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Phylogeographic and systematic studies of selected bat taxa of the western part of the Old World

Fylogeografické a systematické studium vybraných taxonů netopýrů západní části Starého světa

Doctoral thesis

Supervisor: doc. RNDr. Petr Benda, PhD.

Prague, 2023

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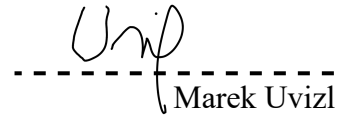

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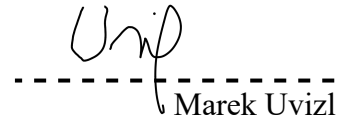

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Abstract

Bats attract attention due their extraordinary adaptations including their ability to actively fly and echolocate, and extended lifespan, phenotypic diversity, etc. The phylogeny was analysed using cutting-edge molecular methods. However, the molecular revision of several species and species groups is still pending, especially those with wide distribution ranges or cryptic species complexes, even in the western part of the Old World. This specification encompasses Europe, the Middle East, Central Asia, and Africa and it represents the traditional research area for Central European (Czech and Czechoslovak) bat researchers. In my PhD thesis, I aimed to revise the phylogenetic and phylogeographic relationships of six less studied species and/or species groups of bats, using a combination of molecular and morphological phylogenetic approaches. The sequences of both mitochondrial and nuclear genetic markers were generated from over 10 species. These sequences were used to construct phylogenetic trees, haplotype networks, and estimate the time of divergence of studied species. The main results of my PhD thesis were: (1) filling gaps in the knowledge of the distribution ranges of species from the *M. nattereri* species complex (Vespertilionidae) by including and identifying samples from the Middle East; (2) showing that *Myotis emarginatus* (Vespertilionidae) forms a single species with a wide distribution from Europe to Central Asia and creates three lineages/subspecies; and (3) resurrecting of *Coleura gallarum* (Emballonuridae) in the Arabian peninsula and north-eastern Africa, resulting in a total of four *Coleura* species. Next, the big portion of the PhD thesis was centred on horseshoe bats (Rhinolophidae). The most interesting results included (4) the revision of the *Rhinolophus hipposideros* group, with separating *R. midas* from *R. hipposideros*, (5) the separation of the sub-Saharan populations of the *R. ferrumequinum* group as a separate species *R. acrotis* (instead of *R. clivosus*), and (6) the discovery of a new *Rhinolophus* species from the *R. fumigatus* group in Lesotho. Additionally, I helped with the identification of bat species from Zambia in the collections of the National Museum in Prague. My PhD thesis made a contribution to the knowledge of bat evolutionary history in the western part of the Old World and generated novel data that can be utilised in further bat research.

