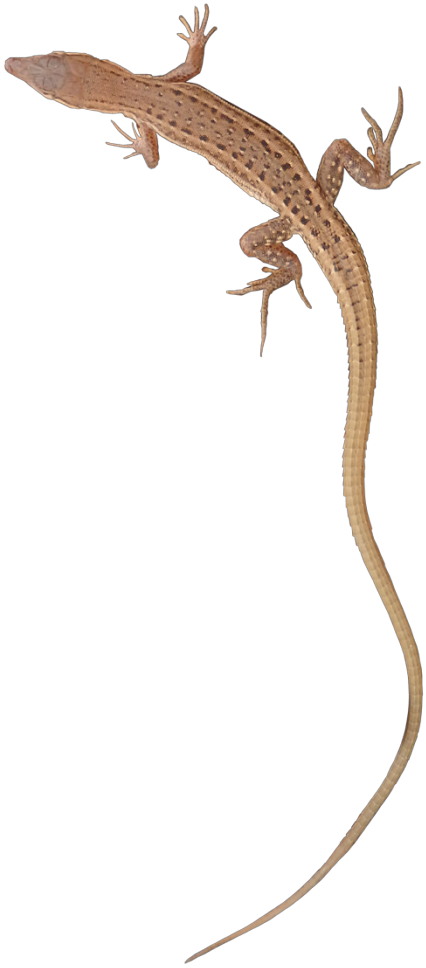

# Phylogeography and systematics of the *Mesalina olivieri* species complex (Squamata: Lacertidae) from North-West Africa


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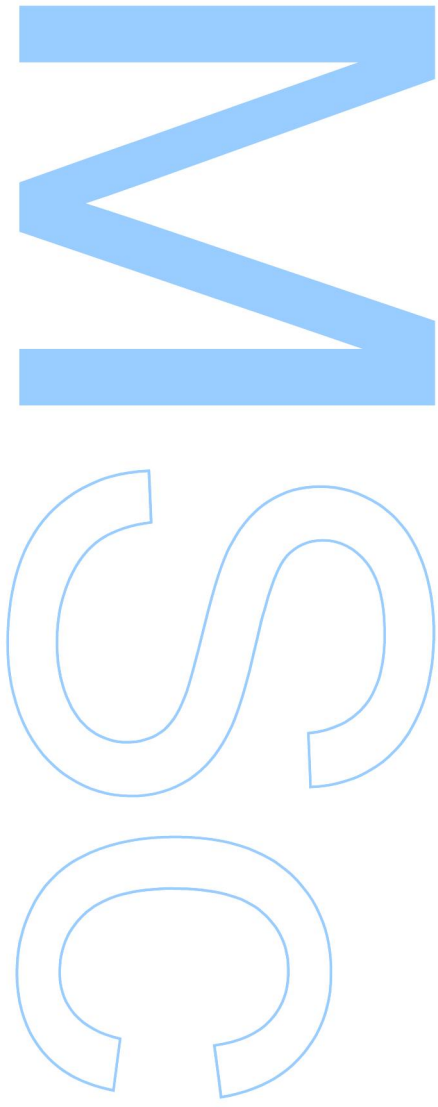

To my parents

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# Abstract

*Mesalina* is a genus of small xeric lizards currently comprising 19 species distributed from West Africa throughout the Saharo-Sindian deserts to the Indo-Iranian plateau. Previous phylogenetic studies highlighted the presence of cryptic diversity within the genus and described new *Mesalina* species from its eastern lineages. In this study, we investigated the taxonomy and systematics within the *Mesalina olivieri* species complex, focusing on the Atlantic Sahara (from Morocco to Mauritania). The species complex is currently represented by three recognised species, all of them present in this region: *M. olivieri*, *M. pasteuri* and *M. simoni*. Using an integrative taxonomy approach based on morphological (pholidotic, coloration and pattern) and molecular (one mtDNA and four nuDNA markers) datasets, we provide robust evidences for the existence of additional taxa within the *M. olivieri* complex is provided, including an undescribed species in Mauritania. *Mesalina* sp. nov. . All *M. olivieri* that cluster together with *M. simoni* were proposed to be included as subspecies of the latter (*M. simoni* ssp. nov.). The clade including *Mesalina* sp. nov. and *M. simoni* diverged from *M. olivieri* and *M. pasteuri* around 9.5 Mya whereas these two-latter species separated 1 or 2 Mya later in the end of the Miocene. The combined analyses supported a new classification of the *Mesalina olivieri* species complex into four extant species. The new species is sympatric with *M. pasteuri* in Mauritania but it is phylogenetically and morphologically divergent from it. Species distribution modelling suggests that the new taxon occurs exclusively in the rocky areas of the Adrar Atar plateau and neighbouring regions. The relatively wide but fragmented distribution of *Mesalina* sp. nov. suggests that its conservation status is Least Concern (LC).

# Keywords

Adrar Atar, Atlantic Sahara, cytonuclear discordance, Ecological model, Integrative Taxonomy

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# Resumo

*Mesalina* é um género de pequenos lagartos xéricos, actualmente constituídos por 19 espécies distribuídas desde a África Ocidental pelos desertos Saara-indianos até ao planalto Indo-Iraniano. Estudos filogenéticos anteriores destacaram a presença de diversidade críptica dentro do género e novas espécies de *Mesalina* foram descritas das linhagens orientais. Neste estudo, investigou-se a taxonomia e a sistemática no complexo de espécies *Mesalina olivieri*, com foco no Saara Atlântico (de Marrocos à Mauritânia). Actualmente, o complexo de espécies é representado por três espécies reconhecidas, todas presentes nesta região: *M. olivieri*, *M. pasteuri* e *M. simoni*. Utilizando uma abordagem de taxonomia integrativa baseada em conjuntos de dados morfológicos (folidose, coloração e padrão) e moleculares (um marcador mtDNA e quatro marcadores nuDNA), são fornecidas evidências robustas da existência de taxa adicionais no complexo *M. olivieri*, incluindo uma espécie não descrita na Mauritânia. *Mesalina* sp. nov. Além disso, a parafilia taxonómica em *M. olivieri* foi resolvida. Todos os indivíduos de *M. olivieri* que agrupam com *M. simoni* foram propostos para serem incluídos como subespécies deste último táxon (*M. simoni* ssp. Nov.). O clado incluindo *Mesalina* sp. nov. e *M. simoni* divergiu de *M. olivieri* e *M. pasteuri* há cerca de 9,5 Ma, enquanto as duas últimas espécies separaram-se cerca de 1 ou 2 Ma depois, no final do Mioceno. As análises combinadas sustentaram uma nova classificação do complexo *Mesalina olivieri* em quatro espécies. A nova espécie é simpática com *M. pasteuri* na Mauritânia mas é filogenética e morfologicamente divergente. A modelação da distribuição sugere que o novo táxon ocorre exclusivamente nas áreas rochosas do planalto de Adrar Atar e nas regiões vizinhas. A distribuição relativamente ampla, porém fragmentada, de *Mesalina* sp. nov. sugere que o estatuto de conservação seja Pouco Preocupante (LC).

# Palavras-chave

Adrar Atar, discordância citonuclear, Modelo ecológico, Saara Atlântico, Taxonomia Integrativa

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# List of Abbreviations

| | |
|---|---|
| **BSP** | Bayesian Skyline plots |
| **B-fib7** | beta fibrinogen intron 7 gene |
| **CCS** | Confirmed Candidate Species |
| **Cyt-b** | Cytochrome b gene |
| **DCL** | Deep Conspecific Lineage |
| **ENM** | Ecological Niche-based Model |
| **ESU** | Evolutionary Significant Units |
| **HPD** | High posterior density |
| **Indel** | Insertion-Deletion |
| **MC1R** | Melanocortin receptor 1 gene |
| **Mya** | Million years ago |
| **mtDNA** | Mitochondrial DNA |
| **MU** | Management Unit |
| **nDNA** | Nuclear DNA |
| **OD** | ornithine decarboxylase gene |
| **OXPHOS** | Oxidative Phosphorylation |
| **PCA** | Principal Component Analysis |
| **PCR** | Polymerase chain reaction |
| **PgD7** | Phosphogluconase dehydrogenase intron 7 gene |
| **PP** | Posterior Probability |
| **scnDNA** | Single-copy nuclear DNA |
| **sp. nov.** | specie nova |
| **ssp. nov.** | subspecies nova |
| **s.s.** | Sensu stricto |
| **UCS** | Unconfirmed Candidate Species |
| **WSS** | West Sahara-Sahel |

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# 1. INTRODUCTION

## 1.1 GLOBAL BIODIVERSITY DECLINE

Biodiversity is defined as the diversity among living organisms based on the hereditarily variation at all levels of organization, from the genes within a single local population or species, to the species composing all or part of a local community, and to the communities themselves that compose the living parts of Earth's ecosystems (Wilson, 1997). Biodiversity is fundamental to ecosystem functioning since it regulates its stability, its fertility, the vulnerability to possible invasive species, and ensures the supplying of all its ecosystem services. There are numerous examples of ecosystem services provided by environments with high biodiversity levels at multiple scales, from the support of the hydrological cycles in the Amazonian forest that are fundamental for water supply and temperature control for most of South America (i.e. with providing pure water, hydroelectric power, and diminish temperature increases; Nobre *et al*., 1991), to the Mexican Long-Tongued Bat (*Choeronycteris mexicana*) that pollinate agave plants from which tequila is produced (Flores-Abreu *et al.,* 2019).

Biodiversity is facing a decline that aggravated in the last decades (Butchard *et al.,* 2010). While previous mass extinctions were caused by catastrophic geological events and consequent climate changes distributed over a vast geological time scale (Hallam & Hallam, 2005), human footprint is currently leading the sixth massive extinction process that will be the faster in the history of this planet (Singh, 2002; UN General Assembly, 2019). Long is the list of the anthropogenic activities that affect biodiversity, such as invasive species introduction, overfishing, bush-meat overhunting, warfare, pollution and greenhouse gasses (Stork, 1997; Hoffmann *et al.,* 2010; Barnosky *et al.,* 2011; Tittensor *et al.,* 2014; Brito *et al.,* 2018). However, the most important causes of biodiversity decline are habitat loss and fragmentation together with global warming and climate change (Stork, 1997; Pimm, 2008).

According to the *Catalogue of Life* (2018) around 8.7 million of species are now living on our planet and just 20% of them have been described, of which the highest concentration is recorded in the tropical biomes (Mace, 2005). Biodiversity hotspots are high priority conservation areas (Reid, 1998) selected based on their species richness and percentage of rare species concentration (Myers *et al.*, 2000). At both local and global scales, quantifying species richness, their distribution and extinction rate is therefore a priority measure to safeguard the biodiversity of an ecosystem and of the services provided by it. Species identification is especially important for conservations purposes given that it allows the identification of Conservation Units that will help to preserve the species themselves, the ecological role that it carry out, and the ecosystem to which they belong

(Mace, 2005). To this respect, the International Union for Conservation of Nature's guidelines for the Red List of Threatened Species (IUCN Red List) allows to track the health of global biodiversity by providing information about the range, population size, habitat and ecology, use and/or trade, threats, and conservation actions that will help inform necessary conservation decisions and policy options that are critical to protect the natural resources we need to survive (IUCN, 2019).

## 1.2 INTEGRATIVE TAXONOMY

The definition of a species is one of the most intriguing and unresolved biological questions. Most of the scientific community agrees on the fact that species derive from separate evolving lineages of populations or meta-populations (Wiley, 1978). Given that speciation is a continuum process, discordances arrive about which is the threshold after which divergence between separate lineages should allow recognizing them as distinct species (Hey, 2006; Mallet, 2008). Nowadays, we realized that what matters for the study of speciation and to decide the threshold that defines a species from another, is to know what caused the origin of these species and what determined their evolutionary trajectories. This knowledge requires inferences about origin, genetic structure, degree of ecological interchangeability of divergent lineages and, most of the times, the reconstruction of the population history, phylogenetic relationships and biogeographic background of the lineages (Bond *et al.,* 2008; Dávalos *et al.,* 2009; Padial *et al.,* 2010). Therefore, an integrative approach to taxonomy is necessary because it allows studying species boundaries from multiple, complementary perspectives that will address in the most complete way possible the complexity of the biology of a species (Dayrat *et al,* 2005).

Integrative taxonomy is the science that aims to delimit the units of life's diversity in a multidisciplinary perspective, integrating the objectives of taxonomy (description, identification and classification) with the study of phylogeography, comparative morphology, population genetics, ecology, development, and behaviour (Fig. 1.1; Dayrat *et al.,* 2005; Padial *et al.,* 2010). The need of an integrative taxonomy approach starts from the fact that the traditional "Linnaean taxonomy" (based on the identification, description and classification of species using morphological dichotomous characters) just allows the description of what Cain (1953) defined as "morpho-species". Nowadays, we know that this approach is incomplete (Dayrat *et al.,* 2005) to corroborate a species status. Indeed a "morpho-species" should be analysed via different approaches and with different kinds of data, such as DNA variation, which provides strong evidences in evolutionary studies (Ballard & Whitlock, 2004). Mitochondrial (mtDNA) and nuclear (nDNA) DNA differ strongly in the way they evolve and in the modes of inheritance. The mtDNA is a haploid molecule inherited just from the egg cell that accumulates nucleotide substitutions several times faster than a single-copy nuclear DNA (scnDNA) not only because of its effective population size but also because it is

subject to damage from reactive oxygen molecules released as a product during OXPHOS (Oxidative Phosphorylation), and it lacks the repair mechanisms found in the nucleus. Because of its fast-evolutionary rate, mtDNA is particularly useful and accurate to resolve relationships among recently diverged species/populations (Wan *et al.*, 2004). On the contrary, nDNA is inherited from both parents and has much slower evolution rate than mtDNA, and thus give less sharp resolving power for recent divergences (Wan *et al.*, 2004). Given these structural differences between mitochondrial and nuclear markers, the use of both markers is required to obtain a complete picture of the evolutionary history of organisms and accurate delimitation of lineages (e,g, Godinho *et al.*, 2008).

Currently, species described through DNA barcoding approaches are rising in number thanks to the developmental advances in analysing molecular markers and computational methods, such as employing the coalescent theory to produce phylogenetic trees (Jennings *et al.,* 2005). These advances are contributing to the increased use of genetic methods to delimit species and to study intraspecific diversity and phylogeography (Templeton *et al.*, 2001; Hickerson *et al.*, 2010), which brought light to both the “morpho” and “cryptic” (species that differ only at genetic level) species (Malhotra & Thorpe, 2004; Gvoždík *et al.*, 2010; Ghielmi *et al.*, 2016). However, knowing that a species differs from another by a certain amount of molecular or morphological level does not allow studying the life’s diversity *per se*, but just how the organisms differ between each other’s from a genetic or morphological point of view. More information are needed about the ecological role, the effective and potential distribution, and the dispersal rate. To cope with these issues, the use of ecological niche-based models (ENMs) and topographic, environmental and land-cover georeferenced data provide complementary approaches to describe the biology and the ecology of a species (Kozak *et al.*, 2008; Elith, 2009). The use of ENMs allows correlating species observational data (usually presence/absence data) with environmental variables to determine and map the realized niche of a species, predict possible suitable areas outside its range, and projected these predictions into different times (past or future) to forecast putative areas of climatic refugia or ecological corridors (Waltari *et al.*, 2007; Franklin, 2010).

After archived the scientific porpoise of Integrative Taxonomy (delineate and classifying species), it is important to proceed with the naming of species and identifying characters that allow to recognise them in the easiest and fastest way possible (Dyar *et al.,* 2005). Evolutionary Significant Units (ESU) and Management Units (MU) are used now in conservation to characterise populations. ESUs are classified based on the historical population structure and mitochondrial phylogeny, and they are helpful to plan long term conservation needs. On the other hands MUs, are defined based on allele frequencies between populations and then on current population structure and are used for short-term management issues. To integrate the identification of both in an integrative taxonomy workflow is pivotal to define taxa with a more critical conservation status according to the IUCN guidelines.

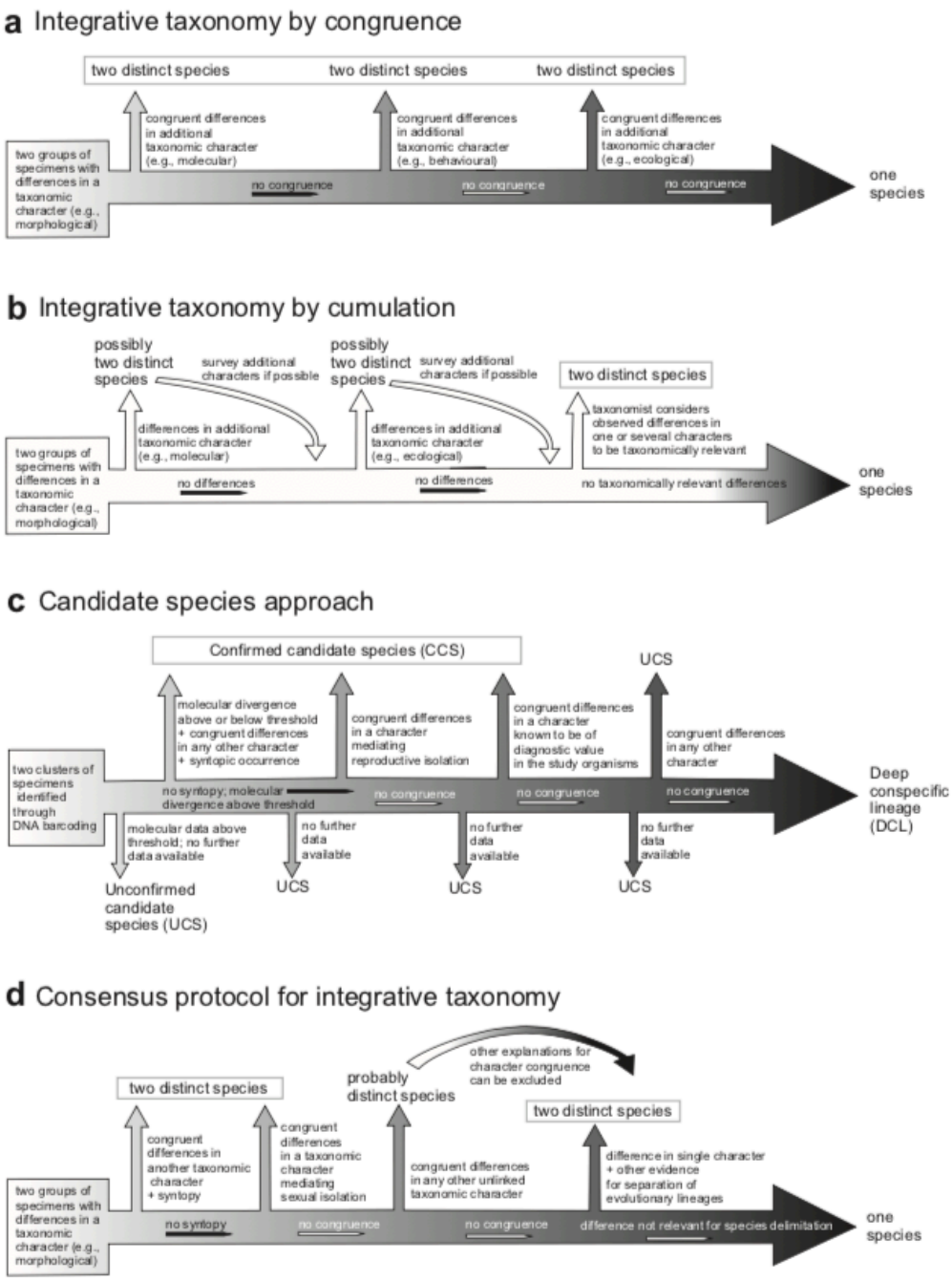


**Fig 1.1.** Schematic representation of work protocols in taxonomy (adapted from Padial *et al.,* 2010). Workflow in (a) integrative taxonomy by congruence and (b) by cumulation; (c) work protocol to define Unconfirmed Candidate Species (UCS), Confirmed Candidate Species (CCS) and Deep Conspecific Lineages (DCL) in an automated approach that starts with DNA barcoding and (d) a general work protocol for integrative taxonomy proposed here that combines advantages of cumulative and congruence approaches. Increasing black colour intensity in a-d represents increasing uncertainty about species status and the need of a more thorough evaluation of data.

# 1.3 BIOGEOGRAPHY OF NORTH AFRICA

Biogeographic realms are large spatial regions within which ecosystems share a broadly similar biological evolutionary history. The realms delineate the large areas of the Earth's surface within which organisms have been evolving in relative isolation over long periods of time, separated from one another by geographic features, such as oceans, broad deserts, or high mountain ranges, that constitute barriers to migration. As such, biogeographic realm designations are used to indicate general groupings of organisms based on their shared biogeography. At the global level, eight terrestrial biogeographic realms are typically recognized, corresponding roughly to the continents. North Africa is situated at the turn of the Palaearctic and the Afro-tropic biogeographical realms. Although is one of the most arid areas on Earth, North Africa is an emblematic biogeographic region inasmuch including nine out of sixteen biomes present in the whole continent (Olson *et al.,* 2001): (i) Coniferous forests, (ii) Mountain grasslands and shrublands, (iii) Mediterranean Forests, Woodlands and Shrublands, (iv) Flooded grasslands and Savannas, (v) Desert and Xeric Shrublands, (vi) Grasslands, Savannas, and Shrublands, (vii) Moist Broadleaf Forests, (viii) Lakes and (ix) Mangroves. Among these regions, the Sahara Desert and the xeric shrublands of the Sahel ecoregions constitute the largest warm desert in the world, covering about 11,230,000 km2 (Dinerstein *et al.,* 2017). 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 desiccation of the Sahara began in the Miocene (7 or 6 Mya) in Chad (Schuster *et al.*, 2006; Kröpelin *et al.*, 2008; Holmes, 2008) or later in the western region (Schuster *et al.*, 2006) when the area was prone to drastic climatic oscillations with a consequent gradual decrease of precipitations and an increase of the dust flow that consequently lead to a vegetation collapse (Waller *et al.*, 2007; Wang *et al.*, 2008; Claussen *et al.*, 2009). Thereby, multiple dry-wet cycles have characterised the Sahara-Sahel ecoregions since the Pleistocene (5.3 to 2.5 Mya) (Le Houérou, 1997). The succession of these dry and humid periods was hypothesized as the main cause of speciation in the Sahara-Sahel, in the absence of adaptation processes (Brito *et al.*, 2014). The population fragmentation due to the contraction and expansion of both dry and humid areas caused long-term isolation and a consequent interruption of gene flow within species which turned out into a series of vicariant events. This allopatric effect led to evolutionarily independent lineages or new species according to the isolation time and their habitat requirements. Xeric species had diversification processes probably during humid periods. On the contrary, mesic species (adapted to arid conditions but still requiring some moisture) had population contraction and diversification events under hyper-arid, and water-dependent species were abundant during wet periods along

permanent or temporary rivers but become extinct or isolated in small wetlands (oases and mountain rock pools) during dry periods (Fig. 1.2 adapted from Brito *et al.*, 2014). Mountains and coastal areas likely played a key role in diversification patterns across the Sahara-Sahel by acting as refugia and/or corridors for many species and facilitating gene flow during favourable climatic conditions (Geniez *et al.*, 2011; Vale *et al.*, 2015; Gonçalves *et al.*, 2012, 2018). Several studies have assessed the strong climatic variations of the Sahara-Sahel and how those variations shaped the biodiversity dynamics (e.g. Wang *et al.*, 2008; Claussen, 2009; Trape *et al.*, 2012; Brito *et al.*, 2014).

Knowledge on biodiversity distribution across the Sahara-Sahel is still scarce if compared to neighbouring areas. Large portions of northern-eastern Mauritania, northern Mali, western Algeria, southern Libya, and almost all mountain regions are under-sampled due to the harsh conditions and the current political instability that characterise these countries (Ewi *eta al.*, 2010; Brito *et al.*, 2014, 2018). In the cases of the Adrar des Ifoghas, Tibesti, Ennedi, and Marra mountains there is scarce or non-existent sampling effort and the current knowledge on local species richness and distribution is particularly low.

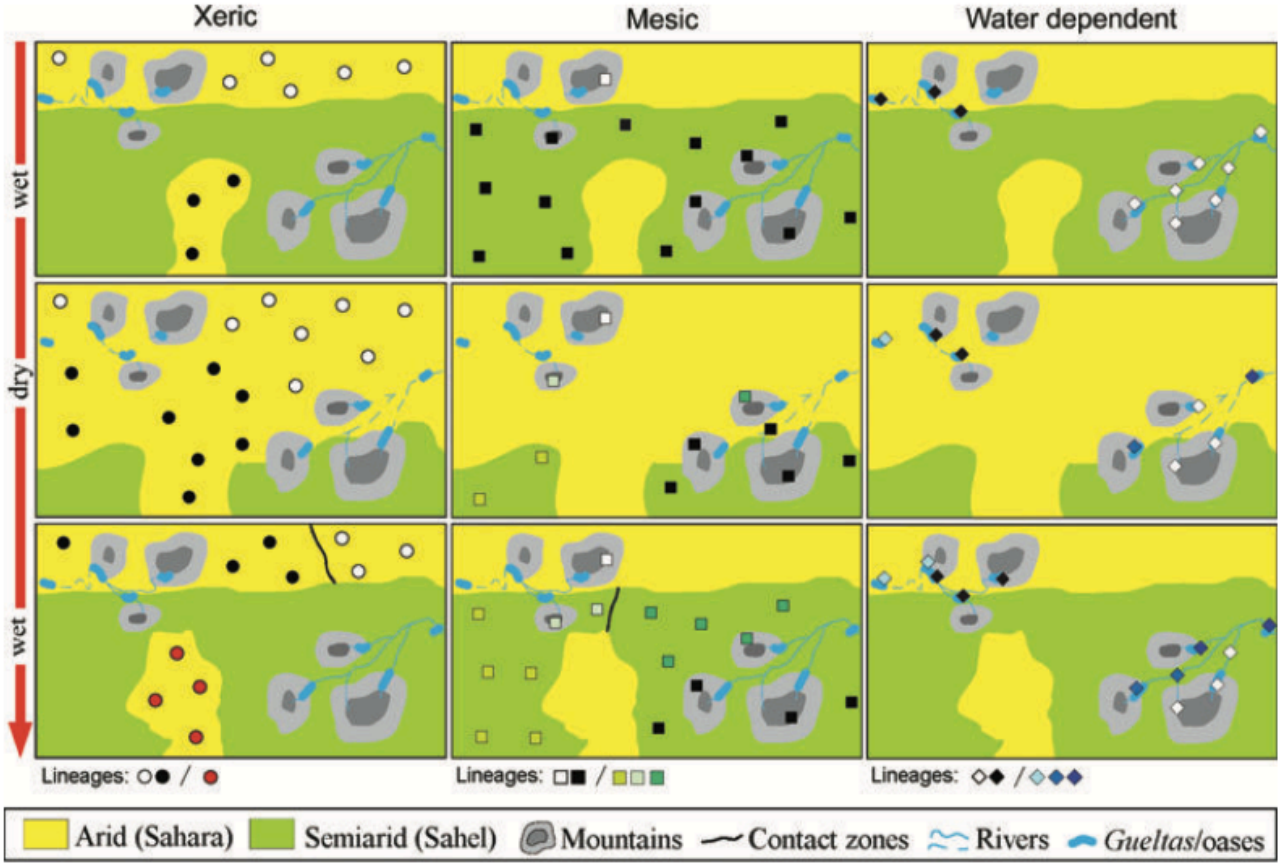


**Fig 1.2.** Summary of hypothetical diversification mechanisms through allopatric processes expected for three types of Sahara- adapted species (adapted from Brito *et al.,* 2014): xeric (circles), mesic (squares) and water-dependent species (diamonds). A time series of climatic cycles is shown from top to bottom. Wet periods associated with a cooler climate lead to expansion of semiarid environments (Sahel) while dry periods, associated with a warmer climate, lead to wider arid environments (Sahara). Cycles of range expansion-contraction lead to the formation of new lineages (colours) and subsequent contact zones between lineages (black lines).

## 1.4 THE LIZARDS OF GENUS *MESALINA*

The genus *Mesalina* (subfamily: *Lacertinae*, family: *Lacertidae,* tribe: *Eremiadini*) has been presented for the first time to the scientific community by Grey, 1838 together with the inclusion in the genus of the first *Mesalina*: *Mesalina lichtensteini* (now *Mesalina rubropunctata* Gray 1845) named after the zoologist Hinrich Lichtenstein (1780-1857), who initially described the species in 1823 (as *Lacerta rubropunctata*). The genus comprises diurnal xeric-adapted small lizards that can be found from the Atlantic Sahara all the way through North Africa, Middle East, and Arabian Peninsula to Pakistan (Sindaco & Jeremcenko, 2008). These fast-moving lizards differ in habitat selection patterns, inhabiting rocky and mountain areas (i.e. *M. guttulata*), sand dunes (i.e. *M. pasteuri*), xeric shrublands and mesic areas along the Mediterranean and Atlantic coasts (i.e. *M. olivieri* and *M. simoni*) (Trape *et al.*, 2012). The genus currently comprises 19 species (Arnold *et al.*, 2007; Uetz *et al.*, 2019), which makes it the third most species-richness genus within the tribe *Eremiadini* (Mayer & Pavlicev, 2007).

Genus *Mesalina* is divided into seven species complexes (Fig. 1.3): (i) *M. watsonana*, (ii) *M. martini*, (iii) *M. olivieri*, (iv) *M. rubropunctata*, (v) *M. adramitana* group, (vi) *M. brevirostris* and (vii) the *M. guttulata* group. Since its restoration at generic status, the taxon, has been largely investigated from a phylogenetic, systematic (Kapli *et al*., 2008; Šmíd & Frynta, 2012; Šmid *et al.*, 2017a; Sindaco *et al*., 2018; Simó-Riudalbas *et al.,* 2019) and biogeographical point of view, using both molecular and morphological approaches (Arnold, 1986; Moravec, 2004; Kapli *et al*., 2015; Hosseinian, 2015; Simó-Riudalbas *et al.,* 2019). The genus provides a case-study to address the influence of geological events and past climatic oscillations in diversification events across the Sahara-Sahel, and other drylands of the Palaearctic. The recent studies of Kapli *et al.* (2015) and Simó-Riudalbas *et al.* (2019) agreed about the evolutionary history of the genus: after diversifying from the genus *Adolfus* (Arnold *et al.,* 2007) in south-west Asia, it was subjected to a first intra-generic split during the early Miocene (*c*.22 Ma) when a temporary connection between the current Arabian Peninsula with the Eurasian continent allowed the dispersal of one lineage into Arabia. A series of tectonic events (described in detail in Popov *et al.,* 2004*;* Agard *et al.,* 2011) contributed then to the geographical isolation of the Eurasian linage that diversified in what is currently recognised as *M. watsonana*. Then, a second major divergence occurred at *c.* 17 Mya between the ancestral forms of *M. martini* and the rest of the *Mesalina*, which successively split again during a third major diversification event into the *M. olivieri* species complex and the ancestral form of the *M. guttulata, M. rubropunctata M. brevirostris*, and *M. adramitana* groups, with the dispersal throughout North Africa of the former species complex while the other four taxa remained in Arabia. Progressively, these four taxa diversified into *M. rubropunctata* and *M. guttulata* (which also dispersed into North Africa during the Late Miocene), *M. brevirostris*, *M. microlepis, M.*

*bernoullii*, *M. saudiarabica*, *M. austroarabica*, *M. arnoldi, M. adrarmitana, M. ayunensis, M. kuri*, and *M. balfouri* (that colonized the Socotra Archipelago at the Miocene-Pliocene boundary). Recent dispersal into Asia took place in the *M. brevirostris* group when *M. bernoullii* and *M. brevirostris* colonized Iran in the Pleistocene (reviewed by Simó-Riudalbas *et al*., 2019).

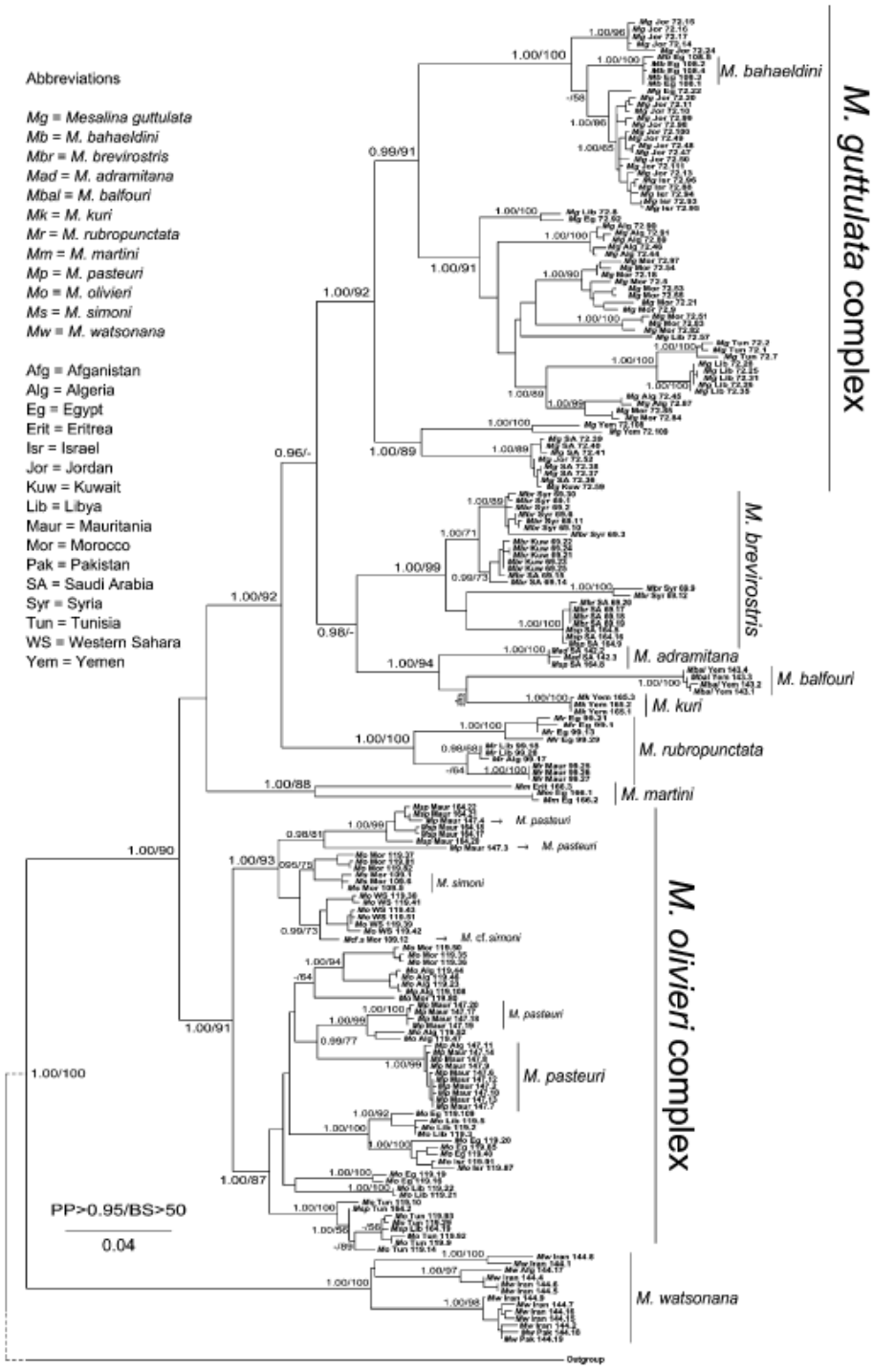


**Fig 1.3.** Phylogenetic relationships of Mesalina from North Africa and the Middle East, according to the maximum likelihood (ML) method adapted from Kapli *et al.* (2015). The numbers on the branches indicate the posterior probabilities (PP) of the Bayesian inference (only values above 0.95 are shown) followed by the bootstrap supports (BS) of the ML method (only values above 50 are shown). Seven samples of the genus Gallotia and two of the genus Eremias were used as outgroup taxa.

After the successful colonisation of North Africa, *M. olivieri* species complex diversified at about 8 Mya into two well-supported lineages (Kapli *et al.,* 2015), one ranging from Morocco and Mauritania through the north-eastern part of the African continent and the second one restricted to Morocco, Mauritania and Atlantic Sahara that diversified into *M. simoni* and *M. pasteuri*. This distribution pattern could be explained by the fact that the species retracted to the mountain of Morocco and Mauritania, which worked as refugia during the humid and arid cycles that characterised the history of Sahara-Sahel (reviewed in Brito *et al.*, 2014). From Kapli *et al.* (2015), the estimation for the isolation of the Tunisian lineage from the "Eastern clade" of the *M. olivieri* species complex was due to a series of orogenetic events that occurred during the Messinian period, which caused vicariance between the Tunisian populations from other North African populations. This hypothesis is corroborated by the occurrence of distinct Tunisian lineages in other North African species (Beddek *et al.,* 2018), such as in genus *Podarcis* and *Hyla* (Recuero *et al*., 2007; Kaliontzopoulou *et al*., 2011). Then, less than 2.5 Mya, a final colonisation of the Middle East by populations of *M. olivieri* started, likely due to the desiccation of the Nile, which occurred in the Pleistocene and lasted about a million years (Baha El Din, 2006). There are currently five recognised species in North West Africa (Fig. 1.4): *M. guttulata, M. rubropunctata* (these two not part of the *M. olivieri* species complex), *M. olivieri, M. pasteuri* and *M. simoni.* Previous studies about the systematics (Arnold *et al.* 2007), phylogeny (Kapli *et al.,* 2008), phylogeography, and biogeography of *Mesalina* (Kapli *et al.,* 2015; Simó-Riudalbas *et al.,* 2019), stressed the huge intraspecific variation that characterise the *M. olivieri* species complex, including the possible presence of at least two new species, one from Mauritania (Fig. 1.4) and another from Tunisia, not yet studied.

## 1.5 OBJECTIVES

The aims of this study are to: (i) investigate the genetic and morphological diversity within the *Mesalina olivieri* species complex; (ii) understand if the genetic and phenotypic variation is spatially structured; (iii) determine how many lineages can be identified; (iv) understand if identified lineages correspond to true species; (v) map their potential distribution; (vi) determine which is their extinction risk. Overall, it is aimed to solve the systematics of Mesalina lineages distributed in the Atlantic Sahara addressing the taxonomy of the species complex through an integrative approach.

