	
	<i>Psammophis mossambicus*</i>	1	Pythonidae	<i>Python sebae</i>	6	
	<i>Psammophis schokari</i>	16		Scincidae	<i>Chalcides boulengeri*</i>	3
	<i>Spalerosophis diadema</i>	4	<i>Chalcides colosii*</i>		1	
	<i>Spalerosophis dolichospilus*</i>	2	<i>Chalcides delislei</i>		2	
	<i>Telescopus fallax*</i>	1	<i>Chalcides montanus*</i>		1	
	<i>Telescopus tripolitanus</i>	5	<i>Chalcides ocellatus*</i>		2	
	Uncertain id.	22	<i>Chalcides parallelus*</i>		1	
Crocodylidae	<i>Crocodylus niloticus*</i>	1	<i>Chalcides polylepis*</i>		1	
	<i>Crocodylus suchus</i>	11	<i>Chalcides pseudostriatus*</i>		1	

	Uncertain id.	1		<i>Chalcides sphenopsiformis</i>	6
Elapidae	<i>Elapechis guentheri</i>	1		<i>Eumeces algeriensis</i> *	1
	<i>Naja haje</i> *	4		<i>Scincopus fasciatus</i>	2
	<i>Naja nigricollis</i>	1		<i>Scincus albifasciatus</i>	3
Gekkonidae	<i>Hemidactylus angulatus</i>	10		<i>Trachylepis affinis</i> *	2
	<i>Hemidactylus turcicus</i> *	1		<i>Trachylepis perrotetii</i>	8
	<i>Stenodactylus mauritanicus</i>	10		<i>Trachylepis quinquetaeniata</i>	8
	<i>Stenodactylus petrii</i>	10	Sphaerodactylidae	<i>Pristurus adrarensis</i>	2
	<i>Stenodactylus sthenodactylus</i>	14	Testudinidae	<i>Centrochelys sulcata</i>	2
	<i>Tropicolotes algericus</i>	7	Typhlopidae	<i>Indotyphlops braminus</i>	2
	<i>Tropicolotes steudneri</i> *	1	Varanidae	<i>Varanus exanthematicus</i>	12
	<i>Tropicolotes tripolitanus</i>	59		<i>Varanus griseus</i>	13
	Uncertain id.	3		<i>Varanus niloticus</i>	10
Lacertidae	<i>Acanthodactylus aureus</i>	13	Viperidae	<i>Bitis arietans</i>	5
	<i>Acanthodactylus boskianus</i>	8		<i>Cerastes cerastes</i>	16
	<i>Acanthodactylus busacki</i>	4		<i>Cerastes vipera</i>	6
	<i>Acanthodactylus dumerilii</i>	16		<i>Echis leucogaster</i>	15
	<i>Acanthodactylus longipes</i>	17		<i>Echis ocellatus</i> *	1
				Uncertain id.	1

## 2.3 Laboratory methods

Previous to DNA extraction, all samples were placed in Phosphate buffered saline (PBS) overnight at room temperature to remove possible contaminants which could act as inhibitors in the following reactions such as the PCR. Total genomic DNA was extracted using the QIAGEN's EasySpin Kit or the QIAGEN's QIAmp® DNA MicroKit for the samples for which the amount of tissue was limited or the QIAGEN'S DNeasy Blood & Tissue Kit for samples that had enough tissue but it was expected lower quality DNA (tissue collected from dead animals or shed skin). The DNA from the museum samples was extracted by a technician from CTM laboratory, Diana Castro, following the protocol optimized by Dabney *et al.*, (2013). Since DNA from museum samples are particularly susceptible to DNA contamination and are usually degraded, these DNA extractions and subsequent procedures (PCR) were performed in sterile and isolated rooms under special conditions optimized for the manipulation of low quality DNA. To evaluate the success of the DNA extractions, both DNA quality and quantity, electrophoresis was performed in 0,8% agarose gel dyed with GelRed™ (Biotium). The obtained DNA and subsequent dilutions were then stored at -20 °C until further use.

A fragment of a mitochondrial gene (ca. 650 bp), cytochrome oxidase subunit I gene (COI) was amplified by polymerase chain reaction (PCR). The COI fragment was amplified using degenerate primers RepCOI-F (primer forward, 5'-TNTTMTCAACNAACCACAAAGA-3') and RepCOI-R (primer reverse, 5'-ACTTCTGGRTGKCCAAARAATCA-3') (Nagy *et al.*, 2012), except for samples of *Pristurus adrarensis* where COI was amplified with the universal

primers LCO1490 (primer forward, 5'-GGTCAACAAATCATAAAGATATTGG-3') and HC02198 (primer reverse, 5'-TAAACTTCAGGGTGACCAAAAATCA-3') (Folmer *et al.*, 1994). PCRs were performed using a total volume of 10  $\mu$ l which contained: 5  $\mu$ l of My Taq™ HS Mix 2X (Bioline), 3.2  $\mu$ l of ultra-pure water, 0.4  $\mu$ l of each primer from a primer solution of 10  $\mu$ M and 1  $\mu$ l of DNA extraction (~50 ng/ $\mu$ l). The amount of DNA used differed for some samples that had less DNA concentration (1.5-4  $\mu$ l). In all the PCRs a negative control was used. A Touchdown (TD) PCR program was used to facilitate the amplification of multiple reptiles' species. TD PCR allows a simple and fast way to optimize PCRs, increasing sensitivity, specificity and yield (Korbie and Mattick, 2008; Don *et al.*, 1991), having a great applicability, particularly when using degenerate primers (Fietto *et al.*, 2002; Levano-Garcia, Verjovski-Almeida and Da Silva, 2005). TD PCR was performed with the following conditions: initial denaturation at 95 °C for 10 min, followed by an initial phase of 9 cycles of 40 s of denaturation at 95 °C, 30 s of annealing at 52°C with a decrease in the annealing temperature by 0.5 °C per cycle until the 48°C and extension at 72 °C for 45 s, followed by a second phase with 31 cycles of 40 s of denaturation at 95 °C, 30 s of annealing at 48°C and elongation during 45 s at 72°C, and a final extension cycle of 10 min at 72 °C. Adjustments of the temperature gradients were done for those species that fail to amplify with the general TD PCR. The final PCR conditions are present in Table 4.

Quality and quantity of PCR products were checked by visual examination in electrophoresis using 2% agarose gel. Successful PCR products were outsourced for sequencing to Beckman Coulter Genomics (Essex, UK). The forward primer was used for sequencing. The sequence chromatograms were visually inspected, assembled, and edited using Geneious Pro v.4.8.5 (Biomatters Ltd.). Sequences were aligned using the MUSCLE version implemented in Geneious Pro v.4.8.5 (Biomatters Ltd.) under default settings (Edgar, 2004). Since a protein coding gene was used, all sequences were translated into amino acids to aid during the alignment, and the original nucleotide sequences were used for later analyses. Gap positions were allowed under strict parameter settings. Sequences were checked for stop codons to detect the presence of nuclear DNA pseudogenes and trimmed to the same length. All COI sequences were transformed into unique haplotype data using the online tool DNA to haplotype collapser and converter implemented in Fabox 1.41 (Villesen, 2007) in order to reduce the computational time necessary for the subsequent analyses.

Different data sets were created in order to achieve the thesis goals. A data set, with all samples within and outside the study area was made in order to identify samples with dubious morphological assignments (e.g. shed skins, bones or tissue collected from dead animals). Once some of the dubious samples were correctly identified, the haplotypes were renamed to the correct species names. This dataset aims at identifying mtDNA lineages and potential cryptic species, as well as to construct a reference barcoding library for the study

region.

Table 4. PCR conditions for the sequenced gene COI for those species that fail to amplify with the general TD PCR.

Species	Amplification step	Temperature (°C)	Duration	N° cycles
<b><i>Latastia longicaudata</i></b>	Initial denaturation	95°C	10 minutes	1
	Denaturation	95°C	40 seconds	40
	Annealing	50°C	30 seconds	
	Extension	72°C	45 seconds	
	Final Extension	72°C	10 minutes	1
<b><i>Acanthodactylus longipes</i> and <i>Acanthodactylus scutellatus</i></b>	Initial denaturation	95°C	10 minutes	1
	Denaturation	95°C	40 seconds	40
	Annealing	52°C	30 seconds	
	Extension	72°C	45 seconds	
	Final Extension	72°C	10 minutes	1
<b><i>Varanus exanthematicus</i></b>	Initial denaturation	95°C	10 minutes	1
	Denaturation	95°C	40 seconds	35
	Annealing	54°C	30 seconds	
	Extension	72°C	45 seconds	
	Final Extension	72°C	10 minutes	1
<b><i>Acanthodactylus dumerilii</i></b>	Initial denaturation	95°C	10 minutes	1
	Denaturation	95°C	40 seconds	11
	Annealing	57°-52° (Touchdown -0.5°C)	30 seconds	
	Extension	72°	45 seconds	
	Denaturation	95°	40 seconds	34
	Annealing	52°	30 seconds	
	Extension	72°	45 seconds	
Final Extension	72°	10 minutes	1	

## 2.4 Phylogenetic analysis

To highlight samples incorrectly identified a Bayesian inference was performed with BEAST v1.8.4 (Drummond *et al.*, 2012) to construct a gene tree accounting for the best fitting nucleotide substitution models for COI (JC+I+G) suggested by jModeltest2 (Darriba *et al.*, 2012) under the Bayesian Information Criteria. XML file was made with BEAUti v1.8.4 interface with the following settings: the closest nucleotide substitution model available in BEAST (HKY). All codon positions partitioned with unlinked base frequencies and substitution rates. An uncorrelated relaxed lognormal clock which allows the molecular rate to vary among lineages, was used. The use of uncorrelated relaxed lognormal clock did not attain convergence in any of the and therefore the Strict Clock was used instead. A speciation Yule Process model was used as tree prior. Operators were auto-optimized, and two independent runs were performed the chain length of Markov Chain Monte Carlo (MCMC) chain was 100 million generations, with a sampling every 10,000 generations. The convergence of the runs was verified by examining the effective sample sizes (ESSs) of all parameters using Tracer v1.6.0 (ESS > 200). Trees obtained from the two independent runs were merged using LogCombiner v1.8.4, where 10% of the trees were discarded as burn-in, the remaining trees were used to obtain the subsequent maximum clade credibility summary tree with posterior probabilities for each node using TreeAnnotator v1.8.4. The resulting consensus tree was visualized on FigTree v1.4.3.

The samples detected as incorrectly identified were renamed according to their position in the obtained tree topology.

## 2.5 Barcoding analysis

### 2.5.1 Distance based analysis

In order to estimate the identification success and, a series of genetic distance (Kimura 2-parameter model) thresholds were applied using the R package SPIDER v.1.3 (Brown *et al.*, 2012). The nearest neighbour (NN) criterion, equivalent to the 'Best Match' method by Meier (Meier *et al.*, 2006). was first employed. This method assigns any query to the species name of its best-matching barcode (reference sequence with lowest distance to that query) independently of the similarity between the query and barcode sequence. If the best-matching specie is the same as the individual being identified, the result is TRUE otherwise is FALSE (Brown and Collins, 2011). The name of the nearest match was recovered by setting the names argument of this function to TRUE.

Using these criteria misidentifications are common and in a way inevitable since some species will not have conspecific barcodes in the database (Will and Rubinoff, 2004). To

overcome this limitation, the threshID and Meier's best close match were also performed. The threshID simulates the "species identification" method used by Bold (Ratnasingham and Hebert, 2007). The threshold based criterion (default of 1%) compares all specimens within the threshold of the query, and then assigns a diagnosis to each identification query: "correct"—within the threshold of the query all matches are the same species; "incorrect"—all matches are different species to the query; "ambiguous"—both correct and incorrect species matches within the threshold; "no id"—no matches to any individual within the threshold. The "best close match" also identifies the best barcode match to the query, similar to NN, but using, a threshold (default of 1%) thus having the same four identification categories as the threshID ("correct, incorrect, ambiguous, no id"). However, this function only looks at the single nearest-neighbour match, instead of all matches within the threshold (as with threshID and thus, the species name of the barcode is only given to the query if the similarity between them is sufficient, otherwise the query will stay as unidentified (Meier *et al.*, 2006). The Barcode of Life Data Systems (BOLD) identification tool, which assigns identities using a threshold of 1% for animal species (Ratnasingham and Hebert, 2007), was also employed.

To evaluate the performance of the COI marker as a barcode in our dataset, a barcoding gap analysis was performed. The DNA barcoding gap, which is the maximum intraspecific distance of each species against its minimum distance to the nearest neighbour, was calculated for all species. To evaluate the presence of barcoding gap using the obtained alignments, a pairwise distance matrix between sequences based on Kimura's two-parameter (K2P) model was created after which the statistics maxInDist (furthest intraspecific distance) and nonConDist (smallest interspecific distance) were applied using the R package SPIDER v.1.3 (Brown *et al.*, 2012).

### **2.5.2 Tree based analysis: species and lineage identification**

The phylogenetic species concept (PSC) (Eldredge and Cracraft, 1980) was employed to delimit putative species. A Bayesian implementation of the Poisson tree processes (bPTP) model for species delimitation, which relies on the branch lengths to infer putative species boundaries on a given phylogenetic input tree, was employed using the bPTP server (Zhang *et al.*, 2013) (<http://species.h-its.org/ptp/>). The parameters used were the following: MCMC, 500,000 generations; thinning, 100; burn-in, 0.1; seed, 123, and convergence was assessed in each case to guarantee the reliability of the results.

Together with the PTP approach, a 9.5 % divergence threshold based on uncorrected genetic distances (p-distances) was applied for determining the species identity in cases of cryptic diversity. Both intra- and interspecific uncorrected p-distances were calculated in MEGA7 (Kumar, Stecher, and Tamura, 2016). With these genetic distance matrix, Excalibar

(Aliabadian *et al.*, 2014) was then employed to retrieve the intra- and interspecific genetic distances. To obtain a final delimitation of reptile phylogenetic units occurring in the West Sahara-Sahel, a threshold of 2.5 % divergence was used to identify mitochondrial lineages.

## 2.6 Distribution of phylogenetic units and diversity

The distribution of the phylogenetic units found in WSS was mapped using ArcGIS Geographical Information System (GIS). Distribution maps were produced depicting the geographic locations of species, including the new candidate species, and their respective lineages. The distribution maps also included additional locations from un-sequenced samples from the respective species/candidate species. These data were also collected by BIODESERTS researchers and collaborators.

The distribution of reptile diversity was quantified for several taxonomy levels: families, genera, species and lineages. Initially, ecoregions occurring in the WSS were extracted from the 2017 version of the Terrestrial Ecoregions of the World (Dinerstein *et al.*, 2017) and mountains were manually digitised from topographic maps. Then, ArcGIS was used to combine both data files and to generate a unique raster file depicting regions within the WSS. In ArcGIS, each sequenced sample was then intersected with the raster of ecoregions/mountains (Figure 3) to extract the respective region where each sample occurs. Finally, it were summarized the number of families, genera, species and lineages that occurs in each of the regions. Mapping of species richness at coarser resolutions has the advantage of diminishing potential biases in sampling efforts and provides relevant understanding and visualization of regional patterns (Graham and Hijmans, 2006).

Given that the available area of each zone is distinct (Table 2), it was plotted the relationships between number of families, genera, species and lineages according to the area of each zone. This approach allowed exploring potential species-area relationships, which may affect the spatial patterns in the distribution of richness (Rosenzweig, 1995; Dengler, 2009).

## 2.7 Gap analysis

Polygons depicting the location of current protected areas in the WSS (Figure 5 and Table 5) were downloaded from the World Database on Protected Areas (IUCN & UNEP-WCMC, 2017) and converted to raster format at 1x1 km grid cell size. This raster file was overlapped with a raster file depicting the location of mountains and ecoregions. The current levels of protection of each region were quantified, which allowed identifying areas accumulating high phylogenetic diversity missing from the current network of protected areas in the WSS, as well as identifying putative areas that should also be classified to conserve reptile phylogenetic diversity.

Figure 5. Location of Protected Areas in the West-Sahara-Sahel distinguishing between areas fully implemented and under discussion, and distinct categories of protection (Adapted from IUCN & UNEP-WCMC, 2017).

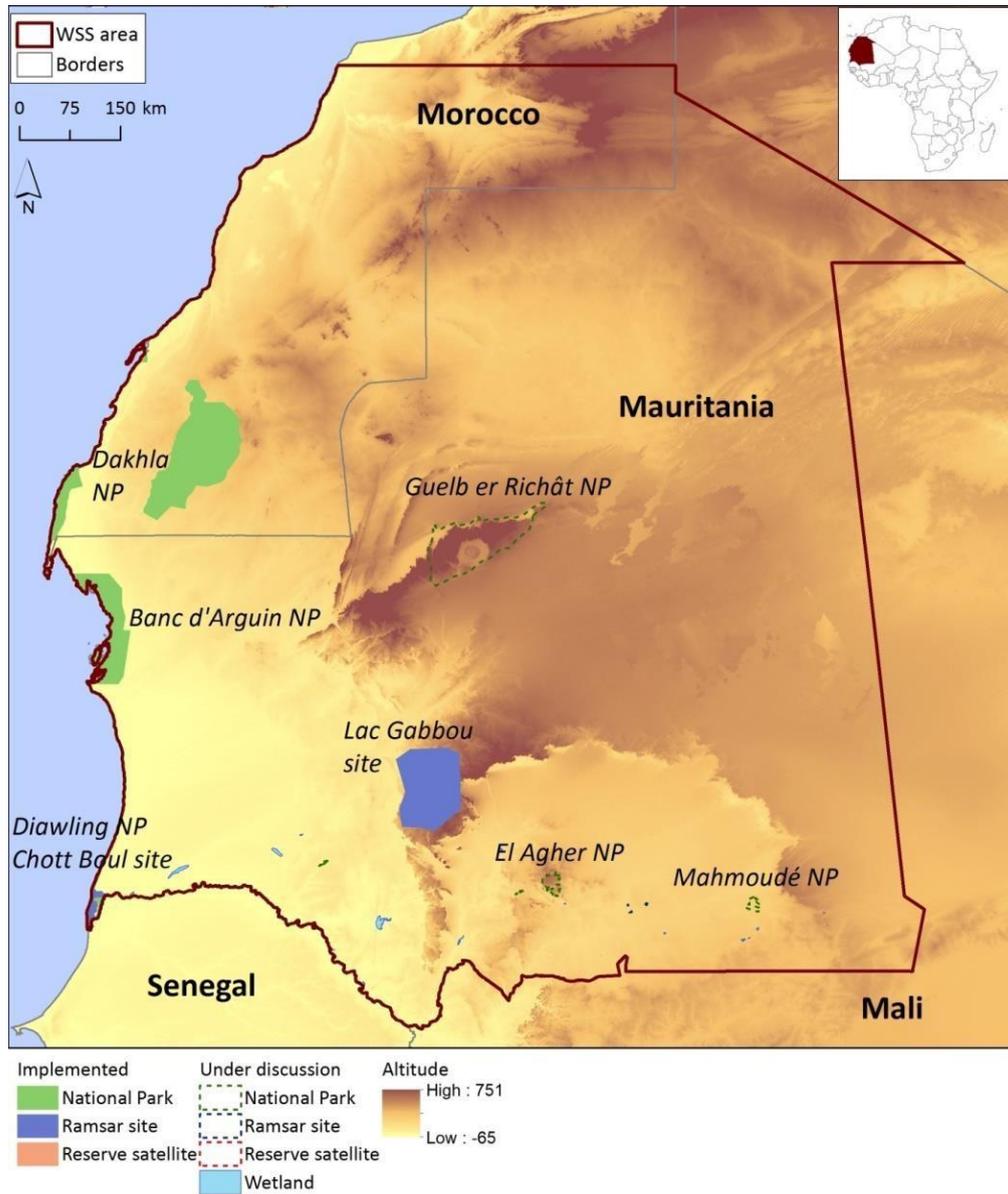


Table 5. List of Protected Areas in the West Sahara-Sahel, including the status (implemented or under discussion), category, name, and area (Adapted from IUCN & UNEP-WCMC, 2017).