Abstrakt

Netopýři přitahují pozornost díky jejich pozoruhodným adaptacím, jako je schopnost letu a echolokace, prodloužená délka života, fenotypová diverzita atd. Jejich fylogeneze byla a je stále studována pomocí nejmodernějších molekulárních metod, ovšem mnoho druhů nebo druhových skupin stále čeká až budou molekulárně zrevidovány. Tato charakteristika platí zejména pro druhy s velkými areály výskytu nebo druhové komplexy s kryptickou diverzitou. Takové taxony může stále nalézt i v západní části Starého světa. Do této oblasti se řadí Evropa, západní Asie a Afrika a jako celek představuje přirozenou oblast výzkumu středoevropských (Českých a Československých) chiropterologů. Tato disertační práce je zaměřená na revizi fylogenetických a fylogeografických vztahů šesti méně studovaných druhů a/nebo druhových skupin netopýrů za použití jak molekulárních, tak morfologických metod. Mitochondriální a jaderné sekvence genetických markerů byly generovány pro více než 10 druhů a dále byly použity pro tvorbu fylogenetických stromů, haplotypových sítí a odhadu doby divergence námi studovaných druhů netopýrů. Mezi hlavní výsledky této disertační práce patří: (1) doplnění dat a tím upřesnění distribučních areálů druhů, které patří do druhového komplexu netopýra řasnatého (Vespertilionidae: *Myotis nattereri*) a to díky přidání a identifikaci vzorků z Blízkého Východu; 2) zjištění, že netopýr brvitý (*M. emarginatus*) tvoří geneticky jednolitý druh v celém svém areálu rozšíření od západní Evropy po Střední Asii, který je dále rozdělený na tři linie/poddruhy; nebo 3) povýšení populací z Arábie a severovýchodní Afriky rodu *Coleura* (Emballonuridae) na samostatný druh *Coleura gallarum* a tím zvýšení počtu druhů v rodu na čtyři. Velká část disertační práce pak zahrnuje studium vrápenců (Rhinolophidae). Mezi nejdůležitější výsledky, které se podařilo vyzkoumat, patří: 4) oddělení ománských populací vrápence malého (*Rhinolophus hipposideros*) na samostatný druh *R. midas*; 5) rozdělení vrápence pouštního (*R. clivosus*) na dva druhy, *R. clivosus* ze severní Afriky a Arabského poloostrova a *R. acrotis* ze subsaharské Afriky; a 6) objevení nového druhu vrápence z Lesotha, který patří do druhové skupiny *fumigatus*. K tomu se v rámci této práce pomohlo k určení netopýrů Zambie, kteří se nachází ve sbírkách Národního Musea. Tato disertační práce tak přispěla ke znalosti evoluční historie netopýrů rozšířených v západní části Starého světa. Navíc se podařilo vygenerovat množství dat, které bude možné využít v dalším výzkumu netopýrů.

Contents

Introduction

Among all mammals, bats possess exceptional adaptations enabling them to secure the second most speciose mammalian order and to conquer the whole world apart from polar areas (Simmons, 2005; Teeling et al. 2018). The unique attribute defining this mammalian group is their capability of self-powered flight. Consequently, bats can surpass boundaries confining other mammals and allowed them to even inhabit distant islands inaccessible to terrestrial mammals. Another ability, although not completely unique but uniquely evolved, is the ability of laryngeal echolocation to navigate through dark spaces and during the night. Bats are also renowned for their extended longevity, with lifespan much longer compared to other animals of similar body size; their unique immune system that enables them to coexist with viruses that cause deadly diseases to other animals (such as Ebola, SARS, MERS etc.); and large phenotypic diversity allowing them to feed on insects and small vertebrates as well as on nectar, fruit, pollen, or even blood (for further details see the review in Teeling et al., 2018).

The boom of genetic methods started the new era of biologic discoveries and the pace of describing new species does not seem to slow down. New discoveries span the entire tree of life (Donoghue and Alverson 2000). Even among mammals, the one of the well-known group of animals, the number of species has increased by more than 20% over the past 20 years (Wilson & Reeder, 2005; Mammal Diversity Database, 2023). The unexplored regions are progressively diminishing, leading to more and more species are elevated from subspecies ranks through genetic revisions. Additionally, numerous species form cryptic species complexes when morphologically uniform lineages are constituted from several genetic lineages (Burgin et al., 2018). The number of bat species has increased by over 80 species in just five years, bringing the total to 1469 bat species (Burgin et al., 2018; Simmons & Cirranello, 2023). Moreover, molecular data has led to widely accepted hypothesis of two suborders Yinpterochiroptera and Yangochiroptera (Hutcheon et al., 1998; Springer et al., 2001). The first suborder consists of non-echolocating Pteropodidae family and the echolocating superfamily Rhinolophoidea. The second suborder includes the remaining echolocators from the original microbats (superfamilies Vespertillionoidea, Noctillionoidea, and Emballonuroidea) (Teeling et al., 2005). Molecular data has also supported raising some subfamilies to family rank, including Miniopteridae (Van Den Bussche & Hoofer, 2004) and Rhinonycteridae (Foley et al., 2015). In some cases, species may also be divided into newly recognised families, such as Cistugidae (Lack et al., 2010).