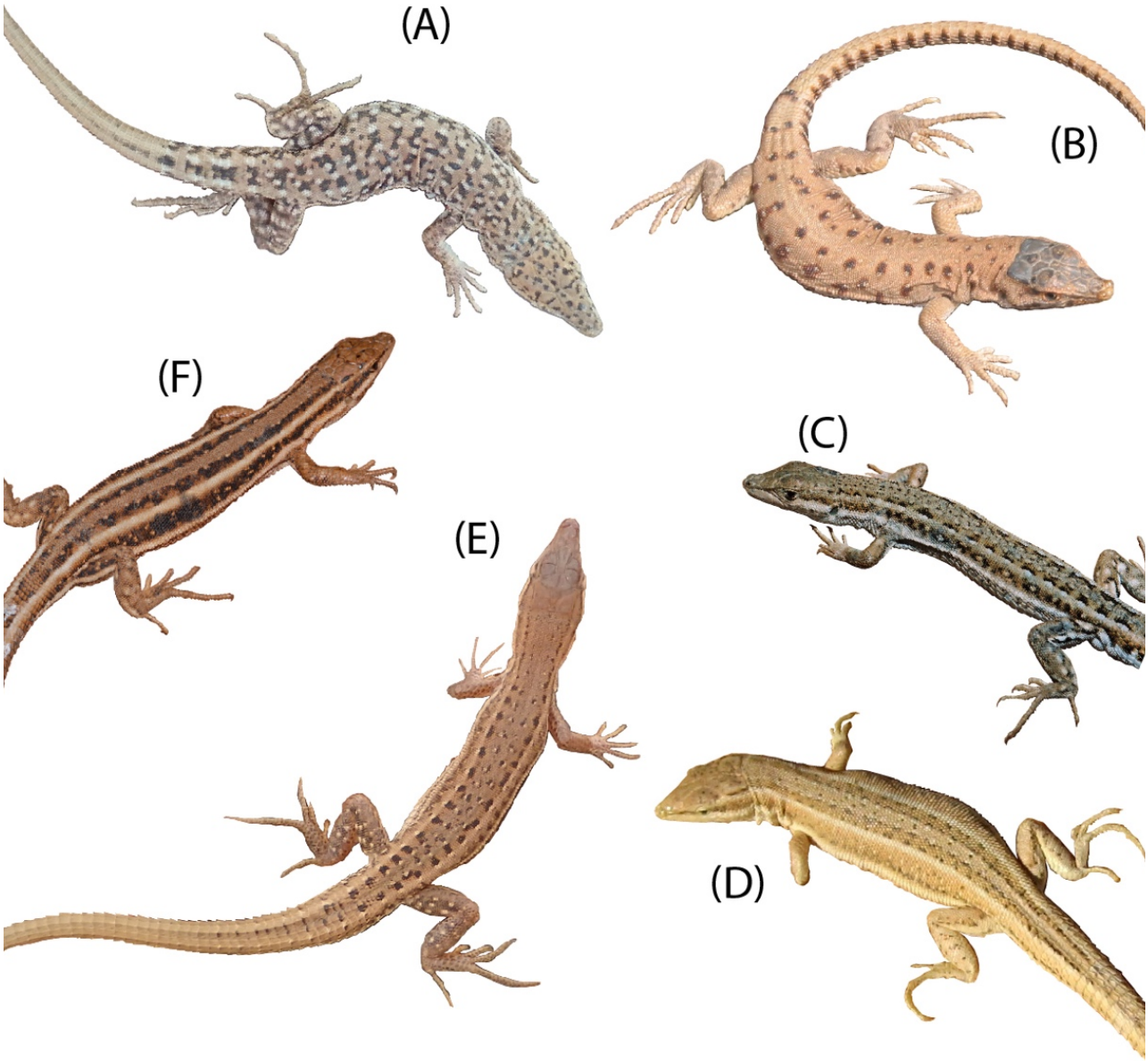


**Fig 1.4.** Examples of *Mesalina* lizards from the *M. olivieri* species complex in North Africa. In a clockwise direction: (A) *M. guttulata,* (B) *M. rubropunctata,* (C) *M. simoni,* (D) *M. pasteuri,* (E) *Mesalina* sp. (Kapli et al., 2015) (F) *M. olivieri.* Pictures from José Carlos Brito and Philippe Geniez

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# 2. PHYLOGEOGRAPHY AND SYSTEMATICS OF THE *MESALINA OLIVIERI* SPECIES COMPLEX (SQUAMATA: LACERTIDAE) FROM NORTH-WEST AFRICA WITH THE DESCRIPTION OF A NEW SPECIES AND SUBSPECIES

This work is under preparation to be submitted as manuscript to a scientific journal:
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## 2.1. INTRODUCTION

In Africa, the Sahara Desert and the neighbouring Sahel are ecoregions where past climatic oscillations have left strong imprints on current biodiversity patterns. The origin of the current Sahara is set approximately 7 to 6 million years ago (Mya) in Chad (Schuster et al., 2006; Kröpelin et al., 2008; Holmes, 2008) or even more recently further west (Schuster et al., 2006), when a gradual decrease in precipitation and increase of the dust flow led to vegetation collapse (Waller et al., 2007; Wang et al., 2008; Claussen et al., 2009). After the Pleistocene (5.3 to 2.5 Mya), multiple dry-wet cycles impacted the region and induced a succession of dry-humid periods. Population fragmentation and connectivity were induced by episodes of contraction and expansion of both dry and humid areas. These episodes likely caused long term isolation and interruption of gene flow within ancestral forms, which promoted diversification of evolutionarily independent lineages and speciation, depending on isolation time and habitat requirements (Brito et al., 2014). For instance, mountains and coastal areas have played a key role in diversification patterns across the Sahara-Sahel by acting as refugia and/or corridors for many species and facilitating gene flow during favourable climatic conditions (e.g. Gonçalves et al., 2018a,b; Velo-Antón et al., 2018). Several studies assessed the effects of past climatic variation in the Sahara-Sahel and how such climatic shifts shaped current biodiversity patterns (reviewed in Brito et al., 2014). However, large portions of northern-eastern Mauritania, northern Mali, western Algeria or southern Libya, and almost all mountain regions, are under-sampled due to their remote character and long-term regional instability (Brito et al., 2014, 2018). Consequently, in comparison to other biomes, knowledge

about biodiversity distribution across the Sahara-Sahel is still relatively scarce (Brito and Pleguezuelos, 2019).

The lizards of the genus *Mesalina* (Lacertidae*,* Lacertinae, Eremiadini) provide a case-study to address the influence of geological events and past climatic oscillations on diversification events across the Sahara-Sahel and other drylands of the Palaearctic. The genus comprises diurnal xeric-adapted small lizards that can be found from the Atlantic Sahara all the way through North Africa, the Middle East, and the Arabian Peninsula to Pakistan (Sindaco & Jeremcenko, 2008). These fast-moving lizards differ in habitat selection, inhabiting rocky and mountain areas (i.e. the *M. guttulata* group), sand dunes (*M. pasteuri*) or xeric shrublands and mesic areas along the transition between the Sahara and the Mediterranean and Atlantic coasts (i.e. the *M. olivieri* group, Trape et al., 2012). The genus currently comprises 19 species (Uetz et al., 2019), subdivided into seven species complexes: (i) *M. watsonana*, (ii) *M. martini*, (iii) *M. olivieri*, (iv) *M. rubropunctata*, (v) *M. adramitana* group, (vi) *M. brevirostris* and (vii) *M. guttulata* group (see also Kapli et al., 2008, 2015; Simó-Riudalbas et al., 2019). Previous studies have addressed the phylogeny, systematics and biogeography of the genus (Arnold, 1986; Moravec, 2004; Kapli et al., 2008; Hosseinian and Pouyani, 2015; Kapli et al., 2015; Šmíd and Frynta, 2008; Šmíd et al., 2017; Sindaco et al., 2018; Simó-Riudalbas et al., 2019) using both molecular and morphological approaches. The studies of Kapli et al. (2015) and Simó-Riudalbas et al. (2019) agreed about the evolutionary history of the genus *Mesalina*: it diverged from the sister genus *Adolfus* (Arnold et al., 2007) in south-west Asia and started to diversify during the early Miocene (*c.*22 Mya) when a temporary connection between the current Arabian Peninsula with the Eurasian continent allowed the dispersal of one lineage into Arabia. A series of tectonic events (described in detail in Popov et al., 2004; Agard et al., 2011) contributed then to the geographical isolation of the Eurasian linage that diversified into what is currently recognised as *M. watsonana*. Then, a second major divergence occurred at *c.* 17 Mya between the ancestral form of *M. martini* and the rest of the *Mesalina*. A subsequent split occurred during a third major diversification event into the *M. olivieri* species complex and the ancestral form of the *M. guttulata, M. rubropunctata*, *M. brevirostris*, and *M. adramitana* groups, with the dispersal throughout North Africa of the former species complex while the later four taxa remained in Arabia. Progressively, these four taxa diversified into: *M. rubropunctata* and *M. guttulata* (which both also dispersed into North Africa during the Late Miocene); *M. brevirostris*, *M. microlepis, M. bernoullii*, *M. saudiarabica*, *M. austroarabica*, *M. arnoldi, M. adrarmitana, M. ayunensis, M. kuri*, and *M. balfouri* (the last two colonised the Socotra Archipelago at the Miocene-Pliocene boundary). Recent dispersal into Asia took place in the *M. brevirostris* group when *M. bernoullii* and *M. brevirostris* colonized Iran in the Pleistocene (reviewed in Simó-Riudalbas et al., 2019). After the successful colonisation of North Africa, *M. olivieri* species complex diversified at about *c.* 8 Mya (Kapli et al., 2015) into two well-supported lineages, one restricted to Morocco through Atlantic Sahara to Mauritania and one ranging from Israel to Mauritania, Atlantic Sahara and southern

Morocco (Fig. 2.1). This distribution pattern could be explained by a retraction of the distribution to the refugia present in the area (as the Atlantic coast or the mountain areas of Morocco and Mauritania) during the humid and arid cycles that characterised the history of Sahara-Sahel (reviewed in Brito et al., 2014). Arnold et al. (2007), Kapli et al. (2008; 2015) or Simó-Riudalbas et al. (2019) all stressed the wide distribution and huge intraspecific variation of the *M. olivieri* species complex, suggesting the presence of several undescribed species. In addition, numerous paraphyletic clades have been found within *M. olivieri* and *M. pasteuri,* including: i) one *olivieri* lineage from Algeria clustering with one lineage of *M. pasteuri* from Mauritania; ii) one *olivieri* lineage from the Atlantic Sahara and iii) one *olivieri* lineage from the Atlas Mountains in Morocco, clustering with the Moroccan endemic *M. simoni* and iv) a *pasteuri* specimen from the Tagant region in Mauritania embedded in the *olivieri*-like specimens of the same region. Last, the *olivieri*-like specimens from Mauritania constitutes a divergent lineage restricted to this country and sister to *M. simoni*; it has been suggested to constitute a new species by Kapli et al. (2015).

The aim of this study is to investigate the genetic and morphological variation within the *Mesalina olivieri* species group, aiming to clarify the paraphyletic situation within the complex by revising its systematics. This required formally describing the new species suggested by Kapli et al. (2015) and revising the status of the *M. olivieri* populations from the Atlas Mountains, Atlantic Sahara and south-west Morocco.

## 2.2. MATERIALS AND METHODS

### *2.2.1 Sampling and study area*

In this study, we were mainly interested on the population status of the *M. olivieri* complex from Mauritania and south-western Morocco (including Atlantic Sahara = Western Sahara). For specimens outside our study area, we only selected samples from each major lineage identified by Kapli et al. (2015). A total of 128 samples were sequenced for this work from *M. olivieri*, 57 from *M. pasteuri*, 23 from *M. simoni*, and 28 from the undescribed *Mesalina* lineage from Mauritania (Fig. 2.1). The complete list of all the new specimens sequenced for this work plus those sequences derived from GENEBANK (a total of 450 for the genetic analysis and 104 for the morphological analysis) with their collecting localities are given in Tables S2 and S4.

### *2.2.2. GENETIC ANALYSES*

#### *2.2.2.1 DNA extraction and amplification*

DNA was extracted from ethanol-preserved tissue using a proteinase K (10 mg/ml) digestion followed by a standard salt-extraction protocol (Bruford et al., 1992). Amplifications were performed in 5 µL of 2× MyTaq Mix and 0.4 µM of each primer. The PCR conditions adopted for

every primer are specified in Table S1. Some samples required minor adjustments on the temperature and time of annealing to the reported conditions. We amplified one fragments from the mitochondrial (mtDNA) cytochrome b gene (cyt-b, 400 bp; appositely designed for the *Mesalina* genus by Kapli et al., 2015), and four nuclear DNA (nDNA) gene fragments from the beta fibrinogen intron 7 (B-fib7, 600 bp), ornithine decarboxylase gene (OD, 396 bp), phosphogluconase dehydrogenase intron 7 (PgD7, 420 bp) and melanocortin receptor 1 gene (MC1R, 630 bp, Table S2). PCR products were cleaned using ExoSAP, and the purification and sequencing was outsourced to GENEWIZ Leipzig, Germany.

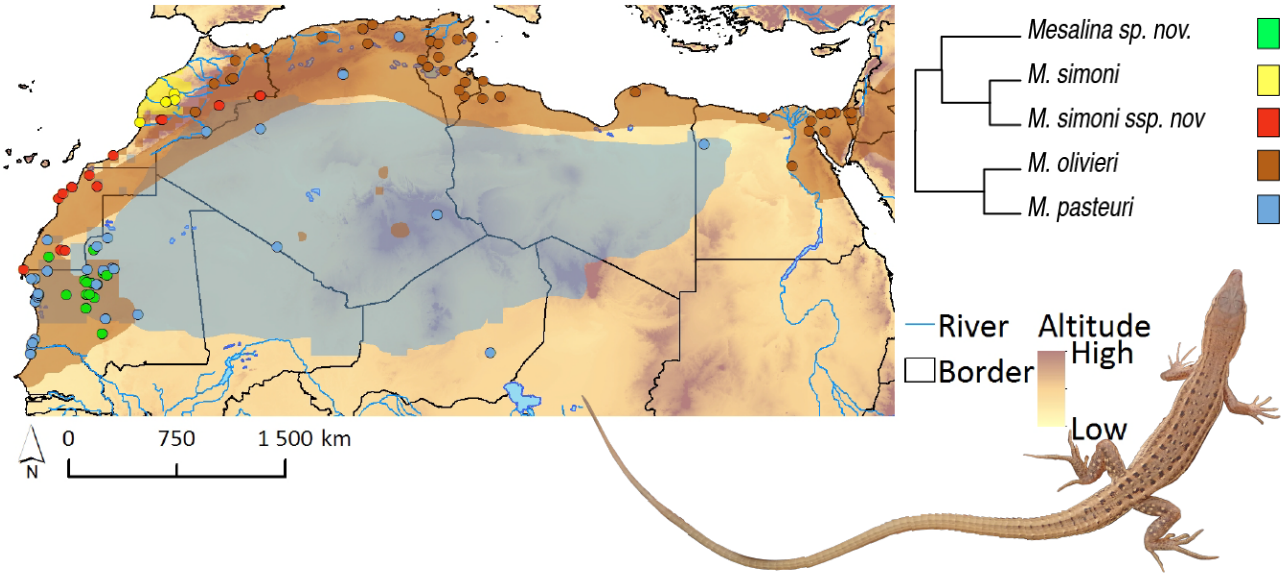


**Fig. 2.1.** Localities of the *Mesalina olivieri* species complex samples included in this study, polygons correspond to the relative species distribution from Roll *et al.* (2017) (left) and species tree (upper right). All the branches of the tree display PP >95%. Inset picture shows a specimen of the new species from Mauritania.

### 2.2.2 Genetic analyses

Amplified fragments were sequenced for the forward strand only. Obtained DNA sequences were edited and checked for errors manually using Codon-Code Aligner (v. 2.0.6, Codon Code Corporation). Heterozygote positions of nuclear sequences were coded using IUPAC ambiguity codes. MEGA7 was used to check for stop codons. DNA sequences were aligned using MAFFT v.7 (Katoh and Standley, 2013) applying parameters by default (Auto strategy, Gap opening penalty: 1.53, Offset value: 0.0). Homozygous indels were coded with dashes to maintain the overall alignment. As there is no IUPAC ambiguity code for heterozygous indels, they were encoded with dashes. In all analyses were kept just the number if dashes necessary to maintain the alignment in the portions with missing data (indels), the rest were discarded. All newly determined sequences in the present study were deposited in GenBank (access codes XXX-XXX; Table S2).

*2.2.2.2 Phylogenetic analyses and haplotype networks*

A mitochondrial (cyt-b) dataset was first created by adding sequences produced in this study and sequences from Kapli et al. (2015) for a preliminary analysis to assess the morphological identification of the samples as recorded in the field. The tree was rooted with four different outgroups sequences from Kapli et al. (2015): *Podarcis peloponnesiacus* (AY896116), *Psammodromus algirus* (DQ150365) *Eremias brenchleyi* (EF490071) *Gallotia atlantica* (KF003365).

Different approaches were then used to infer phylogenetic relationships. First, cyt-b and nuclear (four concatenated loci: B-fib7, MC1R, PgD7, and OD) datasets were analysed separately, then the two datasets were merged in a single concatenated alignment. For all datasets, Bayesian inferences (BI) were performed with BEAST 1.10.4 (Drummond et al., 2012). The best-fitting model of nucleotide substitutions for each gene was determined with PartitionFinder v.1.1.1 (Lanfear et al., 2012). The program was set as follows: branch lengths unlinked, only models available in BEAST were evaluated, initial partitions by gene, BIC model selection criterion applied and all partition schemes analysed. The partition scheme and models of sequence evolution selected were B-fib7: HKY+G; MC1R: HKY+I; OD: HKY; PgD7: K80+G; and Cyt-b: HKY+G+I. No partition by codon position was selected for any of the genes.

To test whether the genes studied evolve in a clock-like manner (strict clock) a preliminary run was done using a strict-clock for all the markers. The results were then analysed in TRACER 1.6. (http://tree.bio.ed.ac.uk/software/tracer). The strict-clock model was rejected when the ucld.mean parameter was different from zero. Then a Speciation Yule Process model was selected assuming a constant lineage birth rate for each branch in the tree. Three independent MCMC runs of 100 million generations were implemented for each analysis for each dataset, sampling every 10,000 generations, and 10% of the trees were discarded as burn-in. The convergence of chains and effective sample sizes (ESSs) for all parameters was verified using TRACER 1.6. (http://tree.bio.ed.ac.uk/software/tracer; ESSs higher than 400 for all parameters). Log and tree files of the three independent runs were combined using LOGCOMBINER. The subsequent maximum clade credibility summary tree with posterior probabilities for each node, using the median values, was obtained in TREEANOTATOR (both available in the BEAST package). The resulting tree was visualized and edited with FIGTREE 1.4.1 (http://tree.bio.ed.ac.uk/software/figtree). Nodes were considered as strongly supported if they received a posterior probability (PP) ≥ 0.95. Independent haplotype networks were constructed in TCS (Clement et al., 2000) with a 95% parsimony threshold for the nuclear genes: B-fib7 (186 sequences), MC1R (152 sequences), OD (120 sequences), and PgD7 (124 sequences). The PHASE algorithm, implemented in DNASP 5.10.01 (Librado and Rozas, 2009), was used to reconstruct the haplotypes of nuclear data. The algorithm was run five times, for 10,000 iterations, with a burn-in of 1,000. The most probable reconstructed haplotypes were used to create the

haplotype network. For this analysis, the aim was to highlight differences between the *M. olivieri* lineage from Mauritania and all the other lineages without addressing the diversity within the species *M. olivieri* and *M. pasteuri*. The Cyt-b sequences produced for this study were previously aligned with those from by Kapli *et al.* (2015) available in GENEBANK. This dataset (Dataset 0) has been used to ensure the quality of the sequences produced for this study. Three additional datasets were then produced: i) Dataset 1: a single-locus (Cyt-b) mitochondrial dataset including all sequences available from the most recent publications from Kapli et al. (2015), Šmíd et al. (2017), Sindaco et al. (2018) and Simó-Riudalbas et al. (2019) for a total of 402 sequences; ii) Dataset 2: a concatenated multilocus nuclear dataset with a total of 78 sequences with (1700 bp), and iii) Dataset 3: a concatenated cytonuclear Dataset with a total of 87 concatenated sequences (1986 bp). The dataset including only samples with at least three genes out of five and has been used to calculate the phylogeny of the species complex and the time of divergence adding *Gallotia atlantica, Psammodromus algirus, Podarcis pityusensis* and *Podarcis lilfordi* as outgroup for this last analysis. For the specific of the analysis where the datasets have been used see Table S3.

*2.2.2.3. Species-tree*

The phylogeny of the complex was also investigated using a coalescent-based species-tree estimation in *BEAST (Bouckaert et al., 2019). The data set for this analysis contained only specimens with as many gene sequences as possible. A total of 83 samples were used in the analysis (Table S2). The preliminary species subdivision was based on the results of the separate analysis on the mitochondrial and nuclear datasets. The four-putative species were: *M. olivieri*, *M. pasteuri*, *M. simoni* (subdivided into two lineages, *M. simoni s.s.* and the other *M. olivieri* from the Atlantic Sahara and Atlas Mountains), and the new potential species from Mauritania. The subdivision in two lineages for *M. simoni* is due to the genetic divergence between the lineage from the Atlas Mountain and Atlantic Sahara and the formal *M. simoni* (Table 1). Substitution, clock and tree models were unlinked across all partitions. Since the genes and the samples used for this analysis were the same as in the previous ones, the substitution models and partitions used have been kept the same. The strict-clock model was used for the four nuclear markers, meanwhile an uncorrelated relaxed clock was used for the mitochondrial marker. Speciation Yule Process model were used as tree priors. Three independent MCMC runs of 100 million generations were implemented for each analysis for each dataset, sampling every 10,000 generations, and 10% of the trees were discarded as burn-in. The convergence of the chains and effective sample sizes (ESSs) for all parameters were verified using TRACER 1.6. Log and tree files of the three independent runs were combined using LOGCOMBINER. The subsequent maximum clade credibility summary tree with posterior probabilities for each node, using the median values, was obtained in TREEANOTATOR (both available in the BEAST package). The resulting tree was

visualized and edited with FIGTREE 1.4.1 (http://tree.bio.ed.ac.uk/software/figtree). Nodes were considered strongly supported if they received a posterior probability (PP) ≥ 0.95.

*2.2.2.4. Time of divergence*

To infer the time of divergence between the new potential species and *M. simoni* a concatenated cytonuclear dated tree has been produced in BEAST 1.10.4. To root and calibrate the tree four outgroups were used: *Gallotia atlantica, Psammodromus algirus, Podarcis pityusensis* and *Podarcis lilfordi*. Inferred models of sequence evolution are described in Table S3. The calibration points and priors applied to the divergence time analysis are the same as Kapli et al. (2015) and Simó-Riudalbas et al. (2019) and are: i) the split between *Gallotia atlantica* and *Psammodromus algirus* (age of the of the Canary Islands Fuerteventura and Lanzarote; normal distribution, mean 18, stdev 2); and ii) the split between *Podarcis pityusensis* and *Podarcis lilfordi* (endemic to the Balearic Islands) at the end of the Messinian Salinity Crisis (normal distribution, mean 5.32, stdev 0.05). A Speciation Yule Process model was used as tree priors. Three independent runs of 100 million generations were implemented, sampling every 10000 generations and 10% of the trees discarded as burn-in (1000 trees). TRACER v.1.6 (Rambaut and Drummond, 2007) was used to verify that the sampling achieved stationarity (ESSs higher than 300 for all parameters). Samples from each run were combined using the software LogCombiner v.1.7.5. (available in the BEAST package), and a consensus chronogram with node height distribution was generated and visualized using TreeAnnotator v.1.7.5. (available in the BEAST package) and FigTree v.1.3.1.

*2.2.2.5. Genetic distances*

Computation of sequence divergence (uncorrected *p*-distances, distance is the proportion "*p*" of nucleotide sites at which two sequences being compared are different) for the Cyt-b fragment was performed in MEGA 10 (Kumar *et al.*, 2013) to provide an overview of the genetic divergence among taxa. The grouping referred to the topology of the Cyt-b tree built using a database containing the sequences from this study and those published by Kapli *et al.* (2015).

*2.2.2.6. Historical demography*

Mitochondrial DNA (Cyt-b) was used to recover the historical demography of the species complex. Clades were selected based on the preliminary phylogenetic analysis conducted with the same dataset and the results of the haplotype networks. A coalescent-based Bayesian skyline plot (BSP) approach implemented in BEAST 1.10.4 was used to provide a temporal perspective on the historical changes in population size for the *M. olivieri* species complex. The Coalescent Bayesian Skyline divides the time between the present and the root of the tree (the tMRCA) into segments,

and estimates a different effective population size for each segment. The endpoints of segments are tied to the branching times (also called coalescent events) in the tree, and the size of segments is measured in the number of coalescent events included in each segment. The Coalescent Bayesian Skyline groups coalescent events into segments and jointly estimates the population size and the size of each segment. No outgroups were included in the analysis for each and all clades combined, as BEAST estimates the root of the individual gene trees (Heled and Drummond, 2010). The best-fitting model of nucleotide substitutions was determined with PartitionFinder v.1.1.1 (Lanfear et al., 2012). The program was set as follows: branch lengths unlinked, only models available in BEAST were evaluated, initial partitions by gene, BIC model selection criterion applied and all partition schemes analysed. The partition scheme and models of sequence evolution selected was HKY+G+I. The strict clock and a Coalescent Bayesian Skyline model were used as tree priors. The final MCMC simulation ran for 100 000 000 generations, sampling every 10 000 generations and a 10% burn-in was used. TRACER v.1.6 was used to check for convergence and stationarity of the effective population sizes.

### *2.2.3 MORPHOLOGICAL ANALYSES*

#### *2.2.3.1. Morphological dataset*

A total of 32 morphological variables were measured in 252 specimens, 19 for the lineage of the potential new species from Mauritania, 109 of *M. olivieri*, 36 of *M. pasteuri*, 21 of *M. simoni*, 17 of *M. rubropunctata*, and 50 of *M. guttulata* (the latter two species not used in the analysis, see Tables S4, S5 and S6). Variables were selected according to their relevance as diagnostic characters in the genus *Mesalina* (Trape et al., 2012) or based on our own examination of specimens of the various lineages. We scored each individual for (i) five quantitative biometric variables, *SVL* = snout-vent length (mm), *TL* = tail length (mm), *HL* = head length (mm), *HW* = head width (mm), *HH* = head height (mm); (ii) eleven quantitative pholidotic variables, *D* = number of longitudinal rows of dorsal scales counted around mid-body, *V* = number of transverse rows of ventral plates, *G* = number of gular scales in one straight line from the collar to the infralabials (collar included), *Pf* = number of femoral pores on the right and left sides (*Dx* and *Sx*); *Lam* = number of lamellae beneath the fourth toe; *NTS* = number of rows of temporal granulae (average of left and right side); *TR* = number of scales around the tail at the 10$^{th}$ scale ring; *SL* = number of supralabials in contact with the subocular on the right and left sides (*Dx* and *Sx*); *IL* = number of infralabials on the right and left sides (*Dx* and *Sx*); *Col* = number of enlarged scales forming a collar; *EL* = number of enlarged scales on the lower eyelid (forming the palpebral disks, average of left and right side); (iii) ten semi-quantitative (ordinal) chromatic variables: *EBL* = black line surrounding the eyelids scales (0 = absent; 1 = present); *DBF* = dark bands along the flanks (0 =

absent; 1 = fragmented; 2 = continuous); *PSDBF*= pale spots inside the dark bands along the flanks (0 = absent; 1 = small pale spots 2 = ocelli); *PDLL* = pale dorso-lateral line (0 = absent; 1 = fragmented; 2 = continuous); *SPDLL* = number of scales included into the width of the pale dorso-lateral line; *DDSLL* = Dark supra-dorsolateral line (0 = absent; 1 = fragmented; 2 = continuous); *SDDLB* = number of scales included into the width of the dark supra-dorsolateral line; *PSDDSLL* = Pale spots within the dark supra-dorsolateral line (0 = absent; 1 = small pale spots 2 = ocellus); *DSO* = small ocelli arranged in rows along the mid dorsum 0 = absent; 1 = small pale spots 2 = ocelli); *TC* = under tail coloration (0 = white; 1 = yellowish; 2 = yellow) and (iii) four semi-quantitative (ordinal) variables describing morphological states: *TE* = tail enlargement (from 0 to 2); *RN* = raised nostrils (from 0 to 2); *PS* = pointed snout (from 0 to 2), *DS* = shape of the dorsal scales (0=flat; 1 = pointed; 2 = weakly carenated (tectiform); 3 = well carenated). Scale nomenclature, scale counts, and measurements follow Bons and Geniez (1995). All morphological data were obtained by the same observer (C.P.).

*2.3.2 Morphological variation*

The main objective was to identify diagnostic characters and to quantity the amount of morphological differentiation between the potential new species from Mauritania and the other species within the *olivieri* complex. Analyses were restricted to adult specimens to avoid errors due to the strong ontogenic modifications in colour patterns in genus *Mesalina*. Both sexes were treated separately, as preliminary analyses revealed significant sexual dimorphism for many variables in every clade (results not shown). Due to the reduced sample size, statistical tests were not conducted. Three datasets were created to analyse separately biometric (Table S4), pholidotic (Table S5) and coloration (Table S6). An exploratory investigation was first performed with Excel using a heating map to facilitate an immediate visualization of the most relevant interspecific differences. Then, PCAs were run on each database for a pairwise comparison of the potential new species with the other species of the complex. The data on pholidosis and colorations were normalised (to zero mean and unit variance) prior to PCAs. All analyses were run on PAST 3.24 Software (Hammer *et al.,* 2001).

*2.2.4 HABITAT COMPARISON AND DISTRIBUTION MODELLING OF THE POTENTIAL NEW SPECIES*

The percentage of presences of each lineages of the *M. olivieri* species complex in each land cover unit was taken as a measure of the biogeographic affinities of each group (Brito et al., 2009). Selection among land cover units was quantified from the percentages of training observations using the Standardized Levin's B measure of niche breadth: Bs ¼ B_1/n_1, where B is the Levin's index and n the total number of land cover units. B is given by 1/P(p2), where p is the proportion of observations in each land cover unit. The standardized index was used because of unbalanced

sample size among groups. Eleven land cover units were selected: yellow dunes (14.6%), white dunes (8.2%), orange dunes (20.3%), compact sand (8%), compact soil (6.1%), rocky plateaus (16.8%), bare rocks (6.3%), gravel and sand floodplains (1.9%), grasslands (9.1%), savannah (2.9%), croplands (5.7%).

An ecological niche model (ENM) was used to assess the potential distribution and characterise the realized ecological niche of the new species from Mauritania. The study area encompasses an area of approximately 1,979,127km2, varying between 28°N, 14°N, 17°W and 4°W, comprising southern Morocco, south-western Algeria, full extent of Mauritania, south-western Mali and north-eastern Senegal. Models were based on the 28 presence records available based on genetic or morphological assignment. Morphological identifications of specimens not sequenced were based on pictures of live unvouchered animals or from the direct examination of specimens deposited in museum collections. Only records based on adult specimens exhibiting distinctive characteristics of the new species were treated as valid. To remove duplicated observations from the same geographic locations, data were thinned reducing to 20 the number of observations to build models. Models were built using a spatial resolution of 1km.

Variables used for the modelling were: i) Terrain Roughness Index (TRI) calculated by upscaling a digital elevation model (USGS, 2006) from 90 meters to 1 km; ii) 18 land cover categories (Table S7) for the West Sahara-Sahel, adapted from Campos and Brito (2018) and up-scaled from 30m to 1km; iii) four uncorrelated bioclimatic variables, maximum temperature of warmest month (BIO5), minimum temperature of coldest month (BIO6), temperature annual range (BIO7), and annual total precipitation (BIO12) from Hijmans et al. (2005).

The models were developed using the Maximum Entropy approach implemented in Maxent v.3.3 (Phillips et al., 2006) with the following settings: 5,000 maximum number of iterations; regularization multiplier equal to 1; 10,000 maximum number of background points; 20 replicates selected by bootstrap. The area under the receiver-operating curve (AUC) of each replicate run was taken as a measure of model accuracy and ensemble models were generated by averaging the 20 model replicates. Response curves and jackknife analyses were performed to assess the importance of each variable in each model replicate (e.g. Vale et al., 2014). Finally, the minimum training presence threshold was applied to the ensemble model given that less restrictive thresholds should be applied for conservation purposes (Liu et al., 2005). The resulting binary map (depicting presence/absence areas) was used to calculate the extent of occurrence and area of occupancy following IUCN guidelines for assessing Red List categories (IUCN, 2017).

### *2.2.5. TAXONOMIC RANKING*

This study adhered to the principles of the Biological Species Concept (de Queiroz, 2007) and regard the evolution of reproductive isolation as the prime criterion for recognizing independent lineages as valid species. We thus treated sympatric or parapatric lineages which do not exchange genes when they are not isolated by geographic barriers as species. For allopatric lineages, we used level of genetic divergence as a proxy for taxonomic ranking and treated independent evolutionary lineages that are as divergent as related valid species as species. Moreover, this study adopts the framework of integrative taxonomy based on the assumption that divergences in any of the attributes can provide evidence for the species' existence (Dayrat, 2005; Padial et al. Vences, 2010). We have divided our dataset into five categories that can each be regarded as distinct lines of evidences. They can be combined and compared in order to assess the congruence of the putative species limits among the *M. olivieri* species complex: (i) the mtDNA data can be used to test the criteria of reciprocal monophyly and the presence/absence of barcoding gaps, (ii) the multilocus nDNA data can be used to test, independently from the mtDNA set, the criteria of reciprocal monophyly, notably in coalescence theory framework, (iii) the phenotypic data allows to test the criteria of morphological divergence, and (iv) habitat and (v) distribution range data-set can be used as a complementary approach to test the criteria of ecological divergence.

## 2.3. RESULTS

### *2.3.1 PHYLOGENETIC ANALYSES*

A total of 79, 70, 85, 71 and 69 samples were successfully sequenced for Cyt-b (286 bp), B-fib7 (384 bp), MC1R (582 bp), PgD7 (309 bp) and OD (425 bp).

All three runs of BEAST for each dataset converged with ESS values >400 for all parameters indicating adequate mixing of the MCMC analyses. The Bayesian Inference results for Dataset 3 are shown in Fig. 2.2, those for Datasets 0, 1 and 2 are showed in Figures S2 and S3. Since not all samples were successfully amplified for all genes and the data sets were pruned to contain only specimens with as many genes sequenced as possible, some samples were discarded in the concatenate analysis.

All the trees produced displayed a similar topology although the number of samples included in the analysis differs between them (Figs. 2.1, 2.2, S2, S3 and S4). *Mesalina watsonana* branch (when present) was located as basal of all the other *Mesalina.* The other species of *Mesalina* used as outgroups always clustered outside the *M. olivieri* species complex, except for *M. martini.* This later species has been recorded to be basal of the *M. olivieri* species complex in the results for datasets 1 and 3, and to all the *Mesalina* except *M. watsonana* for dataset 0, and sister taxa to the *M.*

*simoni* clade for dataset 2. *Mesalina olivieri* species complex is divided in two main deep well supported clades, one including the potential new species from Mauritania and the *M. simoni* group (including *M. simoni* plus the two lineages of *M. olivieri* from the Atlantic Sahara and Atlas Mountains), and the other clade including the remaining *M. olivieri* and *M. pasteuri*.

*Mesalina olivieri* presents four paraphyletic clades: the potential new species from Mauritania; the two lineages (above mentioned) from North West Africa recorded to be sister taxa of *M. simoni* (in all results); one lineage from Algeria (samples BEV.9225 and BEV.T3038) that fall within the continental lineage of *M. pasteuri* when mtDNA is analysed alone (datasets 0 and 1; Figs. S2 and S3) and basal to the clade including *M. pasteuri* and the *M. olivieri* from the West Morocco until Israel in the nuclear and cytonuclear trees (results for dataset 2 and dataset 3). The lineage of the potential new species from Mauritania resulted to be paraphyletic to the remaining *M. olivieri* with high support (posterior probability [PP] = 100%) in all trees produced. However, this lineage represents another case of cytonuclear discrepancy: it is basal to the entire *M. olivieri* species complex (that here include *M. martini*) in the results for dataset 2 and it was recovered as sister to *M. simoni* and the Alas Mountain and Atlantic Sahara lineages of *M. olivieri* in all trees that included mtDNA in the analysis (datasets 0, 1 and 3). The two individuals previously recognised as *M. pasteuri* (Codes 147.4 and 147.3) in Kapli et al. (2015), grouped with the lineage of the potential new species from Mauritania.