Country	Category	Name	Area (km <sup>2</sup> )	%
<b>Implemented</b>				
Mauritania	National Park	Banc d'Arguin	4274	0.417
		Diawling	146	0.014
Morocco	National Park	Dakhla	11875	1.159
		Baie d'Ad-Dakhla	85	0.008
Mauritania	Reserve satellite	Cap Blanc	5	0.001
	Ramsar site	Chott Boul	366	0.036
		Lac Gabbou	7245	0.707

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**Under discussion**

Mauritania	National Park	El Agher	442	0.043
		Guelb er Richât	6750	0.659
		Lac de Mâl	14	0.001
		Tâmourt Bougâri	7	0.001
		Mahmoudé	191	0.019
	Reserve satellite	Baie de l'Etoile	8	0.001
	Ramsar site	Tâmourt Chlim	1	0.000
		Gaât Sawana	7	0.001
		Tâmourt Oum Lelli	1	0.000
	Wetland	Mare de Boubleine	6	0.001
		Oued Kankossa	16	0.002
		Lac d'Aleg	51	0.005
		Mare de Chôgar	1	0.000
		Foum Gleita	120	0.012
		Koundel	3	0.000
		Toulel	3	0.000
		Barrage de Melga	1	0.000
		Tâmourt Dendaré	1	0.000
		Tâmourt Kerk	1	0.000
		Tâmourt Touf	2	0.000
		Tâmourt Tourh	6	0.001
		Tombaré	1	0.000
		Vani	7	0.001
		Tâmourt Tîntâne	1	0.000
	Tâmourt Zoueina	1	0.000	
	Lac de R'Kiz	82	0.008	
	TOTAL Protected		31719	3.096

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## 3. Results

### 3.1 Laboratory methods

From the initial 755 samples, 684 were extracted and amplified. A total of 542 COI sequences were successfully amplified (ca. 80% of the samples), with 624 base pairs were retrieved from 96 reptile species (of which 63 are from the WSS). With the primers RepCOI-F and RepCOI-R 540 fragments were obtained from the amplified samples whereas LCO and HCO was only used for the amplification of two samples and both were successfully amplified.

For the following species no COI fragment was recovered due to PCR failure: these included the outgroup *Crocodylus niloticus* and four species that occur in the WSS, *Acanthodactylus aureus*, *Acanthodactylus scutellatus*, *Dasypeltis sahelensis* and *Rhamphiophis oxyrhynchus*. In other species there was failure in recovering readable sequences of COI fragments: these included outgroups *Agama tassiliensis*, *Chalcides montanus*, *Chalcides polylepis*, *Gongylophis colubrinus*, *Hemidactylus turcicus*, *Mesalina symoni*, *Trachylepis affinis* and *Tropicolotes steudneri*. For *Elapechis guentheri* and *Varanus exanthematicus* amplifications were obtained but sequences were not readable. In the cases of the outgroups *Chalcides ocellatus*, *Acanthodactylus maculatus*, *Acanthodactylus margaritae*, *Agama paragama*, *Chalcides colosii*, *Chalcides parallelus*, *Chamaeleo gracilis*, *Crotaphopeltis hotamboeia*, *Echis ocellatus*, *Eumeces algeriensis*, *Hemorrhoids hippocrepis*, *Psammophis mossambicus* and *Trapelus mutabilis* only a single sequence was obtained. In three species occurring in the WSS, *Acanthodactylus longipes*, *Centrochelys sulcata* and *Myriopholis algeriensis*, only a single sequence was retrieved from a sample collected outside the study area.

No insertions, deletions or stop codons were detected, supporting the absence of nuclear pseudogene amplification (Pereira and Baker, 2004).

### 3.2 Phylogenetic analysis

The Bayesian inference allowed to identify 27 samples from 18 different species: two samples from two outgroups species, eight samples from five species collected outside the study area, and 17 samples from WSS belonging to 11 species as morphologically incorrectly identified. One of the samples collected in the study area, putatively identified in the field as *Agama boueti*, in fact clustered with *Agama impalearis* from northern Morocco, suggesting the presence of a new species in the WSS. A total of 28 samples from 17 species (four samples from three outgroups + seven samples from outside the study area + 17 samples from 10 species of the WSS) had uncertain identification, which was solved by identifying them according to the species to which they clustered in the tree obtained.

### 3.3 Barcoding analyses

#### 3.3.1 Distance based analyses

The rates of identification success varied among the different distance-based methods applied (Near Neighbour, ThreshID, best close match and BOLD best ID) (Annexes, Table A 1) and between the analyses using all haplotypes and the ones excluding singletons.

Concerning the Near Neighbour criteria, identification success ranged from 92% to 99% when excluding singletons, with only four sequences recovered as incorrectly identified. Regarding the threshID criteria, when applying a 1% threshold the identification success varied from 69% to 74% (excluding singletons) with zero sequences incorrectly identified in both cases. When using a 10% threshold 75% to 80% (excluding singletons), eight sequences were incorrectly identified but seven of them were singletons. Since these species are only represented by a single sequence, their match with a conspecific sequence was not possible. The best close match criterion showed an identification success rate from 69% to 74% (excluding singletons) but no sequence resulted incorrect or ambiguous. The BOLD best ID only was able to identify 10% of the specimens, highlighting the lack of COI barcode for the reptiles from WSS.

The barcoding gap was largely present in our dataset (Figure 6), highlighting the effectiveness of this COI fragment as a barcode marker. However, no barcoding gap was observed for *Rhagerhis moilensis*, *Tarentola hoggarensis*, *Chalcides delislei*, *Mesalina sp. nov.* and *Latastia longicaudata*.

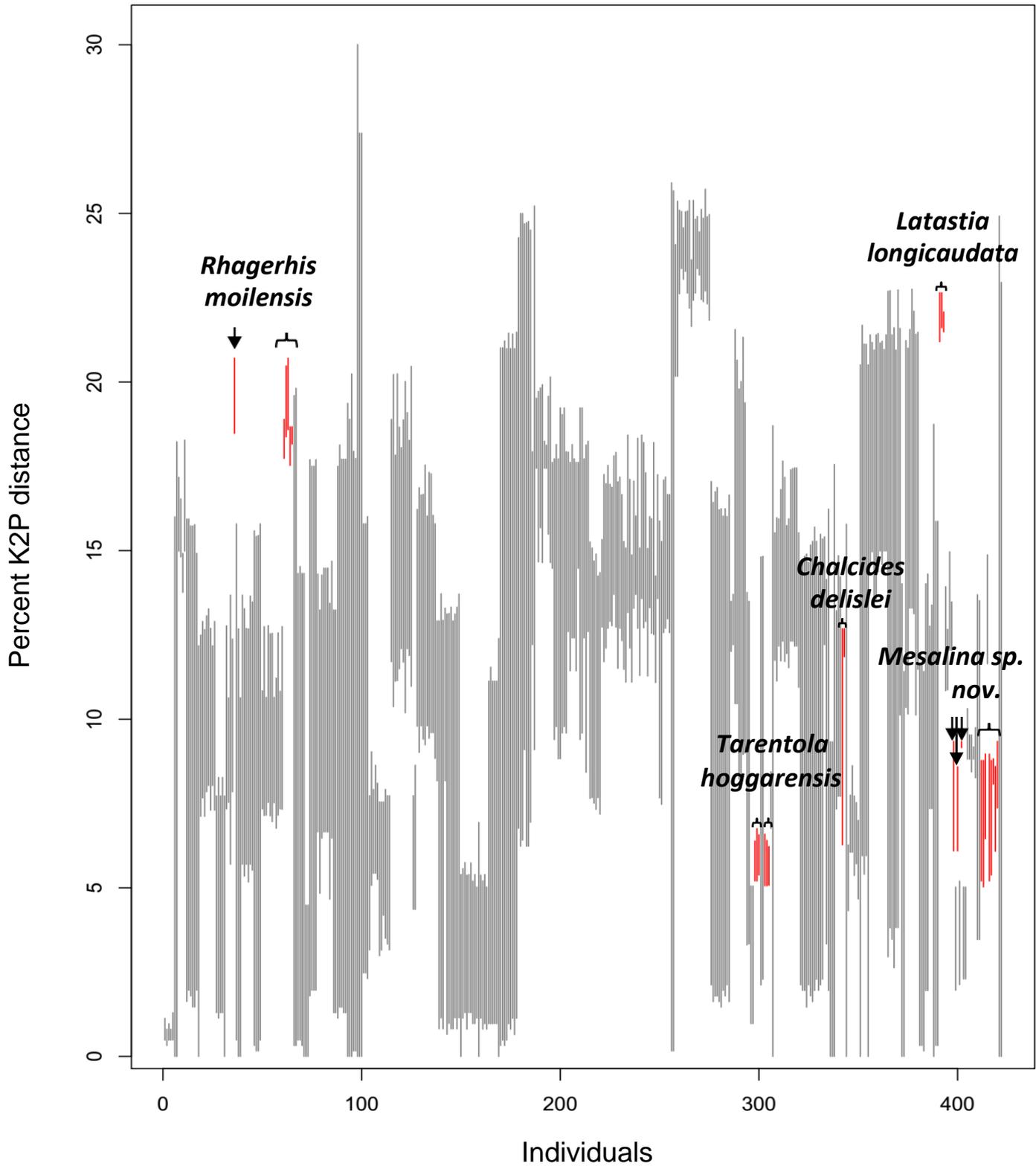


Figure 6. Line-plot of the barcode gap for all Reptile haplotypes as generated by Spider (Brown *et al.*, 2012). For each individual in the dataset, the grey lines represent the furthest intraspecific distance (bottom of line value), and the closest interspecific distance (top of line value). The red lines show where this relationship is reversed, and the closest non-conspecific is actually closer to the query than its nearest conspecific (i.e. no barcoding gap).

### 3.3.2 Barcoding analyses

From the 422 unique mitochondrial haplotypes, the bPTP approach (Annexes Figure A1 ) identified 193 putative species. However, only 121 of these were highly supported (Bayesian posterior probability >0.95). *Tropicolotes tripolitanus* was the species with more genetic structure, with 11 estimated putative species, yet only four had high statistical support (p.p. = 0.97-1). Similarly, *Acanthodactylus boskianus* and *Tarentola parvicarinata* exhibited six putative species, but only four (p.p. = 0.98-1) and two, respectively, were highly supported. *Agama boulengeri* comprised five distinctive putative species with two of them highly supported (p.p. = 0.96-1). *Lytorhynchus diadema* was split into four putative species, all highly supported (p.p. = 0.971-1). *Agama agama* was also divided into four putative species but only three of these, belonging to three different haplotypes were highly supported (p.p. = 0.993-1). *Ptyodactylus oudrii*, *Trachylepis perrotetii*, *Chalcides delislei*, *Trapelus mutabilis*, and *Acanthodactylus maculatus* were divided into two putative species (p.p. = 1). For *Rhagerhis moilensis* and *Psammophis schokari* bPTP estimated that each of them had a single haplotype genetically divergent from the other haplotypes, belonging to a new putative species, and both were highly supported (p.p. = 1).

Bayesian inference analysis of the mitochondrial dataset, with the 2.5% threshold, allowed the identification of 165 genetically divergent lineages (Figure 7 to Figure 12). The monophyly of most of these lineages was highly supported (posterior probability > 0.95; Figure 7 to Figure 12). The 9.5% threshold was used to delimitate species. It allowed the identification of 102 species units, 93 already described as species and nine as putative species. A total of five of the candidate species occur outside the study area, namely *Ptyodactylus cf. oudrii* North and *Ptyodactylus cf. oudrii* South (14% divergence), *Stenodactylus cf. mauritanicus* (10-11% divergence), *Trachylepis cf. perrotetii* (11% divergence), and *Rhagerhis moilensis* (13-15% divergence) (Figure 12).

The gecko *Tropicolotes tripolitanus* was the species with the highest number of mitochondrial lineages (7 lineages) (Figure 9) followed by *Tarentola parvicarinata* (Figure 8) and *Acanthodactylus boskianus*, (Figure 10) similarly to what was observed with the bPTP approach. More than 50% of the species belonging to the sub-order Sauria (commonly named as lizards) had more than one mitochondrial lineage, whereas more than 80% of species of the Ophidia sub-order (snakes) exhibited one single mitochondrial lineage. From the 93 mitochondrial lineages that occur in the WSS (Figure 7 to Figure 12), 57 described species and four candidate species were retrieved: the snake *Lytorhynchus cf. diadema* (10-11% divergence) (Figure 12), two geckos *Stenodactylus cf. mauritanicus* Central (10% divergence) and *Stenodactylus cf. sthenodactylus* (10%) (Figure 9), the latter comprising two different genetic lineages, and lastly the lizard *Latastia cf. longicaudata* (16% divergence) (Figure 10). The species with more genetic distinct lineages in the WSS was also

*Tropicolotes tripolitanus*, with six of the seven identified lineages occurring in the study area (Figure 9). *Tarentola parvicarinata* and *Tarentola chazaliae* (Figure 8), *Tropicolotes algericus* (Figure 9), *Chalcides sphenopsiformis* and *Scincus albifasciatus* (Figure 10) and *Agama boulengeri* (Figure 11) exhibited three distinct mitochondrial lineages in the WSS, while *Stenodactylus petrii* (Figure 9), *Trachylepis quinquetaeniata*, *Trachylepis perrotetii*, *Chalcides delislei*, *Mesalina pasteuri*, *Mesalina guttulata* (Figure 10), *Acanthodactylus boskianus* (Figure 10) and *Agama boueti* (Figure 11) exhibited two lineages. The snakes *Psammophis schokari*, *Lytorhynchus diadema* and *Hemorrhhois algirus* (Figure 12) were the only ones to exhibit two different lineages within the study area. In all other species, only one lineage was detected in WSS.

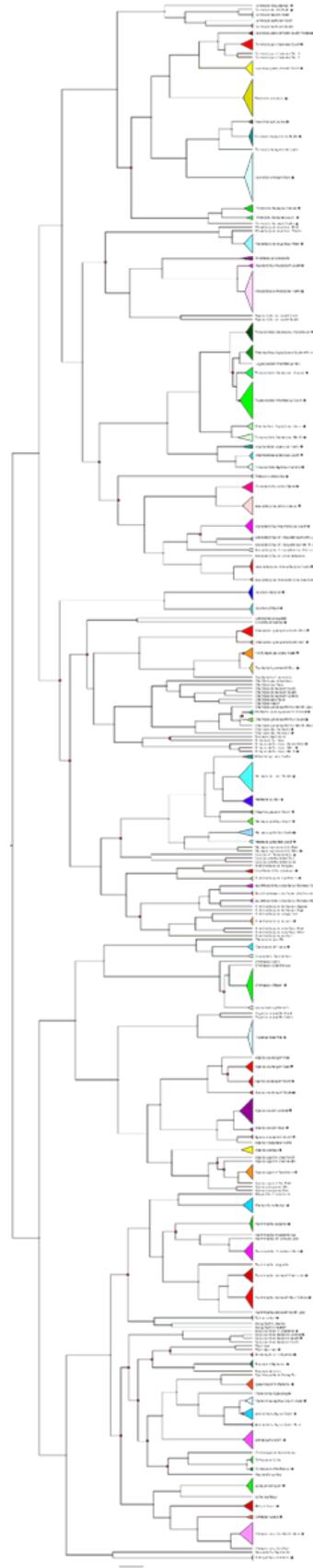


Figure 7. Bayesian COI tree for all reptiles. Species delimitation using 9.5% distance threshold are represented by different colours and the respective lineages are represented using different shades of the same colour. Lineages with more than one specimen were collapse in triangles. Stars indicate species and lineages that occur in the WSS. Black dots indicate posterior probability values equal or higher that 0.95 and the red dots represent lower posterior probability values. The new putative cryptic species are named in green.

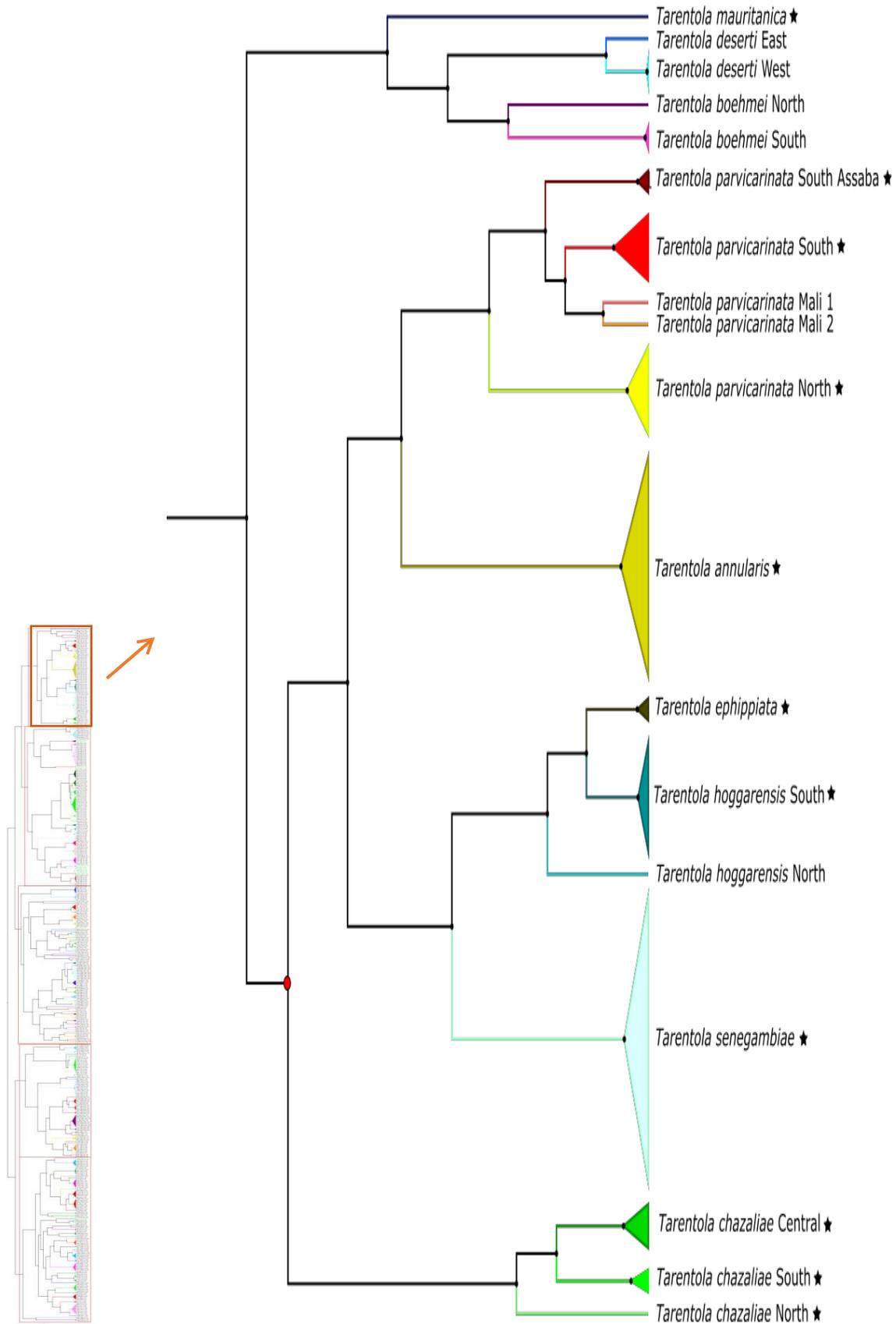


Figure 8. First zoom of the Bayesian COI tree for all reptiles. Clade comprises all species from the genus *Tarentola*. Lineages with more than one specimen were collapse in triangles. Stars indicate species and lineages that occur in the WSS. Black dots indicate posterior probability values equal or higher that 0.95 and the red dots represent lower posterior probability values. The new putative cryptic species are named in green.