Recent findings demonstrate that the phylogeny of bats remains incomplete and each phylogenetic study at every taxonomic level within the Chiroptera order sheds further light on bat evolution. This thesis contributed to our bit to the evolutionary puzzle of bats' phylogeny and to knowledge of bats' diversity in general. It was focused on species and/or species groups distributed in the western part of the Old World, namely Europe, western Asia, and Africa.

The western part of the Old World

The study area of this thesis spans the western part of the Old World. This region is of particular interest to scientists from Central Europe. Historically, Czech zoologists have concentrated their

interest on eastern Europe, the Balkans, and Central Asia as they were accessible for study with relative ease. After the collapse of communism and the opening of borders, the research focus shifted towards the eastern Mediterranean and the Middle East (e.g., Gaisler, 1970; Gaisler et al., 1972; Hanák & Elgadi, 1984; Benda & Horáček, 1995, 1998; Horáček et al., 1998; Benda & Tsytsulina, 2000; Hanák et al., 2001; Benda et al., 2003, 2004, 2006, 2007, 2009, 2010, 2011, 2012, 2016; Hulva et al., 2004, 2007; Benda & Vallo, 2009). Due to the multiple wars in the surrounding areas, such as Syria and Yemen, visiting certain countries has become increasingly difficult in recent years. Therefore, African countries have become a more accessible destination for research expeditions (Šklíba et al., 2007; Benda et al., 2012, 2019; Puechmaille, et al., 2014). After years of meticulous research, the National Museum in Prague houses an extensive collection of samples stretching from Europe to Africa and thus, it is possible to study different species or species groups with very wide sampling. This allows for the discovery of new insights into phylogeny, biogeography, and evolution of bats across the entire region.

The Old World refers to the historical global regions of Eurasia and Africa (while the term New World denotes the Americas). These regions can be divided into three primary biogeographical regions – Palaearctic, Indomalayan, and Afrotropical. The Palaearctic region is the most extensive, encompassing entire Europe, northern Africa, Middle East, and Asia north of Himalayas including Japan. The Afrotropical and Indomalayan (or Oriental) regions comprise the remaining parts of Africa, and Asia, respectively (Sclater, 1858; Wallace, 1872). Although they are capable of flight, certain groups of bats were unable to disperse to the New World and therefore are endemic to the Old World. This is particularly true for the entire suborder Yinpterochiroptera, which includes the families Craseonycteridae, Hipposideridae, Megadermatidae, Pteropodidae, Rhinolophidae, Rhynonyc-teridae, and Rhinopomatidae. Four families (Cistugidae, Miniopteridae, Myzopodidae, Nycteridae) of the suborder Yinpterochiroptera are only found in the Old World, and subfamilies/tribes/clades from other families (Emballonuridae, Mollosidae, Vespertilionidae) can also be found in the Old World (Horáček et al., 2000; Simmons, 2005; Simmons & Cirranello, 2023; Mammal Diversity Database, 2023).

The study area of this thesis encompasses the western part of the Old World, incorporating the Afrotropical and western Palaearctic regions. This area's eastern boundary is defined by the eastern limits of the Middle East and Central Asia or on the western boundaries of the Indomalayan region (Fig. 1). The bat families Cistugidae and Myzopodidae, as well as most of the species from Rhinopomatidae family, are endemic to this region (Bonaparte, 1838; Thomas, 1904; Lack et al., 2010). Other higher Old World taxa are limited to clade/species groups that may be restricted to Africa or the western Palaearctic. For example, the Afro-Palaearctic clade of the genus *Rhinolophus* in the family Rhinolophidae, the Ethiopian clade of the genus *Myotis* in the family Vespertilionidae, the Afrotropical clade of the family Miniopteridae, or five African tribes of the subfamily Rousettinae in the family Pteropodidae (Csorba et al., 2003; Stadelmann et al., 2004; Kingdon, 2015; Amador et al., 2018; Simmons & Cirranello, 2023; Mammal Diversity Database, 2023).