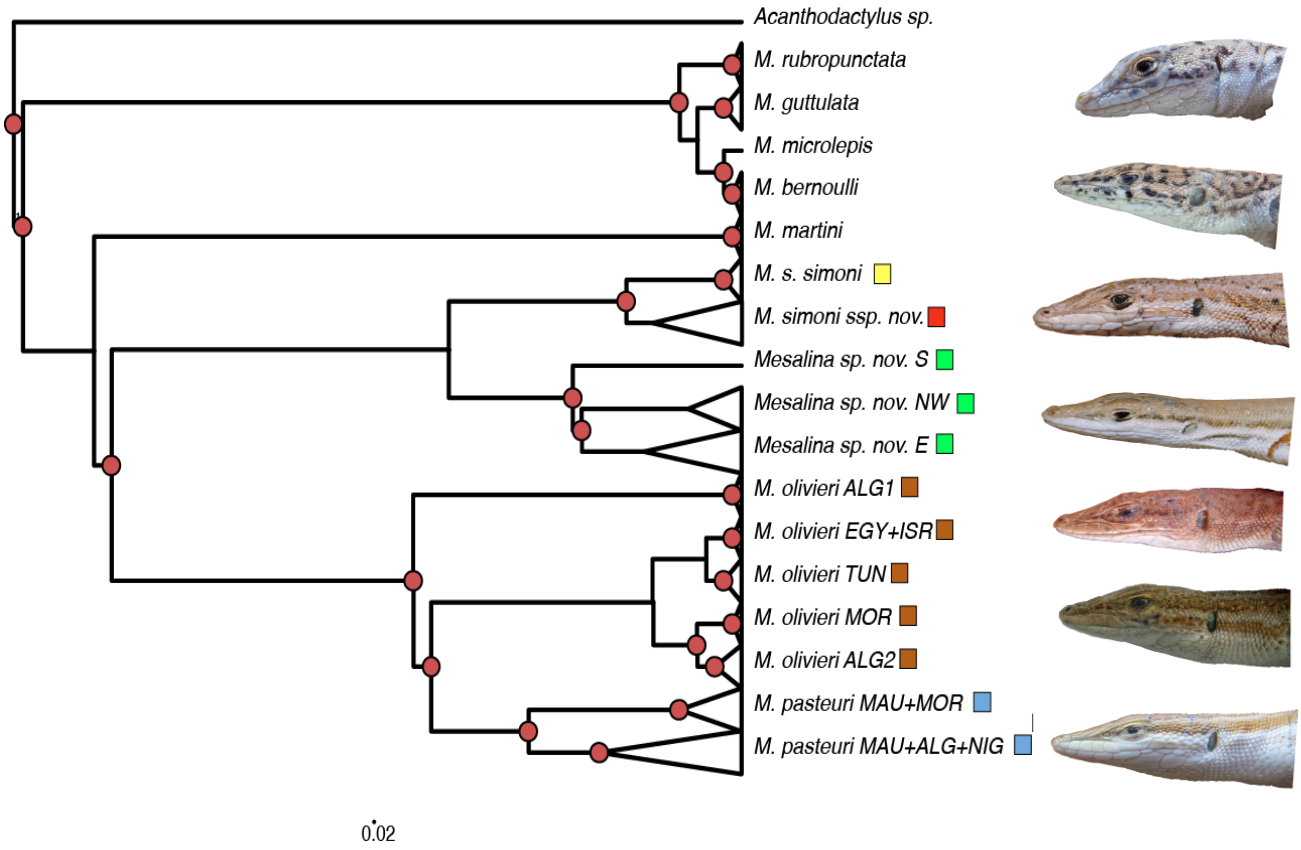


**Fig. 2.2.** Results of the Bayesian Inference analysis on the concatenate cytonuclear dataset (Dataset 3). Red dots on the notes indicate a PP>95%. Colours correspond to those in the maps and haplotype networks. Pictures on the side represent the phenotypic variability of the genus *Mesalina* in North West Africa. From the top, respectively: *M. rubropunctata, M. guttulata, M. simoni simoni, M. simoni* ssp. nov.*, Mesalina* sp. nov., *M. olivieri*, and *M. pasteuri.*

### *2.3.2 HAPLOTYPE NETWORKS AND GENETIC DIVERGENCE*

Allele network reconstructions (Fig. 2.3) for the four nDNA genes indicates that all haplotypes of the new species here described (marked in green in Fig. 2.3) are private and not connected with any other haplotype in genes β-fib7, OD and PgD7. A connection with the other lineages is observed in gene MC1R where the potential new species is separated from at least three mutation steps from the nearest haplotype (*M. olivieri* lineage from the Atlantic Sahara). *Mesalina simoni* and the two *M. olivieri* lineages from Atlas Mountains and Atlantic Sahara confirm to belong to the same cluster in agreement with the phylogenetic results. All haplotypes from this clade are private and show a complete separation from the other lineages in β-fib7 and PgD7 networks. A connection between this lineage and the others is recorded in MC1R and OD where they still diverge from *M. olivieri* by one and two mutational steps respectively. In the network analysis for

MC1R, *M. simoni* show isolation from *M. olivieri* from the Atlas and the Atlantic Sahara diverging by five mutational steps.

All lineages are seemingly parapatric or sympatric (Fig. 2.3). The new potential species has been observed only in the rocky highlands of the Adrar Atar in Mauritania and surrounding refugia being sympatric with *M. pasteuri* and parapatric with the Atlantic Sahara linage of *M. olivieri*.

**Table 2.1** Estimates of evolutionary divergence over sequence pairs between *Mesalina* species present in NW Africa (above) and between the different lineages of *M. simoni* (below). The number of base substitutions per site from averaging over all sequence pairs between groups are shown. Analyses were conducted using the Maximum Composite Likelihood model [1]. This analysis involved 118 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 286 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [2]. Distances in *M. simoni* include the two subspecies.

| | *Mesalina* sp. nov. | *M. olivieri* | *M. pasteuri* | *M. simoni* | *M. guttulata* |
|---|---|---|---|---|---|
| *M. olivieri* | 0.14 | | | | |
| *M. pasteuri* | 0.13 | 0.11 | | | |
| *M. simoni* | 0.08 | 0.11 | 0.11 | | |
| *M. guttulata* | 0.20 | 0.19 | 0.22 | 0.18 | |
| *M. rubropunctata* | 0.21 | 0.19 | 0.18 | 0.21 | 0.16 |
| | | *M. s. simoni* | | *M. simoni* ssp.nov. *AM* | |
| *M. simoni* ssp.nov. *AM* | | 0.03 | | | |
| *M. simoni* ssp.nov. *AS* | | 0.04 | | 0.05 | |

The level of genetic divergence (p-distance) between the potential new species and *M. pasteuri* estimated from the analysis on the mitochondrial (Cyt-b) genetic divergence is 13.7%, while it ranged from 8 to 14.7% between the potential new *Mesalina* and the other members of the *M. olivieri* species complex. The lowest intraspecific genetic divergence within the group was observed between the *M. olivieri* from the Atlas Mountain and Atlantic Sahara and *M. simoni* (Table 2.1).

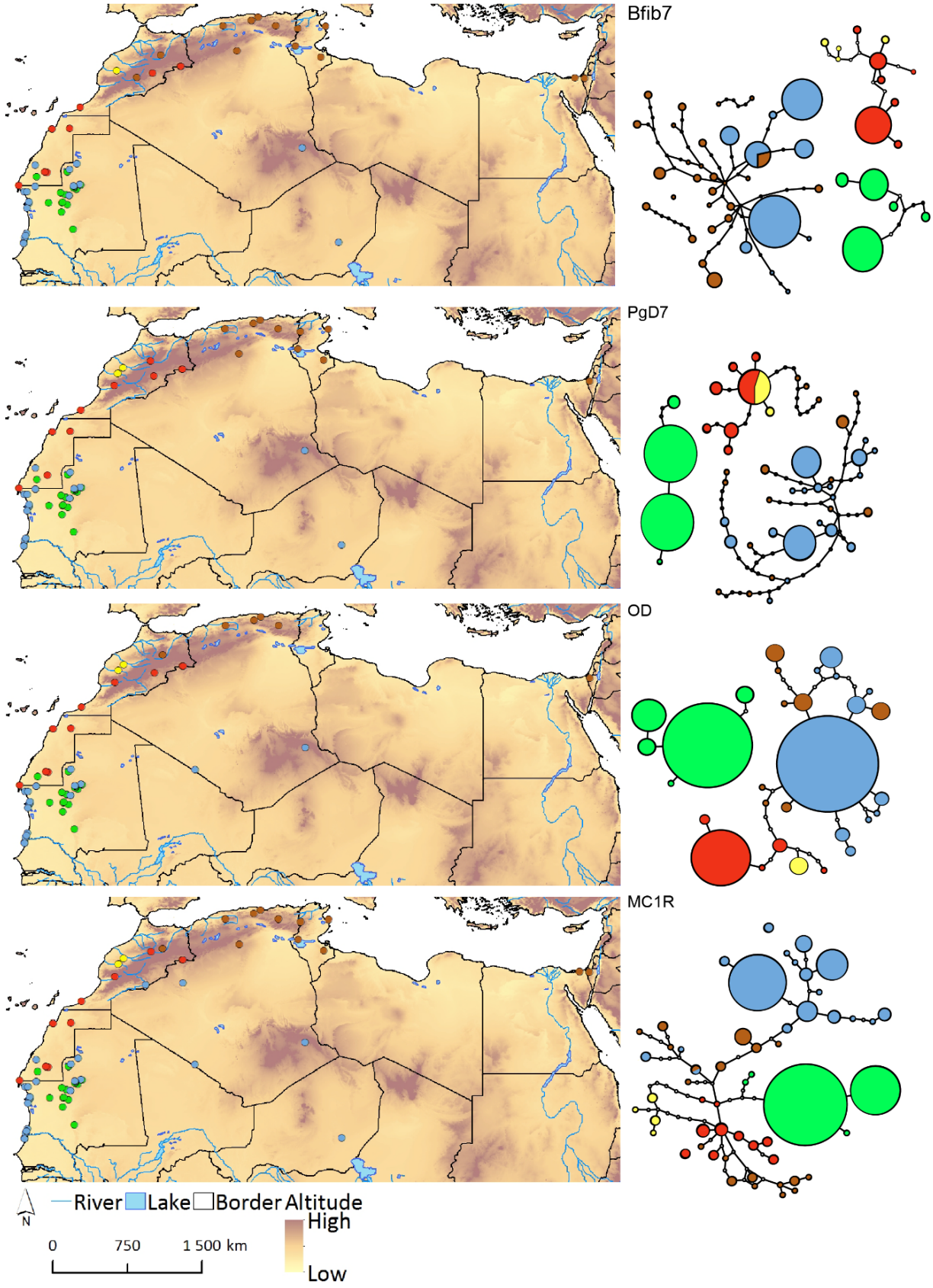


**Fig. 2.3** Results geographical distribution of the *Mesalina olivieri* species complex and unrooted haplotype networks of the nuclear markers (B-fib7, PgD7, OD, MC1R) analysed for the *M. olivieri* species complex. Circle size is proportional to the number of alleles with colours corresponding to those shown in Fig. 2.1. Small circles show the number of mutational steps between two haplotypes.

### *2.3.3 TIME DIVERGENCE AND HISTORICAL DEMOGRAPHIC ANALYSIS*

The divergence time estimation analysis using dataset 3 and including the outgroups (see 2.2.2.4) indicates that the *M. olivieri* species complex started to diversify in the Late Miocene, c. 9.6 Mya (6.92-12.87 Mya, 95% highest posterior densities [HPD]; Fig. S4). In this period, two main groups of lineages were split. One group including *M. olivieri* and *M. pasteuri* and the other group including the potential new species and *M. simoni*. The latter two species also diversified in the same period (c. 8.94 Mya; 6.30-12.8 Mya 95% HPD). Most of the intraspecific diversification in the four species of the complex occurred in the Plio-Pleistocene transition. For instance, the two lineages of *M. olivieri* clustering together with *simoni* split at c. 3.5 Mya (2.05-4.37 Mya 95% HPD) and the eastern/north-western and southern lineages of the potential new *species* c. 3.17 Mya (2.01-4.54 Mya 95%).

The Bayesian Skyline plots (BSP) showed the effective population size throughout time (Mya) and detected signs of expansion and contractions for each of the analysed clades (Fig. 2.4). A general expansion of the population size in the genus started around c. 6 or 7 Mya (Fig. 2.4 A) that later shrink during a bottleneck event that lasted from 2.5 Mya to 500.000 years ago, and then expanded again until today. The individual clade BSP showed the same trend for each different lineage. The analysis regarding all clades merged revealed an expansion that started between 7.5 and 10 Mya. This expansion was preceded by population stabilization, during the last 22 Mya years.

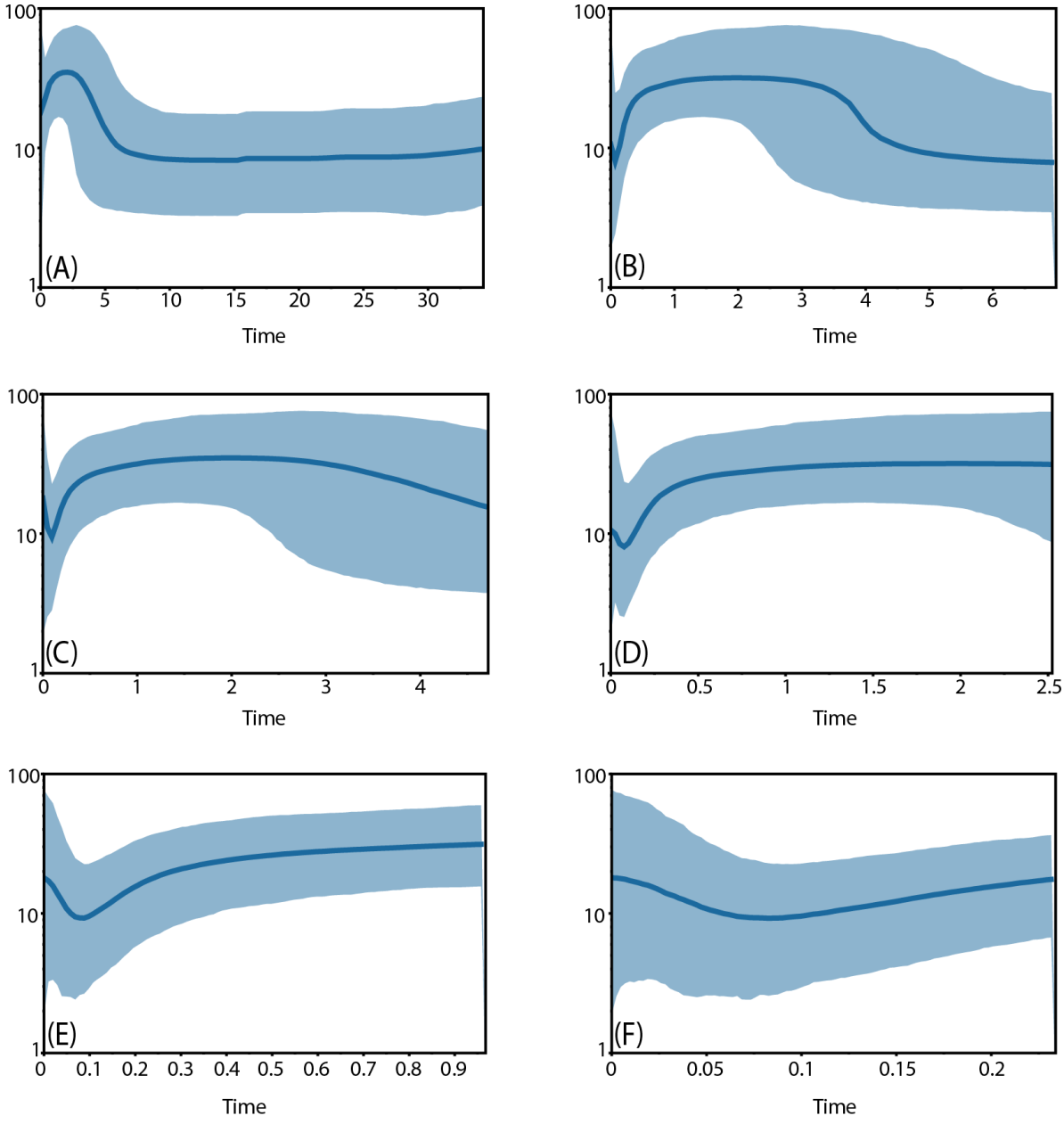


**Fig. 2.4** Bayesian skyline plots for mitochondrial DNA (Dataset 1) for the species included into the *M. olivieri* species complex. The median estimate (black line) and 95% highest posterior density limits (blue background) are indicated. The y-axis represents the scaled population size and the x-axis indicates measures time in substitutions per site per million years. (A) All *Mesalina,* (B) *M. olivieri*, (C) *M. pasteuri*, (D) *Mesalina* sp. nov., (E) *M. simoni simoni*, (F) *M. simoni* ssp. nov.

### *2.3.4 MORPHOLOGICAL ANALYSES*

Measurements of all individuals examined as well as those obtained from the literature are given in Table S4, S5 and S6. Descriptive statistics of the morphological data are shown in Table 2.2 . Morphological differences were found between the *M. olivieri* lineage from Mauritania and the remaining species of the complex inhabiting its distribution (Table 2.2, 2.3, 2.4 and 2.5; Fig. S5, S6

and S7). For instance, individuals of Mauritanian *M. olivieri* tend to have a narrower and pointed snout, more dorsal and gular scales, more femoral pores, and higher number of lamellae beneath the fourth toe than the other species of the complex (Table 2.2). The nostrils of Mauritanian *M. olivieri* have been recorded to be more exposed than those of *M. simoni* and *M. pasteuri.* Principal Component Analysis for the biometry also shows a clear separation on the PC1 axes between males of the Mauritanian *M. olivieri*, *M. simoni* and *M. pasteuri* (Fig. S5). This result highlighted that the Mauritanian *M. olivieri* have a more narrowed snout and raised nostrils than *M. simoni*, and they are smaller than *M. pasteuri* (Fig. S5).

Females of the Mauritanian *M. olivieri* differ from the other *M. olivieri* from the number of scales composing the tail ring, higher in the latter; from the females of *M. simoni* in number of ventral scales, lower for the Mauritanian *M. olivieri*; from the females of *M. pasteuri* in head height, higher in *M. pasteuri*.

Shape and disposition of scales that compose the eyelid in both sexes of the new species is resemble *M. guttulata* and *M. simoni* (5-6 large scales composing the eyelids), but without dark line separating the scales. Major differences were also found in the dorsal coloration. PCAs on these characters between *M. olivieri* and the new species shows main differences in the presence of pale dorsal spots on the dorsum (PSDDSLL), i.e. more evident in both male and females of the Mauritanian *M. olivieri*, and on the dark band on the dorsum (SDDLB) and on the flanks (DBF), i.e. thicker and better defined in the other *M. olivieri* than in the Mauritanian lineage. Similar results were obtained in the comparison between *M. pasteuri* and the Mauritanian *M. olivieri* where the former also presents a pattern composed mostly by defined and continuous lines in the dorso-lateral part of the body when the latter presents fragmented dark supra-dorsolateral line and a mostly shaded pale dorso-lateral line. Differences were recorded also in the background colorations from pictures of live specimens: ochre-bronze brown in the Mauritanian *M. olivieri*, orange fire for *M. simoni,* from orange to yellowish in *M. pasteuri,* and brownish in the other *M. olivieri*.

**Table 2.2** Minimum-maximum value, mean and standard deviation (n=sample size) for selected characters in the *Mesalina olivieri* species complex.

| | | *Mesalina* sp. nov. | | | *M. simoni* ssp. nov. | | | *M. simoni* | | *M. olivieri* | | | *M. pasteuri* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | M | F | J | M | F | J | M | F | M | F | J | M | F | J |
| *SVL* | min-max | 33-38 | 41-53 | 25-25 | 36-43 | 42.46-45.75 | - | 43-51 | 43-51 | 33-50 | 36-47 | 20-42 | 48-48 | 40-42 | 28-28 |
| | mean ± SD | 34±2.3 | 42±5.54 | 25±0 | 42±3.03 | 44.10±2.32 | - | 45±2.81 | 45±2.61 | 41±4.39 | 42±3.3 | 34±7.01 | 45±2.9 | 41±1.01 | 28±0 |
| | N | (4) | (3) | (1) | (4) | (45.75) | - | (4) | (6) | (24) | (21) | (8) | (7) | (2) | (1) |
| *HL* | min-max | 9-11 | 9-10 | 7-7 | 8-10 | 8.47-9.45 | - | 10.29-11.32 | 8.77-10.3 | 8.07-12.17 | 8.27-11.04 | 5.86-10.42 | 9.95-12.01 | 8.94-10.09 | 7.19-7.19 |
| | mean ± SD | 10±1.07 | 9±0.62 | 7±0 | 9±0.7 | 9±0.69 | - | 11±0.39 | 10±0.48 | 10±1.03 | 9±0.64 | 8±1.22 | 11±0.72 | 10±0.81 | 7.19±0 |
| | N | (4) | (3) | (1) | (4) | (9.45) | - | (8) | (8) | (25) | (21) | (15) | (7) | (2) | (1) |
| *HW* | min-max | 5.57-6.65 | 4.92-6.13 | 4.41-4.41 | 5.28-6.26 | 4.86-6.75 | - | 6.61-7.44 | 5.62-6.67 | 4.45-8.84 | 4.78-6.92 | 3.29-6.69 | 5.57-7.74 | 5.56-5.59 | 4.54-4.54 |
| | mean ± SD | 6±0.47 | 5±0.54 | 4.41±0 | 5.77±0.69 | 5.9±0.73 | - | 7.05±0.26 | 6.28±0.3 | 6.28±1.09 | 5.98±0.53 | 4.88±0.96 | 6.71±0.84 | 5.58±0.02 | 4.54±0 |
| | N | (4) | (4) | (1) | (2) | (5) | - | (8) | (8) | (25) | (21) | (15) | (7) | (2) | (1) |
| *HH* | min-max | 3.09-3.62 | 3-4.26 | 3.9-3.9 | 3.17-4.89 | 3.6-4.36 | - | 3.76-4.6 | 4.52-5.43 | 2.88-5.49 | 3.31-5.08 | 2.21-4.29 | 3.9-5.63 | 4.16-4.25 | 2.99-2.99 |
| | mean ± SD | 3.28±0.23 | 3.73±0.57 | 3.9±0 | 4.21±0.63 | 3.98±0.53 | - | 4.28±0.3 | 4.82±0.29 | 4.13±0.85 | 4.16±0.45 | 3.65±0.55 | 4.55±0.71 | 4.21±0.06 | 2.99±0 |
| | N | (4) | (4) | (1) | (5) | (2) | - | (8) | (8) | (20) | (21) | (15) | (7) | (2) | (1) |
| *TE* | min-max | 2-3 | 1-2 | - | 1-3 | 1-2 | 1-2 | 2-3 | 1-2 | 1-3 | 1-2 | 1-2 | 1-3 | 1-2 | 2-2 |
| | mean ± SD | 3±1 | 1±1 | - | 2.28±0.75 | 2.28±0.70 | 1.5±0.70 | 3±0.48 | 1±0.46 | 2±0.55 | 1±0.47 | 2±0.51 | 2±0.55 | 1±0.51 | 2±0 |
| | N | (4) | (4) | - | (5) | (2) | (2) | (10) | (8) | (32) | (22) | (15) | (17) | (8) | (3) |
| *RN* | min-max | 1-2 | 1-2 | 1-2 | 1-2 | 1-2 | 1-3 | 1-2 | 1-2 | 1-2 | 1-2 | 1-1 | 1-2 | 1-2 | 1-2 |
| | mean ± SD | 2±0.5 | 2±0.54 | 2±0.57 | 1±0.53 | 2±0.57 | 2±1 | 1±0.51 | 1±0.48 | 2±0.43 | 1±0.4 | 1±0 | 1±0.51 | 2±0.5 | 2±0.57 |
| | N | (11) | (5) | (3) | (7) | (3) | (3) | (10) | (10) | (34) | (25) | (3) | (21) | (9) | (3) |
| *PS* | min-max | 1-3 | 3-3 | 1-3 | 1-3 | 1-1 | 2-3 | 1-3 | 1-3 | 1-3 | 1-3 | 1-3 | 1-3 | 1-3 | 2-3 |
| | mean ± SD | 3±0 | 3±0 | 2±1.15 | 2±0.81 | 1±0 | 3±0.57 | 2±0.7 | 1±0.58 | 2±0.7 | 1±0.58 | 2±0.67 | 2±0.65 | 3±0.72 | 2±0.53 |
| | N | (10) | (5) | (3) | (7) | (2) | (3) | (10) | (25) | (35) | (25) | (15) | (21) | (9) | (3) |
| *V* | min-max | 27-33 | 32-34 | 31-32 | 28-34 | 34-34 | 31-32 | 29-33 | 33-35 | 27-35 | 27-35 | 25-35 | 28-33 | 30-34 | 26-34 |

| | | *Mesalina* sp. nov. | | | *M. simoni* ssp. nov. | | | *M. simoni* | | *M. olivieri* | | | | *M. pasteuri* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | M | F | J | M | F | J | M | F | M | F | J | M | F | J |
| | mean ± SD | 30.14±2.03 | 33.25±0.95 | 31.67±0.57 | 30.6±2.19 | 34±0 | 31.5±0.7 | 30.56±1.23 | 34±1.41 | 30.22±1.78 | 31.55±1.73 | 30±2.56 | 30.17±1.68 | 31.13±1.6 | 30.33±4.04 |
| | N | (7) | (4) | (3) | (5) | (2) | (2) | (8) | (5) | (22) | (21) | (16) | (18) | (6) | (3) |
| *D* | min-max | 45-56 | 44-52 | 52-52 | 34-54 | 39-46 | - | 42-49 | 36-50 | 40-64 | 37-60 | 36-50 | 39-43 | 38-39 | 42-42 |
| | mean ± SD | 50.5±4.65 | 47±3.82 | 52±0 | 44.57±10.06 | 42.33±3.51 | - | 45.63±2.06 | 42.5±4.78 | 45.54±5.64 | 45.38±5.16 | 42.79±4.19 | 40.43±1.51 | 38.5±0.7 | 42±0 |
| | N | (4) | (4) | (1) | (3) | (3) | - | (8) | (8) | (24) | (21) | (13) | (7) | (2) | (1) |
| *TR* | min-max | 14-17 | 12-15 | 16-16 | 15-1 | 15-15 | - | 15-18 | 13-16 | 14-20 | 12-18 | 14-18 | 15-18 | 14-16 | 14-14 |
| | mean ± SD | 15.75±1.25 | 14±1.41 | 16±0 | 15.2±0.44 | 15±0 | - | 15.88±0.99 | 14.38±0.91 | 16.63±1.66 | 16.22±1.84 | 15.31±1.6 | 16±1.15 | 15±1.41 | 14±0 |
| | N | (4) | (4) | (1) | (5) | (2) | - | (8) | (8) | (21) | (15) | (13) | (7) | (2) | (1) |
| *SL (Sx)* | min-max | 4-5 | 4-4 | - | 4-5 | 4-4 | - | 4-5 | 4-4 | 4-6 | 3-5 | 4-5 | 5-7 | 4-4 | 5-5 |
| | mean ± SD | 4.2±0.44 | 4±0 | ± | 4.29±0.48 | 4±0 | - | 4.09±0.3 | 4±0 | 4.35±0.56 | 4.09±0.42 | 4.24±0.43 | 5.33±0.7 | 4±0 | 5±0 |
| | N | (5) | (4) | () | (7) | (2) | - | (11) | (11) | (24) | (22) | (17) | (9) | (2) | (1) |
| *SL (Dx)* | min-max | 4-5 | 4-4 | 4-5 | 4-5 | 4-4 | 4-4 | 4-5 | 3-4 | 4-6 | 4-5 | 3-6 | 4-6 | 4-6 | 5-6 |
| | mean ± SD | 4.27±0.46 | 4±0 | 4.33±0.57 | 4.25±0.5 | 4±0 | 4±0 | 4.13±0.35 | 3.88±0.35 | 4.31±0.53 | 4.29±0.46 | 4.06±0.55 | 4.91±0.42 | 5±0.5 | 5.33±0.57 |
| | N | (11) | (5) | (3) | (4) | (2) | (2) | (8) | (8) | (32) | (24) | (17) | (22) | (9) | (3) |
| *IL(Sx)* | min-max | 7-9 | 7-9 | 7-8 | 7-9 | 7-8 | 8-8 | 7-9 | 7-8 | 7-9 | 7-9 | 6-10 | 6-11 | 8-9 | 6-8 |
| | mean ± SD | 7.8±1.09 | 7.5±1.41 | 7.5±0.7 | 7.83±0.75 | 7.5±0.7 | 8±0 | 7.7±0.67 | 7.2±0.42 | 7.93±0.79 | 7.86±0.57 | 7.41±0.93 | 7.73±1.48 | 8.33±0.57 | 7±1.41 |
| | N | (5) | (4) | (2) | (6) | (2) | (1) | (10) | (10) | (29) | (21) | (17) | (11) | (3) | (2) |
| *IL(Dx)* | min-max | 6-9 | 7-8 | 7-8 | 7-9 | 7-7 | 7-7 | 8-9 | 6-8 | 6-10 | 6-9 | 5-9 | 6-10 | 6-9 | 6-8 |
| | mean ± SD | 7.11±0.92 | 7.6±0.54 | 7.33±0.57 | 7.8±0.83 | 7±0 | 7±0 | 8.25±0.46 | 7.38±0.74 | 7.91±1.05 | 7.68±0.69 | 7.41±0.93 | 7.32±0.99 | 7.22±0.97 | 7±1 |
| | N | (9) | (5) | (3) | (5) | (2) | (2) | (8) | (8) | (32) | (24) | (17) | (22) | (9) | (3) |
| *G* | min-max | 21-32 | 24-28 | 24-27 | 24-28 | 26-28 | 22-30 | 21-27 | 20-24 | 20-34 | 21-33 | 17-29 | 22-31 | 24-28 | 25-30 |
| | mean ± SD | 27.63±2.82 | 25.75±2.06 | 25.33±1.52 | 25.4±1.67 | 27±1.41 | 26±5.65 | 23.43±2.12 | 22.86±1.6 | 25.73±3.29 | 24.77±2.8 | 23.41±2.98 | 26.46±2.56 | 26.5±1.73 | 29±2.64 |
| | N | (8) | (4) | (3) | (5) | (2) | (2) | (8) | (8) | (30) | (22) | (17) | (15) | (4) | (3) |
| *Col* | min-max | 10-11 | 7-11 | 11-12 | 6-12 | 8-9 | 11-11 | 9-12 | 8-12 | 6-14 | 7-12 | 8-14 | 8-12 | 7-11 | 7-12 |
| | mean ± SD | 10.88±0.35 | 9.33±2.08 | 11.33±0.57 | 8.6±2.19 | 8.5±0.7 | 11±0 | 10.5±1.19 | 9.75±1.28 | 9.72±1.98 | 9.36±1.29 | 9.75±1.43 | 10.13±1.12 | 9.8±1.64 | 9.5±3.53 |

| | | *Mesalina* sp. nov. | | | *M. simoni* ssp. nov. | | | *M. simoni* | | *M. olivieri* | | | *M. pasteuri* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | M | F | J | M | F | J | M | F | M | F | J | M | F | J |
| | N | (8) | (3) | (3) | (5) | (2) | (2) | (8) | (8) | (29) | (22) | (16) | (15) | (5) | (2) |
| *EL* | min-max | 5-6 | 5-5 | 5-6 | 6-10 | 8-5 | - | 9-12 | 5-12 | 3-10 | 5-12 | 4-8 | 1-7 | 5-6 | 5-5 |
| | mean ± SD | 5.11±0.33 | 5±0 | 5.33±0.577 | 7.5±1.6 | 6.5±2.12 | - | 10.5±1.19 | 8.38±2.44 | 6±1.78 | 6.86±2.03 | 5.83±1.32 | 4.83±1.64 | 5.25±0.5 | 5±0 |
| | N | (9) | (5) | (3) | (8) | (2) | - | (8) | (8) | (17) | (14) | (6) | (12) | (4) | (1) |
| *NTS* | min-max | 11-13 | 10-13 | 10-13 | 8-10 | 10-11 | 8-11 | 9-15 | 8-12 | 7-13 | 8-12 | 7-10 | 6-11 | 7-10 | 8-9 |
| | mean ± SD | 12.36±0.67 | 11.6±1.14 | 12±1.73 | 8.71±0.75 | 10.5±0.7 | 9.33±1.52 | 10.9±1.91 | 9.7±1.41 | 10±1.06 | 9.68±1.03 | 9.17±0.92 | 8.57±1.43 | 8.89±1.26 | 8.67±0.57 |
| | N | (11) | (5) | (3) | (7) | (2) | (3) | (10) | (10) | (36) | (25) | (18) | (21) | (9) | (3) |
| *Pf (Sx)* | min-max | 12-18 | 12-17 | 14-15 | 10-12 | 11-12 | 12-13 | 10-14 | 10-14 | 11-18 | 10-16 | 9-14 | 11-17 | 10-13 | 9-12 |
| | mean ± SD | 14.6±1.83 | 14.25±2.06 | 14.33±0.57 | 11.6±0.89 | 11.5±0.7 | 12.5±0.7 | 12.44±1.23 | 12±1.19 | 13.19±1.75 | 12.45±1.77 | 12.2±1.69 | 13.44±1.65 | 11.67±1.03 | 11±1.73 |
| | N | (10) | (4) | (3) | (5) | (2) | (2) | (9) | (8) | (27) | (19) | (15) | (18) | (6) | (3) |
| *Pf (Dx)* | min-max | 12-17 | 14-15 | 14-14 | 12-13 | 11-12 | 12-13 | 5-13 | 10-14 | 11-18 | 4-15 | 10-15 | 11-17 | 8-13 | 9-13 |
| | mean ± SD | 14.6±1.83 | 14.25±0.95 | 14±0 | 12.2±0.83 | 11.5±0.7 | 12.5±0.7 | 11.33±2.55 | 12±1.41 | 13.29±1.75 | 11.5±2.41 | 12.38±1.41 | 13.44±1.65 | 11.33±1.75 | 11.33±2.08 |
| | N | (10) | (4) | (3) | (5) | (2) | (2) | (9) | (8) | (28) | (20) | (16) | (18) | (6) | (3) |
| *Lam* | min-max | 18-23 | 20-24 | 23-23 | 22-24 | 21-25 | 23-23 | 18-23 | 19-22 | 17-29 | 16-24 | 10-15 | 11-17 | 8-13 | 16-24 |
| | mean ± SD | 22.14±1.86 | 21.5±1.91 | 23±0 | 22.75±0.95 | 23±2.82 | 23±0 | 20±1.65 | 20.25±1.5 | 21.44±3.05 | 19.5±2.3 | 12.38±1.41 | 13.44±1.65 | 11.33±1.75 | 19.93±2.63 |
| | N | (7) | (4) | (1) | (4) | (2) | (1) | (9) | (4) | (25) | (20) | (16) | (18) | (6) | (15) |

**Table 2.3** Loading scores and percentage of variance explained in the first two principal components extracted according to the Principal Components Analysis using morphological characters comparing male and female individuals of *Mesalina* sp. nov. with the other species of the Mesalina olivieri species complex.

| | *Mesalina* sp. nov. VS *M. olivieri* | | | *Mesalina* sp. nov. VS *M. simoni* | | | *Mesalina* sp. nov. VS *M. pasteuri* | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Males | | | | | |
| Characters | PC 1 | PC 2 | PC 3 | PC 1 | PC 2 | PC 3 | PC 1 | PC 2 | PC 3 |
| SVL | **0.944** | 0.123 | -0.171 | -0.181 | **0.942** | 0.059 | **0.956** | 0.059 | -0.177 |
| HL | 0.211 | 0.155 | 0.262 | -0.030 | 0.194 | -0.008 | 0.214 | -0.058 | 0.037 |
| HW | 0.177 | -0.159 | 0.303 | -0.025 | 0.149 | -0.017 | 0.140 | -0.065 | **0.755** |
| HH | 0.152 | -0.266 | 0.355 | -0.026 | 0.106 | 0.037 | 0.131 | 0.037 | 0.453 |
| TE | 0.021 | 0.069 | **0.785** | -0.006 | 0.058 | -0.026 | -0.019 | **0.863** | 0.010 |
| RN | -0.035 | -0.113 | 0.238 | 0.510 | 0.049 | **0.858** | -0.024 | 0.232 | 0.415 |
| PS | -0.093 | **0.921** | 0.104 | **0.839** | 0.189 | -0.508 | -0.040 | 0.435 | -0.138 |
| % variance | 95.009 | 2.273 | 1.155 | 97.701 | 2.021 | 0.213 | 96.853 | 1.471 | 0.986 |
| | | | | Females | | | | | |
| Characters | PC 1 | PC 2 | PC 3 | PC 1 | PC 2 | PC 3 | PC 1 | PC 2 | PC 3 |
| SVL | **0.985** | -0.032 | -0.130 | 0.015 | **0.956** | 0.214 | **0.980** | 0.113 | -0.034 |
| HL | 0.130 | 0.222 | **0.665** | 0.017 | 0.164 | 0.077 | 0.167 | -0.467 | 0.108 |
| HW | 0.069 | 0.084 | 0.518 | 0.003 | 0.079 | 0.031 | 0.086 | 0.120 | -0.175 |
| HH | 0.045 | -0.238 | 0.475 | -0.003 | 0.017 | 0.064 | 0.037 | **-0.558** | -0.312 |
| TE | -0.046 | 0.142 | 0.163 | -0.006 | 0.002 | -0.014 | -0.042 | 0.524 | -0.371 |
| RN | 0.067 | -0.069 | -0.082 | 0.584 | 0.174 | **-0.791** | 0.033 | 0.007 | **0.821** |
| PS | 0.020 | **0.928** | -0.120 | **0.811** | -0.147 | 0.564 | -0.002 | 0.411 | 0.218 |
| % variance | 90.752 | 4.559 | 2.013 | 97.682 | 2.142 | 0.125 | 98.441 | 0.793 | 0.426 |

**Table 2.4** Loading scores and percentage of variance explained in the first two principal components extracted according to the Principal Components Analysis using polidosis characters comparing male and female individuals of *Mesalina* sp. nov. with the other species of the *Mesalina olivieri* species complex.