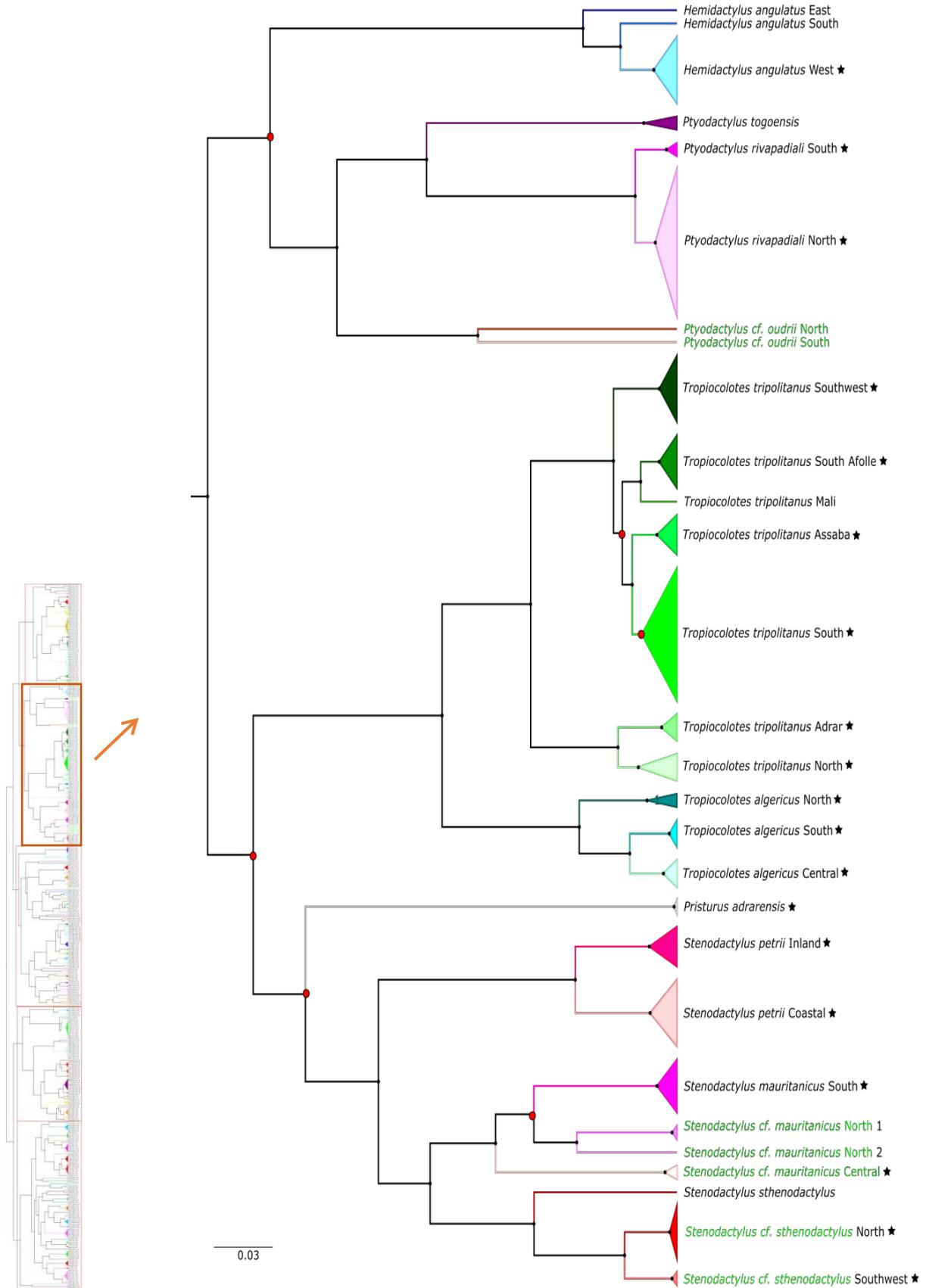


Figure 9. Second zoom of the Bayesian COI tree for all reptiles. Clades comprise all species from the genera *Hemidactylus*, *Ptyodactylus*, *Tropicolotes* and *Stenodactylus*. Lineages with more than one specimen were collapse in triangles. Stars indicate species and lineages that occur in the WSS. Black dots indicate posterior probability values equal or higher that 0.95 and the red dots represent lower posterior probability values. The new putative cryptic species are named in green.

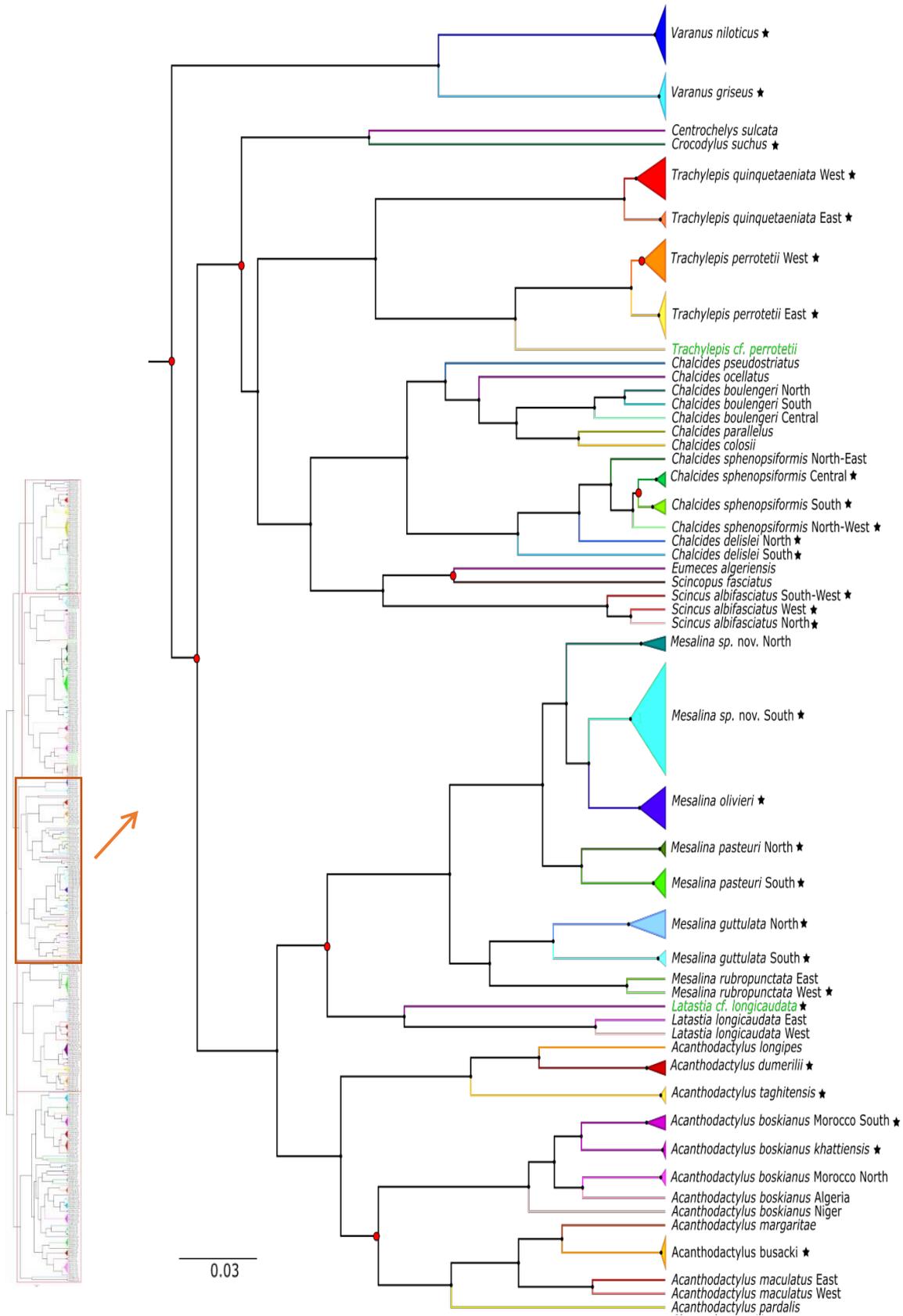


Figure 10. Third zoom of the Bayesian COI tree for all reptiles. Clades comprise all species from the genera *Varanus*, *Centrochelys*, *Crocodylus*, *Trachylepis*, *Chalcides*, *Mesalina*, *Latastia* and *Acanthodactylus*. Lineages with more than one specimen were collapse in triangles. Stars indicate species and lineages that occur in the WSS. Black dots indicate posterior probability values equal or higher than 0.95 and the red dots represent lower posterior probability values. The new putative cryptic species are named in green.

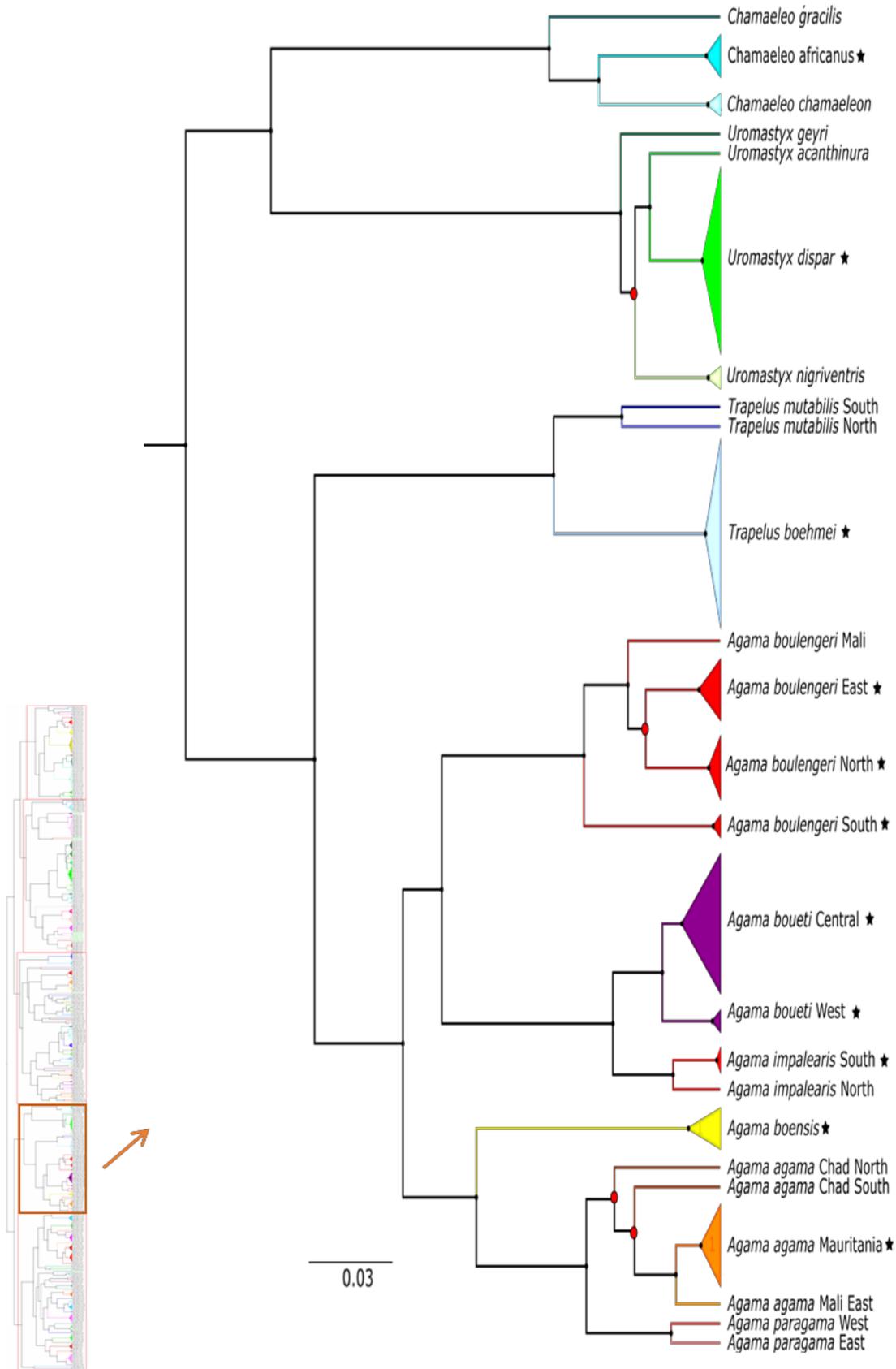


Figure 11. Fourth zoom of the Bayesian COI tree for all reptiles. Clades comprise all species from the genera *Chamaeleo*, *Uromastyx*, *Trapelus* and *Agama*. Lineages with more than one specimen were collapse in triangles. Stars indicate species and lineages that occur in the WSS. Black dots indicate posterior probability values equal or higher that 0.95 and the red dots represent lower posterior probability values. The new putative cryptic species are named in green.

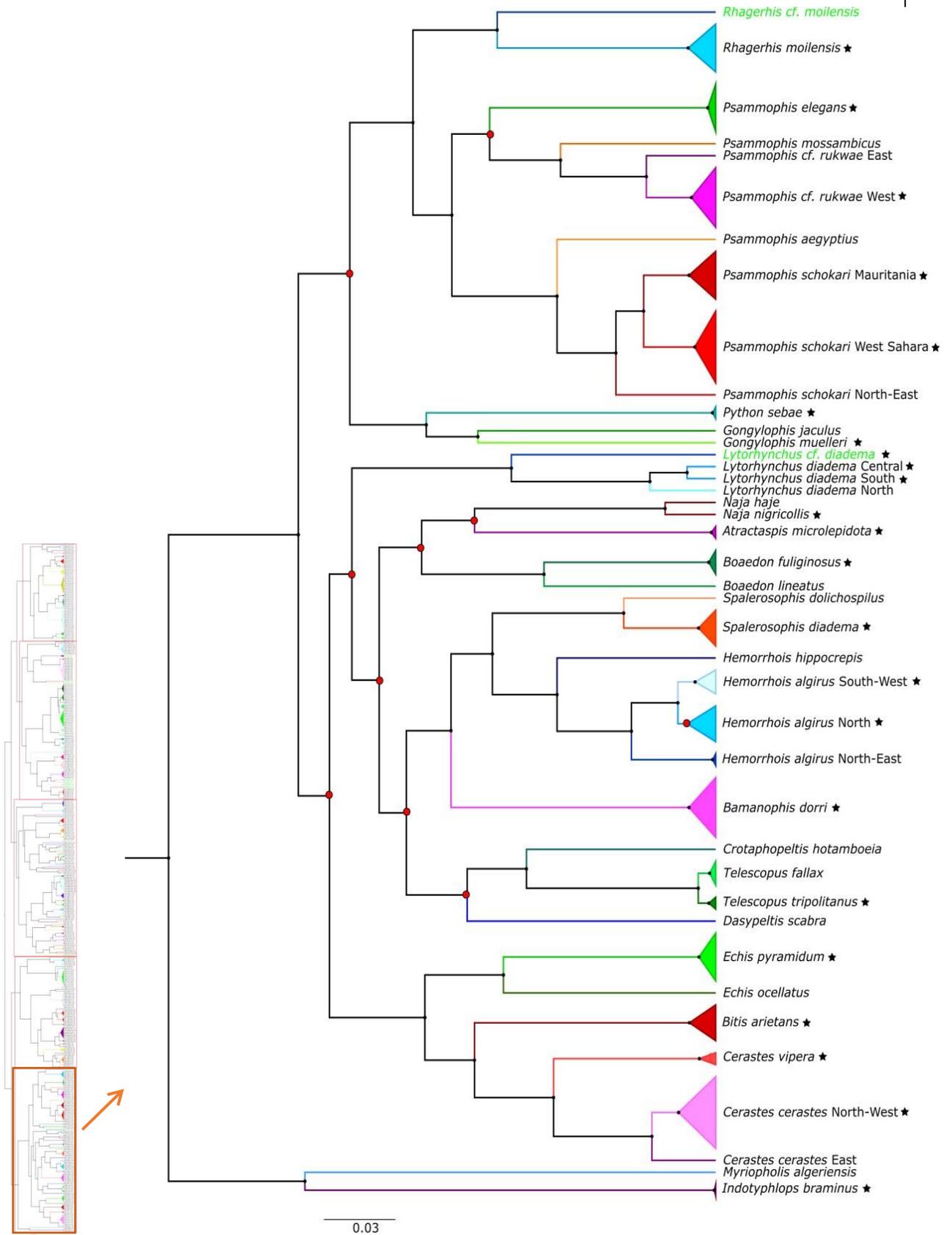


Figure 12. Fifth zoom in the Bayesian COI tree for all reptiles. Clades comprise all snake species from the genera *Rhagerhis*, *Psammophis*, *Python*, *Gongylophis*, *Lytorhynchus*, *Naja*, *Atractaspis*, *Boaedon*, *Spalerosophis*, *Hemorrhhois*, *Bamanophis*, *Crotaphopeltis*, *Telescopus*, *Dasypeltis*, *Echis*, *Cerastes*, *Myriopholis* and *Indotyphlops*. Lineages with more than one specimen were collapse in triangles. Stars indicate species and lineages that occur in the WSS. Black dots indicate posterior probability values equal or higher that 0.95 and the red dots represent lower posterior probability values. The new putative cryptic species are named in green.

Overall, it was possible to confirm that 454 samples, belonging to 90 species, were correctly identified in the field (Table 6). A total of 41 samples were identified as being from 15 different species, whereas 24 samples that had an uncertain identification (corresponded to shed skins or bones) were successfully identified within 15 different species. The more exciting result was the finding of 9 candidate new species, of which four occur in the WSS. All species, candidate species, and respective lineages are alphabetically ordered in Table 7.

Table 6. Summary of successful barcoding results.

Result	N species	N samples	%
Morphological diagnose confirmed	90	454	83.8
Morphological diagnose corrected	15	41	7.6
Morphological uncertainty solved	15	24	4.4
New candidate species identified inside WSS	4	15	2.8
New candidate species identified outside	5	8	1.3
Total		542	

Table 7. List of species, candidate species and respective lineages identified in this study. \* - distribution is mapped in Figure 13 Figure 22.

SPECIES	LINEAGES	CANDIDATE SPECIES
<b>West Sahara-Sahel taxon</b>		
<i>Acanthodactylus boskianus</i>	<i>boskianus</i> Algeria <i>boskianus khattiensis</i> * <i>boskianus</i> Morocco North <i>boskianus</i> Morocco South* <i>boskianus</i> Niger	
<i>Acanthodactylus busacki</i> *		
<i>Acanthodactylus dumerilii</i> *		
<i>Acanthodactylus longipes</i>		
<i>Acanthodactylus pardalis</i>		
<i>Acanthodactylus taghitensis</i> *		
<i>Agama agama</i>	<i>agama</i> Chad North <i>agama</i> Chad South <i>agama</i> Mali East <i>agama</i> Mauritania*	
<i>Agama boensis</i> *		
<i>Agama boueti</i>	<i>boueti</i> Central* <i>boueti</i> West*	
<i>Agama boulengeri</i>	<i>boulengeri</i> East* <i>boulengeri</i> North* <i>boulengeri</i> South*	
<i>Agama impalearis</i>	<i>impalearis</i> North <i>impalearis</i> South*	
<i>Atractaspis microlepidota</i> *		
<i>Bamanophis dorri</i> *		

<i>Bitis arietans</i> *		
<i>Boaedon fuliginosus</i> *		
<i>Centrochelys sulcata</i> *		
<i>Cerastes cerastes</i>	<i>cerastes</i> East <i>cerastes</i> North-West*	
<i>Cerastes vipera</i> *		
<i>Chalcides delislei</i>	<i>delislei</i> North* <i>delislei</i> South*	
<i>Chalcides sphenopsiformis</i>	<i>sphenopsiformis</i> Central* <i>sphenopsiformis</i> North-East <i>sphenopsiformis</i> North-West* <i>sphenopsiformis</i> South*	
<i>Chamaeleo africanus</i> *		
<i>Crocodylus suchus</i> *		
<i>Echis pyramidum</i> *		
<i>Gongylophis muelleri</i> *		
<i>Hemidactylus angulatus</i>	<i>angulatus</i> East <i>angulatus</i> South <i>angulatus</i> West* <i>algirus</i> North* <i>algirus</i> North-East <i>algirus</i> South-West*	
<i>Hemorrhhois algirus</i>		
<i>Indotyphlops braminus</i> *		
<i>Latastia longicaudata</i>	<i>longicaudata</i> East <i>longicaudata</i> West	<i>Latastia cf. longicaudata</i> *
<i>Lytorhynchus diadema</i>	<i>diadema</i> Central* <i>diadema</i> North <i>diadema</i> South*	<i>Lytorhynchus cf. diadema</i> *
<i>Mesalina guttulata</i>	<i>guttulata</i> North* <i>guttulata</i> South*	
<i>Mesalina olivieri</i> *		
<i>Mesalina pasteuri</i>	<i>pasteuri</i> North* <i>pasteuri</i> South*	
<i>Mesalina rubropunctata</i>	<i>rubropunctata</i> East <i>rubropunctata</i> West*	
<i>Mesalina sp. nov.</i>	<i>sp. nov.</i> North <i>sp. nov.</i> South*	
<i>Myriopholis algeriensis</i>		
<i>Naja nigricollis</i> *		
<i>Pristurus adrarensis</i> *		
<i>Psammophis cf. rukwae</i>	<i>cf. rukwae</i> East <i>cf. rukwae</i> West*	
<i>Psammophis elegans</i> *		
<i>Psammophis schokari</i>	<i>schokari</i> Mauritania* <i>schokari</i> North-East <i>schokari</i> West Sahara*	