The western Old World could be further divided into smaller geographical regions, including Europe and Mediterranean including northern Africa, Middle East including Arabian Peninsula, Central Asia, eastern, western, central, and southern Africa (Fig. 1). Samples from all these areas, with exception of western and central Africa, were processed and studied as part of this thesis. As a result, the next subchapter does not include western and central Africa.

Fig. 1 The map of the western Old World. The solid line denotes the borders of biogeographical regions. 'Im' denotes the Indomalayan region. The dashed lines indicate the regions of the western Old World mentioned in the text.

The regions of the western Old World

Europe here refers mostly to the regions located north of the Alps comprising western, central, and northern Europe. As this thesis is based on a limited number of samples from this area, it also includes the Mediterranean region that encompasses southern Europe, the Balkans, the Levant, and northern Africa (Fig. 1). The mediterranean bat fauna is highly diverse and mainly Palaearctic (Dobson, 1998). The dominant species inhabiting the area belong to the Vespertilionidae, Rhinolophidae, and Miniopteridae families. In addition, the sole remaining species present in Europe belongs to the family Molossidae (Arlettaz et al., 2000; Mammal Diversity Database, 2023). The other families, which are mainly distributed in the African Mediterranean include Emballonuridae, Hipposideridae, Nycteridae, Pteropodidae, and Rhinopomatidae, with some of them being only marginally present (Aulagnier et al., 2018; Mammal Diversity Database, 2023).

The Middle East serves as a crossroads between the other regions as it shares borders with Europe and the Mediterranean to the west and north, Central Asia and the Indomalayan region to the east, and Africa to the west and south (Fig. 1). Consequently, this area is home to various bat species, including some that can also be found in other locations. Examples of these bats include those from arid regions of Africa as well as bats from temperate parts of Europe (Harrison & Bates, 1991; Benda et al., 2006, 2012). This area exhibits considerable geographic diversity, ranging from the mountainous Caucasus through the relatively fertile Mesopotamia to the deserts of the Arabian Peninsula. The bat families with highest species diversity are Vespertilionidae and Rhinolophidae. Additionally, families with at least one species distributed in the Middle East include Emballonuridae, Hippo-sideridae, Miniopteridae, Nycteridae, Pteropodidae, and Rhinopomatidae (Mammal Diversity Database, 2023).

Central Asia is located between Caspian Sea in the west and western China and Mongolia in the east (Fig. 1), making it a region where bats from Europe and Asia can be found (Benda et al., 2011; Benda & Gaisler, 2015). However, the number of bat families found in this region is lower compared to other areas. Central Asia is less diverse geographically and ecologically than the Middle East, with the arid and semiarid lowlands being the most common habitats and highlands being distributed on the margins of the region in the foothills of Pamir or Zagros. The families Vespertilionidae and Rhinolophidae are the most species-rich in the area, although they have fewer species compared to neighbouring regions. The only other family distributed in the area is Miniopteridae, which has two species found in Central Asia (Furman et al., 2009, 2010; Šrámek, 2010; Benda et al., 2011; Mammal Diversity Database, 2023).

The final two areas of interest are situated in the Afrotropic region. In eastern Africa, the area from Sudan and Ethiopia to Tanzania and northern Mozambique (Fig. 1), certain bat species may be found common with the neighbouring Mediterranean or Arabian Peninsula, or they could be shared with tropical (western and central) Africa (e.g., Demos et al., 2019; Monadjem et al., 2021). This area is characterized by its high topographic diversity and habitat diversity. The area boasts the highest peaks in Africa, the African rift, the Somali desert, the savannahs of eastern Africa, the equatorial rainforest, and the Indian Ocean coast. Furthermore, the region is home to the rich diversity of Madagascar and other Indian Ocean islands (Linder et al., 2012; Goodman et al., 2015; Demos et al., 2023). This may contribute to the high biodiversity represented by high number of bat species and families. The most specious families are once again Vespertilionidae and Rhinolophidae, along with more tropical families of Emballonuridae, Mollosidae, and Pteropodidae (Mammal