| | *Mesalina* sp. nov. VS *M. olivieri* | | *Mesalina* sp. nov. VS *M. simoni* | | *Mesalina* sp. nov. VS *M. pasteuri* | |
|---|---|---|---|---|---|---|
| | | | Males | | | |
| Characters | PC 1 | PC 2 | PC 1 | PC 2 | PC 1 | PC 2 |
| V | 0.312 | 0.012 | **0.579** | 0.469 | 0.298 | -0.186 |
| D | **-0.714** | -0.051 | 0.486 | **-0.475** | **-0.637** | 0.171 |
| DS | 0.049 | -0.159 | -0.082 | 0.057 | -0.275 | 0.154 |
| TR | 0.213 | 0.179 | 0.221 | 0.066 | 0.194 | 0.018 |
| SL(Sx) | 0.047 | 0.075 | -0.177 | -0.049 | 0.194 | -0.038 |
| SL(Dx) | 0.067 | 0.101 | -0.171 | -0.026 | 0.171 | -0.054 |
| IL(Sx) | 0.052 | 0.050 | 0.134 | 0.103 | 0.079 | 0.139 |
| IL(Dx) | 0.064 | 0.059 | 0.097 | 0.152 | 0.098 | 0.110 |
| G | 0.426 | **-0.694** | -0.397 | 0.283 | 0.266 | -0.354 |
| Col | 0.190 | 0.048 | -0.157 | -0.403 | -0.042 | 0.049 |
| EL | 0.161 | 0.385 | -0.007 | -0.191 | -0.109 | 0.100 |
| NTS | 0.101 | -0.031 | 0.210 | -0.044 | -0.227 | -0.082 |
| Pf(Sx) | -0.024 | 0.112 | -0.107 | -0.034 | 0.035 | -0.380 |
| Pf(Dx) | -0.059 | -0.011 | -0.218 | 0.120 | -0.023 | -0.296 |
| Lam | 0.272 | 0.522 | -0.028 | 0.462 | 0.414 | **0.707** |
| % variance | 43.313 | 17.317 | 92.216 | 3.601 | 46.810 | 18.483 |
| | | | Females | | | |
| Characters | PC 1 | PC 2 | PC 1 | PC 2 | PC 1 | PC 2 |
| V | -0.040 | -0.512 | **0.627** | 0.404 | 0.297 | 0.087 |
| D | **-0.403** | -0.185 | 0.482 | **-0.582** | **-0.624** | -0.243 |
| DS | -0.008 | 0.092 | -0.080 | 0.044 | -0.117 | -0.009 |
| TR | -0.173 | **0.664** | 0.185 | 0.199 | 0.242 | 0.144 |
| SL(Sx) | -0.003 | 0.036 | -0.159 | 0.032 | 0.068 | -0.055 |
| SL(Dx) | 0.017 | 0.109 | -0.169 | 0.030 | 0.221 | -0.031 |
| IL(Sx) | -0.078 | 0.112 | 0.108 | 0.034 | 0.140 | -0.004 |
| IL(Dx) | -0.031 | 0.041 | 0.056 | 0.138 | 0.134 | -0.189 |
| G | 0.392 | 0.388 | -0.373 | -0.164 | 0.418 | -0.347 |
| Col | 0.131 | -0.063 | -0.108 | -0.132 | -0.053 | 0.422 |
| EL | -0.542 | 0.258 | -0.003 | 0.489 | -0.034 | -0.011 |
| NTS | 0.070 | -0.020 | 0.193 | 0.107 | -0.231 | -0.259 |
| Pf(Sx) | 0.346 | -0.013 | -0.157 | 0.253 | -0.064 | **0.486** |
| Pf(Dx) | 0.242 | 0.017 | -0.222 | 0.273 | -0.195 | 0.485 |
| Lam | 0.389 | 0.068 | -0.047 | 0.029 | 0.290 | 0.192 |
| % variance | 27.494 | 20.650 | 94.817 | 2.501 | 51.127 | 20.854 |

**Table 2.5** Loading scores and percentage of variance explained in the first two principal components extracted according to the Principal Components Analysis using coloration characters comparing male and female individuals of *Mesalina* sp. nov. with the other species of the *Mesalina olivieri* species complex.

| | *Mesalina* sp. nov. VS *M. olivieri* | | *Mesalina* sp. nov. VS *M. simoni* | | *Mesalina* sp. nov. VS *M. pasteuri* | |
|---|---|---|---|---|---|---|
| | | | Males | | | |
| Characters | PC 1 | PC 2 | PC 1 | PC 2 | PC 1 | PC 2 |
| EBL | -0.191 | -0.063 | **0.964** | -0.203 | 0.196 | -0.104 |
| DBF | 0.080 | **-0.669** | 0.157 | 0.241 | 0.392 | -0.209 |
| PSDBF | 0.337 | 0.214 | -0.053 | -0.123 | 0.179 | 0.339 |
| PDLL | 0.254 | 0.152 | 0.055 | 0.227 | -0.465 | 0.238 |
| SPDLL | -0.124 | 0.517 | 0.106 | 0.368 | -0.216 | 0.044 |
| DDSLL | -0.047 | -0.215 | 0.089 | 0.287 | -0.336 | **-0.663** |
| SDDLB | -0.254 | -0.136 | 0.087 | **0.768** | **0.552** | 0.012 |
| PSDDSLL | **0.689** | 0.147 | -0.061 | -0.100 | 0.231 | -0.055 |
| DSO | -0.409 | 0.353 | 0.084 | 0.080 | -0.062 | 0.565 |
| TC | -0.233 | 0.029 | 0.051 | -0.100 | 0.196 | -0.104 |
| % variance | 49.928 | 14.493 | 78.780 | 9.658 | 50.541 | 26.395 |
| | | | Females | | | |
| Characters | PC 1 | PC 2 | PC 1 | PC 2 | PC 1 | PC 2 |
| EBL | -0.112 | -0.062 | **0.935** | -0.076 | 0.183 | -0.009 |
| DBF | 0.008 | 0.423 | 0.172 | 0.240 | 0.446 | 0.369 |
| PSDBF | 0.186 | -0.014 | 0.027 | 0.054 | 0.227 | 0.074 |
| PDLL | 0.319 | 0.086 | 0.042 | -0.225 | **-0.487** | -0.243 |
| SPDLL | 0.269 | -0.022 | 0.209 | -0.562 | -0.204 | **0.639** |
| DDSLL | -0.063 | 0.233 | 0.055 | 0.199 | -0.377 | -0.184 |
| SDDLB | **-0.809** | 0.158 | 0.206 | **0.703** | 0.471 | -0.547 |
| PSDDSLL | -0.177 | **-0.594** | -0.013 | 0.180 | 0.182 | -0.119 |
| DSO | 0.180 | 0.505 | -0.004 | 0.020 | 0.078 | 0.072 |
| TC | 0.238 | -0.348 | 0.072 | 0.033 | 0.184 | 0.197 |
| % variance | 39.872 | 16.950 | 79.353 | 11.635 | 55.148 | 25.457 |

### *2.3.5 TAXONOMIC IMPLICATIONS*

Given its genetic, morphological and ecological divergence and lack of gene flow with *M. pasteuri* and *M. simoni*, we assign species level to the new lineage of the *olivieri* complex from Mauritania. Moreover, both mtDNA and nuclear DNA data unambiguously group the populations of "*Mesalina olivieri*" from southern and southwestern Morocco with *M. simoni*, and confirm that *M. simoni* is a valid species that is highly divergent from *M. olivieri* (distributed from eastern Morocco to Israel, see Fig. 2.1). The formal description of these two lineages was not included here to do not compromise the future publication on peer-reviewed scientific journal.

### *2.3.6 DISTRIBUTION MODELLING AND CONSERVATION STATUS*

The 20 replicate ecological models exhibited good predictive accuracy (average AUC=0.906). The most important environmental predictors were terrain ruggedness index (42.6% contribution) and Land-cover (35.4%) (Table S8; Fig. 2.6). The highest probability of occurrence is at an intermediate levels of terrain ruggedness and in bare rock habitats (Fig. S8). The predicted range of *Mesalina* sp. nov. encompassed the mountain rocky areas of the Adrar Atar in Mauritania, further extending to the north up to the plateau around F'derick, to the north-west up to Koudiet Laghnem (Morocco), and to the south down to the central Tagant mountain.

The Area of Occupancy (AOO) calculated from the ecological models was of 34,766 $km^2$, while the Extent of Occurrence (EOO) was of 175,445 $km^2$. Following IUCN guidelines, it is here proposed the status of Near Threatened for *Mesalina* sp. nov. according to the following parameters: i) EOO below 20,000 $km^2$, ii) the high degree of uncertainty in the population size; iii) the rarity of the species, and iv) the general fragmented distribution. The taxon is restricted to the rocky highlands of the Adrar and neighbouring highlands with northern disjunct populations apparently separated from the distribution by wide unsuitable habitats (sandy desert) (Fig. 2.6).

### *2.3.7 HABITAT COMPARISON*

There are significant differences in number of observations in each land-cover category ($\chi^2$; p=0.037; df=39). *Mesalina sp. nov.* selects (70% of observations) a specific land cover habitat that has a restricted availability in the study area (6.3% of study area) being most frequently found in bare rocks units. *Mesalina simoni* was most frequently found in rocky plateau, while *M. olivieri* and *M. pasteuri* occur in almost all units. Bs measure of niche breadth was lower for *Mesalina sp. nov.* and *M. simoni*, and higher for *M. olivieri* and *M. pasteuri*, indicating that the former pair are more specialized than the latter (Table S8).

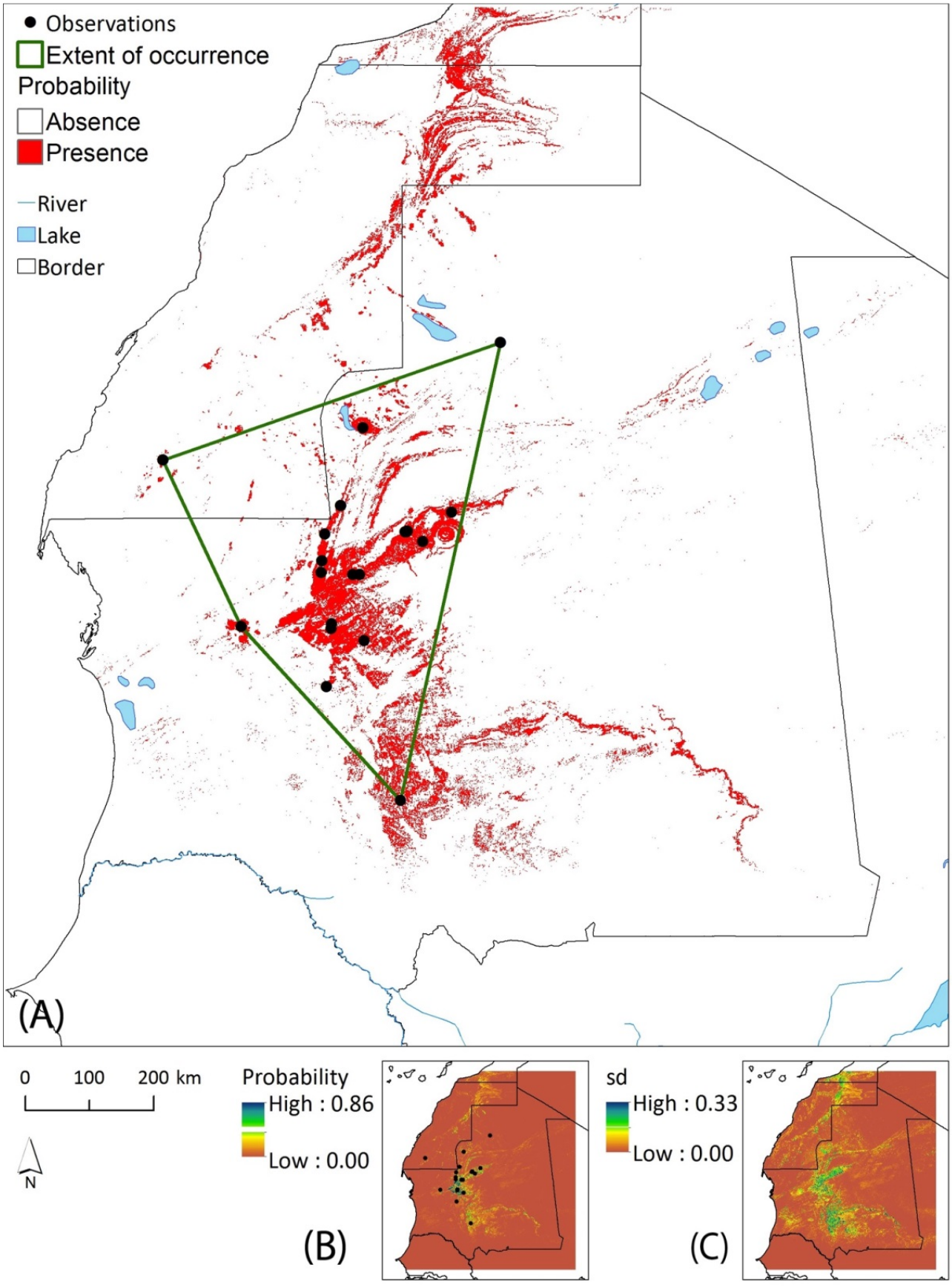


**Fig. 2.5** Ecological modelling of *Mesalina* sp. nov. (A) Binary predictions of suitable habitats for the taxon. (B) Average probability of occurrence and (C) standard deviation from 20 individual model replicates.

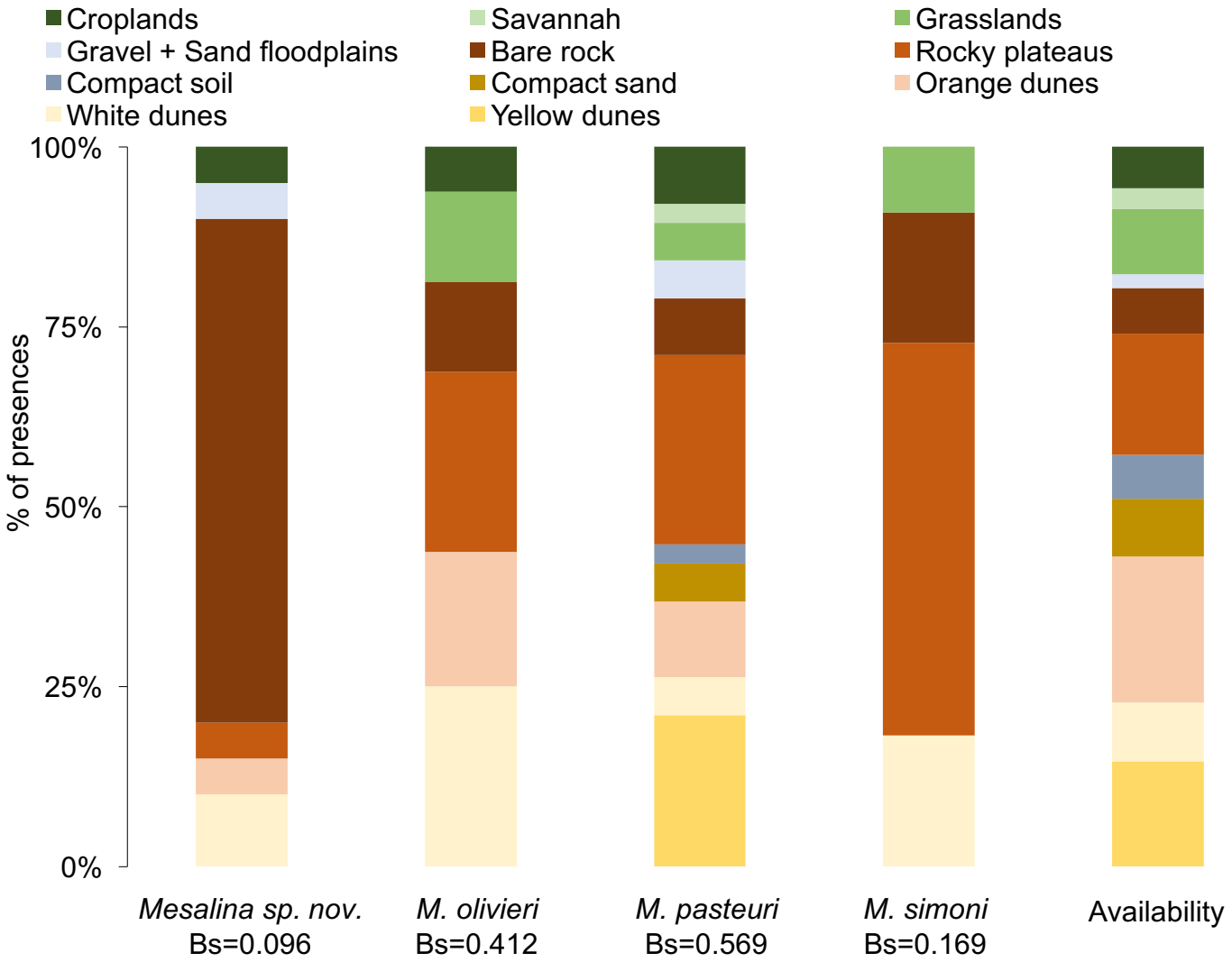


**Fig. 2.6** Results on the habitat analysis on the five species of the *olivieri* complex in North West Africa. Bs is the standardized Levin's measure ranging from 0.0 to 1.0; high B means no discrimination between land-cover units, meanwhile low Bs value means selection among land-cover units.

# 2.4. DISCUSSION

The integrative taxonomy methodology used in this study offers further evidence about how multidisciplinary approaches are pivotal for better understanding and describing a new species. Our study describes a new species within the *M. olivieri* species complex and solves other taxonomic problems within this group. Moreover, this study unveils much more intraspecific diversity within this last two species than previously described. Our integrative taxonomy assessment on *Mesalina sp. nov.* and *M. simoni* provide additional evidences about how highlands, hydrographic systems, and coastal areas can work as refugia and centre of diversification during the humid and dry cycles that characterised the Sahara Desert (Brito et al., 2014; Gonçalves et al., 2018a,b; Velo-Antón et al., 2018).

### *2.4.1 PHYLOGENETIC RELATIONSHIPS AND SYSTEMATICS OVERVIEW*

Our phylogenetic results unveil *Mesalina sp. nov,* as an independent evolving lineage, while the *olivieri* lineages from the Atlas Mountain and Atlantic Sahara evolved together with *M. simoni* as another independent evolving lineage. However, we found mitonuclear discordances in *M. martini* and the Algerian *M. olivieri*. *Mesalina martini* resulted to be basal to the *olivieri* species complex when more data were included in the analysis, higher number of genes in the cytonuclear tree (dataset 3) or higher number of samples in the mitochondrial tree including all the species of the genus available in GeneBank (dataset 1). The unclear phylogenetic situation of *M. martini* was previously highlighted by other studies. It was for instance found to be basal to the *M. olivieri* species complex (Kapli et al., 2008; 2015), but also basal to the other species, except for *M. watsonana* in Sindaco et al. (2018) and Simó-Riudalbas et al. (2019). Seen the low support recorded in any of the cases, this result could be due to incomplete lineage sorting caused by the discrepancy between the amount of data present for *M. martini* included in the analysis. Despite the efforts here developed to sequence additional nDNA genes in comparison to previous studies, further research is needed to better clarify the relativeness of *M. martini* with the other species of the genus, including samples covering the complete distribution area of the species and possibly more genes, both mitochondrial and nuclear.

Regarding the cytonuclear discordance for the *olivieri* clade from Algeria (*M. olivieri* ALG1 in Fig 2.2 and S2; samples BEV.9225 and BEV.T3038 in Fig. S3), just two were the studies including this clade in their Bayesian inference phylogenetic analysis. The study of Kapli et al. (2015) and in the study of Simó-Riudalbas et al. (2019). Both the studies

analysed the same fragments of mitochondrial DNA (*16S* and *Cyt-b*). As it is recorded in this study, the Algerian clade is recovered to be sister taxa of the *M. pasteuri* from Mauritania. This discordance between the nuclear and mitochondrial scenario, could be due to an higher number of variable characters in mtDNA that may consequently express cases of homoplasy. Another potential explanation could be a a relatively recent hybridization event that led to gradual mitochondrial introgression between the Algerian *M. olivieri* and a lineage of *M. pasteuri* from Mauritania, Niger and Algeria. Incongruences on the genetic divergence between mtDNA and nDNA have been also reported in other taxa, like *Drosophila simulans, Podarcis muralis* and *Tarentola mauritanica* (Ballard et al., 2007; Pinho et al., 2007; Rato et al., 2010) explained by incomplete lineage sorting or ongoing gene flow between the two lineages (García-Paris et al., 2008; Rato et al., 2010). The results of the cytonuclear concatenated tree (Fig. 2.2) agree with the nuclear DNA results where the Algerian lineage results to be an independent evolving lineage, basal to *M. olivieri* and *M. pasteuri*. Sampling gaps from the central region of North Africa, especially from the contact zones between *M. olivieri* and *M. pasteuri*, hamper the validation of a putative broader contact zone between *M. pasteuri* and *M. olivieri* over southern Algeria, north-eastern Mauritania, Mali and Niger, and/or the presence of a new species from Algeria.

### *2.4.2 SPATIAL STRUCTURE OF GENETIC VARIABILITY*

#### *2.4.2.1 Biogeography of Mesalina sp. nov.*

The *M. olivieiri* species complex started to diversify in the Late Miocene (ca. 12.08 - 6.38 Mya). This dating is congruent with the beginning of the arid period that originated the current Sahara Desert (Holmes, 2008). Mountain chains, highland and coastal areas worked as refugia during the arid and humid cycles of the Sahara-Sahel (Brito et al., 2014; Gonçalves et al., 2018a,b; Velo-Antón et al., 2018). The progressing desertification in the area likely forced the ancestor of the *M. olivieri* species complex to contract its distribution in the several refugia present in the Sahara (e.g. Adrar-Atar highlands and the Atlantic coastal area). This contraction could have leaved the ancestor of *Mesalina sp. nov.* and *M. simoni* isolated from the other species of *M. olivieri.* This hypothesis is supported by the low genetic divergence between these two taxa and the high divergence observed with the remaining species included in our study (Table 2.1). *Mesalina sp. nov.* and *M. simoni* started their indipend evolution around 8.9 Mya. The high genetic divergence between the Atlantic lineage of *M. simoni ssp. nov.* and *Mesalina sp. nov.* could indicate an early split

between the *M. simoni* group and *Mesalina sp. nov.* occurred in that area (north to the current distribution of *Mesalina sp. nov.* ). It is still unclear what was the geological situation of the area at that time. Signs, as the low elevation above the sea level and the presence of sand in the area even before the Sahara's desertification started (Swezey, 2008), point to the possibility that the area was probably flooded and that the today's mountain chains and plateau (i.e. Adar-Atar) were islands. This potential scenario matches with the phylogeographycal pattern of this new species and *M. simoni* and some species of reptiles as *Agama sp.* (Gonçalves at al., 2018a). These species may have contracted their distribution in the Adrar-Atar and Tagant mountains or to the Atlas Mountain chain respectively to than expand again once the ocean retracted. This diversification could be also explained by the vicariance effect due to the formation of numerous hydrological networks in the Western Sahara (e.g. Tamanrasset paleo river; Skonieczny et al., 2015) that worked as genetic barrier between the two species. This large hydrological system likely acted as barrier to dispersal in some species (e.g. *Psammophis schokari*, Gonçalves et al., 2018b), cutting the region longitudinally with its massive amount of water during the humid periods, and the vast sandy area left behind it during the arid periods. The Atlantic coast of the Sahara Desert has also been proposed to act as Trans-Sahara corridor and refugia for several species (Brito et al., 2014, 2016; Gonçalves et al., 2018b; Velo-Antón et al., 2018) given its stable climate, high humidity and vegetation cover when compared to the continental areas (Dinerstein et al., 2017). However, the shifts in the local hydrological regime have likely worked as landscape barriers that isolated different populations, which promoted high diversification levels through vicariant effects (Gonçalves et al., 2018b; Velo-Antón et al., 2018) and leaded to the formation of multiple endemic taxa (Crochet et al., 2003; Vogel et al., 2006; Sindaco and Jeremcenko, 2008; Ndiaye et al., 2012; Trape et al., 2012), among which *M. simoni* (Schleich et al., 1996). The latitudinal genetic structure recorded for *Mesalina sp. nov.* and *M. simoni* could be due to more recent contraction and expansion of the lineages due to adverse and favourable climatic condition (Swezey, 2009; Brito et al., 2014; Gonçalves et al., 2018a) and/or because of the isolation by temporary geographical features as rivers or dry river basins (Gonçalves et al., 2018b; Velo-Antón et al., 2018).

Moreover, the historical demography results show an overall reduction in the population density of *Mesalina sp. nov.* starting around 2.5 Mya in concomitance with the beginning of the Sahara Green Phase occurred during the Holocene. This shrink in the population density matches also in time with results on the time of divergence that show a high

intraspecific diversification in the four species of the complex during the Plio-Pleistocene transition.

*2.4.2.2 The hidden diversity within M. olivieri and M. pasteuri*

For what concerns the *M. olivieri* lineages, the North African genetic diversity highlighted in this study matches the continuous latitudinal distribution of the species. From the results, at least five potential different lineages can be discerned: i) one from the Atlas Mountain in the eastern part of Morocco; ii) a well-supported (PP>95%) lineage from the western part of Algeria basal to both *M. olivieri* and *M. pasteuri* and iii) another Algerian lineage from the eastern part of the country; iv) one from Tunisia and v) one including the *M. olivieri* from Egypt and Israel. Regarding *M. pasteuri* two well supported lineages (PP=100%) have been unveiled in North West Africa: i) one mostly distributed on the Atlantic Sahara region; and ii) a second one including specimens from the inland of Mauritania, Niger and Algeria. This noteworthy amount of diversity has been already hypnotised in numerous studies (Arnold et al., 1986, 2007; Kapli et al., 2015). Although this study increases the resolution of the systematic background of *M. olivieri* and *M. pasteuri*, additional data are needed for an integrative approach on the description of these lineages.

*2.4.3 HABITAT COMPARISON AND DISTRIBUTION OF MESALINA SIMONI AND MESALINA SP. NOV.*

The habitat comparison among the four species of the complex gives additional support to the validity of *Mesalina sp. nov.* as a new species, showing that the latter selects among land cover units and exhibits narrow niche breadth (low Bs). For instance, *Mesalina sp. nov* dwells almost exclusively on steep and rocky areas (Fig. 2.6 Table S8), contrary to *M. pasteuri* and *M. olivieri* that are more generalist species (Table S8 and Fig. 2.6) and mostly occur in sandy areas (Bons, 1960; Sindaco and Jeremcenko, 2008; Trape *et al.,* 2012). The relatively low Levin's measure recorded for *M. simoni* indicates that the latter is also a habitat selective species. As *Mesalina sp. nov.*, *M. simoni* also prefers rocky areas but contrary to the former it inhabits plateaus with sparse vegetation, from sea level to above 900 meters. *Mesalina simoni* is an endemic species from the central Atlantic coast of Morocco. It has been previously considered as a subspecies of the Moroccan lineage of *M. olivieri* (Shcherbak, 1975) and later elevated to species level (Schleich et al., 1996) based on morphological features. The new *M. simoni* subspecies distribution matches with the hypothesised Trans-Sahara Atlantic corridor that worked as refugia and centre of lineage diversification for mesic species (e.g. Gonçalves et al., 2018b; Velo-Antón et al., 2018), from the South-Eastern part of the Atlas Mountains, along the Atlantic coast of Sahara, to the border with Mauritania.

### *2.4.4 CONCLUSIONS AND FUTURE RESEARCH*

This study allowed a better understanding of the systematic and phylogeography of the *M. olivieri* species complex, providing also new observation for all the species of the complex extending their current distributions. Here it is demonstrated that the use of a combination of molecular, morphological and environmental data is crucial to clarify taxonomy, ecology and distribution of a species, and unveiling from a molecular point of view the existence of previously undescribed lineages (Arnold, 1986). This study also provides evidences that the single use of mtDNA or nDNA could lead to misleading results. Moreover, while assessing the diversification within the *M. olivieri* species complex, this study gives additional evidences on the validity of the Trans-Sahara Atlantic corridor and the Adrar and Tagant highlands as valid refugia during the climatic oscillation since the Miocene. With the inclusion of more samples and molecular markers, future research will be conducted to understand the taxonomic situation of the *Mesalina olivieri* species complex. These studies will be focused on the clarification of the phylogeographic pattern highlighted in this study and on the description of potential new species.

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# 3 DISCUSSION AND FINAL REMARKS

Since the beginning of the Pliocene that North-West Africa has been shaped by arid and humid cycles, which have characterised the history of the Sahara-Sahel ecoregions (Holmes, 2008; Kröpelin *et al.,* 2008; Brito *et al.,* 2014). The complex paleogeographic and climatic events lead to a peculiar topographical, hydrographical and environmental variation that drove the current biodiversity patterns and induced high levels of diversity and endemism in the area (Sindaco *et al.*, 2008; Brito *et al.,* 2014; Velo-Antón *et al.,* 2018). The ever-changing character of the region has attracted the attention of the scientific community. The arid and hot climate and the remoteness of the area always challenged research activities. The most hindering concern currently is the political instability and social insecurity that is growing in the region over the last decades (Brito *et al*., 2018). It has been highlighted that the increasing warfare and politically instability is inversely related to the research efforts in North West Africa (Brito *et al.,* 2014). This, together with remoteness and harsh climatic conditions that characterise the region results in a huge gap of knowledge on the amount of biodiversity inhabiting the area. Fortunately, this gap is being filled by numerous studies that included North West Africa (Geniez, 2004; Sindaco *et al.*, 2008; Brito *et al.,* 2014; Velo-Antón *et al.,* 2018; Freitas *et al.,* 2018; Gonçalves *et al.,* 2018; Karssene *et al.,* 2019; Demos *et al.,* 2019).

Previous studies on the genus *Mesalina* recovered strong patterns of genetic and morphological differentiation (Arnold, 1989; Joger & Mayer, 2002; Moravec, 2004; Kapli *et al.,* 2008; Yousefkhani *et al.,* 2015; Šmíd *et al.,* 2017; Sindaco *et al,* 2018) and suggested that past geological and climatic events had a profound effect in its biogeographic history and speciation of the genus (Kapli *et al.,* 2015; Simó-Riudalbas *eta al.,* 2019). However, no study has deeply addressed the *M. olivieri* species complex leaving a big lack of knowledge on the systematics, morphology and the geographical genetic diversity of the complex. In this work, it was addressed the interspecific variability of the *M. olivieri* species complex using an integrative approach that combined genetic, ecological and morphological variability analyses. This study gives a multidisciplinary overview about the phylogeography, morphology and distribution of two new undescribed taxa from Mauritania and Atlantic Sahara, validating the status of a new species and as new subspecies of *M. simoni*.

Integrative taxonomy is a powerful tool that allows obtaining broader and resolute pictures of the ecological role, natural history and the evolution of a species (Padial *et al.,* 2010; Crottini *et al.,* 2011; Heinrichs *et al.,* 2015; Ghielmi *et al.,* 2016; Sloan *et al.,* 2017; Šmíd *et al.,* 2017; Sindaco *et al.,* 2018). Here it was also provided for the first time information regarding high diversity of the *M. olivieri* species complex in North West Africa, increasing knowledge on genetic variability. Despite

the new data regarding the interspecific and intraspecific patterns of genetic variation in the complex, more studies are needed on its morphological and ecological variation.

## 3.1. INTERSPECIFIC DIVERSITY WITHIN *MESALINA OLIVIERI* SPECIES COMPLEX

### *3.1.1. GENETICS*

The evidences given by the accumulation of the phylogenetic results (concatenated cytonuclear Bayesian tree and species tree), genetic divergences, and haplotype networks give strong genetic support to the recognition of the lineage of *M. olivieri* from Mauritania as a valid species and the inclusion of the two lineages of *M. olivieri* from the Atlantic Sahara and Atlas Mountain as subspecies of *M. simoni*. These results expand the currently known distribution from Figuig in North-eastern Morocco until the North-western border of Mauritania.

The results obtained here also provide evidences on the high intraspecific diversity within the *Mesalina olivieri* species complex putting forward the possibility of an additional valid species from Algeria. Moreover, this study also adds evidences that the single use of mtDNA or nDNA could lead to misleading results.

### *3.1.2. MORPHOLOGY*

The results for the PCA pointed to some morphologic differences between the species of the complex and the new species from Mauritania, as for instance the number, shape and disposition of the scales composing the eyelid and in the number of rows of the temporal granulae (Fig. S6). This species is also discernible from its fragmented but linear and organized dorsal pattern and, In vivo, its pearly coloration stronger on the ventral part of the body (Fig. 3.1 and 3.2). Unfortunately, also the most updated dichotomic key available for the genus *Mesalina* (Trape *et al.,* 2012; Yousefkhani *et al.,* 2015) were found to be not completely accurate or of little use for the species belonging to the *M. olivieri* species complex (e.g. Trape et al., 2012). Future studies to define dichotomous characters that can better discern the species within this complex are necessary. It would be interesting to take into consideration also internal characters, such as hemipenis anatomy because differences in their length have already been highlighted by Arnold (1986a and 1986b).

**Fig 3.1.** Dorsal and ventral prospective of an adult of the new species from the Adrar Atar (Mauritania). Pictures from P.-A. Geniez

Despite this, it is here highlighted the strong morphologic modification during the ontogenesis that characterise individuals of this new species. The evidences given by the phylogenetic analysis and haplotype networks (Fig. 2.2 and 2.3) recovered the two juvenile specimens (147.3 and 147.4; Fig. 3.2) previously misrecognised as *M. pasteuri* in Kapli *et al.* (2015), as belonging to this new species from Mauritania and neighbouring regions. This finding provides a good overview of what most likely are the morphological steps that characterise the ontogenesis of this new species, but also solves the paraphyly of *M. pasteuri* and extends the known distribution of the species (previously restricted to the plateau of the Adrar Atar in Mauritania) to the southern Tagant plateau.


**Fig 3.2.** Juveniles of the new species previously recognized as *M. pasteuri* on the left and in the middle (Codes from Kapli *al.,*2015) and an adult from the Adrar on the right. The specimen on the left is a new-born displaying the typical striped coloration meanwhile on the right a juvenile. It is evident how the striped-like pattern is stronger in the first stage of the ontogenesis to than shade first in the juveniles and totally disappear in the adults. Pictures from Cristian Pizzigalli (left) and José Carlos Brito (middle and right).

### *3.1.3. ECOLOGICAL ANALYSIS AND CONSERVATION STATUS*

The ecological niche-based analysis situates the new species in habitats characterised by intermediate levels of ruggedness and bare rocks. The predicted distribution encompasses the mountain rocky areas of the Adrar Atar in Mauritania, further extending to the north up to the plateau around F'derick, to the north-west up to Koudiet Laghnem (Morocco), and to the south down to the central Tagant mountain. The new species is confirmed to be sympatric to *M. pasteuri, M. rubropunctata* and *M. guttulata* (Trape *et al.,* 2012; Sindaco *et al.,* 2018). While *M. pasteuri* is

well known to inhabit mostly sand dunes, the latter two species share the same habitats with the new species in some localities (Trape *et al.*, 2012), but haplotype networks confirm that they do not hybridise (results not showed). The fine scale distribution of *Mesalina* sp. nov. and *M. pasteuri* in the Adrar Atar plateau in relation to main categories of land-cover (Fig. 3.2) shows that the former species is exclusively found on the upper rocky parts of the plateau (sometimes isolated by kilometres of sandy desert), being absent from the lower sandy and dune valleys where the latter species occurs (Bons, 1960; Sindaco and Jeremcenko, 2008). Although no evident contact zones are known, additional local studies are needed to better understand habitat partition between the two species and potential interactions with the other species of the genus *Mesalina* present in the region.

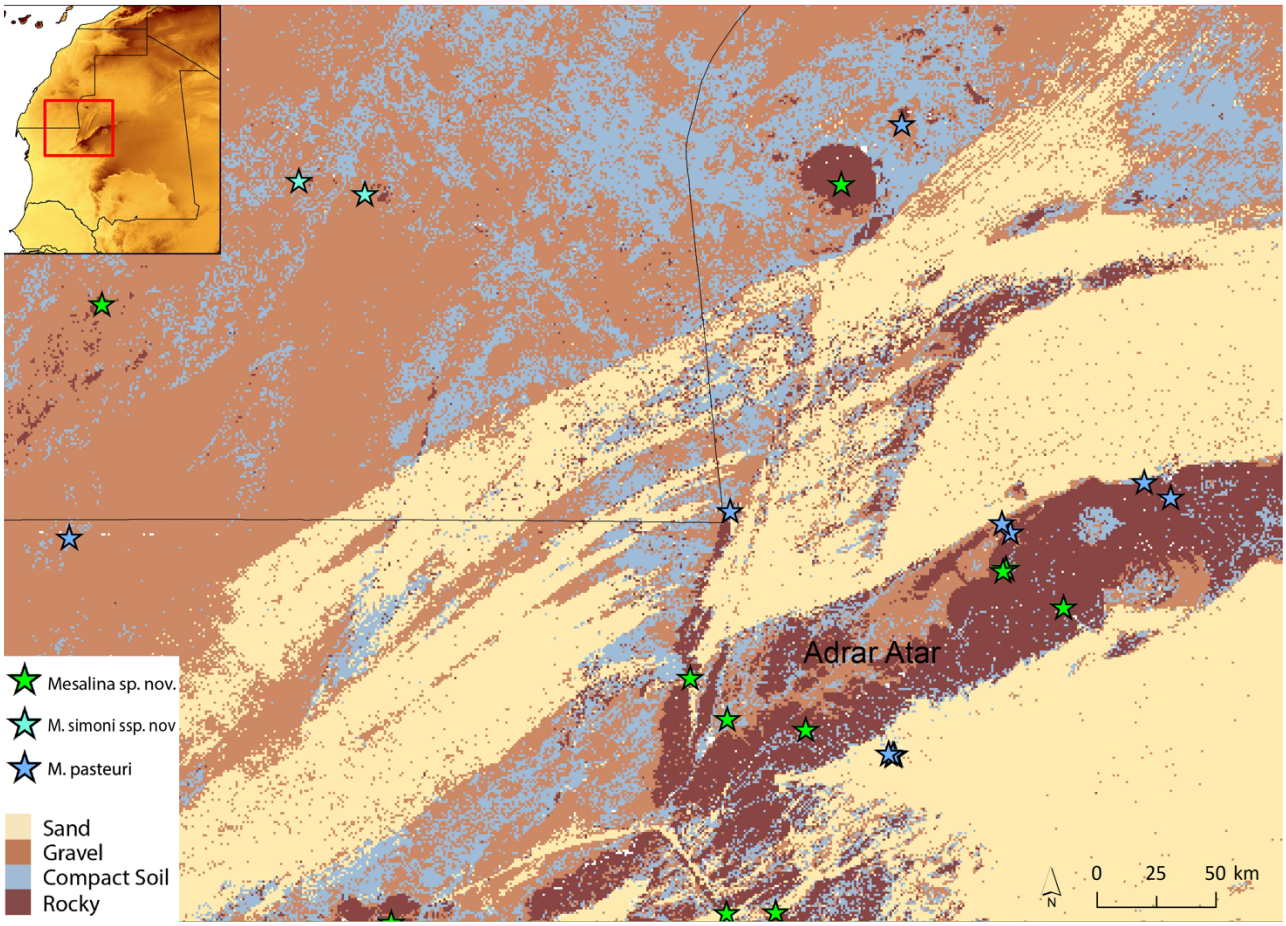


**Fig 3.3** Distribution of *Mesalina* sp. nov. (light green), *M. simoni* ssp. nov. (light blue), *M. pasteuri* (dark blue), and main land-cover categories (adapted from Campos and Brito, 2018) in the Adrar Atar (Mauritania) and adjacent northern regions.