<i>Ptyodactylus rivapadiali</i> sp. nov.	<i>rivapadiali</i> North* <i>rivapadiali</i> South*	
<i>Ptyodactylus togoensis</i>		
<i>Python sebae</i> *		
<i>Rhagerhis moilensis</i> *		<i>Rhagerhis cf. moilensis</i>
<i>Scincopus fasciatus</i>		
<i>Scincus albifasciatus</i>	<i>albifasciatus</i> North* <i>albifasciatus</i> South-West* <i>albifasciatus</i> West*	
<i>Spalerosophis diadema</i> *		
<i>Stenodactylus mauritanicus</i>	<i>mauritanicus</i> South*	<i>Stenodactylus cf. mauritanicus</i> Central <i>Stenodactylus cf. mauritanicus</i> North (including two lineages)
<i>Stenodactylus petrii</i>	<i>petrii</i> Coastal* <i>petrii</i> Inland*	
<i>Stenodactylus sthenodactylus</i>	<i>sthenodactylus</i> North* <i>sthenodactylus</i> Southwest*	<i>Stenodactylus cf. sthenodactylus</i>
<i>Tarentola annularis</i> *		
<i>Tarentola chazaliae</i>	<i>chazaliae</i> Central* <i>chazaliae</i> North* <i>chazaliae</i> South*	
<i>Tarentola ephippiata</i> *		
<i>Tarentola hoggarensis</i>	<i>hoggarensis</i> North <i>hoggarensis</i> South*	
<i>Tarentola parvicarinata</i>	<i>parvicarinata</i> Mali 1 <i>parvicarinata</i> Mali 2 <i>parvicarinata</i> North* <i>parvicarinata</i> South*	
<i>Tarentola senegambiae</i> *		
<i>Telescopus tripolitanus</i> *		
<i>Trachylepis perrotetii</i>	<i>perrotetii</i> East <i>perrotetii</i> West*	<i>Trachylepis cf. perrotetii</i>
<i>Trachylepis quinquetaeniata</i>	<i>quinquetaeniata</i> East <i>quinquetaeniata</i> West*	
<i>Trapelus boehmei</i> *		
<i>Tropicolotes algericus</i>	<i>algericus</i> Central* <i>algericus</i> North* <i>algericus</i> South*	
<i>Tropicolotes tripolitanus</i>	<i>tripolitanus</i> Adrar* <i>tripolitanus</i> Assaba* <i>tripolitanus</i> Mali <i>tripolitanus</i> North* <i>tripolitanus</i> South* <i>tripolitanus</i> South Afolle* <i>tripolitanus</i> Southwest*	
<i>Uromastyx dispar</i> *		
<i>Uromastyx nigriventris</i>		

*Varanus griseus*\**Varanus niloticus*\***Outgroup taxon**


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<i>Acanthodactylus maculatus</i>	<i>maculatus</i> East <i>maculatus</i> West	
<i>Acanthodactylus margaritae</i>		
<i>Agama paragama</i>	<i>paragama</i> West <i>paragama</i> East	
<i>Boaedon lineatus</i>		
<i>Chalcides boulengeri</i>	<i>boulengeri</i> Central <i>boulengeri</i> North <i>boulengeri</i> South	
<i>Chalcides colosii</i>		
<i>Chalcides ocellatus</i>		
<i>Chalcides parallelus</i>		
<i>Chalcides pseudostriatus</i>		
<i>Chamaeleo chamaeleon</i>		
<i>Chamaeleo gracilis</i>		
<i>Crotaphopeltis hotamboeia</i>		
<i>Dasypeltis scabra</i>		
<i>Echis ocellatus</i>		
<i>Eumeces algeriensis</i>		
<i>Gongylophis jaculus</i>		
<i>Hemorrhois hippocrepis</i>		
<i>Naja haje</i>		
<i>Psammophis aegyptius</i>		
<i>Psammophis mossambicus</i>		
<i>Ptyodactylus oudrii</i>		<i>Ptyodactylus cf. oudrii</i> North <i>Ptyodactylus cf. oudrii</i> South
<i>Spalerosophis dolichospilus</i>		
<i>Tarentola boehmei</i>	<i>boehmei</i> North <i>boehmei</i> South	
<i>Tarentola deserti</i>	<i>deserti</i> East <i>deserti</i> West	
<i>Tarentola mauritanica</i>		
<i>Telescopus fallax</i>		
<i>Trapelus mutabilis</i>	<i>mutabilis</i> North <i>mutabilis</i> South	
<i>Uromastyx acanthinura</i>		
<i>Uromastyx geyri</i>		

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### 3.4. Distribution of phylogenetic units and diversity

#### 3.4.1 Distribution of phylogenetic units

Mapping of the distribution of phylogenetic units indicates overall geographic coherence in the distribution of species and lineages (Figure 13 to Figure 22). In the case of species where multiple lineages were detected, their distribution tends to be allopatric. This pattern is best illustrated in the case of *Tropicolotes tripolitanus* (the species with the highest number of distinct mitochondrial lineages), where each of the six lineages tend to occupy distinct geographical areas (Figure 22). The same pattern is observable in many other species exhibiting more than one lineage in the WSS, such as *Acanthodactylus boskianus* (Figure 13) or *Chalcides delislei* (Figure 15). The single exception to the pattern of geographic coherence in the distribution of lineages was observed in *Tarentola chazaliae*, where the distribution of the lineages apparently does not follow a latitudinal gradient (Figure 20).

Contact zones in the distribution of distinct lineages were observable and in some cases they could be ascribed to particular areas. These are the cases of the: 1) Djouk valley for the lineages of *Agama boulengeri* (Figure 14) and *Tarentola parvicarinata* (Figure 21); 2) southern Assaba mountain for *Ptyodactylus rivapadiali* (Figure 19); 3) the area of the Cap Blanc peninsula for *Psammophis schokari* (Figure 19) and *Stenodactylus cf. sthenodactylus* (Figure 20); and 4) Southern Guidimaka for *Trachylepis perrotetii* (Figure 21).

Distinct distribution patterns with geographic coherence were observable in groups of species. These patterns can be grouped in six cases:

- 1) species restricted to the Sahara ecoregion, such as *Acanthodactylus taghitensis* (Figure 13), *Mesalina guttulata* (Figure 17) and *Mesalina rubropunctata* (Figure 18);
- 2) species restricted to the Sahel ecoregion, such as *Atractaspis microlepidota*, *Bamanophis dorri* (Figure 14), *Crocodylus suchus*, *Gongylophis muelleri* (Figure 16), *Naja nigricollis*, *Psammophis cf. rukwae* and *Psammophis elegans* (Figure 18);
- 3) species endemic to the WSS that are restricted to mountain areas, such as *Agama boulengeri* (Figure 14), *Mesalina sp. nov.*, *Pristurus adrarensis* (Figure 18), *Ptyodactylus rivapadiali sp. nov.* (Figure 19), and *Tarentola parvicarinata* (Figure 21);
- 4) species restricted to coastal areas, such as *Chalcides sphenopsiformis* (Figure 15), *Stenodactylus mauritanicus* (Figure 20), *Tarentola chazaliae* (Figure 20) and *Tropicolotes algericus* (Figure 22);
- 5) species widespread across the WSS that lack genetic substructuring, such as *Cerastes vipera* (Figure 15), *Tarentola annularis* (Figure 20) and *Varanus griseus* (Figure 22), or exhibiting more than one mitochondrial lineage but only one of them occurring in the WSS, such as *Boaedon fuliginosus*, *Cerastes cerastes* (Figure 15), *Rhagerhis moilensis* (Figure 19), *Tarentola hoggarensis* (Figure 21), and *Trapelus boehmei* (Figure 22);
- 6) species widespread across the WSS exhibiting distinct lineages in the study area, where

one of them occurs in the Palaearctic realm and the other one occurs in the Afro-tropic realm, such as *Acanthodactylus boskianus* (Figure 13), *Mesalina pasteuri* (Figure 17), and *Psammophis schokari* (Figure 19), or one of them occurs in coastal areas and the other one occurs in inland areas, such as *Scincus albifasciatus* (Figure 19) and *Stenodactylus petrii* (Figure 20).

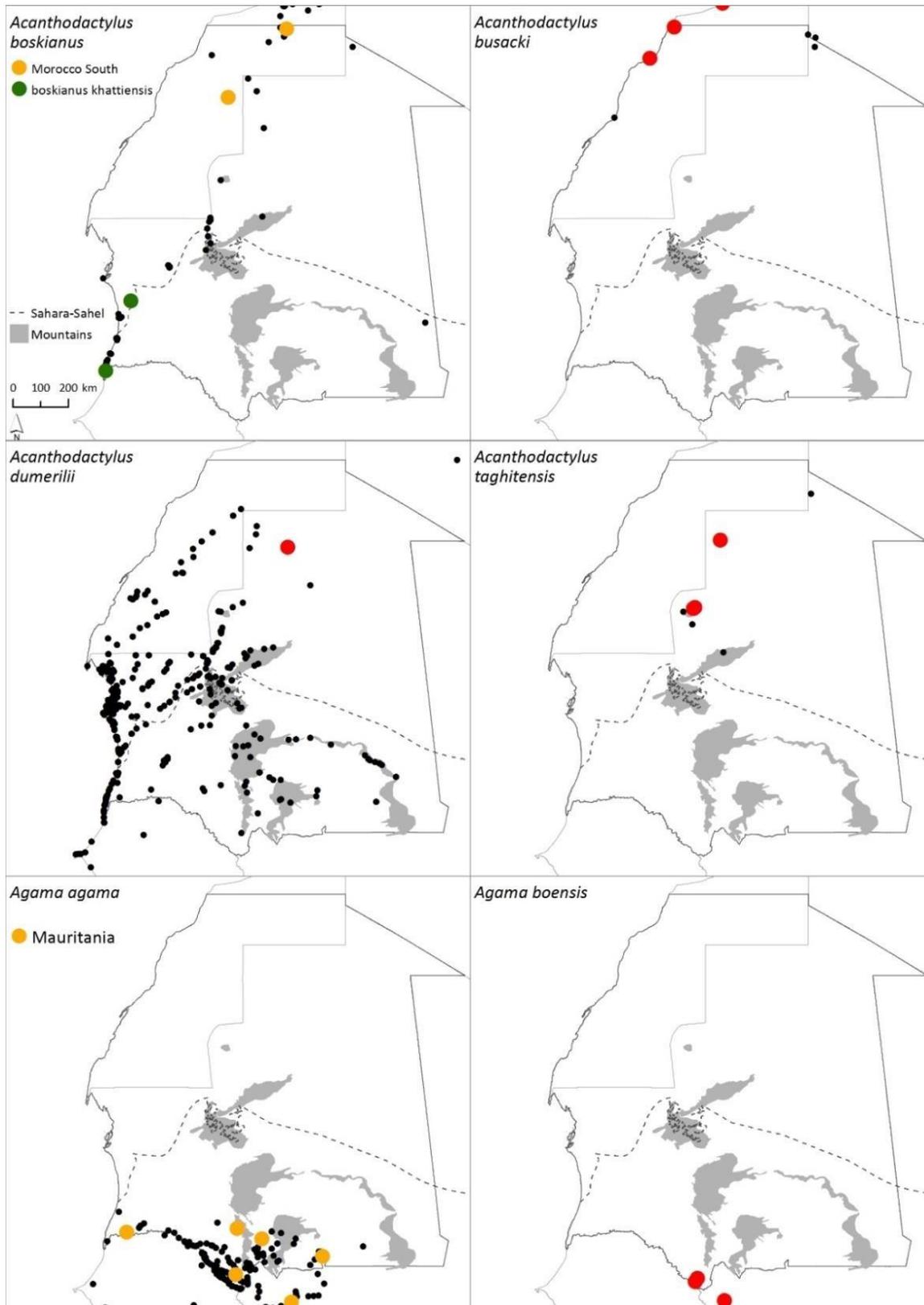


Figure 13. Map of the distribution of reptile species and their lineages in the West Sahara-Sahel: *Acanthodactylus boskianus*, *A. busacki*, *A. dumerilii*, *A. taghitensis*, and *Agama boensis*. Blue, green and yellow dots represent sequenced samples of lineages within the same species. Red dots correspond to sequenced samples from monophyletic species. Black dots represent occurrence records from un-sequenced samples.

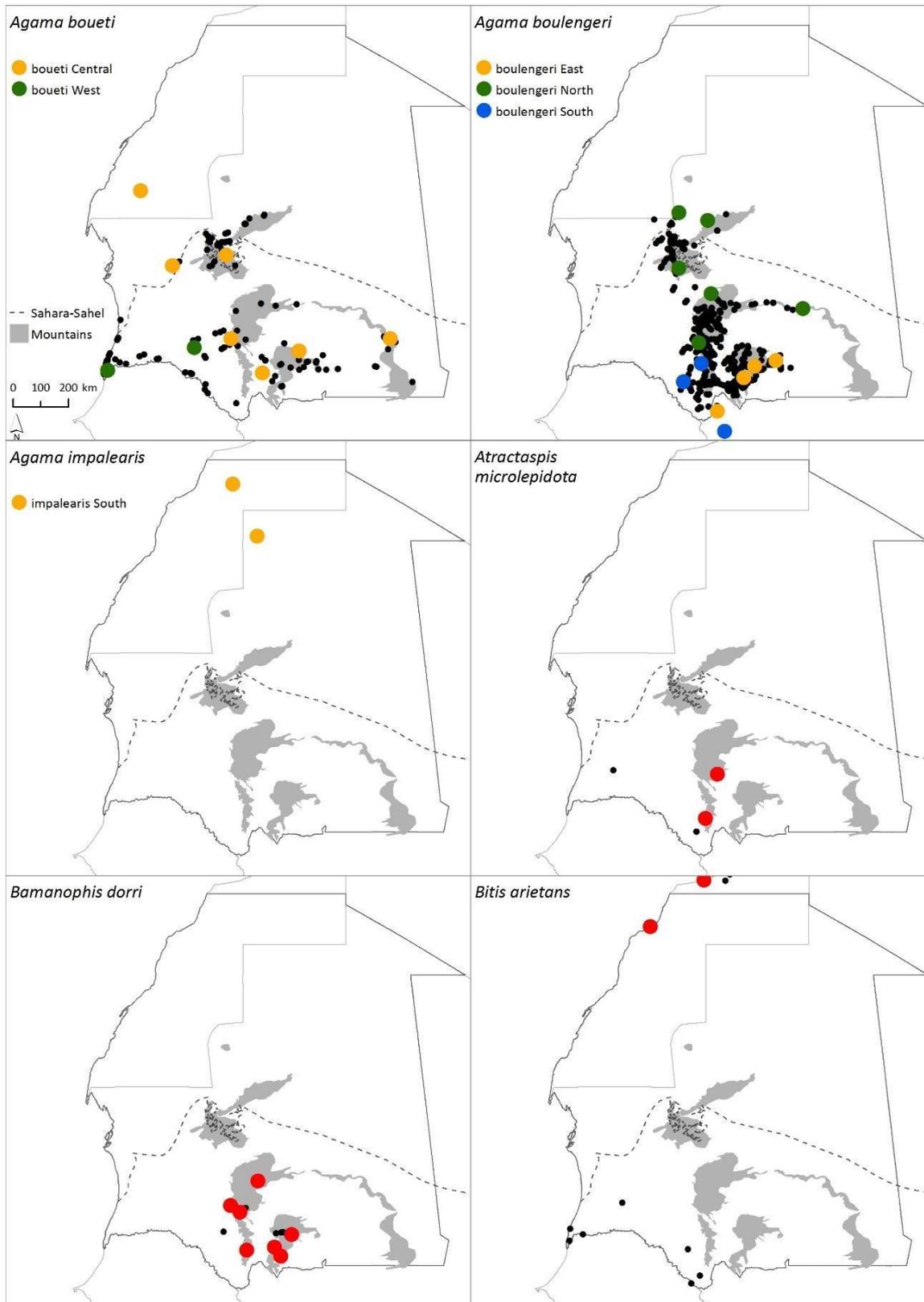


Figure 14. Map of the distribution of reptile species and their lineages in the West Sahara-Sahel: *Agama boueti*, *A. boulengeri*, *A. impalearis*, *Atractaspis microlepidota*, *Bamanophis dorri* and *Bitis arietans*. Blue, green and yellow dots represent sequenced samples of lineages within the same species. Red dots correspond to sequenced samples from monophyletic species. Black dots represent occurrence records from un-sequenced samples.

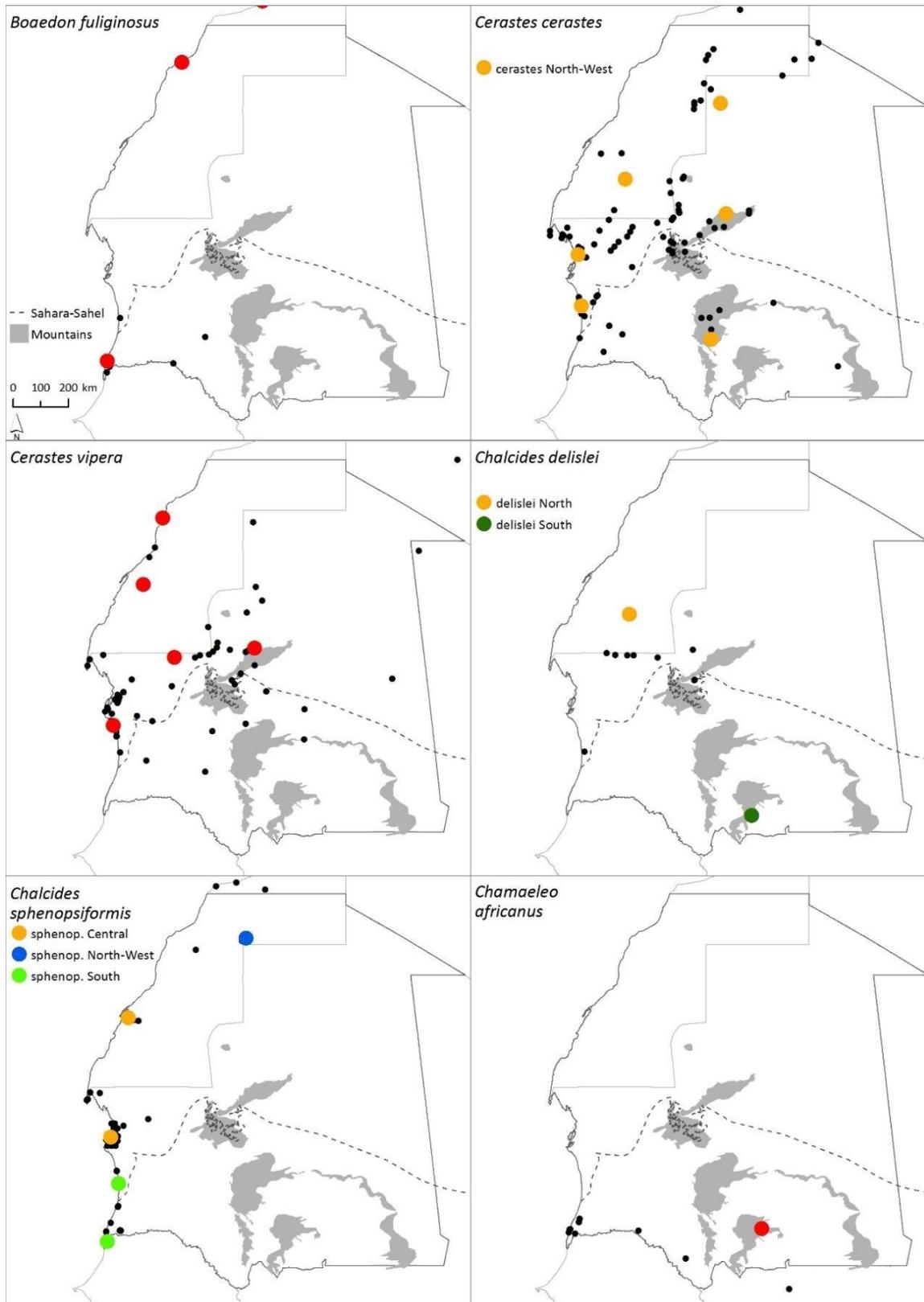


Figure 15. Map of the distribution of reptile species and their lineages in the West Sahara-Sahel: *Boaedon fuliginosus*, *Cerastes cerastes*, *C. vipera*, *Chalcides delislei*, *C. sphenopsiformis* and *Chamaeleo africanus*. Blue, green and yellow dots represent sequenced samples of lineages within the same species. Red dots correspond to sequenced samples from monophyletic species. Black dots represent occurrence records from un-sequenced samples.