Diversity Database, 2023). The greatest diversity of Miniopteridae and endemic Myzopodidae is found in Madagascar (Monadjem et al., 2020a; Demos et al., 2023; Simmons & Cirranello, 2023). Additional bat families in eastern Africa include Hipposideridae, Rhinonycteridae, Rhinopomatidae, Nycteridae, and Megadermatidae (Mammal Diversity Database, 2023).

In southern Africa, which spans from Zambia and Namibia to South Africa (Fig. 1), bats predominantly interact with fauna from eastern and central Africa, and to a lesser extent with those from western Africa (Linder et al., 2012). While eastern Africa is generally more diverse than southern Africa, the latter still presents distinct differences between its arid western region and its wetter eastern region, which can be observed in their distribution ranges (Stuart, 2015). The habitats found in southern Africa are composed of Namib desert, Mediterranean-like Karoo and Fynbos, mountainous Drakensberg range, arid bushland, wetter savannahs, and even forests (Rutherford et al., 2006). The bat fauna in southern Africa is as rich in bat families as in eastern Africa, albeit less diverse in bat species (Mammal Diversity Database, 2023). The Vespertilionidae and Rhinolophidae families are among the most specious bat families, accompanied by Hipposideridae, Miniopteridae, Mollosidae, Nycteridae, and Pteropodidae (Mammal Diversity Database, 2023). The bat families Emballonuridae, Rhinonycteridae, and endemic to southern Africa Cistugidae represent less numerous bat families with only 1-2 species each (Lack et al., 2010; Mammal Diversity Database, 2023).

Order Chiroptera

The phylogeny of bats, the order Chiroptera, has undergone notable transformations due to the rise of molecular methods in recent years. Bats were initially classified in Archonta with Dermoptera, Scadentia, and Primates based on morphological analyses (Gregory, 1910; Wible & Novacek, 1988; Simmons, 1994; Miyamoto, 1996) and/or in Volantia together with Dermoptera (Simmons, 1993; Szalay & Lucas, 1993, 1996). Sometimes, there have been suggestions that pteropodids (megabats) are more closely related to primates than to the remaining bats (microbats) implying an independent evolution of flight within both groups of bats (reviewed in Teeling et al., 2016; Wang et al., 2017). However, molecular studies have supported the monophyly of bats placing them within Laurasiatheria (Murphy et al., 2001; Meredith et al., 2011; Tsagkogeorga et al., 2013). The most recent study focused on genomic data proposed that Chiroptera holds a sister position to Fereuungulata comprised of orders Carnivora, Cetartiodactyla, Perrisodactyla, and Pholidota (Jebb et al., 2020).

The modifications occurred also in relationships among the groups of bats that now appear to be widely accepted (Simmons & Cirranello, 2023). Morphological studies have divided bats into megabats, which are incapable of laryngeal echolocation, and echolocating microbats (e.g., Simmons, 1998; Simmons & Geisler, 1998; Gunnell & Simmons, 2005). Conversely, the molecular evidence supports an alternative hypothesis regarding the classification of bats, identifying two infraorders – Yinpterochiroptera and Yangochiroptera (Fig. 2; Hutcheon et al., 1998; Teeling et al., 2000, 2002, 2005; Springer et al., 2001; Van Den Bussche & Hoofer, 2004). The first infraorder, Yinpterochiroptera, consists of megabats (Pteropodoidea/Pteropodidae) and rhinolophoid microbats (Rhinolophoidea – Craseonycteridae, Hipposideridae, Megadermatidae, Rhinolophidae, Rhinopomatidae, and Rhinonycteridae) (Teeling et al., 2000, 2002, 2005; Springer et al., 2001; Van Den Bussche & Hoofer, 2004). The second infraorder, Yangochiroptera, comprises of all the remaining