The conservation status of the new species can be considered as Least Concern according to the IUCN Red List categories (2019). This classification was based on the high values recorded for the area of occupancy (AOO = 34.766 km$^2$) and extend of occurrence (EOO = 175,445 km$^2$). Still, it is important to consider the scarce currently available knowledge about the new species, the

remoteness of its distribution area, and its apparent extreme habitat fragmentation. Thus, local studies are needed to confirm the correct assignment of the conservation status.

## 3.2 PAST CLIMATIC CHANGES AS DRIVERS FOR SPECIATION WITHIN *MESALINA OLIVIERI* SPECIES COMPLEX.

The analysis on the time of divergence show that high intraspecific diversification occurred during the Pliocene-Pleistocene transition, and the same pattern was found in the mtDNA historical demography simulations. A demographic contraction was predicted to have started around 4 Mya and a bottleneck period from 2 Mya until 0.1 Mya. This initial contraction was preceded by a period population stabilisation and followed by an expansion starting at about 0.1 Mya. The climatic oscillations that characterised the Sahara-Sahel during the Pliocene and Pleistocene affected the region after the beginning of the desertification process. These climatic oscillations resulted in arid and humid periods that drove the population expansion (during dry periods) and contraction (during humid periods) of the xeric species inhabiting the area, leading to the formation of new lineages and contact zones (Brito *et al.,* 2014). This pattern has been found in several reptile species, including *Tarentola* sp. (Rato *et al., 2012*), *Stenodactylus* sp. (Metallinou *et al.*, 2012), and *Agama* sp. (Gonçalves *et al.,* 2018). Although additional data analysis are needed to support this hypothesis, the new species from Mauritania could be considerate another good model to test the diversification through aridity-induced vicariance in North-West Africa and how highlands, hydrographic systems, and coastal areas can work as refugia and centers of diversification for xeric species during the humid and dry cycles that characterised the Sahara Desert. Moreover, the assessment of the diversification within the *M. olivieri* species complex developed in this study gave additional evidence on the validity of the Trans-Sahara Atlantic corridor and the Adrar and Tagant highlands as valid refugia during the climatic oscillation from the Miocene until now.

Still, it remains to be clarified the taxonomic situation of the other species of the *M. olivieri* species complex, understand their biogeographic histories, and describe the potential new species highlighted in this work.

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# 4. APPENDIX SUPPLEMENTARY MATERIAL

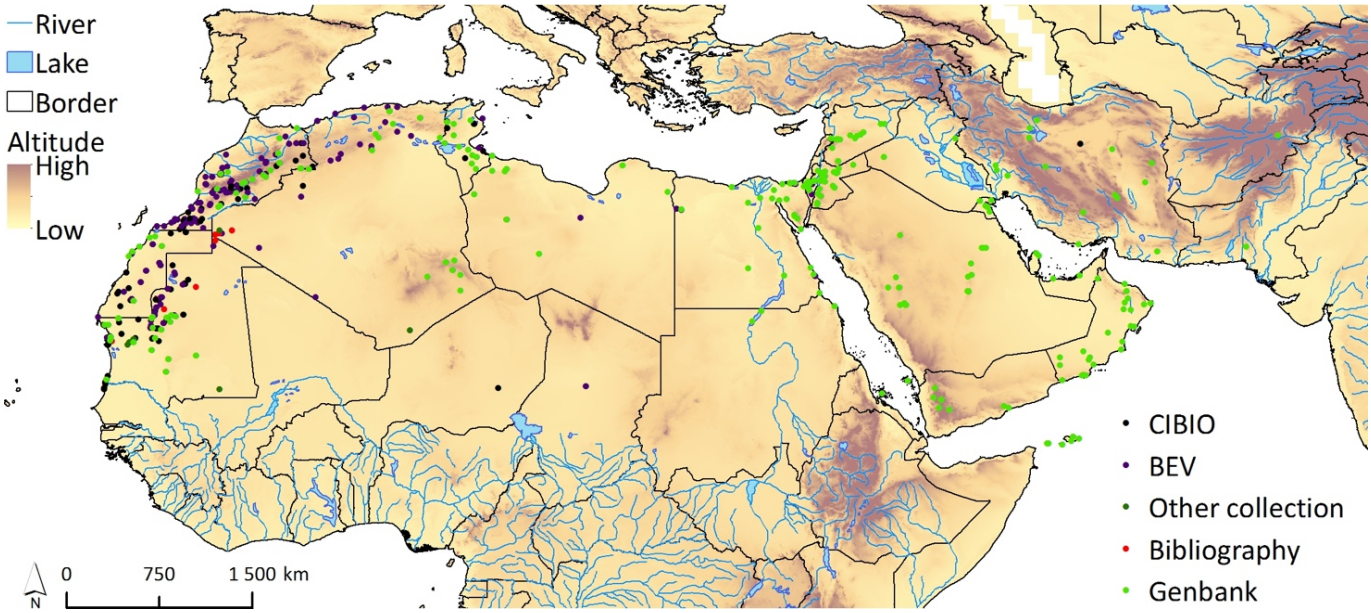


**Fig S1.** Sampling localities and sources of the *Mesalina* specimens used in this study.

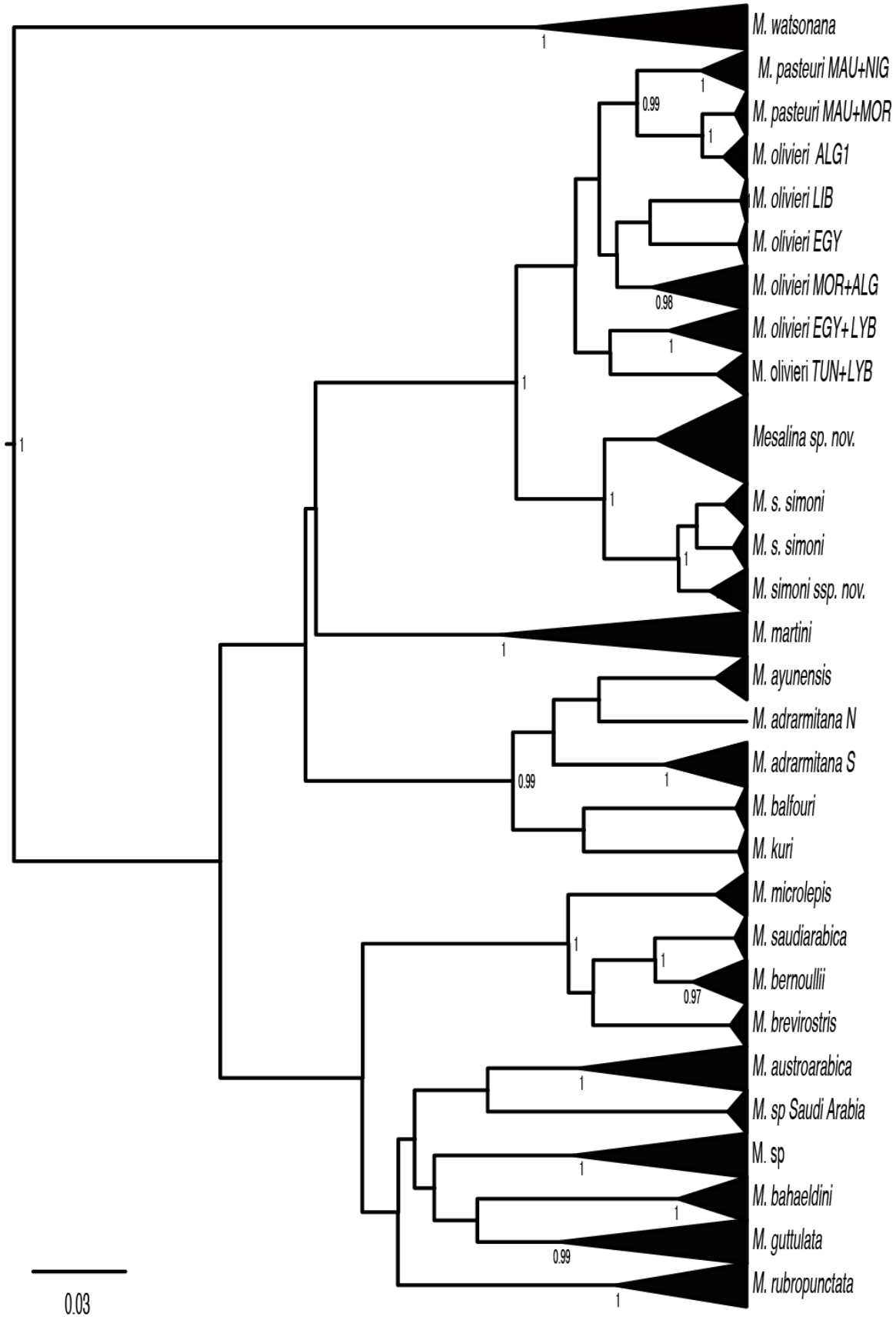


**Fig S2.** Results of the BI analysis of mtDNA dataset 1. Only posterior probabilities ≥ 0.95 are shown in the branches. Sample codes correspond to those in Table S1.

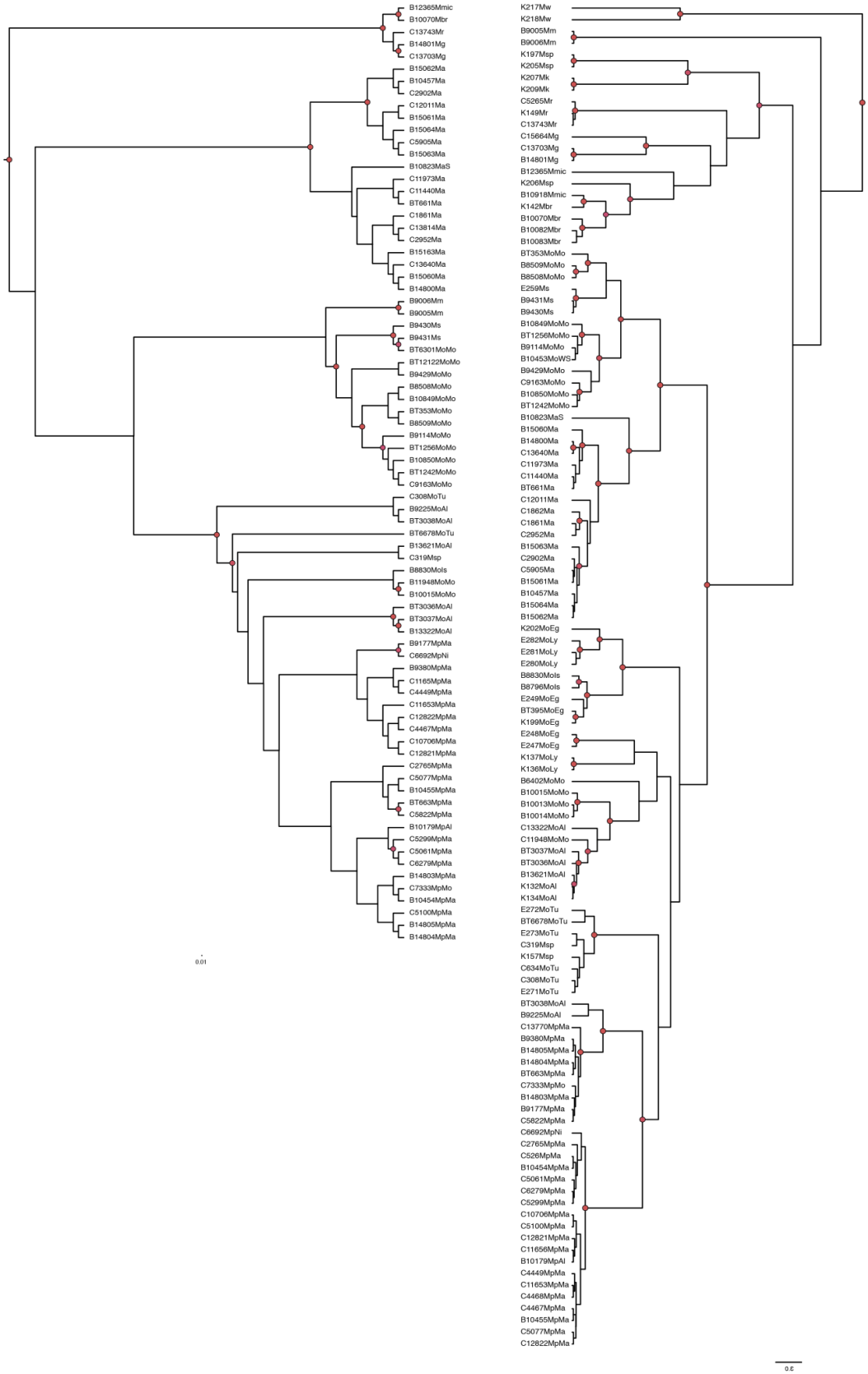


**Fig S3.** Mirrored results of the BI analysis of multilocus nuclear dataset 0 (left) and mitochondrial dataset 2 (right). Only posterior probabilities ≥ 0.95 are shown in the branches. Sample codes correspond to those in Table S1.

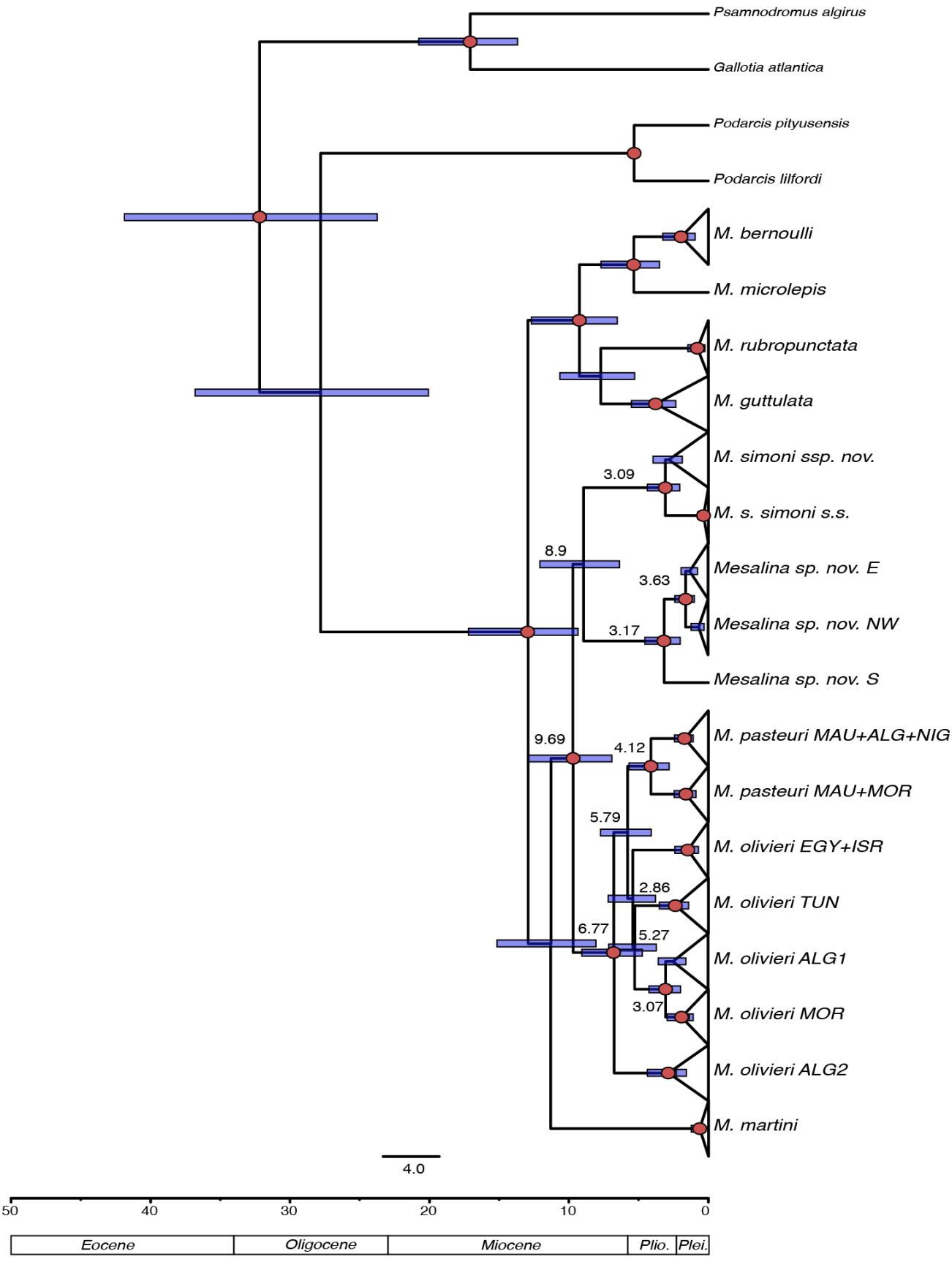


**Fig S4.** Calibrated phylogenetic tree obtained from cytonuclear Dataset 3. Support values represent posterior probability values from BEAST (PP<95% not showed). Blue horizontal bars represent the 95% confidence intervals for age estimate.

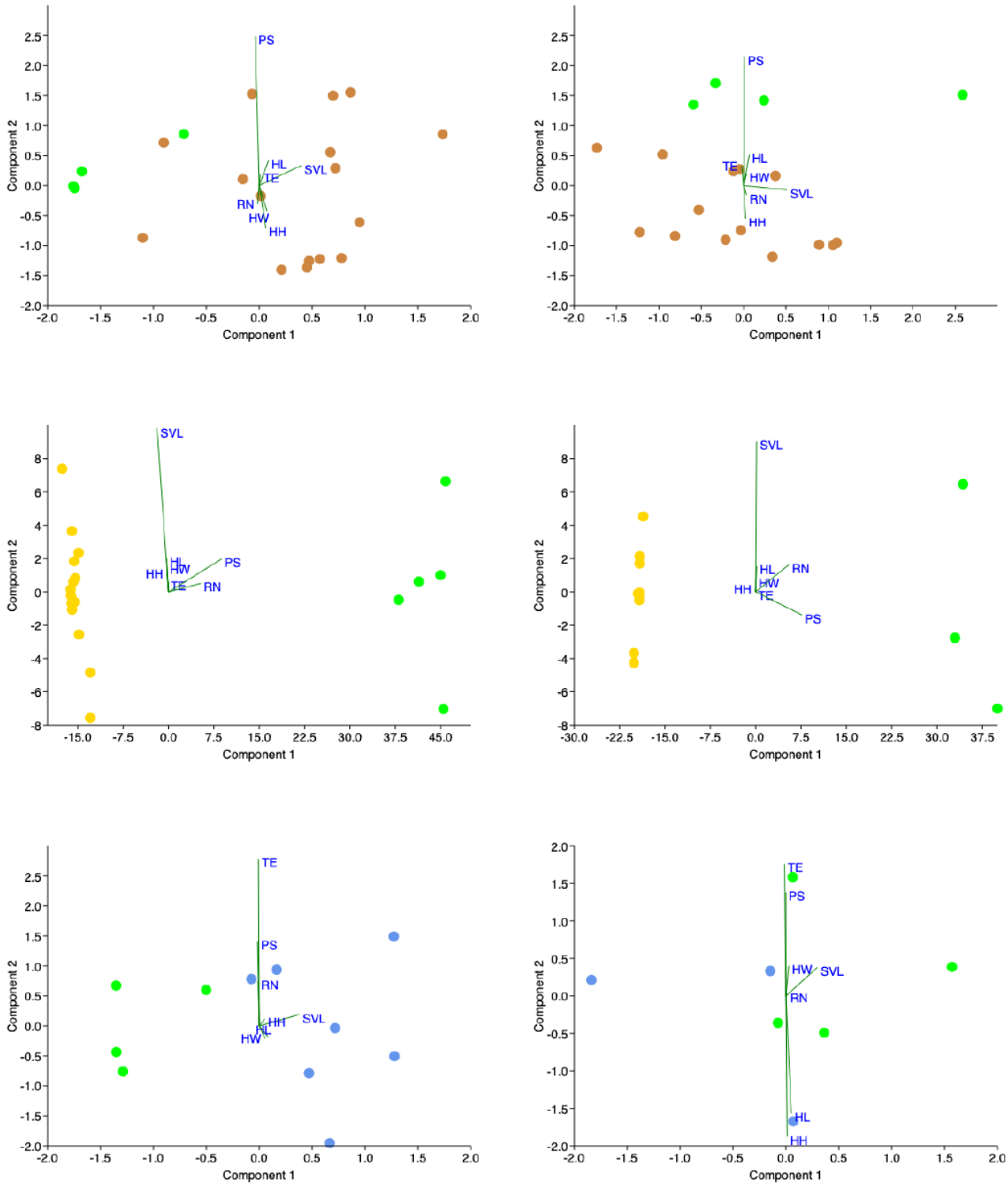


**Fig. S5.** Principal Component Analysis (PCA) of the morphological variables selected from the heating map analysis from male (above) and female (below) individuals of *Mesalina olivieri* species complex. The main morphological variables are superimposed to the graphs. Colours are the same previously used in Fig 2.1, and represent *M. olivieri* from Mauritania (green), *M. pasteuri* (blue), *M.simoni* (yellow), and *M.olivieri* (brown).

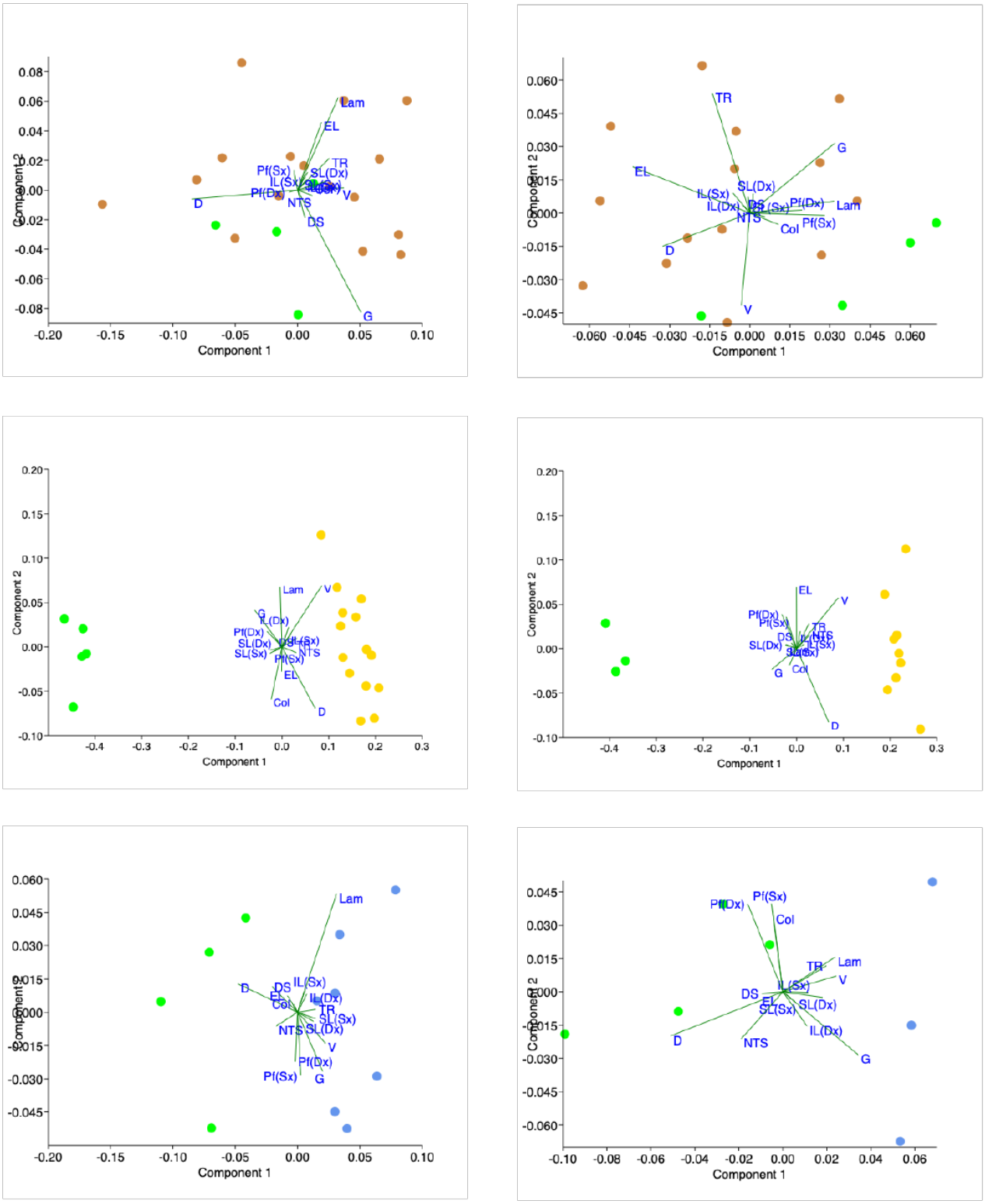


**Fig. S6.** Principal Component Analysis (PCA) of the pholidosis variables selected from the heating map analysis from male (above) and female (below) individuals of *Mesalina olivieri* species complex. The main morphological variables are superimposed to the graphs. Colours are the same previously used in Fig 2.1, and represent *M. olivieri* from Mauritania (green), *M. pasteuri* (blue), *M. simoni* (yellow), and *M.olivieri* (brown).

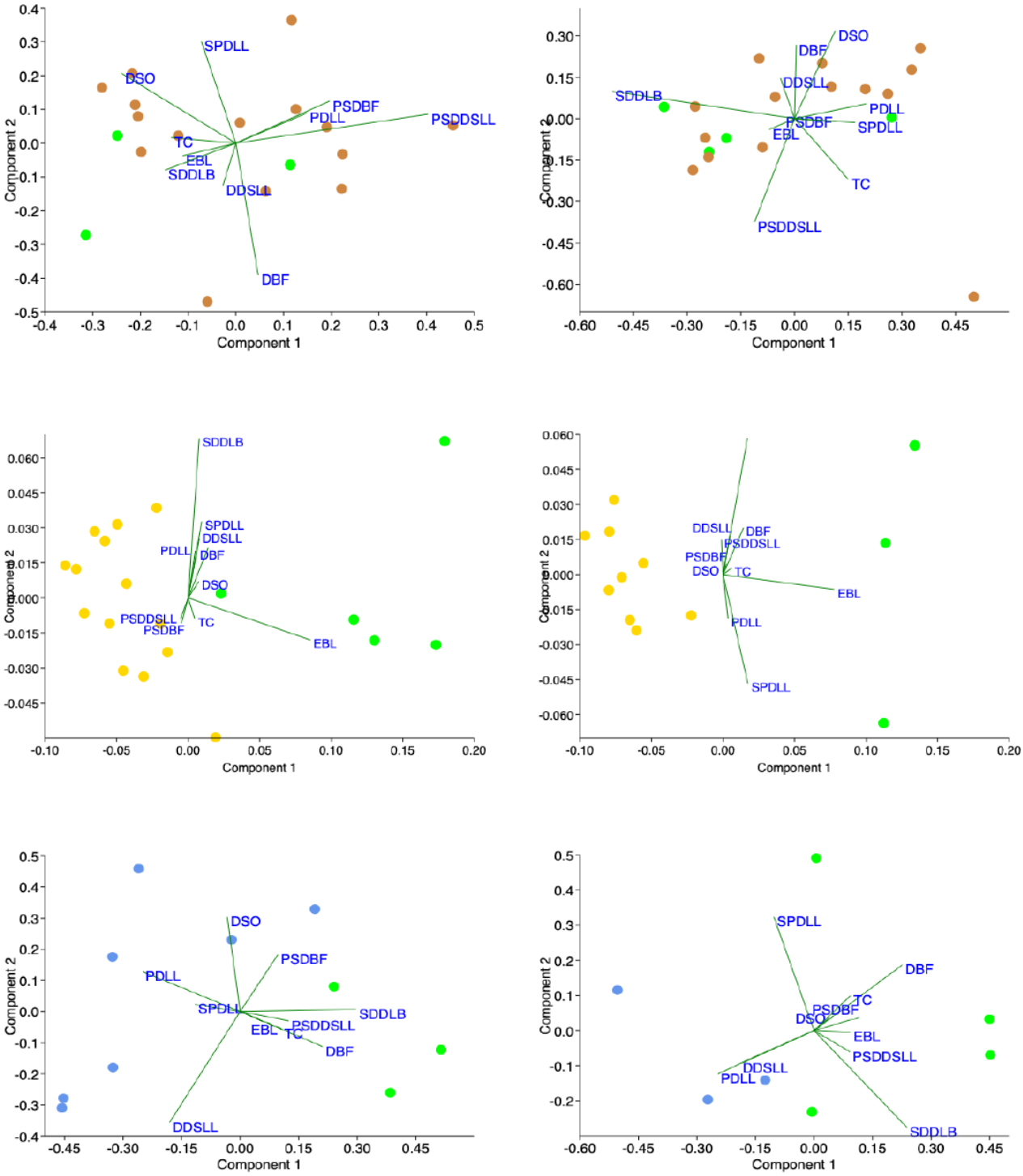


**Fig. S7.** Principal Component Analysis (PCA) of the coloration variables selected from the heating map analysis from male (above) and female (below) individuals of *Mesalina olivieri* species complex. The main morphological variables are superimposed to the graphs. Colours are the same previously used in Fig 2.1, and represent *M. olivieri* from Mauritania (green), *M. pasteuri* (blue), *M. simoni* (yellow), and *M.olivieri* (brown).

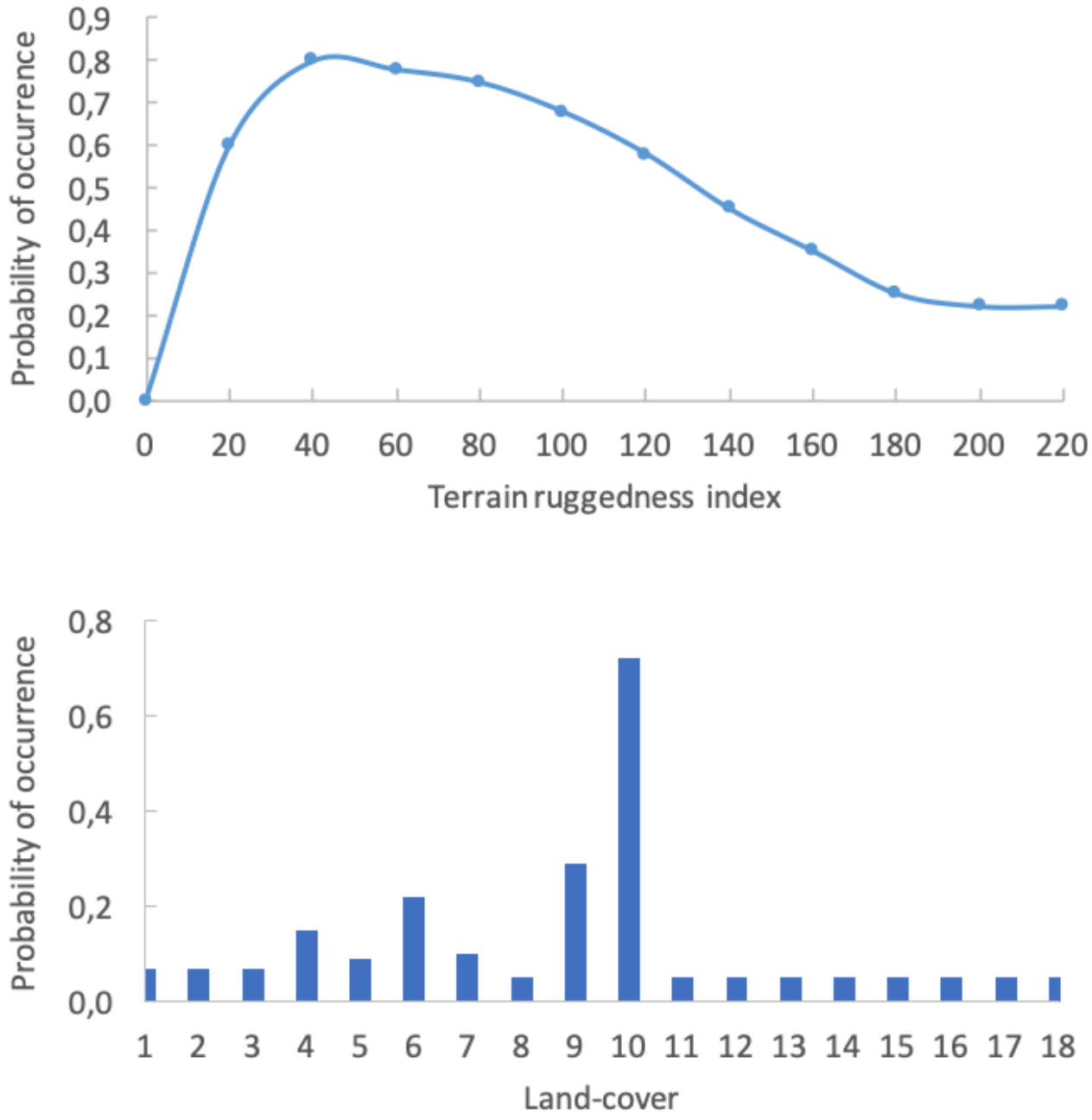


**Fig S8.** Response curves for the most important environmental predictors related to the distribution of *Mesalina* sp. nov. .