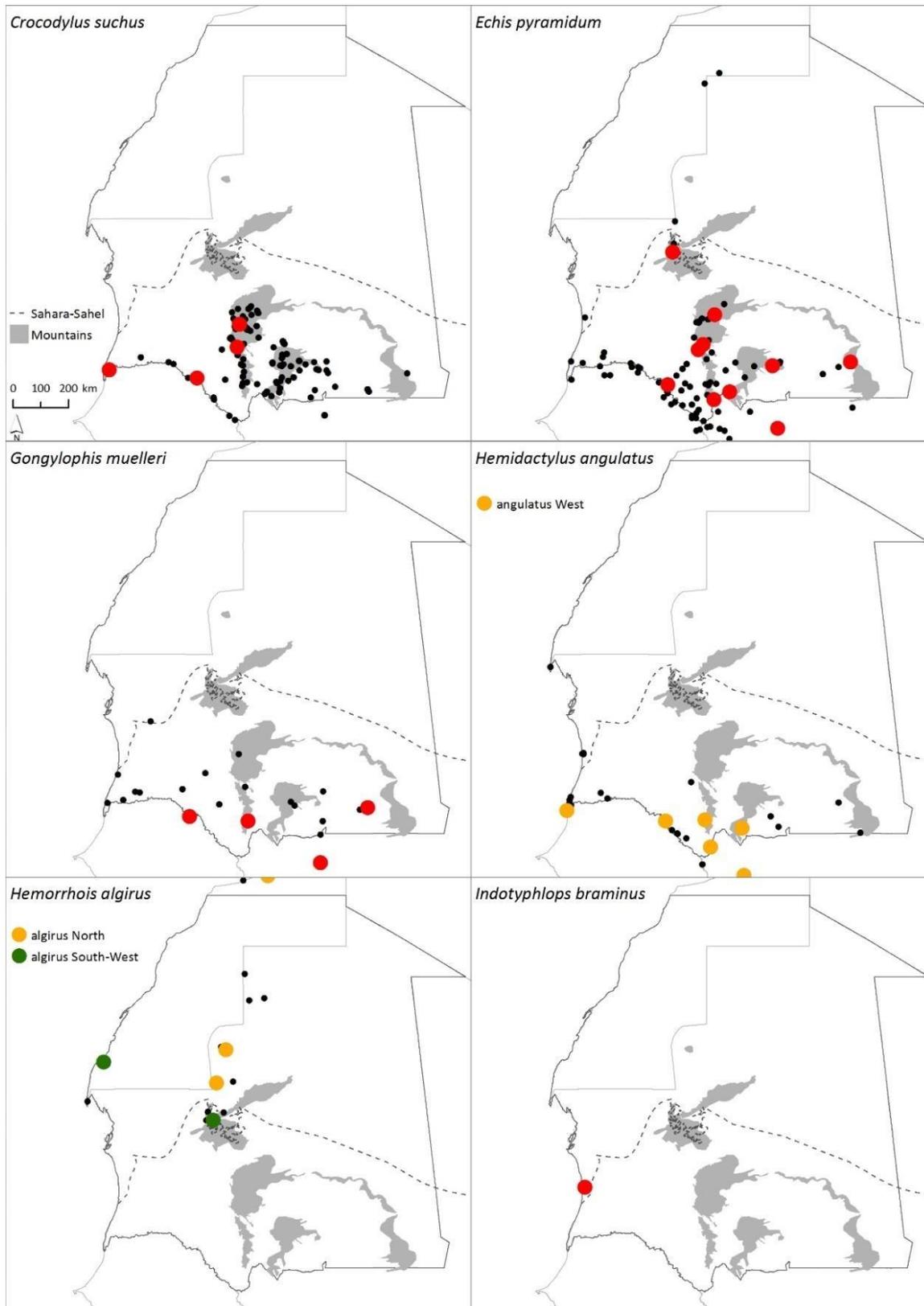


Figure 16. Map of the distribution of reptile species and their lineages in the West Sahara-Sahel: *Crocodylus suchus*, *Echis pyramidum*, *Gongylophis muelleri*, *Hemidactylus angulatus*, *Hemorrhhois algirus* and *Indotyphlops braminus*. Blue, green and yellow dots represent sequenced samples of lineages within the same species. Red dots correspond to sequenced samples from monophyletic species. Black dots represent occurrence records from un-sequenced samples.

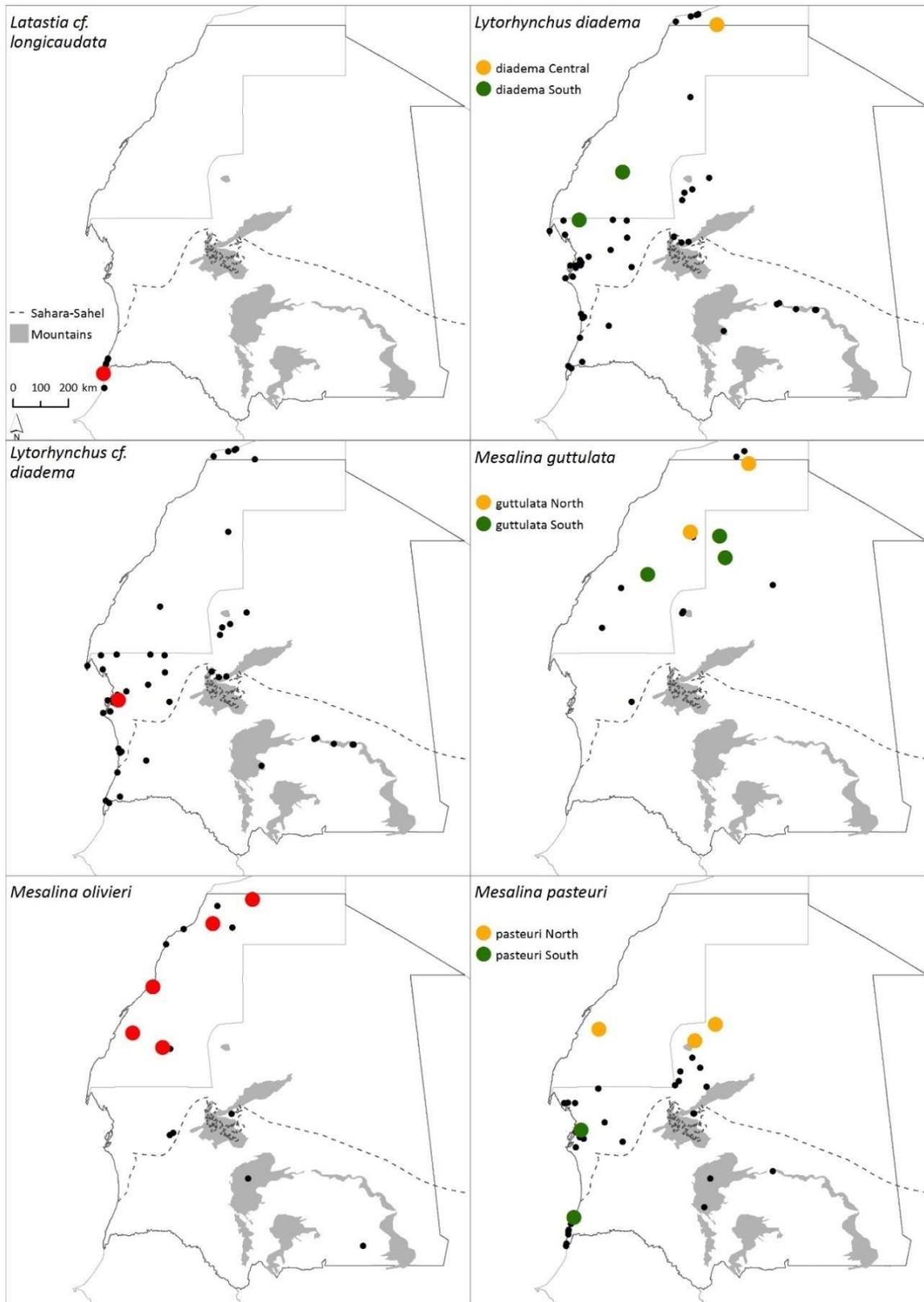


Figure 17. Map of the distribution of reptile species and their lineages in the West Sahara-Sahel: *Latastia cf. longicaudata*, *Lytorhynchus diadema*, *L. cf. diadema*, *Mesalina guttulata*, *M. olivieri* and *M. pasteuri*. Blue, green and yellow dots represent sequenced samples of lineages within the same species. Red dots correspond to sequenced samples from monophyletic species. Black dots represent occurrence records from un-sequenced samples.

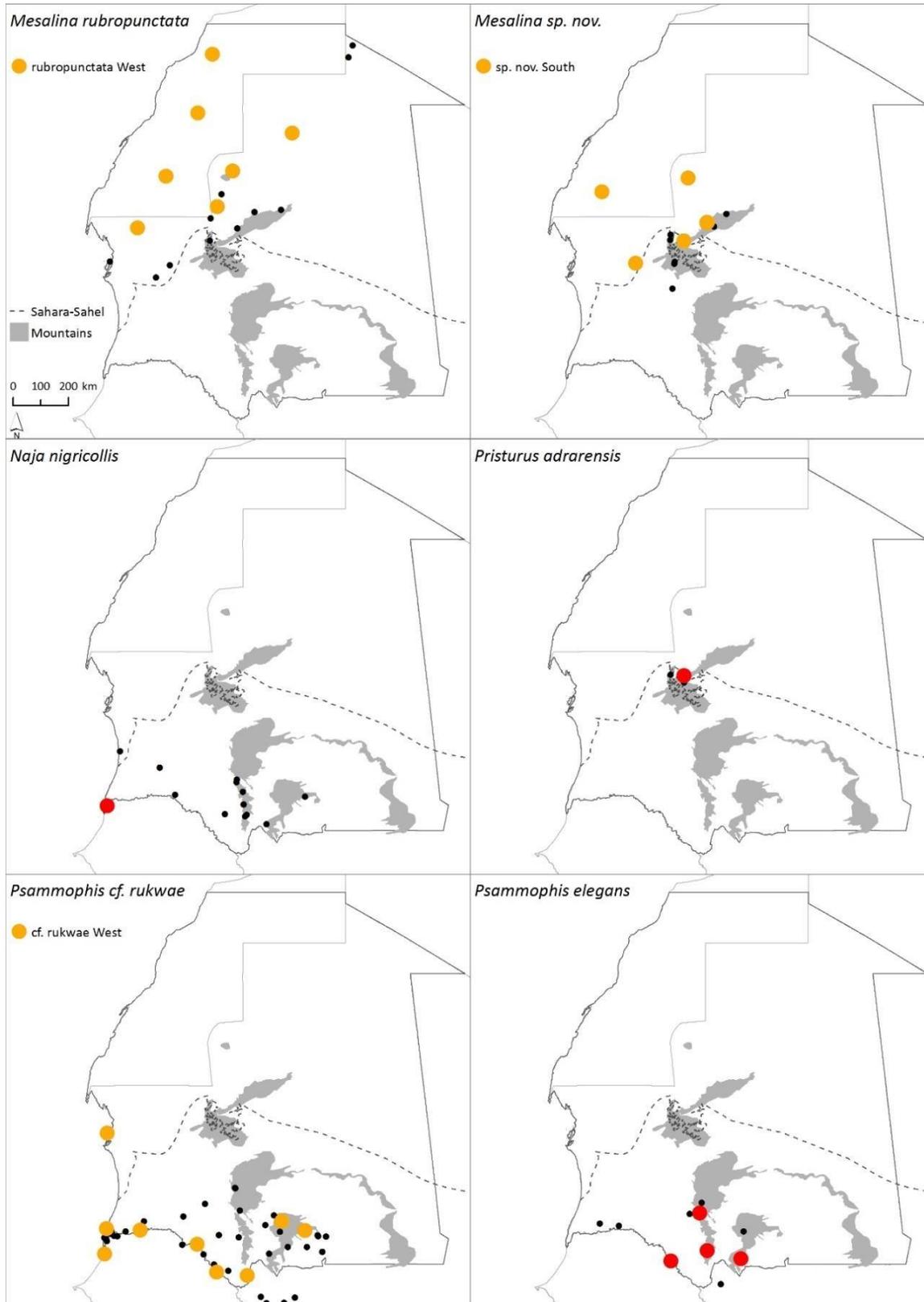


Figure 18. Map of the distribution of reptile species and their lineages in the West Sahara-Sahel: *Mesalina rubropunctata*, *M. sp. nov.*, *Naja nigricollis*, *Pristurus adrarensis*, *Psammophis cf. rukwae* and *P. elegans*. Blue, green and yellow dots represent sequenced samples of lineages within the same species. Red dots correspond to sequenced samples from monophyletic species. Black dots represent occurrence records from un-sequenced samples.

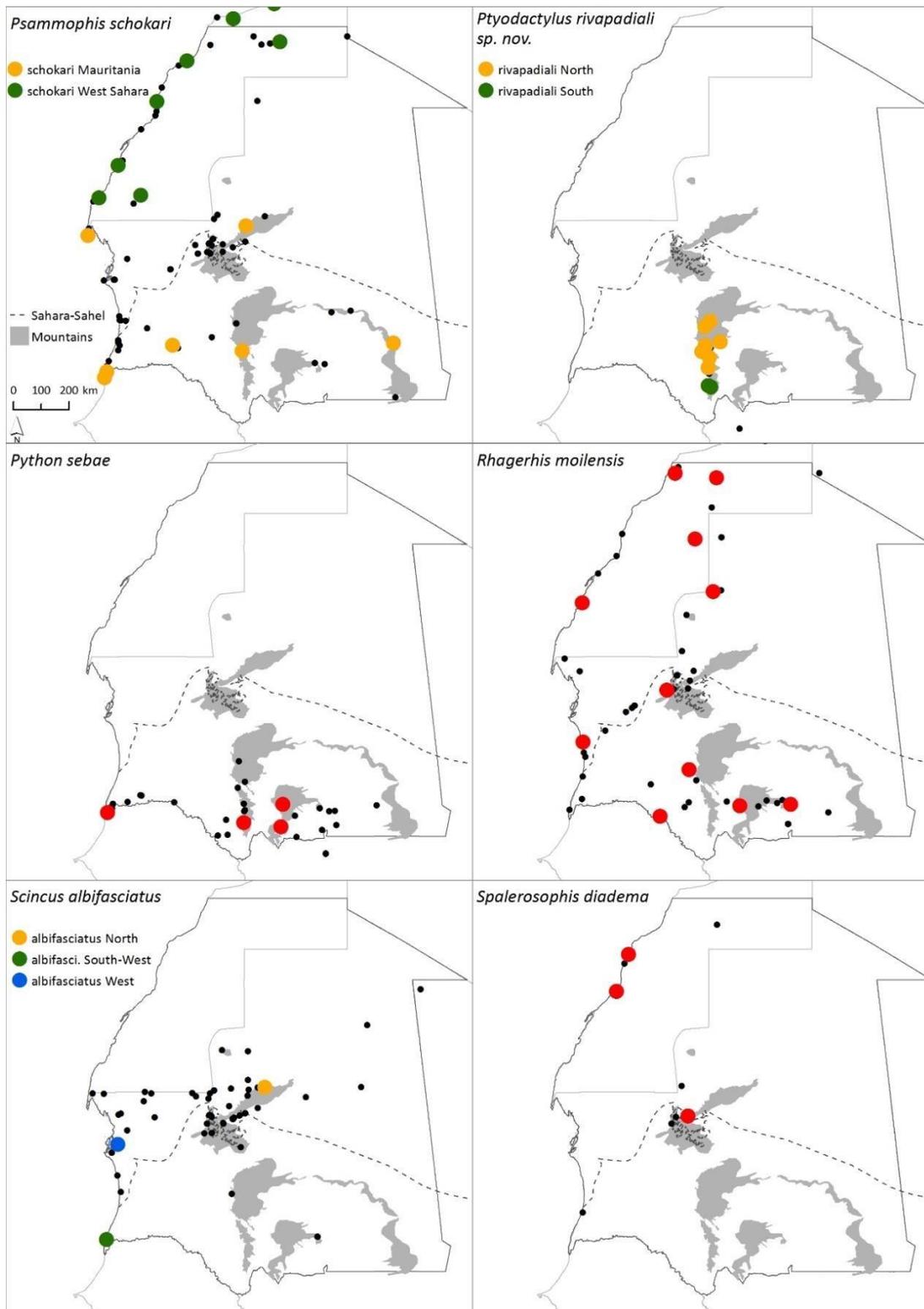


Figure 19. Map of the distribution of reptile species and their lineages in the West Sahara-Sahel: *Psammophis schokari*, *Ptyodactylus rivapadiali* sp. nov., *Python sebae*, *Rhagerhis moilensis*, *Scincus albifasciatus* and *Spalerosophis diadema*. Blue, green and yellow dots represent, sequenced samples of lineages within the same species. Red dots correspond to sequenced samples from monophyletic species. Black dots represent occurrence records from un-sequenced samples.

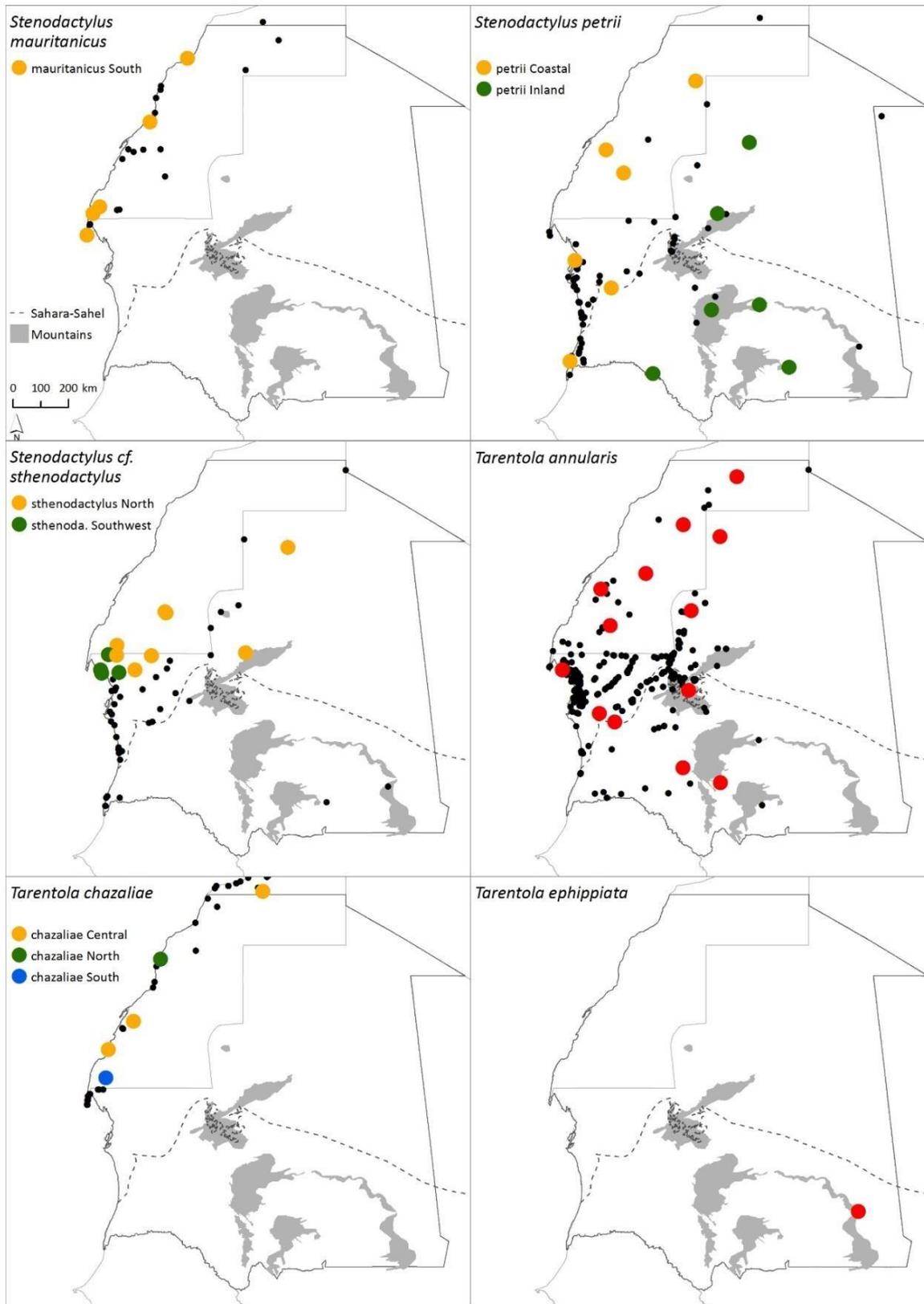


Figure 20. Map of the distribution of reptile species and their lineages in the West Sahara-Sahel: *Stenodactylus mauritanicus*, *S. petrii*, *S. cf. sthenodactylus*, *Tarentola annularis*, *T. chazaliae* and *T. ehippiata*. Blue, green and yellow dots represent sequenced samples of lineages within the same species. Red dots correspond to sequenced samples from monophyletic species. Black dots represent occurrence records from un-sequenced samples.