microbats belonging to superfamilies Emballonuroidea, Noctilionoidea, and Vespertilionoidea) (Teeling et al., 2000, 2002, 2005; Springer et al., 2001; Van Den Bussche & Hoofer, 2004; Hermsen & Hendricks, 2008; O'Leary et al., 2013). Subsequently, a combined analysis of molecular and morphological data of extant and fossil bats supported the hypothesis of a single origin of echolocation with later loss in pteropodids (Springer et al., 2001, 2004).

The studies of this thesis centre on chosen bat taxa from two infraorders and three superfamilies (Fig. 2). The selection criteria encompassed two factors: first, longstanding ambiguities in their phylogeny and the need for molecular revision, and second, availability of samples for molecular analysis from the depositories of the National Museum in Prague. Utilising these criteria, six species or species groups have been chosen for molecular studies. Samples of *Myotis nattereri* species complex from Europe and the eastern Mediterranean were used to adress gaps in knowledge regarding the phylogeny and distribution of the species within this species complex (**Paper 1**). Samples of *M. emarginatus* from Europe, the Mediterranean, the Middle East, and Central Asia were included in the revision of this widely distributed species (**Paper 2, Paperand 3**). Both taxa belong to the family Vespertilionidae, superfamily Vespertilionoidea. Then, the relationships among Palaearctic species from the family Emballonuridae, superfamily Emballonuroidea, were studied using samples collected in the Middle East (**Paper 4**). Subsequent studies focused on the family Rhinolophidae, superfamily Rhinolophoidea, and infraorder Yinpterochiroptera. Samples of *Rhinolophus hipposideros*, mainly from the Middle East, were used to study population relationships within this species (**Paper 5**). The phylogeny revision within the *R. ferrumequinum* group included four to five species from their entire distribution across Europe, Mediterranean, the Middle East, Central Asia, eastern, and southern Africa (**Paper 6**). Additionally, the utilisation of *Rhinolophus* samples from Lesotho led to discovery and description of completely new species distributed in the mountainous parts of southern Africa. This new species was placed into the *R. fumigatus* group (**Paper 7**). Finally, the description of Zambian bats from the collections of the National Museum in Prague required their molecular identification (**Paper 8**). The higher taxa of the chosen bats will be presented below.