**Table S1.** Data on the gene fragments used in this study, including their lengths and the primers used (with their orientation, sequences, references and PCR conditions).

| Gene | Primers | Reference | Sequence 5'- 3' | PCR cycling |
|---|---|---|---|---|
| *MC1R* | MC1RF | Pinho et al. (2009) | GGCNGCCATYGTCAAGAACCGGAACC | 95° (5 :00) ; [94° (0 :30) ; 58° (1 :30)- 72° (1 :00)] for 40 cycles ; 72° (7 :00) |
| | MC1RR | | CTCCGRAAGGCRTAAATGATGGGGTCCAC | |
| *PgD7* | PgDP8R | Pinho et al. (2008) | GAG TCC AGC TCA GTC TTA TTC CAC | 95° (5 :00) ; [94° (0 :30) ; 58° (1 :30)- 72° (1 :00)] for 40 cycles ; 72° (7 :00) |
| | PgDP7F | | GAC ATG CAG CTG ATC TGT GAG GCC | |
| *OD* | ODlez R | | CCACCAATATCAAGCAGGTAC | 95° (5 :00) ; [94° (0 :30) ; 56 to 58° (1 :30)- 72° (1 :00)] for 40 to 42 cycles ; 72° (7 :00) |
| | ODlez F | | GCTACACTAAAAACCAGCAG | |
| *Cyt b* | Mes_cytb_F | Kapli et al. (2015) | CGWAAACAACACCCVATCCT | 95° (5 :00) ; [95° (0 :30) ; 50 to 53° (0:45)- 72° (1 :00)] for 45 cycles ; 60° (10 :00) |
| | Mes_cytb_R | Kapli et al. (2015) | GATATTTGTCCTCADGGHA | |
| *B-fib7* | BFXF | Sequeira et al. (2008) | CAGGGAGAGCTACTTTTGATTAGAC | 95° (10 :00) ; [95° (0 :30) ; 52° (0:30)- 72° (1 :00)] for 38 cycles ; 60° (10 :00) |
| | BF8 | Pinho et al. (2006) | CACCACCGTCTTCTTTGGAACACTG | |

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**Table S2.** Information about the samples used for the genetic analyses including Genbank accession codes.

| Sample code | Species | Country | Latitude | Longitude | *Cyt-b* | *B-fib7* | *OD* | *MC1R* | *PgD7* |
|---|---|---|---|---|---|---|---|---|---|
| CN1 [a b c] | *M. adramitana* | Oman | 23.7861 | 57.7977 | MK551691 | -- | -- | MK551616 | -- |
| CN10639 [a b] | *M. adramitana* | Oman | 22.365 | 59.7011 | --- | -- | -- | --- | -- |
| CN10649 [b] | *M. adramitana* | Oman | 22.365 | 59.7011 | --- | -- | -- | MK551662 | -- |
| CN10674 [a b] | *M. adramitana* | Oman | 22.3937 | 59.6855 | --- | -- | -- | MK551663 | -- |
| CN10693 [b] | *M. adramitana* | Oman | 22.365 | 59.7011 | --- | -- | -- | MK551664 | -- |
| CN10712 [a b] | *M. adramitana* | Oman | 22.365 | 59.7011 | --- | -- | -- | MK551665 | -- |
| CN2632 [a b] | *M. adramitana* | Oman | 22.4405 | 57.822 | --- | -- | -- | MK551618 | -- |
| CN3819 [b] | *M. adramitana* | Oman | 20.6824 | 58.5296 | --- | -- | -- | MK551619 | -- |
| CN4089 [b] | *M. adramitana* | Oman | 22.3098 | 59.2202 | --- | -- | -- | MK551615 | -- |
| CN7493 [b] | *M. adramitana* | Oman | 21.9253 | 57.719 | --- | -- | -- | MK551617 | -- |
| CN7835 [b] | *M. adramitana* | Oman | 21.8839 | 58.2575 | --- | -- | -- | MK551614 | -- |
| OM04/2010-101 [b] | *M. adramitana* | Oman | 23.2543 | 57.9315 | --- | -- | -- | MK551622 | -- |
| OM04/2010-98 [b] | *M. adramitana* | Oman | 23.2543 | 57.9315 | --- | -- | -- | MK551621 | -- |
| S3219 [a b] | *M. adramitana* | Oman | 22.7238 | 58.014 | --- | -- | -- | MK551620 | -- |
| SPM001457U [b] | *M. adramitana* | UAE | 24.16 | 55.84 | --- | -- | -- | MK551623 | -- |
| 577 [b] | *M. adramitana* | Saudi Arabia | 22.2371 | 41.8428 | --- | -- | -- | --- | -- |
| CN7935 [a b c] | *M. adramitana* | Oman | 18.8911 | 54.8119 | MK551683 | -- | -- | MK551606 | -- |
| CN8005 [a b] | *M. adramitana* | Oman | 18.2627 | 55.1938 | MH040005 | -- | -- | MH040050 | -- |
| CN8009 [a b] | *M. adramitana* | Oman | 18.4416 | 55.2722 | MK551684 | -- | -- | MK551607 | -- |
| CN8021 [a b] | *M. adramitana* | Oman | 17.8778 | 53.0862 | MK551685 | -- | -- | MK551608 | -- |
| CN8360 [a b] | *M. adramitana* | Oman | 18.464 | 53.0678 | MK551690 | -- | -- | MK551613 | -- |
| NHMC80.3.142.2 [a] | *M. adramitana* | Saudi Arabia | 22.2445 | 41.432 | KM411197 | -- | -- | --- | -- |
| NHMC80.3.142.3 [a] | *M. adramitana* | Saudi Arabia | 22.2421 | 41.4478 | KM411196 | -- | -- | --- | -- |
| NHMC80.3.164.8 [a c] | *M. adramitana* | Saudi Arabia | 22.2525 | 41.8796 | KM411205 | -- | -- | --- | -- |
| S2807 [a b] | *M. adramitana* | Oman | 19.5626 | 57.6233 | KY967144 | -- | -- | KY967099 | -- |
| S7640 [a b] | *M. adramitana* | Oman | 20.7264 | 58.2622 | MK551686 | -- | -- | MK551609 | -- |
| S7642 [a b] | *M. adramitana* | Oman | 19.0311 | 57.5285 | MK551687 | -- | -- | MK551610 | -- |
| S7916 [b] | *M. adramitana* | Oman | 20.2584 | 56.581 | MK551688 | -- | -- | MK551611 | -- |
| S7976 [a b] | *M. adramitana* | Oman | 19.0311 | 57.5285 | MK551689 | -- | -- | MK551612 | -- |
| BEV.10457 | *Mesalina* sp. nov. | Mauritania | 21.015000 | -11.718000 | Pending | Pending | Pending | Pending | Pending |
| BEV.10823 | *Mesalina* sp. nov. | Mauritania | 17.398228 | -12.030510 | Pending | Pending | Pending | Pending | Pending |
| BEV.14800 | *Mesalina* sp. nov. | Mauritania | 22.608563 | -12.556853 | Pending | Pending | Pending | Pending | Pending |
| BEV.15060 | *Mesalina* sp. nov. | Mauritania | 20.553695 | -12.691632 | Pending | Pending | Pending | Pending | Pending |
| BEV.15061 | *Mesalina* sp. nov. | Mauritania | 21.159582 | -11.936230 | Pending | Pending | Pending | Pending | Pending |
| BEV.15062 | *Mesalina* sp. nov. | Mauritania | 21.159582 | -11.936230 | Pending | Pending | Pending | Pending | Pending |

| Sample code | Species | Country | Latitude | Longitude | *Cyt-b* | *B-fib7* | *OD* | *MC1R* | *PgD7* |
|---|---|---|---|---|---|---|---|---|---|
| BEV.15063 | *Mesalina* sp. nov. | Mauritania | 21.159582 | -11.936230 | Pending | Pending | Pending | Pending | Pending |
| BEV.15064 | *Mesalina* sp. nov. | Mauritania | 21.159582 | -11.936230 | Pending | Pending | Pending | Pending | Pending |
| BEV.15163 | *Mesalina* sp. nov. | Mauritania | 20.553695 | -12.691632 | Pending | Pending | Pending | Pending | Pending |
| BEV.T661 | *Mesalina* sp. nov. | Mauritania | 20.748540 | -13.127550 | Pending | Pending | Pending | Pending | Pending |
| CIBIO11973 | *Mesalina* sp. nov. | Mauritania | 19.826485 | -14.255477 | Pending | Pending | Pending | Pending | Pending |
| CIBIO11440 | *Mesalina* sp. nov. | Morocco | 22.155680 | -15.346780 | Pending | Pending | Pending | Pending | Pending |
| CIBIO1861 | *Mesalina* sp. nov. | Mauritania | 19.797180 | -12.998011 | Pending | Pending | Pending | Pending | Pending |
| CIBIO12011 | *Mesalina* sp. nov. | Mauritania | 21.159582 | -11.936230 | Pending | Pending | Pending | Pending | Pending |
| CIBIO12018 | *Mesalina* sp. nov. | Mauritania | 21.150235 | -11.962342 | Pending | Pending | Pending | Pending | Pending |
| CIBIO13640 | *Mesalina* sp. nov. | Mauritania | 22.608563 | -12.556853 | Pending | Pending | Pending | Pending | Pending |
| CIBIO13814 | *Mesalina* sp. nov. | Mauritania | 19.628933 | -12.534387 | Pending | Pending | Pending | Pending | Pending |
| CIBIO1862 | *Mesalina* sp. nov. | Mauritania | 19.863228 | -12.990913 | Pending | Pending | Pending | Pending | Pending |
| CIBIO2902 | *Mesalina* sp. nov. | Mauritania | 21.428225 | -11.313882 | Pending | Pending | Pending | Pending | Pending |
| CIBIO2952 | *Mesalina* sp. nov. | Mauritania | 18.984935 | -13.064730 | Pending | Pending | Pending | Pending | Pending |
| CIBIO5865 | *Mesalina* sp. nov. | Mauritania | 20.553695 | -12.691632 | Pending | Pending | Pending | Pending | Pending |
| CIBIO5905 | *Mesalina* sp. nov. | Mauritania | 21.152082 | -11.946980 | Pending | Pending | Pending | Pending | Pending |
| JEM015 [a] | *M. arnoldi* | Yemen | 15.36 | 44.47 | MH040006 | -- | -- | --- | -- |
| JEM4 [a c] | *M. arnoldi* | Yemen | 15.38 | 44.45 | MH040007 | -- | -- | MH040051 | -- |
| MCCI-R890 [a c] | *M. arnoldi* | Yemen | 15.51 | 43.88 | MH040008 | -- | -- | --- | -- |
| S3615 [a c] | *M. arnoldi* | Yemen | 14.78 | 44.28 | MH040009 | -- | -- | MH040052 | -- |
| S4049 | *M. arnoldi* | Yemen | 14.78 | 44.28 | MH040010 | -- | -- | MH040053 | -- |
| CN11196 [a] | *M. austroarabica* | Saudi Arabia | 16.7543 | 41.9985 | MK551727 | -- | -- | MK551656 | -- |
| CN11197 [a] | *M. austroarabica* | Saudi Arabia | 16.7543 | 41.9985 | MK551728 | -- | -- | MK551657 | -- |
| CN11198 [a] | *M. austroarabica* | Saudi Arabia | 16.7543 | 41.9985 | MK551729 | -- | -- | MK551658 | -- |
| CN11199 [a c] | *M. austroarabica* | Saudi Arabia | 16.7543 | 41.9985 | MK551730 | -- | -- | MK551659 | -- |
| CN7392 [a] | *M. austroarabica* | Oman | 17.1494 | 54.9757 | MH040011 | -- | -- | MH040054 | -- |
| CN7638 | *M. austroarabica* | Oman | 17.1597 | 54.8069 | MH040012 | -- | -- | MH040055 | -- |
| CN7641 | *M. austroarabica* | Oman | 17.1494 | 54.9757 | MH040013 | -- | -- | MH040056 | -- |
| JEM109 [a c] | *M. austroarabica* | Yemen | 14.9 | 49.03 | MH040014 | -- | -- | MH040057 | -- |
| JIR70 [a] | *M. austroarabica* | Oman | 16.8014 | 53.2783 | MH040015 | -- | -- | MH040058 | -- |
| NHMC80.3.72.108 [a c] | *M. austroarabica* | Yemen | 16.2333 | 43.9667 | KM411144 | -- | -- | --- | -- |
| NHMC80.3.72.109 [a c] | *M. austroarabica* | Yemen | 14.65 | 45.05 | KM411145 | -- | -- | --- | -- |
| S2421 | *M. austroarabica* | Oman | 17.1139 | 54.7137 | MH040016 | -- | -- | MH040059 | -- |
| S2599 [a] | *M. austroarabica* | Oman | 17.1139 | 54.7137 | MH040017 | -- | -- | MH040060 | -- |
| S2701 | *M. austroarabica* | Oman | 17.1139 | 54.7137 | MH040018 | -- | -- | MH040061 | -- |
| S2725 [a] | *M. austroarabica* | Oman | 17.1139 | 54.7137 | MH040019 | -- | -- | MH040062 | -- |
| S2838 [a] | *M. austroarabica* | Oman | 17.1139 | 54.7137 | MH040020 | -- | -- | MH040063 | -- |

| Sample code | Species | Country | Latitude | Longitude | *Cyt-b* | *B-fib7* | *OD* | *MC1R* | *PgD7* |
|---|---|---|---|---|---|---|---|---|---|
| S7324 [a c] | *M. austroarabica* | Oman | 17.1214 | 54.714 | MH040021 | -- | -- | MH040064 | -- |
| JEM634 [a b] | *M. ayunensis* | Yemen | 14.78 | 49.37 | MK551693 | -- | -- | MK551625 | -- |
| JIR104 [a b c] | *M. ayunensis* | Oman | 17.9335 | 55.5275 | MK551692 | -- | -- | MK551624 | -- |
| HUJR-19066 [a] | *M. bahaeldini* | Israel | 31.254 | 35.163 | MH040022 | -- | -- | MH040065 | -- |
| HUJR-TAIL-26 [a] | *M. bahaeldini* | Israel | 31.987 | 35.436 | MH040023 | -- | -- | --- | -- |
| HUJR-TAIL-27 [a] | *M. bahaeldini* | Israel | 31.208 | 34.771 | MH040024 | -- | -- | MH040066 | -- |
| HUJR-TAIL-28 [a] | *M. bahaeldini* | Israel | 31.204 | 34.793 | MH040025 | -- | -- | MH040067 | -- |
| HUJR-TAIL-29 [a] | *M. bahaeldini* | Israel | 31.327 | 35.228 | MH040026 | -- | -- | MH040068 | -- |
| HUJR-TAIL-30 | *M. bahaeldini* | Israel | 31.327 | 35.228 | MH040027 | -- | -- | --- | -- |
| J66/04 [a] | *M. bahaeldini* | Jordan | 30.7622 | 36.6803 | MH040028 | -- | -- | MH040069 | -- |
| NHMC80.3.108.1 | *M. bahaeldini* | Egypt | 28.5408 | 33.981 | EF555285 | -- | -- | --- | -- |
| NHMC80.3.108.2 [a] | *M. bahaeldini* | Egypt | 28.5408 | 33.981 | EF555286 | -- | -- | --- | -- |
| NHMC80.3.108.3 [a] | *M. bahaeldini* | Egypt | 28.5408 | 33.981 | EF555287 | -- | -- | --- | -- |
| NHMC80.3.108.4 [a] | *M. bahaeldini* | Egypt | 28.5408 | 33.981 | EF555288 | -- | -- | --- | -- |
| NHMC80.3.108.5 [a] | *M. bahaeldini* | Egypt | 28.7064 | 33.748 | EF555283 | -- | -- | --- | -- |
| NHMC80.3.72.10 [a] | *M. bahaeldini* | Jordan | 31.2531 | 35.6135 | EF555293 | -- | -- | --- | -- |
| NHMC80.3.72.100 [a] | *M. bahaeldini* | Jordan | 31.5615 | 35.7827 | KM411182 | -- | -- | --- | -- |
| NHMC80.3.72.11 [a] | *M. bahaeldini* | Jordan | 31.2531 | 35.6135 | EF555294 | -- | -- | --- | -- |
| NHMC80.3.72.111 [a] | *M. bahaeldini* | Jordan | 30.3289 | 35.4426 | KM411201 | -- | -- | --- | -- |
| NHMC80.3.72.13 [a] | *M. bahaeldini* | Jordan | 30.7022 | 35.5841 | EF555295 | -- | -- | --- | -- |
| NHMC80.3.72.14 [a] | *M. bahaeldini* | Jordan | 31.9116 | 36.6168 | EF555317 | -- | -- | --- | -- |
| NHMC80.3.72.15 [a] | *M. bahaeldini* | Jordan | 31.9116 | 36.6168 | EF555318 | -- | -- | --- | -- |
| NHMC80.3.72.16 [a] | *M. bahaeldini* | Jordan | 31.9116 | 36.6168 | EF555319 | -- | -- | --- | -- |
| NHMC80.3.72.17 | *M. bahaeldini* | Jordan | 31.9116 | 36.6168 | EF555320 | -- | -- | --- | -- |
| NHMC80.3.72.20 [a] | *M. bahaeldini* | Jordan | 31.2531 | 35.6135 | EF555292 | -- | -- | --- | -- |
| NHMC80.3.72.22 [a] | *M. bahaeldini* | Egypt | 29.9651 | 33.1606 | EF555284 | -- | -- | --- | -- |
| NHMC80.3.72.24 [a] | *M. bahaeldini* | Jordan | 29.5704 | 35.4113 | EF555321 | -- | -- | --- | -- |
| NHMC80.3.72.47 [a] | *M. bahaeldini* | Jordan | 31.2149 | 35.9653 | KM411174 | -- | -- | --- | -- |
| NHMC80.3.72.48 [a] | *M. bahaeldini* | Jordan | 31.2149 | 35.9653 | KM411175 | -- | -- | --- | -- |
| NHMC80.3.72.49 [a] | *M. bahaeldini* | Jordan | 31.5989 | 35.9934 | KM411176 | -- | -- | --- | -- |
| NHMC80.3.72.50 [a] | *M. bahaeldini* | Jordan | 31.8773 | 35.6834 | KM411177 | -- | -- | --- | -- |
| NHMC80.3.72.88 [a] | *M. bahaeldini* | Israel | 30.6242 | 34.8233 | KM411100 | -- | -- | --- | -- |
| NHMC80.3.72.93 [a] | *M. bahaeldini* | Israel | 30.7077 | 34.7845 | KM411093 | -- | -- | --- | -- |
| NHMC80.3.72.94 [a] | *M. bahaeldini* | Israel | 30.7077 | 34.7845 | KM411094 | -- | -- | --- | -- |
| NHMC80.3.72.95 [a] | *M. bahaeldini* | Israel | 31.0648 | 34.8406 | KM411095 | -- | -- | --- | -- |
| NHMC80.3.72.96 [a] | *M. bahaeldini* | Israel | 31.0648 | 34.8406 | KM411096 | -- | -- | --- | -- |

| Sample code | Species | Country | Latitude | Longitude | *Cyt-b* | *B-fib7* | *OD* | *MC1R* | *PgD7* |
|---|---|---|---|---|---|---|---|---|---|
| NHMC80.3.72.98 [a] | *M. bahaeldini* | Jordan | 29.6864 | 35.426 | KM411180 | -- | -- | --- | -- |
| NHMC80.3.72.99 [a] | *M. bahaeldini* | Jordan | 29.647 | 35.434 | KM411181 | -- | -- | --- | -- |
| S10345 [a c] | *M. bahaeldini* | Saudi Arabia | 27.3243 | 41.43 | MH040029 | -- | -- | MH040070 | -- |
| S2496 [a c] | *M. bahaeldini* | Egypt | 28.5528 | 33.9492 | MH040030 | -- | -- | MH040071 | -- |
| S2835 | *M. bahaeldini* | Egypt | 28.7946 | 33.7321 | MH040031 | -- | -- | MH040072 | -- |
| S3746 [a] | *M. bahaeldini* | Jordan | 30.168 | 35.673 | MH040032 | -- | -- | --- | -- |
| TAU.R16256 [a] | *M. bahaeldini* | Israel | 31.19 | 34.805 | KY967145 | -- | -- | KY967100 | -- |
| TAU.R16263 [a] | *M. bahaeldini* | Israel | 31.256 | 35.171 | MH040033 | -- | -- | MH040073 | -- |
| TAU.R16293 [a] | *M. bahaeldini* | Israel | 30.863 | 34.442 | MH040034 | -- | -- | MH040074 | -- |
| TAU.R16294 [a c] | *M. bahaeldini* | Israel | 30.85 | 34.451 | MH040035 | -- | -- | MH040075 | -- |
| NHMC80.3.143.1 [a] | *M. balfouri* | Yemen | 12.65 | 54.0333 | KM411213 | -- | -- | --- | -- |
| NHMC80.3.143.2 [a] | *M. balfouri* | Yemen | 12.65 | 54.0333 | KM411214 | -- | -- | --- | -- |
| NHMC80.3.143.3 [a] | *M. balfouri* | Yemen | 12.65 | 54.0333 | KM411215 | -- | -- | --- | -- |
| NHMC80.3.143.4 [a] | *M. balfouri* | Yemen | 12.65 | 54.0333 | KM411216 | -- | -- | --- | -- |
| S2500 [a b] | *M. balfouri* | Yemen | 12.1224 | 53.2748 | MH040036 | -- | -- | MH040076 | -- |
| S5187 [a b] | *M. balfouri* | Yemen | 12.546 | 54.4974 | MK551681 | -- | -- | MK551604 | -- |
| S5292 [a b] | *M. balfouri* | Yemen | 12.1224 | 53.2748 | MK551680 | -- | -- | MK551603 | -- |
| S5650 [a b c] | *M. balfouri* | Yemen | 12.3635 | 53.9329 | KY967146 | -- | -- | KY967101 | -- |
| I01 [a c] | *M. bernoullii* | Iran | 34.3893 | 45.4705 | KY967154 | -- | -- | KY967112 | -- |
| I02 [a] | *M. bernoullii* | Iran | 34.3893 | 45.4705 | KY967155 | -- | -- | KY967113 | -- |
| IRA600 [a] | *M. bernoullii* | Iran | 32.03333 | 48.5 | KY967156 | -- | -- | --- | -- |
| MB_Azraq [a] | *M. bernoullii* | Jordan | 31.8333 | 36.8167 | KY967157 | -- | -- | --- | -- |
| MB_Azraq2 [a] | *M. bernoullii* | Jordan | 31.8333 | 36.8167 | KY967158 | -- | -- | --- | -- |
| MB03 [a] | *M. bernoullii* | Jordan | 31.8333 | 36.8167 | KY967159 | -- | -- | --- | -- |
| MB04 [a] | *M. bernoullii* | Jordan | 30.9233 | 36.569 | KY967159 | -- | -- | --- | -- |
| MB05 [a] | *M. bernoullii* | Jordan | 32.4582 | 38.0368 | KY967160 | -- | -- | --- | -- |
| MB07 [a] | *M. bernoullii* | Syria | 34.628 | 38.5609 | KY967162 | -- | -- | --- | -- |
| MB08 | *M. bernoullii* | Syria | 34.628 | 38.5609 | KY967161 | -- | -- | --- | -- |
| MB09 [a] | *M. bernoullii* | Syria | 32.7221 | 36.9376 | KY967165 | -- | -- | --- | -- |
| MB10 | *M. bernoullii* | Syria | 32.7221 | 36.9376 | KY967166 | -- | -- | --- | -- |
| MB11 [a] | *M. bernoullii* | Syria | 34.3111 | 36.9072 | KY967167 | -- | -- | --- | -- |
| MB14 [a] | *M. bernoullii* | Syria | 34.2667 | 37.0667 | KY967163 | -- | -- | --- | -- |
| MB15 | *M. bernoullii* | Syria | 34.2667 | 37.0667 | KY967168 | -- | -- | --- | -- |
| MB16 [a] | *M. bernoullii* | Jordan | 31.9667 | 35.9667 | KY967169 | -- | -- | --- | -- |
| MB17 [a] | *M. bernoullii* | Jordan | 31.9667 | 35.9667 | KY967170 | -- | -- | --- | -- |
| MB19 [a] | *M. bernoullii* | Iraq | 33.0212 | 40.2769 | --- | -- | -- | --- | -- |

| Sample code | Species | Country | Latitude | Longitude | *Cyt-b* | *B-fib7* | *OD* | *MC1R* | *PgD7* |
|---|---|---|---|---|---|---|---|---|---|
| MB21 | *M. bernoullii* | Iraq | 33.0212 | 40.2769 | --- | -- | -- | --- | -- |
| NHMC80.3.69.1 [a] | *M. bernoullii* | Syria | 34.3619 | 38.174 | EF555302 | -- | -- | --- | -- |
| NHMC80.3.69.10 [a] | *M. bernoullii* | Syria | 35.4268 | 40.0278 | EF555307 | -- | -- | --- | -- |
| NHMC80.3.69.11 [a] | *M. bernoullii* | Syria | 35.4268 | 40.0278 | EF555308 | -- | -- | --- | -- |
| NHMC80.3.69.14 [a] | *M. bernoullii* | Saudi Arabia | 26.4185 | 47.4792 | KM411190 | -- | -- | --- | -- |
| NHMC80.3.69.15 [a] | *M. bernoullii* | Saudi Arabia | 26.4147 | 47.4773 | KM411191 | -- | -- | --- | -- |
| NHMC80.3.69.2 [a] | *M. bernoullii* | Syria | 34.6 | 37.8315 | EF555303 | -- | -- | --- | -- |
| NHMC80.3.69.21 [a] | *M. bernoullii* | Kuwait | 29.3678 | 47.8102 | KM411234 | -- | -- | --- | -- |
| NHMC80.3.69.22 [a] | *M. bernoullii* | Kuwait | 29.0144 | 47.9773 | KM411235 | -- | -- | --- | -- |
| NHMC80.3.69.23 [a] | *M. bernoullii* | Kuwait | 29.8433 | 48.1131 | KM411236 | -- | -- | --- | -- |
| NHMC80.3.69.24 | *M. bernoullii* | Kuwait | 29.3794 | 47.8422 | KM411237 | -- | -- | --- | -- |
| NHMC80.3.69.25 [a] | *M. bernoullii* | Kuwait | 29.8236 | 47.2502 | KM411183 | -- | -- | --- | -- |
| NHMC80.3.69.3 [a] | *M. bernoullii* | Syria | 34.6 | 37.8315 | EF555304 | -- | -- | --- | -- |
| NHMC80.3.69.30 [a] | *M. bernoullii* | Syria | 33.6831 | 36.4953 | KM411142 | -- | -- | --- | -- |
| NHMC80.3.69.6 [a] | *M. bernoullii* | Syria | 34.8142 | 38.7897 | EF555305 | -- | -- | --- | -- |
| S15 [a] | *M. bernoullii* | Syria | 34.5247 | 38.2856 | KY967172 | -- | -- | KY967114 | -- |
| S4 | *M. bernoullii* | Syria | 34.3111 | 36.9072 | KY967160 | -- | -- | KY967115 | -- |
| S5 [a] | *M. bernoullii* | Syria | 34.3111 | 36.9072 | KY967173 | -- | -- | KY967115 | -- |
| S7 [a] | *M. bernoullii* | Syria | 34.3111 | 36.9072 | KY967174 | -- | -- | KY967111 | -- |
| S8 [a c] | *M. bernoullii* | Syria | 34.3111 | 36.9072 | KY967175 | -- | -- | KY967115 | -- |
| S9 [a] | *M. bernoullii* | Syria | 34.3111 | 36.9072 | KY967164 | -- | -- | KY967110 | -- |
| Sherif10708 [a] | *M. bernoullii* | Egypt | 27.7472 | 34.2287 | KY967171 | -- | -- | --- | -- |
| 868 [a c] | *M. brevirostris* | Iran | 26.6878 | 54.4221 | --- | -- | -- | --- | -- |
| Mb_UAE [a] | *M. brevirostris* | UAE | 24.3865 | 54.547 | --- | -- | -- | --- | -- |
| QAT1 | *M. brevirostris* | Qatar | 26.0112 | 51.3901 | --- | -- | -- | --- | -- |
| QAT2 [a] | *M. brevirostris* | Qatar | 25.8234 | 51.5739 | --- | -- | -- | --- | -- |
| SPM001455U [a c] | *M. brevirostris* | UAE | 24.1719 | 52.6109 | KY967153 | -- | -- | KY967109 | -- |
| SPM002959(97) [a] | *M. brevirostris* | Bahrain | | | KY967152 | -- | -- | KY967108 | -- |
| BEV.10070 | *M. brevirostris* | Kuwait | 28.584400 | 18.167300 | Pending | Pending | Pending | Pending | Pending |
| BEV.10082 | *M. brevirostris* | Kuwait | 29.555900 | 47.741000 | Pending | Pending | Pending | Pending | Pending |
| NHMC80.3.109.12 [a] | *M. cf. simoni* | Morocco | 30.6811 | -8.2834 | KM411125 | -- | -- | --- | -- |
| NHMC80.3.72.1 [a c] | *M. guttulata* | Tunisia | 33.5225 | 9.9925 | EF555310 | -- | -- | --- | -- |
| NHMC80.3.72.18 [a] | *M. guttulata* | Morocco | 31.0882 | -6.4673 | EF555299 | -- | -- | --- | -- |
| NHMC80.3.72.2 [a] | *M. guttulata* | Tunisia | 33.5225 | 9.9925 | EF555311 | -- | -- | --- | -- |
| NHMC80.3.72.21 [a] | *M. guttulata* | Morocco | 31.7146 | -4.9221 | EF555300 | -- | -- | --- | -- |
| NHMC80.3.72.25 [a] | *M. guttulata* | Libya | 32.1245 | 12.8076 | KM411130 | -- | -- | --- | -- |

| Sample code | Species | Country | Latitude | Longitude | *Cyt-b* | *B-fib7* | *OD* | *MC1R* | *PgD7* |
|---|---|---|---|---|---|---|---|---|---|
| NHMC80.3.72.26 [a] | *M. guttulata* | Libya | 32.1247 | 12.8068 | KM411129 | -- | -- | --- | -- |
| NHMC80.3.72.28 [a] | *M. guttulata* | Libya | 31.9756 | 12.6724 | KM411131 | -- | -- | --- | -- |
| NHMC80.3.72.31 [a] | *M. guttulata* | Libya | 32.0601 | 12.7227 | KM411133 | -- | -- | --- | -- |
| NHMC80.3.72.35 [a] | *M. guttulata* | Libya | 32.1245 | 12.8076 | KM411135 | -- | -- | --- | -- |
| NHMC80.3.72.44 [a c] | *M. guttulata* | Algeria | 25.3483 | 8.3791 | KM411165 | -- | -- | --- | -- |
| NHMC80.3.72.45 [a] | *M. guttulata* | Algeria | 34.4176 | 3.4798 | KM411167 | -- | -- | --- | -- |
| NHMC80.3.72.46 [a] | *M. guttulata* | Algeria | 25.35 | 8.3911 | KM411173 | -- | -- | --- | -- |
| NHMC80.3.72.5 [a] | *M. guttulata* | Morocco | 32.0472 | -4.4088 | EF555297 | -- | -- | --- | -- |
| NHMC80.3.72.51 [a c] | *M. guttulata* | Morocco | 32.586 | -3.7605 | KM411178 | -- | -- | --- | -- |
| NHMC80.3.72.53 [a] | *M. guttulata* | Morocco | 29.3698 | -8.1993 | KM411210 | -- | -- | --- | -- |
| NHMC80.3.72.54 [a] | *M. guttulata* | Morocco | 30.3918 | -6.8817 | KM411211 | -- | -- | --- | -- |
| NHMC80.3.72.55 [a] | *M. guttulata* | Morocco | 29.4523 | -8.0597 | KM411212 | -- | -- | --- | -- |
| NHMC80.3.72.57 [a] | *M. guttulata* | Libya | 28.4433 | 12.78 | KM411222 | -- | -- | --- | -- |
| NHMC80.3.72.7 [a] | *M. guttulata* | Tunisia | 33.1502 | 10.2899 | EF555312 | -- | -- | --- | -- |
| NHMC80.3.72.8 [a] | *M. guttulata* | Libya | 30.4659 | 24.5366 | EF555296 | -- | -- | --- | -- |
| NHMC80.3.72.82 [a] | *M. guttulata* | Morocco | 33.2894 | -3.8437 | KM411092 | -- | -- | --- | -- |
| NHMC80.3.72.83 [a] | *M. guttulata* | Morocco | 33.2894 | -3.8437 | KM411088 | -- | -- | --- | -- |
| NHMC80.3.72.84 [a] | *M. guttulata* | Morocco | 32.1193 | -1.5799 | KM411089 | -- | -- | --- | -- |
| NHMC80.3.72.85 [a c] | *M. guttulata* | Morocco | 32.1193 | -1.5799 | KM411090 | -- | -- | --- | -- |
| NHMC80.3.72.87 [a] | *M. guttulata* | Algeria | 34.6805 | 3.2499 | KM411099 | -- | -- | --- | -- |
| NHMC80.3.72.89 [a] | *M. guttulata* | Algeria | 24.4441 | 9.4138 | KM411101 | -- | -- | --- | -- |
| NHMC80.3.72.9 [a] | *M. guttulata* | Morocco | 31.4018 | -5.7276 | EF555298 | -- | -- | --- | -- |
| NHMC80.3.72.90 [a] | *M. guttulata* | Algeria | 25.5012 | 8.9996 | KM411102 | -- | -- | --- | -- |
| NHMC80.3.72.91 [a] | *M. guttulata* | Algeria | 23.313 | 9.4302 | KM411103 | -- | -- | --- | -- |
| NHMC80.3.72.92 [a] | *M. guttulata* | Egypt | 23.1141 | 35.5882 | KM411091 | -- | -- | --- | -- |
| NHMC80.3.72.97 [a] | *M. guttulata* | Morocco | 30.078 | -6.2365 | KM411097 | -- | -- | --- | -- |
| S3612 [a c] | *M. guttulata* | Libya | 30.3132 | 10.4496 | MH040037 | -- | -- | MH040077 | -- |
| S3907 [a] | *M. guttulata* | Libya | 30.3131 | 10.4495 | MH040038 | -- | -- | --- | -- |
| SPM001477U [a] | *M. guttulata* | Morocco | | | MH040039 | -- | -- | MH040078 | -- |
| SPM002367(7) [a] | *M. guttulata* | Egypt | 22.1831 | 36.6657 | MH040040 | -- | -- | MH040079 | -- |
| SPM002368(93) [a] | *M. guttulata* | Egypt | 22.1831 | 36.6657 | MH040041 | -- | -- | MH040080 | -- |
| SPM002382(8) [a c] | *M. guttulata* | Egypt | 30.832 | 29.2037 | MH040042 | -- | -- | MH040081 | -- |
| SPM003430 [a c] | *M. guttulata* | Morocco | 27.14 | -13.18 | MH040043 | -- | -- | MH040082 | -- |
| SUD12/2010-68 [a] | *M. guttulata* | Sudan | 21.072 | 30.6938 | MH040044 | -- | -- | MH040083 | -- |
| BEV.14801 | *M. guttulata* | Mauritania | 24.462253 | -11.351480 | Pending | Pending | Pending | Pending | Pending |
| CIBIO15664 | *M. guttulata* | Algeria | 30.923680 | -2.031399 | Pending | Pending | Pending | Pending | Pending |

| Sample code | Species | Country | Latitude | Longitude | *Cyt-b* | *B-fib7* | *OD* | *MC1R* | *PgD7* |
|---|---|---|---|---|---|---|---|---|---|
| CIBIO13703 | *M. guttulata* | Mauritania | 25.156463 | -11.534827 | Pending | Pending | Pending | Pending | Pending |
| NHMC80.3.165.1 [a] | *M. kuri* | Yemen | 12.1867 | 52.1528 | KM411207 | -- | -- | --- | -- |
| NHMC80.3.165.2 [a] | *M. kuri* | Yemen | 12.1867 | 52.1528 | KM411208 | -- | -- | --- | -- |
| NHMC80.3.165.3 [a] | *M. kuri* | Yemen | 12.1867 | 52.1528 | KM411209 | -- | -- | --- | -- |
| S5169 [a b c] | *M. kuri* | Yemen | 12.1996 | 52.2658 | MK551682 | -- | -- | MK551605 | -- |
| S5368 [a b] | *M. kuri* | Yemen | 12.1996 | 52.2658 | KY967147 | -- | -- | KY967102 | -- |
| NHMC80.3.166.1 [a c] | *M. martini* | Egypt | 24.9629 | 34.9357 | KM411104 | -- | -- | MK551653 | -- |
| NHMC80.3.166.2 | *M. martini* | Egypt | 24.4929 | 35.1594 | KM411105 | -- | -- | MH040084 | -- |
| NHMC80.3.166.3 [a c] | *M. martini* | Eritrea | 15.8009 | 40.1271 | KM411204 | -- | -- | --- | -- |
| BEV.9005 | *M. martini* | Egypt | 24.361320 | 35.292090 | Pending | Pending | Pending | Pending | Pending |
| BEV.9006 | *M. martini* | Egypt | 24.395590 | 35.237350 | Pending | Pending | Pending | Pending | Pending |
| L32 [a c] | *M. microlepis* | Lebanon | 34.3656 | 36.4026 | KY967149 | -- | -- | KY967105 | -- |
| L33 [a] | *M. microlepis* | Lebanon | 34.3656 | 36.4026 | KY967150 | -- | -- | KY967106 | -- |
| L34 | *M. microlepis* | Lebanon | 34.3656 | 36.4026 | KY967150 | -- | -- | KY967107 | -- |
| MB12 [a] | *M. microlepis* | Syria | 34.3111 | 36.9072 | KY967151 | -- | -- | --- | -- |
| MB13 [a] | *M. microlepis* | Syria | 34.3111 | 36.9072 | KY967151 | -- | -- | --- | -- |
| NHMC80.3.69.12 [a] | *M. microlepis* | Syria | 34.2931 | 36.7655 | EF555309 | -- | -- | --- | -- |
| NHMC80.3.69.9 [a] | *M. microlepis* | Syria | 35.4174 | 40.3198 | EF555306 | -- | -- | --- | -- |
| BEV.10918 | *M. microlepis* | Jordan | 31.598900 | 35.993400 | Pending | Pending | Pending | Pending | Pending |
| BEV.12365 | *M. microlepis* | Lebanon | 34.316359 | 36.413579 | Pending | Pending | Pending | Pending | Pending |
| NHMC80.3.119.10 [a] | *M. olivieri* | Tunisia | 32.1287 | 10.5638 | EF555314 | -- | -- | --- | -- |
| NHMC80.3.119.108 | *M. olivieri* | Algeria | 35.4151 | 4.519 | KM411132 | -- | -- | --- | -- |
| NHMC80.3.119.109 [a] | *M. olivieri* | Egypt | 27.83 | 31.1068 | KM411202 | -- | -- | --- | -- |
| NHMC80.3.119.14 [a] | *M. olivieri* | Tunisia | 33.7531 | 9.335 | EF555315 | -- | -- | --- | -- |
| NHMC80.3.119.16 [a c] | *M. olivieri* | Egypt | 29.9651 | 33.1606 | EF555289 | -- | -- | --- | -- |
| NHMC80.3.119.19 [a c] | *M. olivieri* | Egypt | 29.9651 | 33.1606 | EF555290 | -- | -- | --- | -- |
| NHMC80.3.119.2 [a] | *M. olivieri* | Libya | 32.3912 | 21.2404 | EF555322 | -- | -- | --- | -- |
| NHMC80.3.119.20 [a c] | *M. olivieri* | Egypt | 29.9797 | 32.1187 | EF555291 | -- | -- | --- | -- |
| NHMC80.3.119.21 [a c] | *M. olivieri* | Libya | 32.1247 | 12.8068 | KM411136 | -- | -- | --- | -- |
| NHMC80.3.119.22 [a] | *M. olivieri* | Libya | 32.1247 | 12.8068 | KM411137 | -- | -- | --- | -- |
| NHMC80.3.119.23 [a] | *M. olivieri* | Algeria | 35.4151 | 4.519 | KM411134 | -- | -- | --- | -- |
| NHMC80.3.119.29 [a] | *M. olivieri* | Tunisia | 35.6895 | 10.1501 | KM411225 | -- | -- | --- | -- |
| NHMC80.3.119.3 [a] | *M. olivieri* | Libya | 32.3912 | 21.2404 | EF555323 | -- | -- | --- | -- |
| NHMC80.3.119.35 [a] | *M. olivieri* | Morocco | 33.2894 | -3.8437 | KM411106 | -- | -- | --- | -- |
| NHMC80.3.119.36 [a] | *M. olivieri* | Morocco | 33.2894 | -3.8437 | KM411107 | -- | -- | --- | -- |
| NHMC80.3.119.37 [a] | *M. olivieri* | Morocco | 31.5739 | -4.7417 | KM411108 | -- | -- | --- | -- |