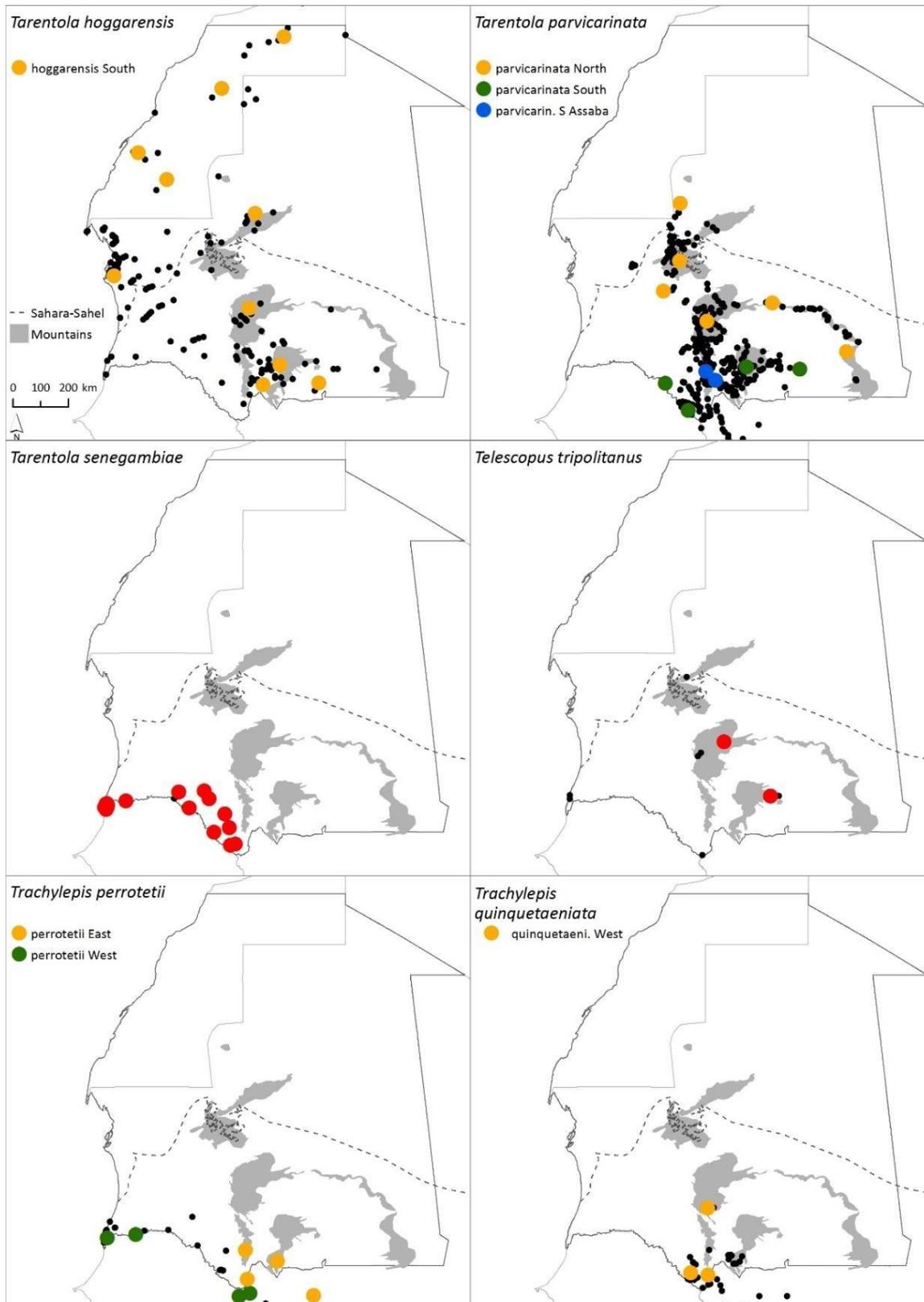


Figure 21. Map of the distribution of reptile species and their lineages in the West Sahara-Sahel: *Tarentola hoggarensis*, *T. parvicarinata*, *T. senegambiae*, *Telescopus tripolitanus*, *Trachylepis perrotetii* and *T. quinquetaeniata*. Blue, green and yellow dots represent sequenced samples of lineages within the same species. Red dots correspond to sequenced samples from monophyletic species. Black dots represent occurrence records from un-sequenced samples.

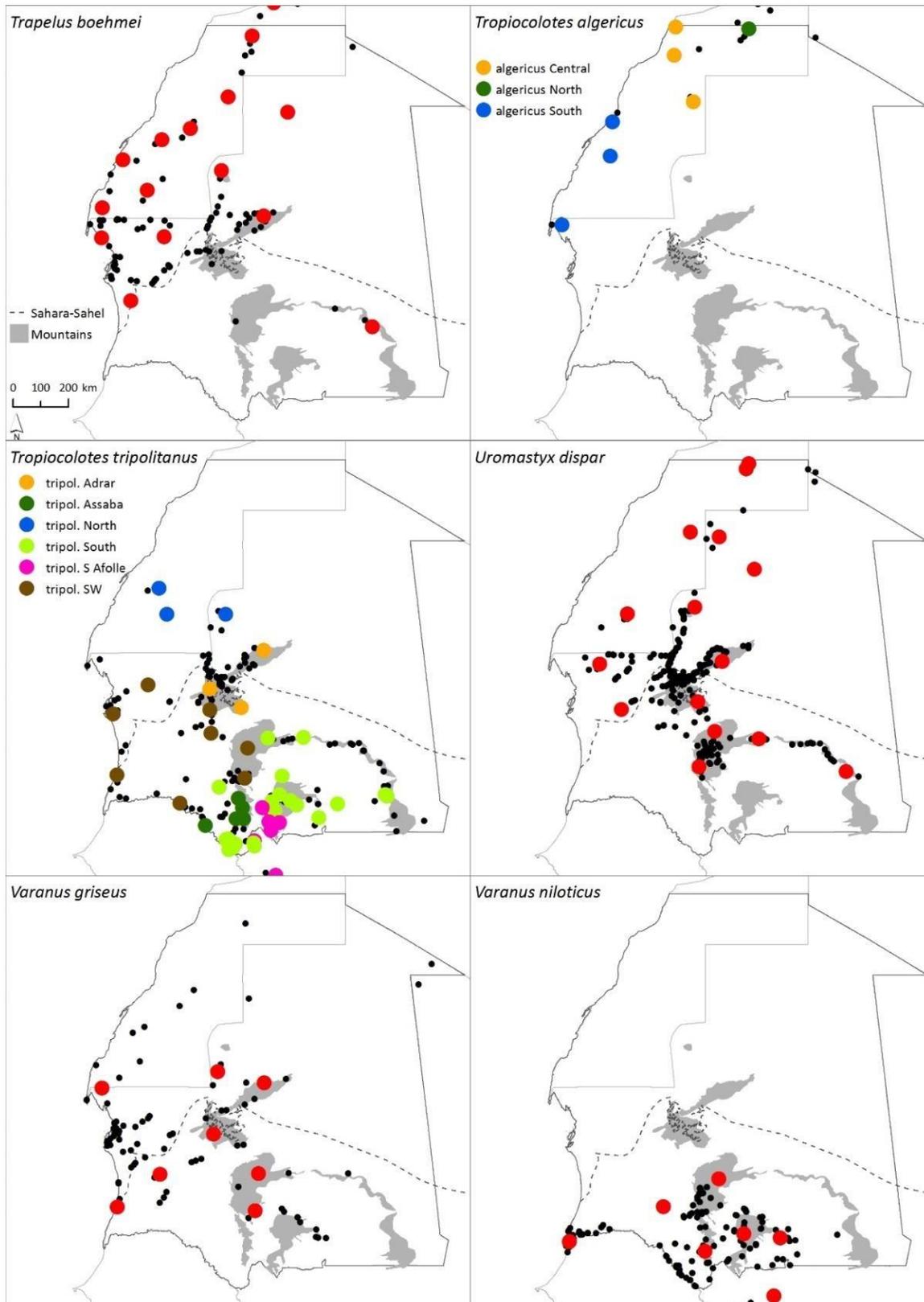


Figure 22. Map of the distribution of reptile species and their lineages in the West Sahara-Sahel: *Trapelus boehmei*, *Tropicolotes algericus*, *T. tripolitanus*, *Uromastyx dispar*, *Varanus griseus* and *V. niloticus*. Blue, green and yellow dots represent sequenced samples of lineages within the same species. Red dots correspond to sequenced samples from monophyletic species. Black dots represent occurrence records from un-sequenced samples.

### 3.4.2 Distribution of reptile diversity

The summarising of the number of families, genera, species and lineages in each mountain and ecoregions indicates that some regions display richness concentrations (Table 8). These are the cases of the ecoregions Sahelian Acacia savannah, Saharan Atlantic coastal desert, and South Sahara desert which, in absolute numbers, accumulate the highest richness's (Annexes; Figure A2). The ecoregion South Sahara desert and the Tagant and Afollé mountains concentrate the highest richness in families, and the former two regions are also important for richness of genera.

Table 8. Number of families (F), genera (G), species (S) and lineages (L) of reptiles occurring in distinct regions of the West Sahara-Sahel.

Regions	NF	NG	NS	NL
<b>Mountain</b>				
Adrar Atar	6	7	9	9
Afollé	9	11	13	14
Assaba	7	9	9	10
Dhar Chinguetti	8	11	11	11
Dhar Néma	3	3	3	3
Dhar Tichit	2	4	5	5
Kédiatt ej Jill	3	3	3	3
Tagant	9	14	16	17
<b>Ecoregion</b>				
Mediterranean Acacia-Argania dry woodlands and succulent thickets	3	3	3	3
North Saharan Xeric Steppe and Woodland	8	13	23	27
Saharan Atlantic coastal desert	10	18	27	30
Saharan halophytics	1	1	1	1
Sahelian Acacia savanna	14	24	36	44
South Sahara desert	9	14	27	31

Contrasting the patterns of distribution of richness according to the available area of mountains and ecoregions within the WSS (Table 2) suggests a trend for higher richness being found in regions covering larger areal extents (Figure A2). This trend is observable in all analysed taxonomic levels, but relationships between richness and area of region are stronger (higher R<sup>2</sup>) in the number of lineages and species.

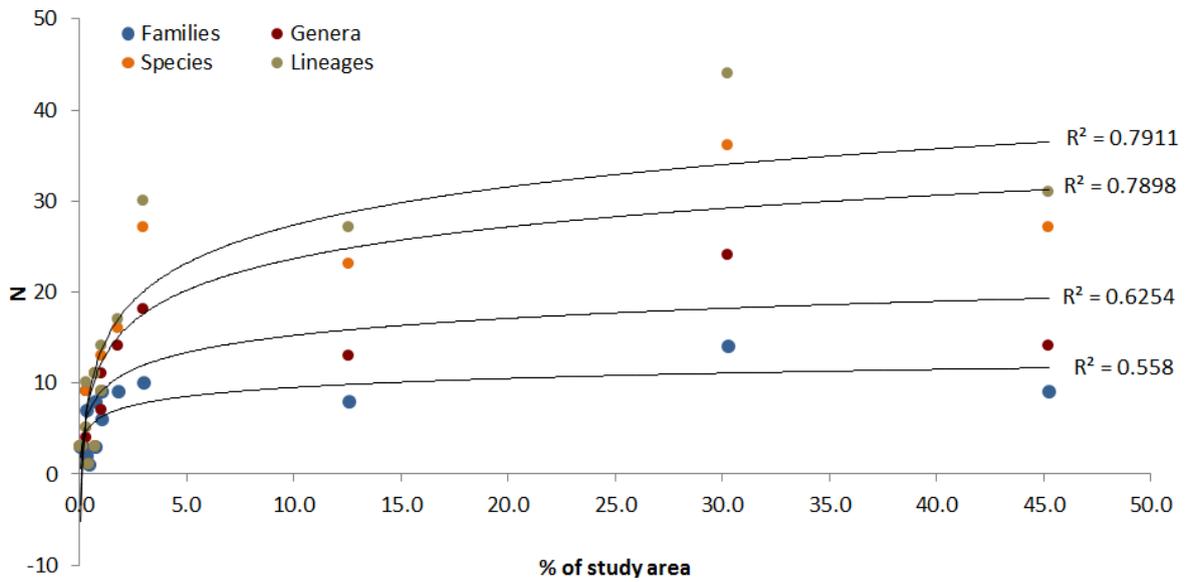


Figure 23. Relationships between area of mountains/ecoregions (% of study area) in the West Sahara-Sahel and the absolute number (N) of families, genera, species and lineages retrieved in each region.

Given that absolute richness is related with the area of regions analysed, it was mapped the relative number of families, genera, species and lineages weighted by the area of each region (Figure 23). The Assaba Mountain accumulates the highest relative richness observed in all analysed taxonomic levels. The mountains of Dhar Chinguetti, Dhar Tîchît, and Afollé are the second most important ones in terms of accumulating relative reptile richness. The Sahara Atlantic coast desert is the single ecoregion identified as important either considering absolute or relative reptile's richness.

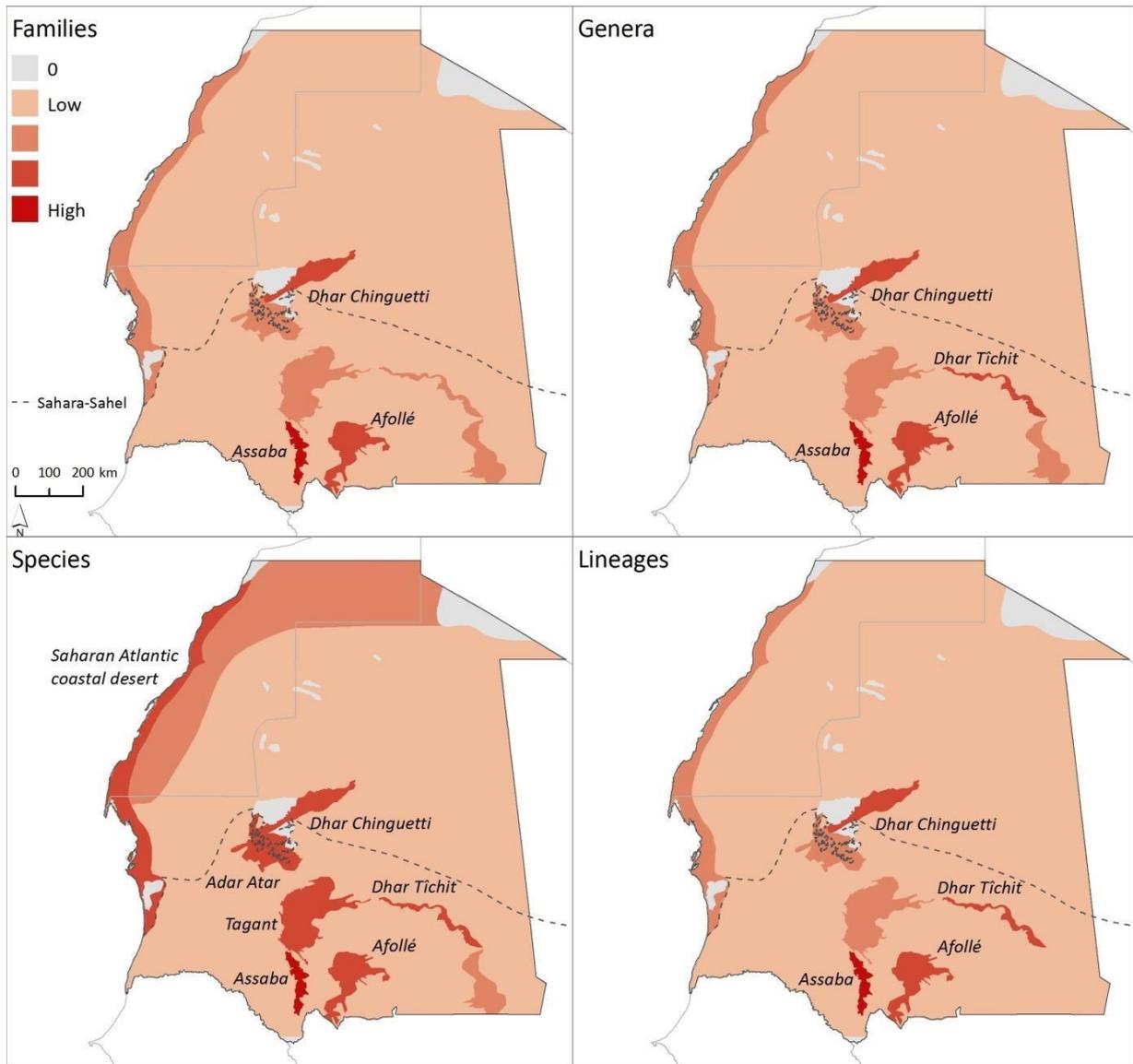


Figure 24. Distribution maps of richness of families, genera, species and lineages in each studied region. Richness is weighted by the area of each region. Grey zones (excluded) indicate regions where no taxa were detected.

### 3.4.3 Gap analysis

The gap analysis illustrated that only a fraction, about 3.1%, of the mountains and ecoregions are protected (Table 9). From the regions identified as accumulating the highest reptile richness, Dhar Chinguetti is the only mountain with more than half of its area protected by the Guelb er Richât National Park (see Figures 3 and 5 for location of regions and protected areas). The Tagant mountain is partially included in the Lac Gabbou RAMSAR site, while the Afollé Mountain is almost unprotected. No single area of the Assaba and Dhar Tîchît Mountain has been included in any of the protected areas of the WSS.

Table 9. Area and percent of protection of mountains and ecoregions of the West Sahara-Sahel

Region	Area Protected (km <sup>2</sup> )	% Protected
<b>Mountain</b>		
Dhar Chinguetti	5527	72.3
Tagant	7127	37.7
Afollé	449	4.1
<b>Ecoregion</b>		
Saharan Atlantic coastal desert	5660	18.3
North Saharan Xeric Steppe and Woodland	6727	5.2
Sahelian Acacia savanna	1140	0.4
South Sahara desert	5081	1.1

## 4. Discussion

This study represents the first comprehensive DNA barcode database for reptiles from the West Sahara-Sahel, including the collection and analysis of 109 species. It demonstrated the ability of DNA barcoding to identify species and it shed new light on local species diversity and distribution. In the following sections it will be discussed the sampling and DNA barcoding identification success, focusing on the achievements and constraints of the study, the diversity of lineages found and their support, and the preliminary distribution patterns of reptile richness found.

### 4.1 Sampling and DNA barcoding identification success

The samples available for this study included 69 reptile species from 20 families found in the WSS, but nine species were missing from the dataset, including representatives of Families Eublepharidae, Pelomedusidae and Trionychidae. As such, the dataset includes representatives of 90% of the overall reptile diversity described for the region. A recent biogeographic study using DNA barcoding revising bat diversification in the Caribbean included 60% of the described bat species in the region (Lim, 2017). Similarly, about 64% of species coverage was the basis for a DNA barcoding assessment of reptiles in Madagascar (Nagy *et al.*, 2012). The sample coverage in the WSS can thus be considered as highly representative, especially because desert regions are usually under-sampled (Brito *et al.*, 2014). Still, further fieldwork should focus in acquiring samples particularly from under-represented species and regions.

The overall amplification success of the 684 samples was 80%. DNA barcodes were not possible to retrieve for six of the 69 species available in the dataset. This amplification success rate is similar to the one reported (84.6%) in other reptile DNA barcoding studies (Nagy *et al.*, 2012). Initially, we tried amplifying COI fragments based on using a new pair of degenerative primers (RepCOI-F and RepCOI-R) designed specifically for squamates and reported as successful for reptiles (Nagy *et al.*, 2012). However, for some WSS species, these primers failed even after optimising PCR conditions. Later in this study, it were used the universal primers LCO1490 and HC02198 (Folmer *et al.*, 1994), which successfully amplified the only two samples available for *Pristurus adraensis*. Hawlitschek *et al.* (2013) used both sets of primer pairs and while the degenerative primers achieved the highest amplification success rates, the universal primers (LCO and HCO) outperformed the former in some species. Although time constrains did not allow testing these primers for other WSS

species in which the use of the specific primers was unsuccessful, our results suggest that both primers should be used in DNA barcoding to achieve high amplification success rates. DNA barcodes of 63 of the 77 recognized species from WSS and for *Agama impalearis* (new species record for the WSS) were recovered, representing more than 80% of the reptile diversity of the WSS in the DNA barcoding library. This rate is considerably higher than other DNA barcoding libraries (50 to 64%; Nagy *et al.*, 2012; Lim, 2017). Complete DNA barcoding has been achieved in particular cases, such as in insular systems (Vasconcelos *et al.*, 2016). As such, our DNA barcoding library provides a valuable tool for identifying and assessing the diversity of WSS reptiles. Future work should aim at increasing sampling across species and regions, together with improvements in the amplification success, to complete the DNA barcoding library.