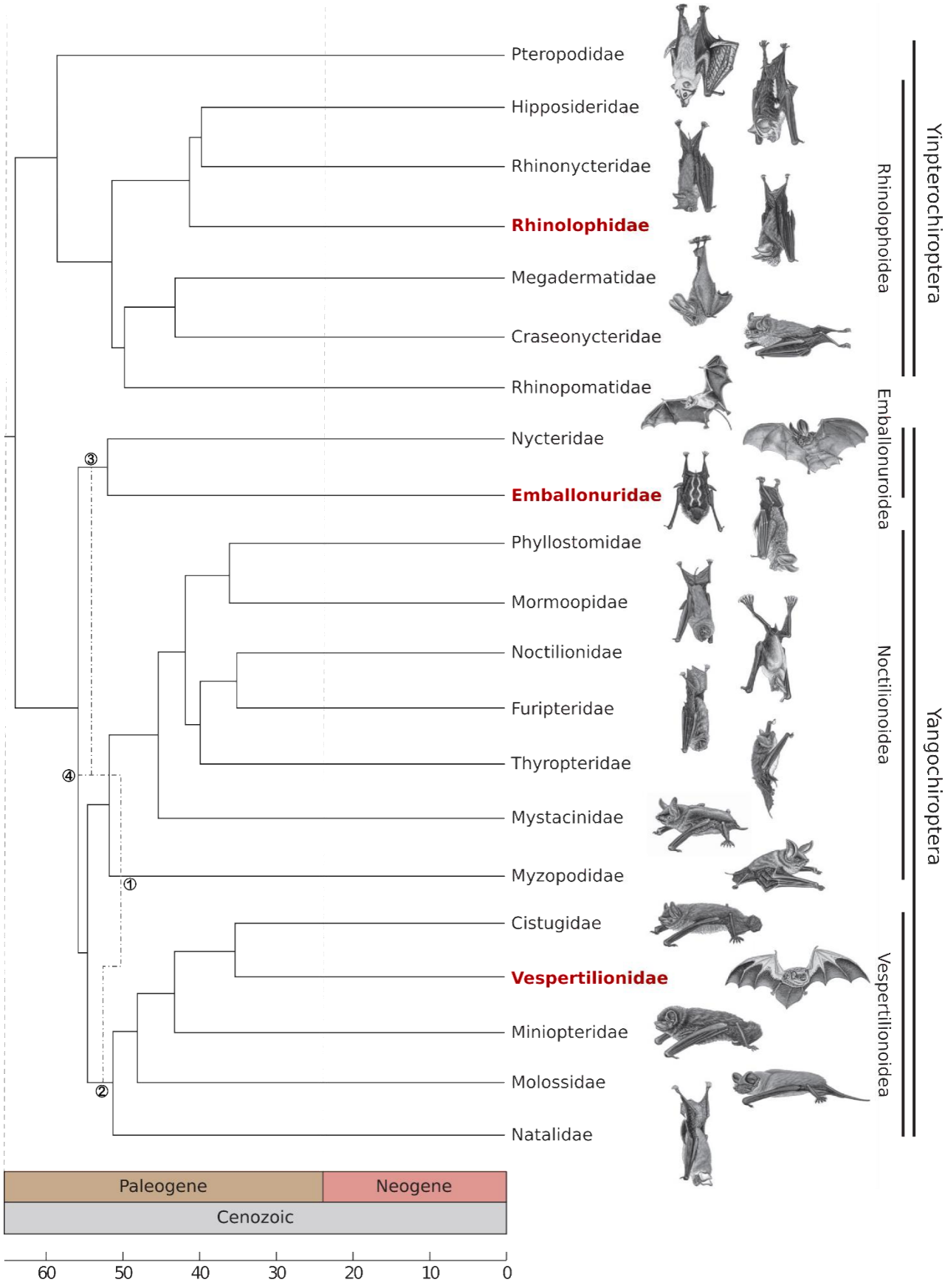

Fig. 2 Molecular phylogeny of the order Chiroptera and divergence times within the order. The red names denote the bat families that are discussed within this PhD thesis. The numbers show alternative positions of Myzopodidae within Yangochiroptera. Modified after Teeling et al. (2018).

Studied bat taxa

This PhD thesis focused on studying bat taxa whose phylogeny remained ambiguous in both chiropteran infraorders, Yangochiroptera and Yinpterochiroptera (Fig. 2). The infraorder Yangochiroptera comprises over 1,000 bat species which accounts for approximately 70 % of all bat species (Simmons & Cirranello, 2023). This infraorder is separated into three superfamilies, with two of them, Vespertillionoidea and Emballonuroidea, found worldwide. The superfamily Vespertiliono-idea comprises approximately 700 species, which is nearly half of all bat species, and encompasses five bat families (Simmons & Cirranello, 2023). In contrast, the superfamily Emballunoroidea, which is largely distributed in the Old World, contains substantially fewer species (~70 species) and two to three families (Teeling et al., 2002, 2005; Agnarsson et al., 2011; Amador et al., 2018; Simmons & Cirranello, 2023). The third superfamily, Noctilionoidea, is only found in the New World (Rojas et al., 2016, 2018; Simmons & Cirranello, 2023) and thus, does not fall within the scope of this thesis.