| Sample code | Species | Country | Latitude | Longitude | *Cyt-b* | *B-fib7* | *OD* | *MC1R* | *PgD7* |
|---|---|---|---|---|---|---|---|---|---|
| NHMC80.3.119.38 [a] | *M. olivieri* | Morocco | 27.2369 | -12.8208 | KM411109 | -- | -- | --- | -- |
| NHMC80.3.119.39 [a] | *M. olivieri* | Morocco | 25.7847 | -14.7328 | KM411110 | -- | -- | --- | -- |
| NHMC80.3.119.40 [a] | *M. olivieri* | Egypt | 30.5965 | 32.2715 | KM411199 | -- | -- | --- | -- |
| NHMC80.3.119.41 [a] | *M. olivieri* | Morocco | 22.5709 | -14.3544 | KM411164 | -- | -- | --- | -- |
| NHMC80.3.119.42 [a] | *M. olivieri* | Morocco | 26.5298 | -12.3364 | KM411166 | -- | -- | --- | -- |
| NHMC80.3.119.43 [a c] | *M. olivieri* | Morocco | 26.4925 | -13.9198 | KM411170 | -- | -- | --- | -- |
| NHMC80.3.119.44 [a] | *M. olivieri* | Algeria | 36.3333 | 4.25 | KM411163 | -- | -- | --- | -- |
| NHMC80.3.119.46 [a] | *M. olivieri* | Algeria | 36.3413 | 4.2509 | KM411168 | -- | -- | --- | -- |
| NHMC80.3.119.5 [a] | *M. olivieri* | Libya | 32.3912 | 21.2404 | EF555324 | -- | -- | --- | -- |
| NHMC80.3.119.50 [a] | *M. olivieri* | Morocco | 33.2894 | -3.8437 | KM411172 | -- | -- | --- | -- |
| NHMC80.3.119.51 [a] | *M. olivieri* | Morocco | 26.1256 | -14.4799 | KM411227 | -- | -- | --- | -- |
| NHMC80.3.119.80 [a c] | *M. olivieri* | Morocco | 31.1755 | -6.2374 | KM411112 | -- | -- | --- | -- |
| NHMC80.3.119.81 [a c] | *M. olivieri* | Morocco | 32.19 | -2.2037 | KM411113 | -- | -- | --- | -- |
| NHMC80.3.119.82 [a] | *M. olivieri* | Morocco | 32.175 | -2.1667 | KM411114 | -- | -- | --- | -- |
| NHMC80.3.119.85 [a c] | *M. olivieri* | Egypt | 31.1319 | 33.8032 | KM411117 | -- | -- | --- | -- |
| NHMC80.3.119.87 [a] | *M. olivieri* | Israel | 30.7077 | 34.7845 | KM411115 | -- | -- | --- | -- |
| NHMC80.3.119.9 [a] | *M. olivieri* | Tunisia | 34.4076 | 7.9448 | EF555313 | -- | -- | --- | -- |
| NHMC80.3.119.91 [a] | *M. olivieri* | Israel | 31.0858 | 34.631 | KM411116 | -- | -- | --- | -- |
| NHMC80.3.119.92 [a] | *M. olivieri* | Tunisia | 35.5667 | 8.4667 | KM411228 | -- | -- | --- | -- |
| NHMC80.3.119.93 [a] | *M. olivieri* | Tunisia | 34.0009 | 8.2847 | KM411229 | -- | -- | --- | -- |
| NHMC80.3.164.19 [a] | *M. olivieri* | Libya | 33.0956 | 11.7626 | KM411157 | -- | -- | --- | -- |
| NHMC80.3.164.2 [a] | *M. olivieri* | Tunisia | 32.9974 | 10.608 | KM411233 | -- | -- | --- | -- |
| S2596 [a] | *M. olivieri* | Tunisia | 32.5349 | 10.2712 | MK551715 | -- | -- | MK551644 | -- |
| S2808 [a] | *M. olivieri* | Egypt | 31.1111 | 33.4399 | MK551708 | -- | -- | MK551639 | -- |
| S3322 [a c] | *M. olivieri* | Morocco | 35.0983 | -2.4593 | MK551716 | -- | -- | MK551645 | -- |
| S3344 [a] | *M. olivieri* | Libya | 32.0055 | 11.727 | MK551723 | -- | -- | --- | -- |
| S3608 [a c] | *M. olivieri* | Egypt | 31.0698 | 33.4523 | MK551712 | -- | -- | --- | -- |
| S3748 [a] | *M. olivieri* | Tunisia | 35.409 | 8.9508 | MK551714 | -- | -- | MK551643 | -- |
| S3765 [a] | *M. olivieri* | Egypt | 31.1111 | 33.4399 | MK551707 | -- | -- | --- | -- |
| S3928 [a c] | *M. olivieri* | Egypt | 31.0698 | 33.4523 | MK551709 | -- | -- | --- | -- |
| S5404 | *M. olivieri* | Egypt | 31.07 | 33.45 | MH040045 | -- | -- | MH040085 | -- |
| S5407 | *M. olivieri* | Egypt | 31.11 | 33.44 | MK551733 | -- | -- | MK551661 | -- |
| SPM002917 [a c] | *M. olivieri* | Egypt | | | MK551705 | -- | -- | MK551637 | -- |
| SPM002920 | *M. olivieri* | Egypt | | | MK551704 | -- | -- | MK551636 | -- |
| SPM002954(28) [a c] | *M. olivieri* | Egypt | 30.832 | 29.2037 | MK551711 | -- | -- | MK551641 | -- |
| SPM002981(55) [a c] | *M. olivieri* | Egypt | 31.0512 | 32.8878 | MK551706 | -- | -- | MK551638 | -- |

| Sample code | Species | Country | Latitude | Longitude | *Cyt-b* | *B-fib7* | *OD* | *MC1R* | *PgD7* |
|---|---|---|---|---|---|---|---|---|---|
| SPM003875 [a] | *M. olivieri* | Morocco | 34.3589 | -3.7495 | MK551717 | -- | -- | MK551646 | -- |
| T31-34_Mesaoliv1 [a c] | *M. olivieri* | Tunisia | 34.5854 | 8.9445 | MK551713 | -- | -- | MK551642 | -- |
| TAU.R16257 [a c] | *M. olivieri* | Israel | 31.124 | 34.809 | MK551710 | -- | -- | MK551640 | -- |
| BEV.10013 | *M. olivieri* | Morocco | 33.186000 | -3.990000 | Pending | Pending | Pending | Pending | Pending |
| BEV.10014 | *M. olivieri* | Morocco | 33.186000 | -3.990000 | Pending | Pending | Pending | Pending | Pending |
| BEV.10015 | *M. olivieri* | Morocco | 33.186000 | -3.990000 | Pending | Pending | Pending | Pending | Pending |
| BEV.10849 | *M. olivieri* | Morocco | 26.529800 | -12.336400 | Pending | Pending | Pending | Pending | Pending |
| BEV.10850 | *M. olivieri* | Morocco | 22.570900 | -14.354400 | Pending | Pending | Pending | Pending | Pending |
| BEV.11948 | *M. olivieri* | Morocco | 32.9297 | -5.0465 | Pending | Pending | Pending | Pending | Pending |
| BEV.13322 | *M. olivieri* | Algeria | 36.6245 | 4.8517 | Pending | Pending | Pending | Pending | Pending |
| BEV.13621 | *M. olivieri* | Algeria | 35.88019 | 1.68412526 | Pending | Pending | Pending | Pending | Pending |
| BEV.5925 | *M. olivieri* | Morocco | 28.350000 | -9.840000 | Pending | Pending | Pending | Pending | Pending |
| BEV.6402 | *M. olivieri* | Morocco | 31.194000 | -6.210000 | Pending | Pending | Pending | Pending | Pending |
| BEV.8508 | *M. olivieri* | Morocco | 32.190030 | -2.203730 | Pending | Pending | Pending | Pending | Pending |
| BEV.8509 | *M. olivieri* | Morocco | 32.175000 | -2.165000 | Pending | Pending | Pending | Pending | Pending |
| BEV.8830 | *M. olivieri* | Israel | 31.085800 | 34.631000 | Pending | Pending | Pending | Pending | Pending |
| BEV.9114 | *M. olivieri* | Morocco | 26.492500 | -13.919800 | Pending | Pending | Pending | Pending | Pending |
| BEV.9225 | *M. olivieri* | Algeria | 33.591400 | 2.950800 | Pending | Pending | Pending | Pending | Pending |
| BEV.9429 | *M. olivieri* | Morocco | 30.707600 | -8.357700 | Pending | Pending | Pending | Pending | Pending |
| BEV.T12122 | *M. olivieri* | Morocco | 28.487590 | -11.336590 | Pending | Pending | Pending | Pending | Pending |
| BEV.T1242 | *M. olivieri* | Morocco | 21.396300 | -16.957900 | Pending | Pending | Pending | Pending | Pending |
| BEV.T1256 | *M. olivieri* | Morocco | 26.492500 | -13.919800 | Pending | Pending | Pending | Pending | Pending |
| BEV.T3036 | *M. olivieri* | Algeria | 36.341300 | 4.250883 | Pending | Pending | Pending | Pending | Pending |
| BEV.T3037 | *M. olivieri* | Algeria | 36.341300 | 4.250883 | Pending | Pending | Pending | Pending | Pending |
| BEV.T3038 | *M. olivieri* | Algeria | 35.858500 | 6.490833 | Pending | Pending | Pending | Pending | Pending |
| BEV.T353 | *M. olivieri* | Morocco | 31.574000 | -4.738000 | Pending | Pending | Pending | Pending | Pending |
| BEV.T395 | *M. olivieri* | Egypt | 31.120000 | 33.760000 | Pending | Pending | Pending | Pending | Pending |
| BEV.T6678 | *M. olivieri* | Tunisia | 35.800637 | 11.036123 | Pending | Pending | Pending | Pending | Pending |
| CIBIO308 | *M. olivieri* | Tunisia | 35.582150 | 8.482633 | Pending | Pending | Pending | Pending | Pending |
| CIBIO9163 | *M. olivieri* | Morocco | 22.621538 | -14.604377 | Pending | Pending | Pending | Pending | Pending |
| NHMC80.3.147.10 [a] | *M. pasteuri* | Mauritania | 20.7972 | -16.2221 | KM411162 | -- | -- | --- | -- |
| NHMC80.3.147.11 [a] | *M. pasteuri* | Algeria | 24.7839 | 8.8719 | KM411171 | -- | -- | --- | -- |
| NHMC80.3.147.12 [a] | *M. pasteuri* | Mauritania | 21.2777 | -15.4703 | KM411230 | -- | -- | --- | -- |
| NHMC80.3.147.13 [a] | *M. pasteuri* | Mauritania | 21.2777 | -15.4703 | KM411231 | -- | -- | --- | -- |
| NHMC80.3.147.14 [a] | *M. pasteuri* | Mauritania | 21.2777 | -15.4703 | KM411232 | -- | -- | --- | -- |
| NHMC80.3.147.17 [a] | *M. pasteuri* | Mauritania | 21.3321 | -11.9512 | KM411118 | -- | -- | --- | -- |
| NHMC80.3.147.18 [a] | *M. pasteuri* | Mauritania | 20.4606 | -12.362 | KM411119 | -- | -- | --- | -- |

| Sample code | Species | Country | Latitude | Longitude | *Cyt-b* | *B-fib7* | *OD* | *MC1R* | *PgD7* |
|---|---|---|---|---|---|---|---|---|---|
| NHMC80.3.147.19 [a] | *M. pasteuri* | Mauritania | 20.4641 | -12.379 | KM411120 | -- | -- | --- | -- |
| NHMC80.3.147.2 [a] | *M. pasteuri* | Mauritania | 20.8061 | -16.4561 | KM411226 | -- | -- | --- | -- |
| NHMC80.3.147.20 | *M. pasteuri* | Mauritania | 20.4641 | -12.379 | KM411121 | -- | -- | --- | -- |
| NHMC80.3.147.3 [a c] | *M. pasteuri* | Mauritania | 17.3982 | -12.0305 | KM411138 | -- | -- | --- | -- |
| NHMC80.3.147.4 [a] | *M. pasteuri* | Mauritania | 18.9849 | -13.0647 | KM411139 | -- | -- | --- | -- |
| NHMC80.3.147.6 [a] | *M. pasteuri* | Mauritania | 16.8484 | -16.3503 | KM411158 | -- | -- | --- | -- |
| NHMC80.3.147.7 [a] | *M. pasteuri* | Mauritania | 19.6851 | -16.0641 | KM411159 | -- | -- | --- | -- |
| NHMC80.3.147.8 [a] | *M. pasteuri* | Mauritania | 19.7795 | -16.039 | KM411160 | -- | -- | --- | -- |
| NHMC80.3.147.9 [a] | *M. pasteuri* | Mauritania | 19.9197 | -16.028 | KM411161 | -- | -- | --- | -- |
| NHMC80.3.164.17 [a] | *M. pasteuri* | Mauritania | 21.015 | -11.718 | KM411140 | -- | -- | --- | -- |
| NHMC80.3.164.18 [a] | *M. pasteuri* | Mauritania | 21.4282 | -11.3139 | KM411141 | -- | -- | --- | -- |
| NHMC80.3.164.20 [a c] | *M. pasteuri* | Mauritania | 20.5936 | -12.9883 | KM411098 | -- | -- | --- | -- |
| NHMC80.3.164.21 [a] | *M. pasteuri* | Mauritania | 19.8652 | -12.8055 | KM411223 | -- | -- | --- | -- |
| NHMC80.3.164.22 [a] | *M. pasteuri* | Mauritania | 19.8652 | -12.8055 | KM411224 | -- | -- | --- | -- |
| RIM113 [a] | *M. pasteuri* | Mauritania | 21.3776 | -12.9764 | MK551722 | -- | -- | MK551651 | -- |
| RIM171 [a] | *M. pasteuri* | Mauritania | 18.5748 | -9.8064 | MK551720 | -- | -- | MK551649 | -- |
| RIM201 [a c] | *M. pasteuri* | Mauritania | 18.3334 | -11.8334 | MK551718 | -- | -- | MK551647 | -- |
| RIM202 [a] | *M. pasteuri* | Mauritania | 18.3334 | -11.8334 | MK551719 | -- | -- | MK551648 | -- |
| SPM002372(98) [a c] | *M. pasteuri* | Egypt | 29.19 | 25.51 | MK551721 | -- | -- | MK551650 | -- |
| NHMC80.3.119.47 [a] | *M. pasteuri* | Algeria | 35.8585 | 6.4908 | KM411169 | -- | -- | --- | -- |
| NHMC80.3.119.52 [a] | *M. pasteuri* | Algeria | 33.4992 | 3.0106 | KM411111 | -- | -- | --- | -- |
| BEV.10179 | *M. pasteuri* | Algeria | 24.783900 | 8.871900 | Pending | Pending | Pending | Pending | Pending |
| BEV.10454 | *M. pasteuri* | Mauritania | 21.277733 | -15.470317 | Pending | Pending | Pending | Pending | Pending |
| BEV.10455 | *M. pasteuri* | Mauritania | 21.277733 | -15.470317 | Pending | Pending | Pending | Pending | Pending |
| BEV.14803 | *M. pasteuri* | Mauritania | 21.486580 | -11.413930 | Pending | Pending | Pending | Pending | Pending |
| BEV.14804 | *M. pasteuri* | Mauritania | 21.296970 | -11.919850 | Pending | Pending | Pending | Pending | Pending |
| BEV.14805 | *M. pasteuri* | Mauritania | 21.296970 | -11.919850 | Pending | Pending | Pending | Pending | Pending |
| BEV.5926 | *M. pasteuri* | Algeria | 30.130000 | -2.170000 | Pending | Pending | Pending | Pending | Pending |
| BEV.5927 | *M. pasteuri* | Morocco | 29.970000 | -5.520000 | Pending | Pending | Pending | Pending | Pending |
| BEV.5928 | *M. pasteuri* | Algeria | 22.800000 | -1.100000 | Pending | Pending | Pending | Pending | Pending |
| BEV.9177 | *M. pasteuri* | Mauritania | 21.332140 | -11.951210 | Pending | Pending | Pending | Pending | Pending |
| BEV.9380 | *M. pasteuri* | Mauritania | 20.460000 | -12.356000 | Pending | Pending | Pending | Pending | Pending |
| BEV.T662 | *M. pasteuri* | Mauritania | 20.456280 | -12.360220 | Pending | Pending | Pending | Pending | Pending |
| BEV.T663 | *M. pasteuri* | Mauritania | 20.464100 | -12.379000 | Pending | Pending | Pending | Pending | Pending |
| CIBIO10706 | *M. pasteuri* | Mauritania | 16.205077 | -16.503377 | Pending | Pending | Pending | Pending | Pending |
| CIBIO11653 | *M. pasteuri* | Mauritania | 16.607197 | -16.441810 | Pending | Pending | Pending | Pending | Pending |
| CIBIO11656 | *M. pasteuri* | Mauritania | 16.655410 | -16.424157 | Pending | Pending | Pending | Pending | Pending |

| Sample code | Species | Country | Latitude | Longitude | *Cyt-b* | *B-fib7* | *OD* | *MC1R* | *PgD7* |
|---|---|---|---|---|---|---|---|---|---|
| CIBIO12821 | *M. pasteuri* | Mauritania | 16.130688 | -16.511242 | Pending | Pending | Pending | Pending | Pending |
| CIBIO12822 | *M. pasteuri* | Mauritania | 16.130688 | -16.511242 | Pending | Pending | Pending | Pending | Pending |
| CIBIO13770 | *M. pasteuri* | Mauritania | 23.369895 | -11.669612 | Pending | Pending | Pending | Pending | Pending |
| CIBIO2765 | *M. pasteuri* | Mauritania | 20.806070 | -16.456107 | Pending | Pending | Pending | Pending | Pending |
| CIBIO4449 | *M. pasteuri* | Mauritania | 17.067378 | -16.255501 | Pending | Pending | Pending | Pending | Pending |
| CIBIO4467 | *M. pasteuri* | Mauritania | 16.848397 | -16.350311 | Pending | Pending | Pending | Pending | Pending |
| CIBIO4468 | *M. pasteuri* | Mauritania | 16.848397 | -16.350311 | Pending | Pending | Pending | Pending | Pending |
| CIBIO5061 | *M. pasteuri* | Mauritania | 19.685135 | -16.064098 | Pending | Pending | Pending | Pending | Pending |
| CIBIO5077 | *M. pasteuri* | Mauritania | 19.779490 | -16.038992 | Pending | Pending | Pending | Pending | Pending |
| CIBIO5100 | *M. pasteuri* | Mauritania | 19.919693 | -16.028018 | Pending | Pending | Pending | Pending | Pending |
| CIBIO5299 | *M. pasteuri* | Mauritania | 20.797217 | -16.222065 | Pending | Pending | Pending | Pending | Pending |
| CIBIO5822 | *M. pasteuri* | Mauritania | 22.835012 | -12.329155 | Pending | Pending | Pending | Pending | Pending |
| CIBIO5849 | *M. pasteuri* | Mauritania | 21.832925 | -12.810607 | Pending | Pending | Pending | Pending | Pending |
| CIBIO6279 | *M. pasteuri* | Mauritania | 19.351447 | -16.200417 | Pending | Pending | Pending | Pending | Pending |
| CIBIO6692 | *M. pasteuri* | Niger | 16.217758 | 12.198542 | Pending | Pending | Pending | Pending | Pending |
| CIBIO7333 | *M. pasteuri* | Morocco | 23.218518 | -15.446822 | Pending | Pending | Pending | Pending | Pending |
| NHMC80.3.99.1 [a] | *M. rubropunctata* | Egypt | 24.4 | 33.017 | EF555316 | -- | -- | --- | -- |
| NHMC80.3.99.13 [a] | *M. rubropunctata* | Egypt | 24.9294 | 30.3897 | KM411122 | -- | -- | --- | -- |
| NHMC80.3.99.17 [a] | *M. rubropunctata* | Algeria | 24.3264 | 7.0058 | KM411203 | -- | -- | --- | -- |
| NHMC80.3.99.18 [a] | *M. rubropunctata* | Libya | 25.9666 | 15.1574 | KM411146 | -- | -- | --- | -- |
| NHMC80.3.99.21 [a] | *M. rubropunctata* | Egypt | 30.0457 | 31.2402 | KM411147 | -- | -- | --- | -- |
| NHMC80.3.99.25 [a] | *M. rubropunctata* | Mauritania | 19.5518 | -14.3706 | KM411148 | -- | -- | --- | -- |
| NHMC80.3.99.26 [a] | *M. rubropunctata* | Mauritania | 19.5518 | -14.3706 | KM411149 | -- | -- | --- | -- |
| NHMC80.3.99.27 [a] | *M. rubropunctata* | Mauritania | 21.2985 | -13.0674 | KM411150 | -- | -- | --- | -- |
| NHMC80.3.99.28 [a] | *M. rubropunctata* | Libya | 25.9666 | 15.1574 | KM411143 | -- | -- | --- | -- |
| NHMC80.3.99.29 [a c] | *M. rubropunctata* | Egypt | 29.8734 | 32.6497 | KM411200 | -- | -- | --- | -- |
| RIM092 | *M. rubropunctata* | Mauritania | 21.4968 | -11.6168 | MK551703 | -- | -- | MK551635 | -- |
| RIM093 [a c] | *M. rubropunctata* | Mauritania | 21.4968 | -11.6168 | MK551702 | -- | -- | MK551634 | -- |
| SPM002355(45) [a] | *M. rubropunctata* | Egypt | 29.7095 | 30.3792 | MK551701 | -- | -- | MK551633 | -- |
| SUD12/2010-56 [a c] | *M. rubropunctata* | Sudan | 21.8008 | 31.3488 | MK551699 | -- | -- | MK551631 | -- |
| SUD12/2010-57 | *M. rubropunctata* | Sudan | 21.8008 | 31.3488 | KY967148 | -- | -- | KY967103 | -- |
| SUD12/2010-58 | *M. rubropunctata* | Sudan | 21.8008 | 31.3488 | MK551700 | -- | -- | MK551632 | -- |
| CIBIO13743 | *M. rubropunctata* | Mauritania | 24.081058 | -10.395780 | Pending | Pending | Pending | Pending | Pending |
| CIBIO5265 | *M. rubropunctata* | Mauritania | 20.975818 | -15.415585 | Pending | Pending | Pending | Pending | Pending |
| 912 | *M. saudiarabica* | Saudi Arabia | 22.2371 | 41.8428 | --- | -- | -- | KY967104 | -- |
| NHMC80.3.164.16 [a c] | *M. saudiarabica* | Saudi Arabia | 22.3952 | 41.7529 | KM411151 | -- | -- | --- | -- |
| NHMC80.3.164.5 [a] | *M. saudiarabica* | Saudi Arabia | 22.4 | 41.7333 | KM411198 | -- | -- | --- | -- |

| Sample code | Species | Country | Latitude | Longitude | *Cyt-b* | *B-fib7* | *OD* | *MC1R* | *PgD7* |
|---|---|---|---|---|---|---|---|---|---|
| NHMC80.3.164.9 [a] | *M. saudiarabica* | Saudi Arabia | 22.2525 | 41.8796 | KM411206 | -- | -- | --- | -- |
| NHMC80.3.69.17 [a] | *M. saudiarabica* | Saudi Arabia | 23.5388 | 40.5893 | KM411192 | -- | -- | --- | -- |
| NHMC80.3.69.18 | *M. saudiarabica* | Saudi Arabia | 23.5038 | 41.3472 | KM411193 | -- | -- | --- | -- |
| NHMC80.3.69.19 | *M. saudiarabica* | Saudi Arabia | 24.2531 | 41.1539 | KM411194 | -- | -- | --- | -- |
| NHMC80.3.69.20 [a] | *M. saudiarabica* | Saudi Arabia | 23.5114 | 41.422 | KM411195 | -- | -- | --- | -- |
| MC57Me1 [a c] | *M. simoni* | Morocco | 30.5194 | -9.6856 | MK551724 | -- | -- | MK551652 | -- |
| NHMC80.3.109.1 [a] | *M. simoni* | Morocco | 31.912 | -7.505 | EF555301 | -- | -- | --- | -- |
| NHMC80.3.109.5 [a] | *M. simoni* | Morocco | 31.8028 | -8.037 | KM411123 | -- | -- | --- | -- |
| NHMC80.3.109.6 | *M. simoni* | Morocco | 31.8028 | -8.037 | KM411124 | -- | -- | --- | -- |
| BEV.9430 | *M. simoni* | Morocco | 31.830100 | -7.982900 | Pending | Pending | Pending | Pending | Pending |
| BEV.9431 | *M. simoni* | Morocco | 31.812900 | -8.013800 | Pending | Pending | Pending | Pending | Pending |
| BEV.T6301 | *M. simoni* | Morocco | 32.301120 | -7.530650 | Pending | Pending | Pending | Pending | Pending |
| CN11193 [a] | *M. sp.* | Saudi Arabia | 24.4441 | 46.6756 | MK551725 | -- | -- | MK551654 | -- |
| CN11194 [a] | *M. sp.* | Saudi Arabia | 24.4441 | 46.6756 | MK551732 | -- | -- | MK551660 | -- |
| CN11195 [a c] | *M. sp.* | Saudi Arabia | 24.4441 | 46.6756 | MK551726 | -- | -- | MK551655 | -- |
| CN11200 [a] | *M. sp.* | Saudi Arabia | 24.3236 | 46.3961 | MK551731 | -- | -- | --- | -- |
| J16/04 | *M. sp.* | Jordan | 32.169 | 37.0078 | MH040047 | -- | -- | MH040087 | -- |
| NHMC80.3.72.36 | *M. sp.* | Saudi Arabia | 26.4278 | 47.3789 | KM411184 | -- | -- | --- | -- |
| NHMC80.3.72.37 [a] | *M. sp.* | Saudi Arabia | 26.4066 | 47.707 | KM411185 | -- | -- | --- | -- |
| NHMC80.3.72.38 | *M. sp.* | Saudi Arabia | 26.4153 | 47.4736 | KM411186 | -- | -- | --- | -- |
| NHMC80.3.72.39 [a] | *M. sp.* | Saudi Arabia | 23.2786 | 46.3534 | KM411187 | -- | -- | --- | -- |
| NHMC80.3.72.40 [a] | *M. sp.* | Saudi Arabia | 23.193 | 46.4223 | KM411188 | -- | -- | --- | -- |
| NHMC80.3.72.41 [a] | *M. sp.* | Saudi Arabia | 23.2417 | 46.4537 | KM411189 | -- | -- | --- | -- |
| NHMC80.3.72.52 [a] | *M. sp.* | Jordan | 31.8817 | 36.9133 | KM411179 | -- | -- | MH040088 | -- |
| NHMC80.3.72.59 [a] | *M. sp.* | Kuwait | 29.4624 | 47.6407 | KM411238 | -- | -- | MH040089 | -- |
| S10332 [a] | *M. spp.* | Saudi Arabia | 25.2681 | 46.6237 | MH040048 | -- | -- | MH040090 | -- |
| CIBIO312 | *M. spp.* | Tunisia | 34.000883 | 8.284733 | Pending | Pending | Pending | Pending | Pending |
| CIBIO319 | *M. spp.* | Tunisia | 32.997433 | 10.607950 | Pending | Pending | Pending | Pending | Pending |
| IR001 [a c] | *M. watsonana* | Iran | 35.1109 | 50.8977 | MK551694 | -- | -- | MK551626 | -- |
| IR006 [a] | *M. watsonana* | Iran | 30.2702 | 57.1231 | MK551696 | -- | -- | MK551628 | -- |
| IR013 [a c] | *M. watsonana* | Iran | 30.0243 | 57.2848 | MK551697 | -- | -- | MK551629 | -- |
| KUSH12 [a] | *M. watsonana* | Iran | 35.1109 | 50.8977 | MK551695 | -- | -- | MK551627 | -- |
| NHMC80.3.144.1 [a] | *M. watsonana* | Iran | 35.6962 | 51.4229 | KM411217 | -- | -- | --- | -- |
| NHMC80.3.144.15 [a] | *M. watsonana* | Iran | 26.1592 | 60.1861 | KM411153 | -- | -- | --- | -- |
| NHMC80.3.144.16 [a] | *M. watsonana* | Iran | 26.1592 | 60.1861 | KM411154 | -- | -- | --- | -- |
| NHMC80.3.144.17 [a c] | *M. watsonana* | Afghanistan | 34.5833 | 68.95 | KM411219 | -- | -- | --- | -- |

| Sample code | Species | Country | Latitude | Longitude | *Cyt-b* | *B-fib7* | *OD* | *MC1R* | *PgD7* |
|---|---|---|---|---|---|---|---|---|---|
| NHMC80.3.144.18 [a] | *M. watsonana* | Pakistan | 26.4948 | 66.6677 | KM411221 | -- | -- | --- | -- |
| NHMC80.3.144.19 [a] | *M. watsonana* | Pakistan | 26.4948 | 66.6677 | KM411220 | -- | -- | --- | -- |
| NHMC80.3.144.2 [a] | *M. watsonana* | Iran | 32.5777 | 59.7978 | KM411218 | -- | -- | --- | -- |
| NHMC80.3.144.4 [a] | *M. watsonana* | Iran | 32.383 | 48.3982 | KM411126 | -- | -- | --- | -- |
| NHMC80.3.144.5 [a] | *M. watsonana* | Iran | 31.6516 | 49.2775 | KM411127 | -- | -- | --- | -- |
| NHMC80.3.144.6 [a] | *M. watsonana* | Iran | 31.6516 | 49.2775 | KM411128 | -- | -- | --- | -- |
| NHMC80.3.144.7 [a] | *M. watsonana* | Iran | 32.3081 | 52.0158 | KM411152 | -- | -- | --- | -- |
| NHMC80.3.144.8 [a] | *M. watsonana* | Iran | 34.4164 | 50.8608 | KM411155 | -- | -- | --- | -- |
| NHMC80.3.144.9 [a] | *M. watsonana* | Iran | 31.1956 | 59.3201 | KM411156 | -- | -- | --- | -- |
| TAB11 [a c] | *M. watsonana* | Iran | 33.5993 | 56.9123 | MK551698 | -- | -- | MK551630 | -- |
| VAZ10 [a c] | *M. watsonana* | Iran | 28.9974 | 54.7813 | MH040049 | -- | -- | MH040091 | -- |
| | *Gallotia atlantica* | | | | DQ298679 | -- | -- | AY151999 | -- |
| | *Psammodromus algirus* | | | | DQ298675 | -- | -- | AY151998 | -- |
| | *Podarcis lilfordi* | | | | KX658188 | -- | -- | EF679323 | -- |
| | *Podarcis pityusensis* | | | | KX658227 | -- | -- | EF679328 | -- |

**Table S3.** Information on all datasets used in the phylogenetic, calibration, and range reconstruction analyses, including partitions, models and parameters.

| Dataset | Data | Analysis | Partition | Model | Clock Model | Tree model | Run Specification |
|---|---|---|---|---|---|---|---|
| 0 | mtDNA | Bayesian Inference [BEAST] | Cyt-b | HKY+G +I | Relaxed Lognormal Uncorrelated | Speciation: Yule Process | 3 runs; 100000000 generations; 10000 sampling frequency; 10% burn-in |
| | mtDNA (outgroups excluded) | Historical demography [BEAST] | Cyt-b | HKY+G +I | Relaxed Lognormal Uncorrelated | Coalescent-based: Bayesian skyline plot (BSP) | 3 runs; 100000000 generations; 10000 sampling frequency; 10% burn-in |
| 1 | mtDNA all GENEBANK | Bayesian Inference [BEAST] | Cyt-b | HKY+G +I | Relaxed Lognormal Uncorrelated | Speciation: Yule Process | 3 runs; 100000000 generations; 10000 sampling frequency; 10% burn-in |
| 2 | nDNA (outgroups included) | Bayesian Inference [BEAST] | B-fib7<br>MC1R<br>OD<br>PgD7 | HKY+G<br>HKY+I<br>HKY<br>K80+G | Strict clock<br>Strict clock<br>Strict clock<br>Strict clock | Speciation: Yule Process | 3 runs; 100000000 generations; 10000 sampling frequency; 10% burn-in |
| | nDNA (outgroups excluded) | Species Tree (*M. olivieri* sp. Complex) [*BEAST] | B-fib7<br>MC1R<br>OD<br>PgD7 | HKY+G<br>HKY+I<br>HKY<br>K80+G | Strict clock<br>Strict clock<br>Strict clock<br>Strict clock | Speciation Yule Process | 3 runs; 100000000 generations; 10000 sampling frequency; 10% burn-in |
| 3 | Concatenated cytonuclear (Dataset 0+2) [BEAST] | Bayesian Inference [BEAST] | B-fib7<br>Cyt-b<br>MC1R<br>OD<br>PgD7 | HKY+G<br>HKY+G +I<br>HKY+I<br>HKY<br>K80+G | Strict clock<br>Relaxed Lognormal<br>Strict clock<br>Strict clock<br>Strict clock | Uncorrelated Speciation Yule Process | 3 runs; 100000000 generations; 10000 sampling frequency; 10% burn-in |
| | Concatenated cytonuclear (Dataset 0+2) [BEAST] | Divergence time estimation | B-fib7<br>Cyt-b<br>MC1R<br>OD<br>PgD7 | HKY+G<br>HKY+G +I<br>HKY+I<br>HKY<br>K80+G | Strict clock<br>Relaxed Lognormal<br>Strict clock<br>Strict clock<br>Strict clock | Uncorrelated Speciation Yule Process | 3 runs; 100000000 generations; 10000 sampling frequency; 10% burn-in |

**Table S4.** Material examined for morphological comparisons and original measurements for each specimen. Codes of morphological characters are given section Material and Methods. M = Male; F = Female.

| Code | Species | Sex | SVL | HL | HW | HH | TE | RN | PS |
|---|---|---|---|---|---|---|---|---|---|
| BEV.10457 | *Mesalina sp. nov.* | F | 53.04 | 11.45 | 6.65 | 3.86 | 1 | 2 | 3 |
| BEV.14800 | *Mesalina sp. nov.* | F | 44.01 | 9.99 | 6.42 | 4.26 | 1 | 1 | 3 |
| BEV.15060 | *Mesalina sp. nov.* | M | 33.23 | 8.44 | 4.92 | 3.16 | 2 | 2 | 3 |
| BEV.15061 | *Mesalina sp. nov.* | F | 40.80 | 9.38 | 5.57 | 3.79 | 1 | 2 | 3 |
| BEV.15062 | *Mesalina sp. nov.* | M | 33.15 | 8.49 | 5.38 | 3.24 | 3 | 2 | 3 |
| BEV.15063 | *Mesalina sp. nov.* | M | 38.05 | 9.78 | 6.13 | 3.62 | 3 | 1 | 3 |
| BEV.15064 | *Mesalina sp. nov.* | M | 33.53 | 8.72 | 5.06 | 3.09 | 2 | 1 | 3 |
| BEV.15163 | *Mesalina sp. nov.* | F | 42 | 9.00 | 6 | 3 | 2 | 1 | 3 |
| BEV.10013 | *M. olivieri* | M | 40.78 | 10.28 | 5.51 | 4.25 | 3 | 1 | 2 |
| BEV.11948 | *M. olivieri* | F | 44.64 | 9.43 | 6.36 | 4.35 | 1 | 1 | 2 |
| BEV.12808 | *M. olivieri* | F | 47.25 | 10.51 | 6.83 | 5.08 | 1 | 2 | 1 |
| BEV.13621 | *M. olivieri* | M | 49.59 | 12.17 | 8.40 | 5.27 | 2 | 1 | 2 |
| BEV.14833 | *M. olivieri* | M | 44.95 | 10.45 | 6.49 | 4.13 | 2 | 1 | 2 |
| BEV.5925 | *M. olivieri* | F | 41.00 | 10.21 | 6.33 | 3.75 | 1 | 1 | 1 |
| BEV.5861 | *M. olivieri* | F | 46.68 | 9.55 | 6.40 | 4.53 | 1 | 1 | 1 |
| BEV.5864 | *M. olivieri* | F | 38.52 | 8.61 | 5.30 | 3.59 | 1 | 1 | 1 |
| BEV.5867 | *M. olivieri* | F | 40.17 | 8.67 | 5.33 | 3.66 | 1 | 1 | 1 |
| BEV.5870 | *M. olivieri* | M | 41.53 | 10.17 | 6.62 | 4.26 | 3 | 2 | 2 |
| BEV.5871 | *M. olivieri* | F | 42.98 | 9.64 | 5.80 | 4.17 | 1 | 1 | 2 |
| BEV.5875 | *M. olivieri* | F | 42.66 | 9.60 | 6.24 | 4.45 | 1 | 1 | 2 |
| BEV.5876 | *M. olivieri* | M | 44.03 | 10.60 | 6.96 | 5.19 | 2 | 1 | 1 |
| BEV.5877 | *M. olivieri* | F | 44.63 | 8.73 | 5.41 | 4.13 | 1 | 2 | 1 |
| BEV.5879 | *M. olivieri* | M | 45.91 | 11.20 | 6.77 | 5.01 | 3 | 1 | 1 |
| BEV.5880 | *M. olivieri* | F | 47.22 | 9.90 | 6.28 | 4.48 | 1 | 2 | 1 |
| BEV.5881 | *M. olivieri* | M | 42.31 | 10.30 | 6.90 | 4.61 | 3 | 2 | 1 |
| BEV.5882 | *M. olivieri* | F | 42.37 | 9.31 | 5.95 | 4.42 | 1 | 1 | 1 |
| BEV.5888 | *M. olivieri* | F | 36.43 | 9.05 | 6.14 | 3.89 | 2 | 1 | 2 |
| BEV.5889 | *M. olivieri* | M | 43.41 | 10.90 | 7.26 | 4.99 | 2 | 1 | 1 |
| BEV.5890 | *M. olivieri* | M | 41.30 | 10.46 | 6.02 | 3.65 | 3 | 1 | 3 |
| BEV.5899 | *M. olivieri* | F | 39.38 | 9.76 | 5.96 | 4.50 | 2 | 1 | 2 |
| BEV.5956 | *M. olivieri* | M | 37.35 | 9.22 | 5.39 | 3.57 | 2 | 1 | 3 |
| BEV.5959 | *M. olivieri* | M | 36.09 | 9.24 | 5.59 | 3.99 | 2 | 1 | 2 |
| BEV.8830 | *M. olivieri* | M | 43.28 | 10.65 | 7.27 | 5.36 | 3 | 1 | 1 |
| BEV.8796 | *M. olivieri* | M | 45.32 | 11.87 | 7.31 | 5.49 | 3 | 1 | 3 |
| BEV.8797 | *M. olivieri* | M | 44.57 | 11.68 | 7.26 | 5.24 | 3 | 1 | 2 |
| BEV.8798 | *M. olivieri* | M | 44.78 | 11.06 | 7.66 | 5.60 | 3 | 1 | 1 |
| BEV.8829 | *M. olivieri* | M | 44.77 | 11.04 | 7.00 | 5.09 | 3 | 1 | 3 |
| BEV.8975 | *M. olivieri* | F | 43.03 | 9.52 | 6.18 | 4.33 | 2 | 2 | 1 |

| Code | Species | Sex | SVL | HL | HW | HH | TE | RN | PS |
|---|---|---|---|---|---|---|---|---|---|
| BEV.10453 | *M. simoni* | M | 35.74 | 8.27 | 4.86 | 3.17 | 2 | 1 | 3 |
| BEV.10849 | *M. simoni* | M | 38.07 | 9.24 | 5.56 | 4.39 | 3 | 2 | 3 |
| BEV.10850 | *M. simoni* | F | 42.46 | 8.47 | 5.28 | 3.60 | 2 | 1 | 1 |
| BEV.11187 | *M. simoni* | M | 42.49 | 10.41 | 6.90 | 4.65 | 3 | 2 | 1 |
| BEV.11586 | *M. simoni* | M | 44.74 | 11.08 | 7.44 | 4.95 | 2 | 1 | 2 |
| BEV.11587 | *M. simoni* | F | 45.71 | 9.17 | 6.38 | 4.02 | 2 | 1 | 2 |
| BEV.11588 | *M. simoni* | F | 46.10 | 9.69 | 6.27 | 4.43 | 1 | 1 | 2 |
| BEV.11589 | *M. simoni* | M | 46.56 | 11.32 | 7.33 | 5.00 | 3 | 1 | 2 |
| BEV.11590 | *M. simoni* | M | 45.23 | 10.45 | 7.11 | 4.70 | 3 | 1 | 3 |
| BEV.11591 | *M. simoni* | F | 45.92 | 9.71 | 6.49 | 4.60 | 1 | 1 | 2 |
| BEV.11594 | *M. simoni* | F | 47.88 | 10.06 | 6.26 | 3.99 | 1 | 1 | 2 |
| BEV.14869 | *M. simoni* | F | 41.77 | 8.77 | 5.62 | 3.76 | 1 | 1 | 1 |
| BEV.5922 | *M. simoni* | M | 50.79 | 11.08 | 7.00 | 5.43 | 3 | 1 | 1 |
| BEV.5923 | *M. simoni* | F | 50.69 | 9.74 | 6.33 | 4.41 | 2 | 2 | 2 |
| BEV.5924 | *M. simoni* | F | 48.25 | 10.30 | 6.67 | 4.50 | 1 | 1 | 2 |
| BEV.8508 | *M. simoni* | M | 43.14 | 9.72 | 6.42 | 4.89 | 3 | 1 | 1 |
| BEV.8509 | *M. simoni* | F | 45.75 | 9.45 | 6.26 | 4.36 | 1 | 2 | 1 |
| BEV.9114 | *M. simoni* | M | 40.70 | 9.62 | 5.90 | 4.20 | 2 | 1 | 2 |
| BEV.9225 | *M. simoni* | M | 42.39 | 10.23 | 7.09 | 5.35 | 3 | 1 | 1 |
| BEV.9429 | *M. simoni* | M | 42.15 | 10.21 | 6.75 | 4.39 | 3 | 1 | 1 |
| BEV.9430 | *M. simoni* | M | 43.89 | 10.29 | 6.83 | 4.68 | 3 | 1 | 2 |
| BEV.9431 | *M. simoni* | M | 43.74 | 10.39 | 7.14 | 4.64 | 3 | 2 | 1 |
| BEV.9432 | *M. simoni* | M | 43.34 | 10.71 | 6.61 | 4.52 | 3 | 1 | 1 |
| BEV.10179 | *M. pasteuri* | M | 48.32 | 11.83 | 7.44 | 5.63 | 2 | 1 | 2 |
| BEV.10454 | *M. pasteuri* | M | 45.41 | 11.04 | 5.98 | 4.10 | 2 | 1 | 3 |
| BEV.10455 | *M. pasteuri* | M | 42.17 | 10.42 | 5.57 | 3.90 | 3 | 1 | 3 |
| BEV.14803 | *M. pasteuri* | M | 48.31 | 12.01 | 7.74 | 5.44 | 3 | 2 | 3 |
| BEV.14804 | *M. pasteuri* | F | 40.38 | 8.94 | 5.59 | 4.16 | 2 | 1 | 3 |
| BEV.14805 | *M. pasteuri* | M | 40.61 | 9.95 | 6.44 | 4.05 | 3 | 1 | 3 |
| BEV.5926 | *M. pasteuri* | M | 43.75 | 11.17 | 6.27 | 4.07 | 2 | 1 | 2 |
| BEV.5927 | *M. pasteuri* | F | 41.81 | 10.09 | 5.56 | 4.25 | 1 | 1 | 2 |
| BEV.9177 | *M. pasteuri* | M | 44.70 | 11.23 | 7.50 | 4.64 | 1 | 1 | 2 |
| BEV.9380 | *M. pasteuri* | F | 27.77 | 7.19 | 4.54 | 2.99 | 2 | 1 | 3 |