The identification success of specimens ranged from 69% to 92% (74% to 99% when excluding singletons) according to the distinct distance-based methods applied. Only one specimen (identified as *Chalcides delislei*) appear as incorrectly identified, when using the ThreshID criterion with a 10% threshold. Only 10% of the samples were assigned to the originally identified species when using the BOLD best ID approach. In some species (e.g. *Tarentola chazaliae*, *Stenodactylus petrii* and *Tropicolotes tripolitanus*), although a positive identification was retrieved, not all specimens were classified as the original species identified. The rates of identification success obtained are similar to the ones reported in other DNA barcoding studies (e.g. Vasconcelos *et al.*, 2016). The only specimen incorrectly identified belongs to a species where only two samples were available and both specimens clustered paraphyletically in clearly independent, parapatric, and well supported highly divergent mitochondrial lineages (9.2%). Other *Chalcides* species were previously reported as paraphyletic, namely *C. polylepis* (Carranza *et al.*, 2008). Although DNA barcoding methods provide a valuable tool to identify species, non-identical sequences may remain unidentifiable or may be unambiguously wrongly placed. When paraphyletic relationships are found, it is impossible to retrieve any taxonomic information with DNA barcoding methods (Will and Rubinoff, 2004). The percentage of identification achieved using the BOLD approach was lower in comparison to other barcoding studies (e.g. 70%; Shen *et al.*, 2016). This result, together with the fact that some haplotypes identified in BOLD approach came with no-match result, illustrates the lack of barcodes for most of the WSS reptiles and the lack of representative barcodes of the genetic diversity contained within them. Overall, our success rate was good compared with other DNA barcoding studies and the barcodes obtained are crucial for understanding the reptile genetic diversity comprised in the WSS. A barcoding gap, i.e. the maximum intraspecific distances were smaller than the minimum interspecific distances, was observed in all cases with the exceptions of *Rhagerhis moilensis*, *Tarentola hoggarensis*, *Chalcides delislei*, *Mesalina sp. nov.*, and *Latastia longicaudata*. An

ideal DNA barcode should display high interspecific together with low intraspecific divergence, presenting different DNA barcoding gaps (Kress and Erickson, 2007; Kress *et al.*, 2005). The use of DNA barcoding to identify species supposes that species are monophyletic units (Hebert *et al.*, 2003). Thus, the lack of a barcoding gap due to low interspecies distances in three species (*T. hoggarensis*, *C. delislei* and *Mesalina sp. nov.*) is probably related with the fact that they appear to be paraphyletic species. Other species from the *Tarentola* genus, such as *T. mauritanica*, have been considered as paraphyletic with strong support (Harris *et al.*, 2004). The paraphyletic clade in *Mesalina sp. nov.* is also supported by previous studies that have shown complex phylogenetic relationships in genus *Mesalina*, with one paraphyletic and three polyphyletic species identified (Kapli *et al.*, 2015). Absence of barcoding gap can also result from cryptic speciation processes (Zhang *et al.*, 2012; Xu, Li, and Jin, 2016), which might be the cases of *R. moilensis* and *L. longicaudata*. The presence of a barcoding gap in most of the studied species suggests that COI is a reliable barcode marker for identifying reptiles in the WSS.

## 4.2 Species and lineage identification

The number of species clusters identified varied according to the delimitation approach used. The bPTP approach (Annexes; Figure A1) identified 193 species units but only 121 of these were strongly supported. The delimitation using the 9.5% threshold applied to the Bayesian inference lead to the identification of 102 species units, only 93 have been described as species. Clustering methods provide a valuable approach to identify cryptic species (Martien *et al.*, 2017). For instance, in the bPTP unresolved nodes have less impact in the delimitations estimated in comparison to the GMYC estimations (Tang *et al.*, 2014). Delimitations estimated with PTP have been demonstrated to be equivalent to the ones retrieved using GMYC, or even to outperform GMYC (Zhang *et al.*, 2013). Still, additional morphological characters and multigene sequence data (Ence and Carstens, 2011) within an integrative taxonomy approach are useful to validate delimitations (Padiál *et al.*, 2010; Sauer and Hausdorf, 2012). Thus, the putative species inferred by bPTP can be used as initial hypotheses that should be carefully examined with other complementary approaches. The combination of bPTP and threshold approaches allows a great support for the delimitation of putative species units. Both barcoding approaches suggested the presence of undescribed diversity within WSS reptiles. However, since bPTP method appears to overestimate the number of delimited species units, greater emphasis is given to the delimitations based in the 9.5% threshold approach.

A total of nine candidate species were found in our dataset, of which four were found in the WSS (the snake *Lytrohynchus cf. diadema*, the geckos *Stenodactylus cf. mauritanicus*

Central and *Stenodactylus cf. sthenodactylus*, and the lizard *Latastia cf. longicaudata*), and five were found outside the WSS. A previous study in Colubrid snakes of western Asia retrieved an average of 10% divergence in the cyt-b gene among good colubrid snake' species (Rajabizadeh, 2013). Given that COI evolves at a much slower rate than cyt-b (Laopichienpong *et al.*, 2016; Lavinia *et al.*, 2016), the 10-11% of divergence found between *Lytorhynchus cf. diadema* and *Lytorhynchus diadema* in our study support *Lytorhynchus cf. diadema* as a candidate species. In *Latastia longicaudata*, 16% divergence and no barcoding gap were found which suggests the occurrence of cryptic diversity; a phenomenon frequently reported in several genera of family Lacertidae (Böhme *et al.*, 2006; Pinho, Harris, and Ferrand, 2007). In a revision of the systematics of *Stenodactylus* genus, it was described a new species with genetic divergence between the different mitochondrial markers lower than the one found in the WSS candidate species (Metallinou *et al.*, 2013), which also supports the occurrence of cryptic diversity in the genus in the WSS. This study allowed uncovering cryptic diversity hidden in multiple taxa, highlighting the undescribed reptile diversity in the WSS. Future work should focus on combining mitochondrial and nuclear marks and detailed morphological analyses to investigate the status of the candidate's species here suggested. More than 50% of the species from the sub-order Sauria (commonly named as lizards) were found to exhibit substructure, whereas 80% of the species included in Ophidia sub-order (Serpents) exhibited only one mitochondrial lineage. It has been demonstrated that lineage richness in reptiles is usually asymmetrically distributed, with the majority of clades exhibiting few lineages and only a small fraction having radiated greatly, especially in Families Colubridae and Scincidae (Pincheira-Donoso *et al.*, 2013). The great diversity suggested in these families is supported in our study by the existence of cryptic diversity undescribed within them.

From the 93 mitochondrial lineages recorded in the WSS, *Tropicolotes tripolitanus* was the species where most lineages were recovered and six of the seven lineages occur in the WSS. Such diversity was here uncovered for the first time. All lineages found within the genus *Tropicolotes* were strongly supported in the Bayesian inference, with the exception of *T. tripolitanus* South. Three strongly supported lineages were found in *Tarentola parvicarinata* and *T. chazaliae* in the WSS, which is supported by previous molecular studies based in 12S gene (Melo, 2016). The three lineages found in *Agama boulengeri* and the two lineages found in *A. boueti* were highly supported in the Bayesian inference and the bPTP approach also suggested undescribed diversity. Intraspecific diversity spatially structured has been reported in these species (Gonçalves *et al.*, 2012): 1) in *A. boulengeri*, two clades have been reported (north of Tagant mountains and Assaba mountains), but the present study with larger geographic coverage of sampling detected a third lineage in the Afollé Mountains; 2) in *A. boueti*, three clusters were previously reported, two of them broadly

matching with the West and Central lineages here found. *Ptyodactylus rivapadiali* sp. nov. is a new species currently under description (Trape, unpublished data), which comprises the Mauritanian populations formerly assigned to *Ptyodactylus togoensis* (Metallinou *et al.*, 2015). The results of the current study further support the new specific status for WSS populations. In *Mesalina pasteuri* and *Mesalina guttulata*, two highly divergent and strongly supported lineages were found (7.5% and 9% respectively), which are supported by previous molecular studies in *Mesalina* lizards (Kapli *et al.*, 2015). In *Acanthodactylus boskianus*, two highly supported lineages were found in the WSS and one of them has been described as subspecies, *Acanthodactylus boskianus khattiensis* (Trape, 2012). The two lineages found in *Psammophis schokari* were strongly supported in the Bayesian approach and a recent study based in mitochondrial and nuclear markers also retrieved two lineages with distributions identical to the ones here reported (Gonçalves *et al.*, 2017). Overall, most of the lineages retrieved in this study were supported by other studies based in other mitochondrial markers and/or including nuclear markers.

#### 4.3 Distribution of reptile diversity in West Sahara-Sahel

Some species were found to be distributed exclusively in the Palaearctic region (e.g. *Acanthodactylus taghitensis* and *Mesalina guttulata*), while others were apparently restricted to the Afro-tropic region (e.g. *Bamanophis dorri* and *Psammophis elegans*). A similar pattern was observed in some widespread species that exhibited distinct lineages in the WSS, where one of them occurs in the Palaearctic and the other one occurs in the Afro-tropic region (e.g. *Mesalina pasteuri* and *Psammophis schokari*). The transition zone between the Palaearctic and Afro-tropic biogeographic realms is located within the WSS (Dinerstein *et al.*, 2017). This transition causes a latitudinal variation in species distribution, where turnover of flora and fauna communities can be observed over reduced geographic scales (Brito *et al.*, 2014). The results here found further support the transition character of the WSS.

Our study suggests that some widespread species exhibit geographic substructure in the distribution of genetic diversity. In fact, recent molecular advances have been suggesting that many widespread species are in fact composed by complexes of cryptic species with small and sometimes fragmented distribution areas (Brito *et al.*, 2014). As previously suggested, biodiversity distribution in Sahara-Sahel is spatially structured and is apparently related to environmental variation (Brito *et al.*, 2016). The present work further contradicts the general perception that deserts are uniform areas exhibiting reduced diversity.

Five endemic species to the WSS mountains were found: *Agama boulengeri*, *Mesalina* sp. nov., *Pristurus adrarensis*, *Ptyodactylus rivapadiali* sp. nov., and *Tarentola parvicarinata*. The biological value of Sahara-Sahel Mountains has been emphasized, given that they display isolated suitable areas for mesic and aquatic species surrounded by very harsh

environments (Brito *et al.*, 2014). They also play a key role in the diversification patterns across the Sahara-Sahel by serving as refugia for several species and facilitating gene flow during favourable climatic conditions. Previous studies also showed that mountains host more than half of all the Sahara-Sahel endemic vertebrates and harbour isolated populations for almost half (45%) of the vertebrate species with non-Saharan origin (Brito *et al.*, 2014). The present study further supports the important biological role of mountains as refugia and as local biodiversity hotspots.

The present study clearly illustrates that most reptile richness is concentrated in WSS Mountains, in particular the southern Assaba Mountain that contains the highest relative richness observed in all taxonomic levels examined. Analyses of the distribution of 1147 terrestrial Sahara-Sahel vertebrates also found concentrations of species richness in the southern Sahel and the north-western Sahara (Brito *et al.*, 2016). Likewise, previous studies on specific taxa also identified the central Sahara-Sahel mountains as containing high levels of species richness (Patiny *et al.*, 2009; García, Cuttelod, and Malak 2010; Anthelme, Abdoukader, and Viane 2011; Sow *et al.*, 2014; Vale, Pimm, and Brito 2015).

The gap analysis suggested that only a small fraction (about 3.1%) of the WSS is protected. Previous studies showed that biodiversity conservation in the Sahara-Sahel has been generally neglected (Davies *et al.*, 2012; Durant *et al.*, 2012; Ezcurra, 2006). Even more worrying is the fact that most of the WSS regions concentrating the highest reptile richness are not at all represented in the small portion currently protected, such as the Assaba Mountain. In the case of the Dhar Chinguetti Mountain, the protected area of Guelb er Richât National Park is still under discussion and not fully implemented. The Tagant Mountain is partly contained within the Lac Gabbou RAMSAR site (Tellería, 2009) but there is no formal protection of species or habitats, and there are no functional management plans. Clearly, a reserve network should be considered for mountains to maximise the representation and persistence of Mauritanian reptile biodiversity in protected areas.

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## 6. Annexes

Table A 1. Specimen identification success of the 422 haplotypes obtained, with the different distance based approaches.

Species	Near Neighbour	NearNeighbour Names=TRUE	threshl D	threshID threshold = 0.1	Best Close Match	BOLD Best ID
<i>Acanthodactylus boskianus</i>	TRUE	Acanthodactylus boskianus	correct	correct	correct	No match
<i>Acanthodactylus boskianus</i>	TRUE	Acanthodactylus boskianus	correct	correct	correct	No match
<i>Acanthodactylus boskianus</i>	TRUE	Acanthodactylus boskianus	correct	correct	correct	No match
<i>Acanthodactylus boskianus</i>	TRUE	Acanthodactylus boskianus	no id	correct	no id	No match
<i>Acanthodactylus boskianus</i>	TRUE	Acanthodactylus boskianus	no id	correct	no id	No match
<i>Acanthodactylus boskianus</i>	TRUE	Acanthodactylus boskianus	no id	correct	no id	No match
<i>Acanthodactylus boskianus</i>	TRUE	Acanthodactylus boskianus	correct	correct	correct	No match
<i>Acanthodactylus boskianus</i>	TRUE	Acanthodactylus boskianus	no id	correct	no id	No match
<i>Acanthodactylus busacki</i>	TRUE	Acanthodactylus busacki	correct	correct	correct	No match
<i>Acanthodactylus busacki</i>	TRUE	Acanthodactylus busacki	correct	correct	correct	No match
<i>Acanthodactylus busacki</i>	TRUE	Acanthodactylus busacki	correct	correct	correct	No match
<i>Acanthodactylus dumerilii</i>	TRUE	Acanthodactylus dumerilii	no id	correct	no id	No match
<i>Acanthodactylus dumerilii</i>	TRUE	Acanthodactylus dumerilii	no id	correct	no id	No match
<i>Acanthodactylus longipes</i>	FALSE	Acanthodactylus dumerilii	no id	no id	no id	No match
<i>Acanthodactylus maculatus</i>	TRUE	Acanthodactylus maculatus	no id	correct	no id	No match
<i>Acanthodactylus maculatus</i>	TRUE	Acanthodactylus maculatus	no id	correct	no id	No match
<i>Acanthodactylus margaritae</i>	FALSE	Acanthodactylus busacki	no id	no id	no id	No match
<i>Acanthodactylus sp.</i>	FALSE	Acanthodactylus margaritae	no id	no id	no id	No match
<i>Acanthodactylus taghitensis</i>	TRUE	Acanthodactylus taghitensis	correct	correct	correct	No match
<i>Acanthodactylus taghitensis</i>	TRUE	Acanthodactylus taghitensis	correct	correct	correct	No match
<i>Agama agama</i>	TRUE	Agama agama	no id	correct	no id	No match
<i>Agama agama</i>	TRUE	Agama agama	no id	correct	no id	No match
<i>Agama agama</i>	TRUE	Agama agama	correct	correct	correct	No match
<i>Agama agama</i>	TRUE	Agama agama	correct	correct	correct	No match
<i>Agama agama</i>	TRUE	Agama agama	correct	correct	correct	No match
<i>Agama agama</i>	TRUE	Agama agama	no id	correct	no id	No match
<i>Agama agama</i>	TRUE	Agama agama	correct	correct	correct	No match
<i>Agama agama</i>	TRUE	Agama agama	correct	correct	correct	No match
<i>Agama agama</i>	TRUE	Agama agama	no id	correct	no id	No match
<i>Agama agama</i>	TRUE	Agama agama	no id	correct	no id	No match
<i>Agama boensis</i>	TRUE	Agama boensis	no id	correct	no id	No match
<i>Agama boensis</i>	TRUE	Agama boensis	correct	correct	correct	No match
<i>Agama boensis</i>	TRUE	Agama boensis	correct	correct	correct	No match



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<i>Cerastes cerastes</i>	TRUE	Cerastes cerastes	correct	correct	correct	No match
<i>Cerastes cerastes</i>	TRUE	Cerastes cerastes	correct	correct	correct	No match
<i>Cerastes cerastes</i>	TRUE	Cerastes cerastes	correct	correct	correct	No match
<i>Cerastes cerastes</i>	TRUE	Cerastes cerastes	correct	correct	correct	No match
<i>Cerastes cerastes</i>	TRUE	Cerastes cerastes	correct	correct	correct	No match
<i>Cerastes cerastes</i>	TRUE	Cerastes cerastes	correct	correct	correct	No match
<i>Cerastes vipera</i>	TRUE	Cerastes vipera	no id	correct	no id	No match
<i>Cerastes vipera</i>	TRUE	Cerastes vipera	no id	correct	no id	No match
<i>Chalcides boulengeri</i>	TRUE	Chalcides boulengeri	no id	correct	no id	No match
<i>Chalcides boulengeri</i>	TRUE	Chalcides boulengeri	no id	correct	no id	No match
<i>Chalcides boulengeri</i>	TRUE	Chalcides boulengeri	no id	correct	no id	No match
<i>Chalcides colosii</i>	FALSE	Chalcides parallelus	no id	incorrect	no id	No match
<i>Chalcides delislei</i>	FALSE	Chalcides sphenopsiformis	no id	incorrect	no id	No match
<i>Chalcides delislei</i>	FALSE	Chalcides sphenopsiformis	no id	no id	no id	No match
<i>Chalcides ocellatus</i>	FALSE	Chalcides parallelus	no id	no id	no id	No match
<i>Chalcides parallelus</i>	FALSE	Chalcides colosii	no id	incorrect	no id	No match
<i>Chalcides pseudostriatus</i>	FALSE	Chalcides boulengeri	no id	no id	no id	No match
<i>Chalcides sphenopsiformis</i>	TRUE	Chalcides sphenopsiformis	no id	ambiguous	no id	No match
<i>Chalcides sphenopsiformis</i>	TRUE	Chalcides sphenopsiformis	no id	ambiguous	no id	No match
<i>Chalcides sphenopsiformis</i>	TRUE	Chalcides sphenopsiformis	no id	ambiguous	no id	No match
<i>Chalcides sphenopsiformis</i>	TRUE	Chalcides sphenopsiformis	correct	ambiguous	correct	No match
<i>Chalcides sphenopsiformis</i>	TRUE	Chalcides sphenopsiformis	no id	ambiguous	no id	No match
<i>Chalcides sphenopsiformis</i>	TRUE	Chalcides sphenopsiformis	correct	ambiguous	correct	No match
<i>Chamaeleo africanus</i>	TRUE	Chamaeleo africanus	correct	correct	correct	Chamaeleo africanus
<i>Chamaeleo africanus</i>	TRUE	Chamaeleo africanus	correct	correct	correct	Chamaeleo africanus
<i>Chamaeleo africanus</i>	TRUE	Chamaeleo africanus	correct	correct	correct	Chamaeleo africanus
<i>Chamaeleo chamaeleon</i>	TRUE	Chamaeleo chamaeleon	correct	correct	correct	Chamaeleo chamaeleon
<i>Chamaeleo chamaeleon</i>	TRUE	Chamaeleo chamaeleon	correct	correct	correct	Chamaeleo chamaeleon
<i>Chamaeleo gracilis</i>	FALSE	Chamaeleo africanus	no id	no id	no id	No match
<i>Crocodylus suchus</i>	FALSE	Acanthodactylus boskianus	no id	no id	no id	Crocodylus niloticus
<i>Crotaphopeltis hotamboeia</i>	FALSE	Telescopus fallax	no id	no id	no id	No match
<i>Dasypeltis scabra</i>	FALSE	Lytorhynchus diadema	no id	no id	no id	No match
<i>Echis ocellatus</i>	FALSE	Echis pyramidum	no id	no id	no id	No match
<i>Echis pyramidum</i>	TRUE	Echis pyramidum	correct	correct	correct	No match
<i>Echis pyramidum</i>	TRUE	Echis pyramidum	no id	correct	no id	No match
<i>Echis pyramidum</i>	TRUE	Echis pyramidum	correct	correct	correct	No match
<i>Echis pyramidum</i>	TRUE	Echis pyramidum	correct	correct	correct	No match
<i>Echis pyramidum</i>	TRUE	Echis pyramidum	correct	correct	correct	No match
<i>Eumeces algeriensis</i>	FALSE	Scincopus fasciatus	no id	no id	no id	No match
<i>Gongylophis jaculus</i>	FALSE	Spalerosophis diadema	no id	no id	no id	No match