**Table S5.** Material examined for pholidotic characters comparisons for each specimen. Codes of morphological characters are given section Material and Methods. M = Male; F = Female.

| Code | Species | Sex | SVL | HL | HW | HH | TE | RN | PS | V | D | DS | TR | SL(Sx) | SL(Dx) | IL(Sx) | IL(Dx) | G | Col | EL | NTS | Pf(Sx) | Pf(Dx) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BEV.10457 | *Mesalina sp. nov.* | F | 53.04 | 11.45 | 6.65 | 3.86 | 1 | 2 | 3 | 34 | 44 | 4 | 15 | 4 | 4 | 7 | 7 | 28 | 11 | 5 | 12 | 14 | 15 |
| BEV.14800 | *Mesalina sp. nov.* | F | 44.01 | 9.99 | 6.42 | 4.26 | 1 | 1 | 3 | 32 | 44 | 3 | 12 | 4 | 4 | 7 | 7 | 24 | 7 | 5 | 12 | 14 | 13 |
| BEV.15060 | *Mesalina sp. nov.* | M | 33.23 | 8.44 | 4.92 | 3.16 | 2 | 2 | 3 | 31 | 45 | 4 | 14 | 4 | 4 | 7 | 8 | 25 | 11 | 5 | 11 | 12 | 13 |
| BEV.15061 | *Mesalina sp. nov.* | F | 40.80 | 9.38 | 5.57 | 3.79 | 1 | 2 | 3 | 33 | 52 | 4 | 14 | 4 | 4 | 7 | 8 | 24 | 10 | 5 | 11 | 12 | 14 |
| BEV.15062 | *Mesalina sp. nov.* | M | 33.15 | 8.49 | 5.38 | 3.24 | 3 | 2 | 3 | 33 | 52 | 4 | 16 | 4 | 4 | 9 | 7 | 29 | 11 | 5 | 12 | 13 | 13 |
| BEV.15063 | *Mesalina sp. nov.* | M | 38.05 | 9.78 | 6.13 | 3.62 | 3 | 1 | 3 | 30 | 56 | 4 | 17 | 4 | 4 | 7 | 7 | 29 | 11 | 5 | 12 | 17 | 17 |
| BEV.15064 | *Mesalina sp. nov.* | M | 33.53 | 8.72 | 5.06 | 3.09 | 2 | 1 | 3 | 31 | 49 | 4 | 16 | 4 | 4 | 9 | 9 | 32 | 11 | 5 | 12 | 15 | 16 |
| BEV.15163 | *Mesalina sp. nov.* | F | 42 | 9.00 | 6 | 3 | 2 | 1 | 3 | 34 | 48 | 4 | 15 | 4 | 4 | 9 | 8 | 27 | 11 | 5 | 10 | 17 | 15 |
| BEV.10013 | *M. olivieri* | M | 40.78 | 10.28 | 5.51 | 4.25 | 3 | 1 | 2 | 29 | 44 | 4 | 15 | 4 | 4 | 8 | 8 | 24 | 7 | 8 | 9 | 13 | 14 |
| BEV.11948 | *M. olivieri* | F | 44.64 | 9.43 | 6.36 | 4.35 | 1 | 1 | 2 | 32 | 50 | 2 | 19 | 4 | 4 | 9 | 8 | 25 | 11 | 12 | 10 | 13 | 13 |
| BEV.12808 | *M. olivieri* | F | 47.25 | 10.51 | 6.83 | 5.08 | 1 | 2 | 1 | 30 | 46 | 4 | 17 | 5 | 4 | 8 | 7 | 27 | 8 | 6 | 10 | 12 | 12 |
| BEV.13621 | *M. olivieri* | M | 49.59 | 12.17 | 8.40 | 5.27 | 2 | 1 | 2 | 30 | 64 | 3 | 16 | 4 | 4 | 8 | 9 | 26 | 9 | 5 | 9 | 15 | 16 |
| BEV.14833 | *M. olivieri* | M | 44.95 | 10.45 | 6.49 | 4.13 | 2 | 1 | 2 | 32 | 44 | 3 | 17 | 4 | 4 | 7 | 8 | 29 | 12 | 3 | 10 | 11 | 12 |
| BEV.5925 | *M. olivieri* | F | 41.00 | 10.21 | 6.33 | 3.75 | 1 | 1 | 1 | 31 | 46 | 0 | 14 | 4 | 4 | 8 | 8 | 25 | 12 | 5 | 10 | 13 | 12 |
| BEV.5861 | *M. olivieri* | F | 46.68 | 9.55 | 6.40 | 4.53 | 1 | 1 | 1 | 31 | 43 | 3 | 17 | 4 | 4 | 8 | 8 | 25 | 9 | 5 | 9 | 13 | 14 |
| BEV.5864 | *M. olivieri* | F | 38.52 | 8.61 | 5.30 | 3.59 | 1 | 1 | 1 | 32 | 43 | 2 | 12 | 4 | 4 | 8 | 7 | 23 | 9 | 5 | 8 | 10 | 10 |
| BEV.5867 | *M. olivieri* | F | 40.17 | 8.67 | 5.33 | 3.66 | 1 | 1 | 1 | 29 | 41 | 3 | 17 | 4 | 5 | 7 | 8 | 26 | 9 | 5 | 8 | 11 | 10 |
| BEV.5870 | *M. olivieri* | M | 41.53 | 10.17 | 6.62 | 4.26 | 3 | 2 | 2 | 30 | 45 | 4 | 16 | 4 | 4 | 7 | 6 | 23 | 11 | 6 | 11 | 15 | 13 |
| BEV.5871 | *M. olivieri* | F | 42.98 | 9.64 | 5.80 | 4.17 | 1 | 1 | 2 | 35 | 48 | 3 | 16 | 3 | 4 | 8 | 9 | 26 | 8 | 8 | 10 | 13 | 13 |
| BEV.5875 | *M. olivieri* | F | 42.66 | 9.60 | 6.24 | 4.45 | 1 | 1 | 2 | 32 | 42 | 3 | 14 | 5 | 5 | 7 | 8 | 25 | 9 | 7 | 10 | 12 | 10 |
| BEV.5876 | *M. olivieri* | M | 44.03 | 10.60 | 6.96 | 5.19 | 2 | 1 | 1 | 32 | 44 | 2 | 16 | 4 | 4 | 8 | 8 | 25 | 12 | 5 | 9 | 12 | 12 |
| BEV.5877 | *M. olivieri* | F | 44.63 | 8.73 | 5.41 | 4.13 | 1 | 2 | 1 | 32 | 40 | 2 | 16 | 4 | 5 | 8 | 8 | 24 | 11 | 5 | 11 | 13 | 12 |
| BEV.5879 | *M. olivieri* | M | 45.91 | 11.20 | 6.77 | 5.01 | 3 | 1 | 1 | 31 | 40 | 3 | 16 | 4 | 4 | 8 | 9 | 24 | 9 | 5 | 10 | 12 | 13 |
| BEV.5880 | *M. olivieri* | F | 47.22 | 9.90 | 6.28 | 4.48 | 1 | 2 | 1 | 33 | 46 | 3 | 15 | 4 | 4 | 8 | 7 | 21 | 9 | 8 | 9 | 11 | 10 |
| BEV.5881 | *M. olivieri* | M | 42.31 | 10.30 | 6.90 | 4.61 | 3 | 2 | 1 | 30 | 47 | 2 | 18 | 4 | 4 | 7 | 8 | 20 | 9 | 5 | 10 | 12 | 11 |
| BEV.5882 | *M. olivieri* | F | 42.37 | 9.31 | 5.95 | 4.42 | 1 | 1 | 1 | 32 | 42 | 4 | 16 | 4 | 4 | 8 | 8 | 22 | 8 | 9 | 10 | 10 | 12 |

| Code | Species | Sex | SVL | HL | HW | HH | TE | RN | PS | V | D | DS | TR | SL(Sx) | SL(Dx) | IL(Sx) | IL(Dx) | G | Col | EL | NTS | Pf(Sx) | Pf(Dx) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BEV.5888 | *M. olivieri* | F | 36.43 | 9.05 | 6.14 | 3.89 | 2 | 1 | 2 | 27 | 45 | 4 | 17 | 4 | 5 | 9 | 7 | 26 | 8 | 8 | 12 | 12 | 12 |
| BEV.5889 | *M. olivieri* | M | 43.41 | 10.90 | 7.26 | 4.99 | 2 | 1 | 1 | 30 | 52 | 1 | 18 | 4 | 4 | 9 | 9 | 28 | 9 | 3 | 10 | 13 | 16 |
| BEV.5890 | *M. olivieri* | M | 41.30 | 10.46 | 6.02 | 3.65 | 3 | 1 | 3 | 33 | 46 | 2 | 19 | 4 | 4 | 7 | 9 | 31 | 7 | 7 | 10 | 16 | 15 |
| BEV.5899 | *M. olivieri* | F | 39.38 | 9.76 | 5.96 | 4.50 | 2 | 1 | 2 | 33 | 46 | 2 | 16 | 4 | 4 | 8 | 8 | 22 | 11 | 7 | 11 | 12 | 11 |
| BEV.5956 | *M. olivieri* | M | 37.35 | 9.22 | 5.39 | 3.57 | 2 | 1 | 3 | 30 | 41 | 3 | 17 | 5 | 5 | 9 | 8 | 30 | 9 | 7 | 11 | 14 | 13 |
| BEV.5959 | *M. olivieri* | M | 36.09 | 9.24 | 5.59 | 3.99 | 2 | 1 | 2 | 30 | 40 | 4 | 17 | 4 | 4 | 7 | 6 | 29 | 10 | 5 | 10 | 11 | 12 |
| BEV.8830 | *M. olivieri* | M | 43.28 | 10.65 | 7.27 | 5.36 | 3 | 1 | 1 | 30 | 48 | 1 | 16 | 5 | 5 | 8 | 8 | 28 | 8 | 6 | 10 | 14 | 13 |
| BEV.8796 | *M. olivieri* | M | 45.32 | 11.87 | 7.31 | 5.49 | 3 | 1 | 3 | 30 | 57 | 1 | 16 | 6 | 5 | 9 | 8 | 27 | 10 | 6 | 11 | 13 | 14 |
| BEV.8797 | *M. olivieri* | M | 44.57 | 11.68 | 7.26 | 5.24 | 3 | 1 | 2 | 30 | 42 | 2 | 20 | 5 | 5 | 9 | 10 | 26 | 14 | 9 | 11 | 13 | 13 |
| BEV.8798 | *M. olivieri* | M | 44.78 | 11.06 | 7.66 | 5.60 | 3 | 1 | 1 | 30 | 48 | 2 | 17 | 4 | 4 | 9 | 8 | 20 | 8 | 6 | 10 | 16 | 16 |
| BEV.8829 | *M. olivieri* | M | 44.77 | 11.04 | 7.00 | 5.09 | 3 | 1 | 3 | 28 | 42 | 2 | 16 | 5 | 6 | 8 | 7 | 23 | 12 | 10 | 10 | 11 | 12 |
| BEV.8975 | *M. olivieri* | F | 43.03 | 9.52 | 6.18 | 4.33 | 2 | 2 | 1 | 30 | 45 | 2 | 17 | 4 | 5 | 8 | 9 | 23 | 9 | 6 | 8 | 12 | 12 |
| BEV.10453 | *M. simoni* | M | 35.74 | 8.27 | 4.86 | 3.17 | 2 | 1 | 3 | 30 | 39 | 2 | 15 | 5 | 4 | 8 | 7 | 26 | 8 | 5 | 8 | 12 | 11 |
| BEV.10849 | *M. simoni* | M | 38.07 | 9.24 | 5.56 | 4.39 | 3 | 2 | 3 | 34 | 42 | 1 | 15 | 4 | 4 | 9 | 8 | 24 | 8 | 8 | 9 | 12 | 12 |
| BEV.10850 | *M. simoni* | F | 42.46 | 8.47 | 5.28 | 3.60 | 2 | 1 | 1 | 34 | 42 | 1 | 15 | 4 | 4 | 8 | 7 | 26 | 8 | 8 | 10 | 12 | 12 |
| BEV.11187 | *M. simoni* | M | 42.49 | 10.41 | 6.90 | 4.65 | 3 | 2 | 1 | 30 | 47 | 2 | 15 | 4 | 4 | 8 | 8 | 22 | 10 | 9 | 9 | 12 | 12 |
| BEV.11586 | *M. simoni* | M | 44.74 | 11.08 | 7.44 | 4.95 | 2 | 1 | 2 | 30 | 45 | 2 | 16 | 4 | 4 | 7 | 8 | 27 | 12 | 7 | 13 | 14 | 12 |
| BEV.11587 | *M. simoni* | F | 45.71 | 9.17 | 6.38 | 4.02 | 2 | 1 | 2 | 31 | 42 | 2 | 15 | 4 | 3 | 7 | 8 | 24 | 8 | 8 | 8 | 11 | 10 |
| BEV.11588 | *M. simoni* | F | 46.10 | 9.69 | 6.27 | 4.43 | 1 | 1 | 2 | 33 | 36 | 2 | 13 | 4 | 4 | 7 | 8 | 24 | 9 | 8 | 9 | 13 | 13 |
| BEV.11589 | *M. simoni* | M | 46.56 | 11.32 | 7.33 | 5.00 | 3 | 1 | 2 | 30 | 49 | 2 | 18 | 4 | 4 | 8 | 8 | 21 | 12 | 10 | 11 | 12 | 12 |
| BEV.11590 | *M. simoni* | M | 45.23 | 10.45 | 7.11 | 4.70 | 3 | 1 | 3 | 29 | 44 | 3 | 15 | 5 | 5 | 8 | 8 | 26 | 11 | 9 | 11 | 10 | 10 |
| BEV.11591 | *M. simoni* | F | 45.92 | 9.71 | 6.49 | 4.60 | 1 | 1 | 2 | 31 | 45 | 4 | 14 | 4 | 4 | 7 | 7 | 24 | 9 | 7 | 12 | 12 | 12 |
| BEV.11594 | *M. simoni* | F | 47.88 | 10.06 | 6.26 | 3.99 | 1 | 1 | 2 | 34 | 50 | 2 | 14 | 4 | 4 | 7 | 6 | 23 | 11 | 5 | 9 | 10 | 10 |
| BEV.14869 | *M. simoni* | F | 41.77 | 8.77 | 5.62 | 3.76 | 1 | 1 | 1 | 35 | 36 | 4 | 16 | 4 | 4 | 7 | 7 | 20 | 9 | 12 | 10 | 12 | 12 |
| BEV.5922 | *M. simoni* | M | 50.79 | 11.08 | 7.00 | 5.43 | 3 | 1 | 1 | 33 | 42 | 4 | 15 | 4 | 4 | 7 | 9 | 23 | 10 | 7 | 9 | 13 | 13 |
| BEV.5923 | *M. simoni* | F | 50.69 | 9.74 | 6.33 | 4.41 | 2 | 2 | 2 | 34 | 42 | 4 | 14 | 4 | 4 | 8 | 7 | 24 | 10 | 6 | 9 | 12 | 12 |

| Code | Species | Sex | SVL | HL | HW | HH | TE | RN | PS | V | D | DS | TR | SL(Sx) | SL(Dx) | IL(Sx) | IL(Dx) | G | Col | EL | NTS | Pf(Sx) | Pf(Dx) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BEV.5924 | *M. simoni* | F | 48.25 | 10.30 | 6.67 | 4.50 | 1 | 1 | 2 | 33 | 43 | 2 | 14 | 4 | 4 | 7 | 8 | 21 | 12 | 10 | 8 | 12 | 13 |
| BEV.8508 | *M. simoni* | M | 43.14 | 9.72 | 6.42 | 4.89 | 3 | 1 | 1 | 31 | 46 | 4 | 15 | 4 | 4 | 8 | 7 | 28 | 9 | 6 | 9 | 12 | 12 |
| BEV.8509 | *M. simoni* | F | 45.75 | 9.45 | 6.26 | 4.36 | 1 | 2 | 1 | 34 | 46 | 4 | 15 | 4 | 4 | 7 | 7 | 28 | 9 | 5 | 11 | 11 | 11 |
| BEV.9114 | *M. simoni* | M | 40.70 | 9.62 | 5.90 | 4.20 | 2 | 1 | 2 | 28 | 34 | 4 | 15 | 4 | 5 | 7 | 9 | 25 | 6 | 5 | 8 | 10 | 13 |
| BEV.9225 | *M. simoni* | M | 42.39 | 10.23 | 7.09 | 5.35 | 3 | 1 | 1 | 27 | 40 | 4 | 15 | 4 | 4 | 9 | 8 | 24 | 7 | 6 | 10 | 11 | 12 |
| BEV.9429 | *M. simoni* | M | 42.15 | 10.21 | 6.75 | 4.39 | 3 | 1 | 1 | 30 | 54 | 4 | 16 | 4 | 4 | 7 | 8 | 24 | 12 | 8 | 9 | 12 | 13 |
| BEV.9430 | *M. simoni* | M | 43.89 | 10.29 | 6.83 | 4.68 | 3 | 1 | 2 | 30 | 46 | 0 | 16 | 4 | 4 | 8 | 8 | 22 | 11 | 6 | 15 | 14 | 5 |
| BEV.9431 | *M. simoni* | M | 43.74 | 10.39 | 7.14 | 4.64 | 3 | 2 | 1 | 32 | 46 | 1 | 16 | 4 | 4 | 8 | 8 | 23 | 9 | 6 | 11 | 12 | 12 |
| BEV.9432 | *M. simoni* | M | 43.34 | 10.71 | 6.61 | 4.52 | 3 | 1 | 1 | 31 | 46 | 2 | 16 | 4 | 4 | 9 | 9 | 22 | 9 | 6 | 11 | 13 | 13 |
| BEV.10179 | *M. pasteuri* | M | 48.32 | 11.83 | 7.44 | 5.63 | 2 | 1 | 2 | 31 | 39 | 1 | 15 | 5 | 5 | 7 | 6 | 30 | 9 | 1 | 7 | 12 | 15 |
| BEV.10454 | *M. pasteuri* | M | 45.41 | 11.04 | 5.98 | 4.10 | 2 | 1 | 3 | 28 | 40 | 4 | 15 | 5 | 5 | 7 | 8 | 29 | 9 | 4 | 10 | 13 | 12 |
| BEV.10455 | *M. pasteuri* | M | 42.17 | 10.42 | 5.57 | 3.90 | 3 | 1 | 3 | 33 | 40 | 0 | 15 | 6 | 6 | 8 | 7 | 27 | 10 | 4 | 11 | 17 | 15 |
| BEV.14803 | *M. pasteuri* | M | 48.31 | 12.01 | 7.74 | 5.44 | 3 | 2 | 3 | 29 | 39 | 1 | 17 | 5 | 5 | 9 | 9 | 28 | 10 | 4 | 7 | 12 | 12 |
| BEV.14804 | *M. pasteuri* | F | 40.38 | 8.94 | 5.59 | 4.16 | 2 | 1 | 3 | 34 | 38 | 3 | 16 | 4 | 6 | 8 | 8 | 24 | 10 | 4 | 7 | 13 | 12 |
| BEV.14805 | *M. pasteuri* | M | 40.61 | 9.95 | 6.44 | 4.05 | 3 | 1 | 3 | 31 | 40 | 3 | 16 | 7 | 5 | 7 | 8 | 26 | 10 | 3 | 10 | 13 | 12 |
| BEV.5926 | *M. pasteuri* | M | 43.75 | 11.17 | 6.27 | 4.07 | 2 | 1 | 2 | 32 | 43 | 0 | 18 | 5 | 5 | 6 | 8 | 31 | 9 | 5 | 9 | 16 | 13 |
| BEV.5927 | *M. pasteuri* | F | 41.81 | 10.09 | 5.56 | 4.25 | 1 | 1 | 2 | 31 | 39 | 4 | 14 | 4 | 5 | 8 | 9 | 27 | 7 | 4 | 10 | 10 | 8 |
| BEV.9177 | *M. pasteuri* | M | 44.70 | 11.23 | 7.50 | 4.64 | 1 | 1 | 2 | 29 | 42 | 0 | 16 | 5 | 5 | 9 | 8 | 27 | 8 | 5 | 9 | 14 | 13 |
| BEV.9380 | *M. pasteuri* | F | 27.77 | 7.19 | 4.54 | 2.99 | 2 | 1 | 3 | 34 | 42 | 0 | 14 | 5 | 6 | 8 | 8 | 29 | 7 | 5 | 8 | 12 | 13 |

**Table S6.** Material examined for colouration characters comparisons for each specimen. Codes of morphological characters are given section Material and Methods. M = Male; F = Female.

| Code | Species | Sex | EBL | DBF | PSDBF | PDLL | SPDLL | DDSLL | SDDLB | PSDDSLL | DSO | TC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BEV.10457 | *Mesalina sp. nov.* | F | 0 | 2 | 1 | 2 | 5 | 1 | 2 | 0 | 1 | 1 |
| BEV.14800 | *Mesalina sp. nov.* | F | 1 | 3 | 1 | 1 | 2 | 2 | 6 | 1 | 1 | 1 |
| BEV.15060 | *Mesalina sp. nov.* | M | 1 | 2 | 1 | 2 | 2 | 1 | 3 | 0 | 2 | 1 |
| BEV.15061 | *Mesalina sp. nov.* | F | 1 | 2 | 2 | 1 | 2 | 1 | 4 | 1 | 0 | 1 |
| BEV.15062 | *Mesalina sp. nov.* | M | 1 | 2 | 2 | 1 | 2 | 1 | 4 | 2 | 0 | 1 |
| BEV.15063 | *Mesalina sp. nov.* | M | 1 | 2 | 0 | 1 | 1 | 2 | 4 | 0 | 1 | 1 |
| BEV.15064 | *Mesalina sp. nov.* | M | 1 | 2 | 2 | 1 | 2 | 1 | 4 | 2 | 0 | 1 |
| BEV.15163 | *Mesalina sp. nov.* | F | 0 | 0 | 0 | 3 | 2 | 1 | 4 | 1 | 1 | 0 |
| BEV.10013 | *M. olivieri* | M | 0 | 4 | 2 | 1 | 2 | 1 | 4 | 2 | 2 | 0 |
| BEV.11948 | *M. olivieri* | F | 0 | 4 | 2 | 3 | 3 | 2 | 5 | 2 | 0 | 2 |
| BEV.12808 | *M. olivieri* | F | 0 | 0 | 2 | 2 | 2 | 0 | 0 | 2 | 0 | 2 |
| BEV.13621 | *M. olivieri* | M | 0 | 2 | 2 | 2 | 3 | 1 | 4 | 0 | 2 | 2 |
| BEV.14833 | *M. olivieri* | M | 0 | 3 | 2 | 2 | 2 | 1 | 4 | 2 | 0 | 0 |
| BEV.5925 | *M. olivieri* | F | 0 | 2 | 2 | 2 | 2 | 1 | 1 | 0 | 1 | 0 |
| BEV.5861 | *M. olivieri* | F | 1 | 2 | 2 | 0 | 3 | 1 | 4 | 2 | 0 | 0 |
| BEV.5864 | *M. olivieri* | F | 0 | 2 | 2 | 2 | 3 | 1 | 5 | 2 | 0 | 0 |
| BEV.5867 | *M. olivieri* | F | 0 | 2 | 2 | 2 | 2 | 1 | 4 | 0 | 2 | 0 |
| BEV.5870 | *M. olivieri* | M | 0 | 2 | 2 | 2 | 2 | 1 | 4 | 2 | 0 | 0 |
| BEV.5871 | *M. olivieri* | F | 0 | 2 | 2 | 2 | 2 | 1 | 1 | 0 | 2 | 0 |
| BEV.5875 | *M. olivieri* | F | 0 | 2 | 2 | 1 | 2 | 1 | 4 | 2 | 1 | 0 |
| BEV.5876 | *M. olivieri* | M | 0 | 2 | 2 | 2 | 2 | 1 | 4 | 2 | 0 | 0 |
| BEV.5877 | *M. olivieri* | F | 0 | 2 | 2 | 2 | 2 | 1 | 3 | 0 | 0 | 0 |
| BEV.5879 | *M. olivieri* | M | 0 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 0 | 0 |
| BEV.5880 | *M. olivieri* | F | 0 | 2 | 2 | 2 | 3 | 1 | 3 | 0 | 2 | 0 |
| BEV.5881 | *M. olivieri* | M | 0 | 2 | 2 | 1 | 2 | 1 | 4 | 2 | 2 | 1 |
| BEV.5882 | *M. olivieri* | F | 0 | 2 | 2 | 2 | 2 | 1 | 2 | 0 | 2 | 2 |

| Code | Species | Sex | EBL | DBF | PSDBF | PDLL | SPDLL | DDSLL | SDDLB | PSDDSLL | DSO | TC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BEV.5888 | *M. olivieri* | F | 0 | 4 | 1 | 3 | 4 | 2 | 2 | 1 | 1 | 0 |
| BEV.5889 | *M. olivieri* | M | 0 | 0 | 2 | 2 | 2 | 1 | 3 | 2 | 2 | 0 |
| BEV.5890 | *M. olivieri* | M | 0 | 2 | 2 | 2 | 3 | 1 | 4 | 2 | 0 | 0 |
| BEV.5899 | *M. olivieri* | F | 0 | 2 | 2 | 2 | 1 | 2 | 5 | 2 | 2 | 0 |
| BEV.5956 | *M. olivieri* | M | 0 | 2 | 2 | 2 | 3 | 1 | 4 | 2 | 1 | 0 |
| BEV.5959 | *M. olivieri* | M | 0 | 4 | 1 | 1 | 1 | 2 | 4 | 0 | 0 | 0 |
| BEV.8830 | *M. olivieri* | M | 1 | 2 | 2 | 1 | 4 | 1 | 7 | 1 | 2 | 1 |
| BEV.8796 | *M. olivieri* | M | 1 | 3 | 2 | 1 | 3 | 1 | 4 | 0 | 2 | 0 |
| BEV.8797 | *M. olivieri* | M | 2 | 3 | 2 | 2 | 4 | 2 | 5 | 1 | 2 | 0 |
| BEV.8798 | *M. olivieri* | M | 1 | 2 | 2 | 2 | 7 | 2 | 6 | 0 | 2 | 0 |
| BEV.8829 | *M. olivieri* | M | 0 | 2 | 2 | 1 | 6 | 1 | 7 | 0 | 2 | 2 |
| BEV.8975 | *M. olivieri* | F | 0 | 2 | 2 | 2 | 2 | 2 | 3 | 0 | 2 | 2 |
| BEV.10453 | *M. simoni* | M | 22 | 3 | 2 | 2 | 3 | 0 | 2 | 2 | 2 | 0 |
| BEV.10849 | *M. simoni* | M | 22 | 3 | 2 | 2 | 2 | 1 | 1 | 0 | 0 | 0 |
| BEV.10850 | *M. simoni* | F | 21 | 0 | 0 | 2 | 2 | 1 | 3 | 0 | 0 | 0 |
| BEV.11187 | *M. simoni* | M | 19 | 0 | 2 | 2 | 2 | 1 | 4 | 2 | 0 | 0 |
| BEV.11586 | *M. simoni* | M | 21 | 0 | 2 | 2 | 2 | 1 | 4 | 2 | 1 | 0 |
| BEV.11587 | *M. simoni* | F | 18 | 0 | 2 | 2 | 3 | 2 | 4 | 1 | 2 | 1 |
| BEV.11588 | *M. simoni* | F | 18 | 3 | 2 | 0 | 0 | 2 | 4 | 0 | 0 | 0 |
| BEV.11589 | *M. simoni* | M | 18 | 2 | 2 | 2 | 2 | 1 | 5 | 2 | 1 | 0 |
| BEV.11590 | *M. simoni* | M | 18 | 0 | 2 | 2 | 3 | 1 | 5 | 2 | 0 | 0 |
| BEV.11591 | *M. simoni* | F | 20 | 0 | 2 | 2 | 2 | 2 | 4 | 1 | 2 | 1 |
| BEV.11594 | *M. simoni* | F | 18 | 2 | 2 | 2 | 2 | 1 | 5 | 2 | 2 | 2 |
| BEV.14869 | *M. simoni* | F | 19 | 2 | 2 | 3 | 4 | 1 | 3 | 2 | 0 | 0 |
| BEV.5922 | *M. simoni* | M | 21 | 0 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 0 |
| BEV.5923 | *M. simoni* | F | 19 | 2 | 0 | 1 | 1 | 2 | 4 | 2 | 2 | 0 |

| Code | Species | Sex | EBL | DBF | PSDBF | PDLL | SPDLL | DDSLL | SDDLB | PSDDSLL | DSO | TC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BEV.5924 | *M. simoni* | F | 22 | 2 | 2 | 0 | 0 | 1 | 2 | 2 | 2 | 0 |
| BEV.8508 | *M. simoni* | M | 23 | 2 | 2 | 2 | 3 | 1 | 8 | 0 | 2 | 0 |
| BEV.8509 | *M. simoni* | F | 25 | 3 | 1 | 2 | 4 | 1 | 4 | 0 | 2 | 0 |
| BEV.9114 | *M. simoni* | M | 24 | 2 | 2 | 0 | 2 | 0 | 1 | 2 | 1 | 1 |
| BEV.9225 | *M. simoni* | M | 21 | 2 | 2 | 2 | 2 | 2 | 3 | 0 | 1 | 2 |
| BEV.9429 | *M. simoni* | M | 21 | 3 | 2 | 2 | 3 | 1 | 6 | 1 | 2 | 0 |
| BEV.9430 | *M. simoni* | M | 20 | 3 | 2 | 2 | 3 | 2 | 6 | 2 | 1 | 0 |
| BEV.9431 | *M. simoni* | M | 19 | 2 | 2 | 2 | 3 | 2 | 6 | 2 | 0 | 1 |
| BEV.9432 | *M. simoni* | M | 21 | 3 | 2 | 1 | 2 | 2 | 5 | 2 | 0 | 2 |
| BEV.10179 | *M. pasteuri* | M | 0 | 0 | 1 | 3 | 2 | 0 | 1 | 0 | 2 | 0 |
| BEV.10454 | *M. pasteuri* | M | 0 | 0 | 2 | 3 | 3 | 2 | 1 | 0 | 2 | 0 |
| BEV.10455 | *M. pasteuri* | M | 0 | 0 | 0 | 3 | 3 | 3 | 1 | 0 | 0 | 0 |
| BEV.14803 | *M. pasteuri* | M | 0 | 0 | 1 | 3 | 2 | 1 | 3 | 1 | 2 | 0 |
| BEV.14804 | *M. pasteuri* | F | 0 | 0 | 2 | 3 | 2 | 2 | 3 | 0 | 0 | 0 |
| BEV.14805 | *M. pasteuri* | M | 0 | 0 | 0 | 3 | 2 | 3 | 1 | 0 | 0 | 0 |
| BEV.5926 | *M. pasteuri* | M | 0 | 0 | 0 | 3 | 2 | 3 | 2 | 0 | 1 | 0 |
| BEV.5927 | *M. pasteuri* | F | 0 | 0 | 0 | 3 | 3 | 3 | 1 | 0 | 0 | 0 |
| BEV.9177 | *M. pasteuri* | M | 0 | 0 | 2 | 3 | 2 | 0 | 5 | 0 | 2 | 0 |
| BEV.9380 | *M. pasteuri* | F | 0 | 0 | 0 | 3 | 2 | 3 | 3 | 0 | 0 | 0 |

**Table S7.** Categories of the variable Land Cover used in the ecological models. Variable derived from Campos and Brito (2018).

| Code | Category | Definition |
|---|---|---|
| LC_01 | Yellow dunes | Frequently fixed dunes composed by yellow sand. Sparse shrubs with isolated Acacia sp. or no vegetation cover |
| LC_02 | White dunes | Mobile dunes composed by white sand (e.g. barchan dunes). No vegetation cover |
| LC_03 | Orange dunes | Frequently fixed dunes composed by orange sand. Sparse shrubs with isolated Acacia sp. or sometimes no vegetation cover |
| LC_04 | Compact sand | Flat areas composed by consolidated sandy soils. Shrubs and sparse trees (e.g. *Acacia* sp.) |
| LC_05 | Gravel + Sand floodplains | Soil composed by similar amounts of gravel and sand. Sparse vegetation or no vegetation cover |
| LC_06 | Gravel floodplains | Large floodplains covered by gravel (locally known as Reg). Usually no vegetation cover |
| LC_07 | Compact soil | Soils frequently composed by silt and/or clay. Sparse vegetation or no vegetation cover |
| LC_08 | Rocky soil | Non-flat areas with soils composed by stones, silt and/or clay. Sparse vegetation or no vegetation cover |
| LC_09 | Rocky plateaux | Flat areas totally covered by stones (locally known as Hammada). Isolated Acacia sp. or no vegetation cover |
| LC_10 | Bare rock | Large rock outcrops |
| LC_11 | Grasslands | Flat flooding areas covered by grasses (e.g. *Cenchrus biflorus*) |
| LC_12 | Savannah | High vegetation cover (grasses, shrubs and trees) |
| LC_13 | Croplands | Areas of crop cultivation (e.g. rice and sorghum) |
| LC_14 | Permanent water | Permanent water features (rivers, lakes and mountain lagoons) |
| LC_15 | Salt pans | Flat areas covered by salt (locally known as Sebkha). No vegetation cover |
| LC_16 | Railroads | Railroads and linear infra-structures |
| LC_17 | Roads | Paved roads |
| LC_18 | Urban | Major cities and villages |

Campos, J.C., Brito, J.C. (2018) Mapping underrepresented land cover heterogeneity in arid regions: The Sahara-Sahel example. ISPRS J. Photogram. Rem. Sens. 146, 211-220.

**Table S8.** Details and metrics of the 20 model replicates developed for *Mesalina sp. nov.*, including average (standard deviation) training and tests Area under curve (AUC), average (standard deviation) percentage contribution of each variable to the model replicates.

| | Maximum temperature of warmest month | Minimum temperature of coldest month | Temperature annual range | Annual total precipitation | Land-cover | Terrain ruggedness index |
|---|---|---|---|---|---|---|
| % contribution | 3.136 (2.180) | 10.022 (3.766) | 0.000 (0.003) | 8.800 (4.505) | 35.416 (11.808) | 42.625 (13.414) |
| Permutation importance | 6.098 (6.733) | 15.762 (12.539) | 0.037 (0.161) | 21.808 (16.403) | 24.962 (13.477) | 31.332 (22.383) |
| Training gain without | 3.069 (0.387) | 2.931 (0.400) | 3.096 (0.387) | 2.892 (0.345) | 2.458 (0.342) | 2.404 (0.446) |
| Training gain with only | 0.323 (0.110) | 0.215 (0.095) | 0.314 (0.101) | 0.760 (0.226) | 1.300 (0.370) | 1.642 (0.307) |
| Test gain without | 3.289 (1.029) | 3.004 (1.192) | 3.274 (1.077) | 3.109 (1.092) | 2.634 (1.041) | 2.662 (1.086) |
| Test gain with only | 0.350 (0.521) | 0.351 (0.223) | 0.348 (0.561) | 0.837 (0.404) | 1.528 (0.913) | 1.694 (0.870) |
| AUC without | 0.979 (0.019) | 0.970 (0.036) | 0.978 (0.022) | 0.974 (0.025) | 0.960 (0.039) | 0.955 (0.051) |
| AUC with only | 0.741 (0.145) | 0.752 (0.090) | 0.756 (0.145) | 0.848 (0.079) | 0.891 (0.107) | 0.913 (0.080) |