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<i>Gongylophis muelleri</i>	FALSE	Psammophis aegyptius	no id	no id	no id	No match
<i>Hemidactylus angulatus</i>	TRUE	Hemidactylus angulatus	correct	correct	correct	No match
<i>Hemidactylus angulatus</i>	TRUE	Hemidactylus angulatus	correct	correct	correct	No match
<i>Hemidactylus angulatus</i>	TRUE	Hemidactylus angulatus	correct	correct	correct	No match
<i>Hemidactylus angulatus</i>	TRUE	Hemidactylus angulatus	no id	correct	no id	No match
<i>Hemidactylus angulatus</i>	TRUE	Hemidactylus angulatus	correct	correct	correct	No match
<i>Hemidactylus angulatus</i>	TRUE	Hemidactylus angulatus	correct	correct	correct	No match
<i>Hemidactylus angulatus</i>	TRUE	Hemidactylus angulatus	correct	correct	correct	No match
<i>Hemidactylus angulatus</i>	TRUE	Hemidactylus angulatus	no id	correct	no id	No match
<i>Hemorrhhois algirus</i>	TRUE	Hemorrhhois algirus	correct	correct	correct	No match
<i>Hemorrhhois algirus</i>	TRUE	Hemorrhhois algirus	no id	correct	no id	No match
<i>Hemorrhhois algirus</i>	TRUE	Hemorrhhois algirus	no id	correct	no id	No match
<i>Hemorrhhois algirus</i>	TRUE	Hemorrhhois algirus	correct	correct	correct	No match
<i>Hemorrhhois algirus</i>	TRUE	Hemorrhhois algirus	correct	correct	correct	No match
<i>Hemorrhhois algirus</i>	TRUE	Hemorrhhois algirus	correct	correct	correct	No match
<i>Hemorrhhois algirus</i>	TRUE	Hemorrhhois algirus	correct	correct	correct	Hemorrhhois algirus
<i>Hemorrhhois algirus</i>	TRUE	Hemorrhhois algirus	correct	correct	correct	No match
<i>Hemorrhhois algirus</i>	TRUE	Hemorrhhois algirus	correct	correct	correct	Hemorrhhois algirus
<i>Hemorrhhois hippocrepis</i>	FALSE	Hemorrhhois algirus	no id	no id	no id	Hemorrhhois hippocrepis
<i>Indotyphlops braminus</i>	TRUE	Indotyphlops braminus	correct	correct	correct	Indotyphlops braminus
<i>Indotyphlops braminus</i>	TRUE	Indotyphlops braminus	correct	correct	correct	Indotyphlops braminus
<i>Latastia longicaudata</i>	FALSE	Trachylepis perrotetii	no id	no id	no id	No match
<i>Latastia longicaudata</i>	TRUE	Latastia longicaudata	no id	correct	no id	No match
<i>Latastia longicaudata</i>	TRUE	Latastia longicaudata	no id	correct	no id	No match
<i>Lytorhynchus diadema</i>	TRUE	Lytorhynchus diadema	no id	correct	no id	No match
<i>Lytorhynchus diadema</i>	TRUE	Lytorhynchus diadema	no id	correct	no id	No match
<i>Lytorhynchus diadema</i>	TRUE	Lytorhynchus diadema	no id	correct	no id	No match
<i>Lytorhynchus diadema</i>	TRUE	Lytorhynchus diadema	no id	no id	no id	No match
<i>Mesalina guttulata</i>	TRUE	Mesalina guttulata	no id	correct	no id	No match
<i>Mesalina guttulata</i>	TRUE	Mesalina guttulata	no id	correct	no id	No match
<i>Mesalina guttulata</i>	TRUE	Mesalina guttulata	correct	correct	correct	No match
<i>Mesalina guttulata</i>	TRUE	Mesalina guttulata	no id	correct	no id	No match
<i>Mesalina guttulata</i>	TRUE	Mesalina guttulata	correct	correct	correct	No match
<i>Mesalina olivieri</i>	TRUE	Mesalina olivieri	correct	ambiguous	correct	No match
<i>Mesalina olivieri</i>	TRUE	Mesalina olivieri	correct	ambiguous	correct	No match
<i>Mesalina olivieri</i>	TRUE	Mesalina olivieri	no id	ambiguous	no id	No match
<i>Mesalina olivieri</i>	TRUE	Mesalina olivieri	correct	ambiguous	correct	No match
<i>Mesalina pasteuri</i>	TRUE	Mesalina pasteuri	correct	correct	correct	No match
<i>Mesalina pasteuri</i>	TRUE	Mesalina pasteuri	correct	ambiguous	correct	No match
<i>Mesalina pasteuri</i>	TRUE	Mesalina pasteuri	correct	ambiguous	correct	No match

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<i>Mesalina pasteuri</i>	TRUE	Mesalina pasteuri	correct	ambiguous	correct	No match
<i>Mesalina pasteuri</i>	TRUE	Mesalina pasteuri	correct	ambiguous	correct	No match
<i>Mesalina rubropunctata</i>	TRUE	Mesalina rubropunctata	no id	correct	no id	No match
<i>Mesalina rubropunctata</i>	TRUE	Mesalina rubropunctata	no id	correct	no id	No match
<i>Mesalina sp. nov.</i>	TRUE	Mesalina sp. nov.	correct	ambiguous	correct	No match
<i>Mesalina sp. nov.</i>	TRUE	Mesalina sp. nov.	correct	ambiguous	correct	No match
<i>Mesalina sp. nov.</i>	TRUE	Mesalina sp. nov.	no id	ambiguous	no id	No match
<i>Mesalina sp. nov.</i>	TRUE	Mesalina sp. nov.	correct	ambiguous	correct	No match
<i>Mesalina sp. nov.</i>	TRUE	Mesalina sp. nov.	correct	ambiguous	correct	No match
<i>Mesalina sp. nov.</i>	TRUE	Mesalina sp. nov.	correct	ambiguous	correct	No match
<i>Mesalina sp. nov.</i>	TRUE	Mesalina sp. nov.	correct	ambiguous	correct	No match
<i>Mesalina sp. nov.</i>	TRUE	Mesalina sp. nov.	no id	ambiguous	no id	No match
<i>Mesalina sp. nov.</i>	TRUE	Mesalina sp. nov.	correct	ambiguous	correct	No match
<i>Mesalina sp. nov.</i>	TRUE	Mesalina sp. nov.	no id	ambiguous	no id	No match
<i>Myriopholis algeriensis</i>	FALSE	Python sebae	no id	no id	no id	No match
<i>Naja haje</i>	TRUE	Naja haje	correct	correct	correct	No match
<i>Naja haje</i>	TRUE	Naja haje	correct	correct	correct	No match
<i>Pristurus adrarensis</i>	TRUE	Pristurus adrarensis	correct	correct	correct	No match
<i>Pristurus adrarensis</i>	TRUE	Pristurus adrarensis	correct	correct	correct	No match
<i>Psammophis aegyptius</i>	FALSE	Psammophis schokari	no id	no id	no id	No match
<i>Psammophis cf. rukwae</i>	TRUE	Psammophis cf. rukwae	correct	correct	correct	No match
<i>Psammophis cf. rukwae</i>	TRUE	Psammophis cf. rukwae	correct	correct	correct	No match
<i>Psammophis cf. rukwae</i>	TRUE	Psammophis cf. rukwae	correct	correct	correct	No match
<i>Psammophis cf. rukwae</i>	TRUE	Psammophis cf. rukwae	no id	correct	no id	No match
<i>Psammophis cf. rukwae</i>	TRUE	Psammophis cf. rukwae	correct	correct	correct	No match
<i>Psammophis cf. rukwae</i>	TRUE	Psammophis cf. rukwae	no id	correct	no id	No match
<i>Psammophis cf. rukwae</i>	TRUE	Psammophis cf. rukwae	correct	correct	correct	No match
<i>Psammophis elegans</i>	TRUE	Psammophis elegans	correct	correct	correct	No match
<i>Psammophis elegans</i>	TRUE	Psammophis elegans	correct	correct	correct	No match
<i>Psammophis elegans</i>	TRUE	Psammophis elegans	correct	correct	correct	No match
<i>Psammophis elegans</i>	TRUE	Psammophis elegans	correct	correct	correct	No match
<i>Psammophis elegans</i>	TRUE	Psammophis elegans	correct	correct	correct	No match
<i>Psammophis mossambicus</i>	FALSE	Psammophis cf. rukwae	no id	no id	no id	No match
<i>Psammophis schokari</i>	TRUE	Psammophis schokari	correct	correct	correct	No match
<i>Psammophis schokari</i>	TRUE	Psammophis schokari	correct	correct	correct	No match
<i>Psammophis schokari</i>	TRUE	Psammophis schokari	correct	correct	correct	No match
<i>Psammophis schokari</i>	TRUE	Psammophis schokari	correct	correct	correct	No match
<i>Psammophis schokari</i>	TRUE	Psammophis schokari	correct	correct	correct	No match
<i>Psammophis schokari</i>	TRUE	Psammophis schokari	no id	correct	no id	No match
<i>Psammophis schokari</i>	TRUE	Psammophis schokari	correct	correct	correct	No match
<i>Psammophis schokari</i>	TRUE	Psammophis schokari	correct	correct	correct	No match

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<i>Psammophis schokari</i>	TRUE	Psammophis schokari	correct	correct	correct	No match
<i>Psammophis schokari</i>	TRUE	Psammophis schokari	no id	correct	no id	No match
<i>Psammophis schokari</i>	TRUE	Psammophis schokari	correct	correct	correct	No match
<i>Psammophis schokari</i>	TRUE	Psammophis schokari	no id	correct	no id	No match
<i>Psammophis schokari</i>	TRUE	Psammophis schokari	correct	correct	correct	No match
<i>Ptyodactylus oudrii</i>	TRUE	Ptyodactylus oudrii	no id	no id	no id	No match
<i>Ptyodactylus oudrii</i>	TRUE	Ptyodactylus oudrii	no id	no id	no id	No match
<i>Ptyodactylus togoensis</i>	TRUE	Ptyodactylus togoensis	correct	correct	correct	No match
<i>Ptyodactylus togoensis</i>	TRUE	Ptyodactylus togoensis	correct	correct	correct	No match
<i>Ptyodactylus togoensis</i>	TRUE	Ptyodactylus togoensis	correct	correct	correct	No match
<i>Ptyodactylus togoensis</i>	TRUE	Ptyodactylus togoensis	correct	correct	correct	No match
<i>Ptyodactylus togoensis</i>	TRUE	Ptyodactylus togoensis	correct	correct	correct	No match
<i>Ptyodactylus togoensis</i>	TRUE	Ptyodactylus togoensis	correct	correct	correct	No match
<i>Ptyodactylus togoensis</i>	TRUE	Ptyodactylus togoensis	no id	correct	no id	No match
<i>Ptyodactylus togoensis</i>	TRUE	Ptyodactylus togoensis	correct	correct	correct	No match
<i>Ptyodactylus togoensis</i>	TRUE	Ptyodactylus togoensis	correct	correct	correct	No match
<i>Ptyodactylus togoensis</i>	TRUE	Ptyodactylus togoensis	no id	correct	no id	No match
<i>Ptyodactylus togoensis</i>	TRUE	Ptyodactylus togoensis	correct	correct	correct	No match
<i>Ptyodactylus togoensis</i>	TRUE	Ptyodactylus togoensis	correct	correct	correct	No match
<i>Ptyodactylus togoensis</i>	TRUE	Ptyodactylus togoensis	correct	correct	correct	No match
<i>Ptyodactylus togoensis</i>	TRUE	Ptyodactylus togoensis	correct	correct	correct	No match
<i>Ptyodactylus togoensis</i>	TRUE	Ptyodactylus togoensis	correct	correct	correct	No match
<i>Ptyodactylus togoensis</i>	TRUE	Ptyodactylus togoensis	correct	correct	correct	No match
<i>Ptyodactylus togoensis</i>	TRUE	Ptyodactylus togoensis	correct	correct	correct	No match
<i>Ptyodactylus togoensis</i>	TRUE	Ptyodactylus togoensis	correct	correct	correct	No match
<i>Python sebae</i>	TRUE	Python sebae	correct	correct	correct	No match
<i>Python sebae</i>	TRUE	Python sebae	correct	correct	correct	No match
<i>Rhagerhis moilensis</i>	FALSE	Psammophis cf. rukwae	no id	no id	no id	No match
<i>Rhagerhis moilensis</i>	TRUE	Rhagerhis moilensis	correct	correct	correct	No match
<i>Rhagerhis moilensis</i>	TRUE	Rhagerhis moilensis	correct	correct	correct	No match
<i>Rhagerhis moilensis</i>	TRUE	Rhagerhis moilensis	correct	correct	correct	No match
<i>Rhagerhis moilensis</i>	TRUE	Rhagerhis moilensis	correct	correct	correct	No match
<i>Rhagerhis moilensis</i>	TRUE	Rhagerhis moilensis	no id	correct	no id	No match
<i>Scincopus fasciatus</i>	FALSE	Eumeces algeriensis	no id	no id	no id	No match
<i>Scincus albifasciatus</i>	TRUE	Scincus albifasciatus	no id	correct	no id	No match
<i>Scincus albifasciatus</i>	TRUE	Scincus albifasciatus	no id	correct	no id	No match
<i>Scincus albifasciatus</i>	TRUE	Scincus albifasciatus	no id	correct	no id	No match
<i>Spalerosophis diadema</i>	TRUE	Spalerosophis diadema	correct	ambiguous	correct	Spalerosophis diadema
<i>Spalerosophis diadema</i>	TRUE	Spalerosophis diadema	correct	ambiguous	correct	Spalerosophis diadema
<i>Spalerosophis diadema</i>	TRUE	Spalerosophis diadema	correct	ambiguous	correct	Spalerosophis diadema









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<i>Tropicolotes tripolitanus</i>	TRUE	Tropicolotes tripolitanus	correct	correct	correct	No match
<i>Tropicolotes tripolitanus</i>	TRUE	Tropicolotes tripolitanus	correct	correct	correct	Tropicolotes tripolitanus
<i>Tropicolotes tripolitanus</i>	TRUE	Tropicolotes tripolitanus	correct	correct	correct	No match
<i>Tropicolotes tripolitanus</i>	TRUE	Tropicolotes tripolitanus	no id	correct	no id	No match
<i>Tropicolotes tripolitanus</i>	TRUE	Tropicolotes tripolitanus	correct	correct	correct	No match
<i>Tropicolotes tripolitanus</i>	TRUE	Tropicolotes tripolitanus	correct	correct	correct	Tropicolotes tripolitanus
<i>Tropicolotes tripolitanus</i>	TRUE	Tropicolotes tripolitanus	no id	correct	no id	No match
<i>Tropicolotes tripolitanus</i>	TRUE	Tropicolotes tripolitanus	correct	correct	correct	No match
<i>Tropicolotes tripolitanus</i>	TRUE	Tropicolotes tripolitanus	correct	correct	correct	No match
<i>Tropicolotes tripolitanus</i>	TRUE	Tropicolotes tripolitanus	correct	correct	correct	No match
<i>Tropicolotes tripolitanus</i>	TRUE	Tropicolotes tripolitanus	correct	correct	correct	No match
<i>Uromastyx acanthinura</i>	FALSE	Uromastyx dispar	no id	incorrect	no id	No match
<i>Uromastyx dispar</i>	TRUE	Uromastyx dispar	correct	ambiguous	correct	No match
<i>Uromastyx dispar</i>	TRUE	Uromastyx dispar	correct	ambiguous	correct	No match
<i>Uromastyx dispar</i>	TRUE	Uromastyx dispar	correct	ambiguous	correct	No match
<i>Uromastyx dispar</i>	TRUE	Uromastyx dispar	correct	ambiguous	correct	No match
<i>Uromastyx dispar</i>	TRUE	Uromastyx dispar	correct	ambiguous	correct	No match
<i>Uromastyx dispar</i>	TRUE	Uromastyx dispar	correct	ambiguous	correct	No match
<i>Uromastyx dispar</i>	TRUE	Uromastyx dispar	correct	ambiguous	correct	No match
<i>Uromastyx dispar</i>	TRUE	Uromastyx dispar	correct	ambiguous	correct	No match
<i>Uromastyx dispar</i>	TRUE	Uromastyx dispar	correct	ambiguous	correct	No match
<i>Uromastyx geyri</i>	FALSE	Uromastyx nigriventris	no id	incorrect	no id	No match
<i>Uromastyx nigriventris</i>	TRUE	Uromastyx nigriventris	correct	ambiguous	correct	No match
<i>Uromastyx nigriventris</i>	TRUE	Uromastyx nigriventris	correct	ambiguous	correct	No match
<i>Varanus griseus</i>	TRUE	Varanus griseus	correct	correct	correct	No match
<i>Varanus griseus</i>	TRUE	Varanus griseus	correct	correct	correct	No match
<i>Varanus griseus</i>	TRUE	Varanus griseus	correct	correct	correct	No match
<i>Varanus griseus</i>	TRUE	Varanus griseus	correct	correct	correct	No match
<i>Varanus niloticus</i>	TRUE	Varanus niloticus	correct	correct	correct	Varanus niloticus
<i>Varanus niloticus</i>	TRUE	Varanus niloticus	correct	correct	correct	Varanus niloticus
<i>Varanus niloticus</i>	TRUE	Varanus niloticus	correct	correct	correct	Varanus niloticus
<i>Varanus niloticus</i>	TRUE	Varanus niloticus	correct	correct	correct	Varanus niloticus
<i>Varanus niloticus</i>	TRUE	Varanus niloticus	correct	correct	correct	Varanus niloticus

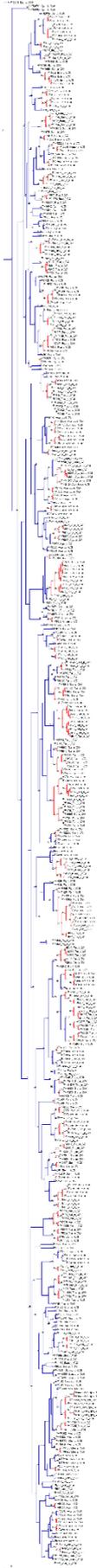


Figure A1. Species delimitation of reptiles by Bayesian Poisson tree processes (bPTP) on the Bayesian COI tree.

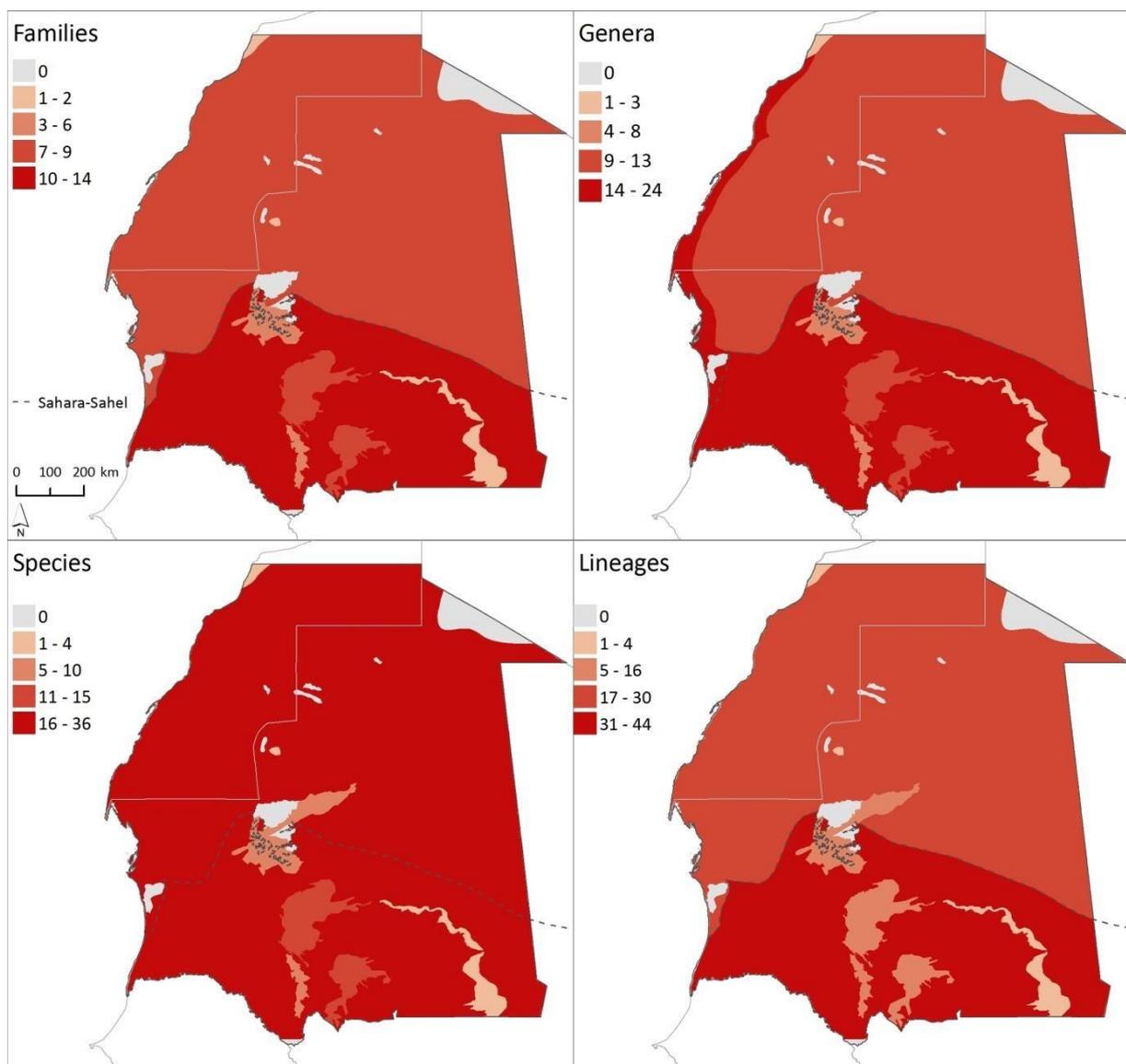


Figure A2. Distribution maps of richness of families, genera, species and lineages in each studied region. Grey zones (excluded) indicate regions where no taxa were detected.