The family Vespertilionidae, belonging to the superfamily Vespertilionoidea, is the most specious bat family with more than 500 species, representing around a third of all bat species (Simmons & Cirranello, 2023). It comprised even more species but recent molecular revisions have resulted in recognition of Cistugidae and Miniopteridae as distinct families (Hoofer & Van Den Bussche, 2003; Van Den Bussche & Hoofer, 2004; Miller-Butterworth et al., 2007; Lack et al., 2010). Currently, there are four subfamilies classified in the family Vespertilionidae: Kerivoulinae, Murininae, Myotinae, and Vespertilioninae (Kawai et al., 2002; Hoofer & Van Den Bussche, 2003; Amador et al., 2018; Simmons & Cirranello, 2023). A part of this thesis focuses on the subfamily Myotinae, which is now the second most abundant subfamily with around 140 species. Initially, it was considered a tribe within the subfamily Vespertilioninae. The increasing evidence provided support for the notion that the *Eudiscopus*, *Myotis*, and *Submyotodon* clade constitute a subfamily (Simmons, 1998; Hoofer & Van Den Bussche, 2003; Stadelmann et al., 2004; Ruedi et al., 2013, 2015; Amador et al., 2018). The genus *Myotis* includes 137 species and, therefore, it belongs among the most specious mammalian genera (Simmons & Cirranello, 2023). This genus was categorised into three subgenera (*Leuconoe*, *Myotis*, and *Selysius*) based on morphology, but the molecular studies indicated their convergent ecomorphs origin (Mayer & von Helversen, 2001; Ruedi & Mayer, 2001; Kawai et al., 2003). Currently, the genus *Myotis* is categorised into three different subgenera – *Chrysopteron*, *Myotis*, and *Pizonyx* (Mammal Diversity Database, 2023). The species under scrutiny, *M. nattereri* and *M. emarginatus*, are assigned to the subgenera *Myotis* and *Chrysopteron*, respectively (Stadelmann et al., 2004; Csorba et al., 2014; Simmons et al., 2021; Mammal Diversity Database, 2023).

The family Emballonuridae, part of superfamily Emballonuroidea, is the largest family within the superfamily with 55 currently recognised species (Simmons & Cirranello, 2023). This circumtropically distributed family is divided into two subfamilies, Emballonurinae (37 species) and Taphozoinae (18 species) (Simmons & Cirranello, 2023). The Emballonurini tribe in the subfamily Emabllonurinae, alongside the entire subfamily Taphozoinae, are restricted to the Old World (Simmons, 2005; Simmons & Cirranello, 2023). The tribe Emballonurini contains four genera, two of which (*Coleura* and *Paremballonura*) are distributed in western part of the Old World (Simmons & Cirranello, 2023). The subfamily Taphozoinae consist of only two genera (*Saccolaimus* and *Taphozous*) and the representatives of both genera are present in the western part of the Old World

(Simmons & Cirranello, 2023). The study that investigated emballonurid bats was focused on the Palaearctic region, where species from both subfamilies occur. *Coleura afra* belongs to the subfamily Emballonurinae, while *Taphozous nudiventris* and *T. perforatus* are members of the subfamily Taphozoinae (Ellerman & Morrison-Scott, 1951; Harrison & Bates, 1991; Horáček et al., 2000).

The second infraorder, Yinpterochiroptera, comprises approximately 430 species, of which over half around 230 species are echolocating rhinolophoid microbats belonging to the superfamily Rhinolophoidea (Simmons & Cirranello, 2023). The superfamily consists of six families, one of which, the subfamily Rhinolophidae, was the focus of this thesis's investigation. This family comprises only one genus, *Rhinolophus*, yet it boasts the hugest number of species (~110) out of all the Rhinolophoidea families (Csorba et al., 2003; Mammal Diversity Database, 2023; Simmons & Cirranello, 2023). The family Rhinolophidae used to include other species, which are now part of the modern families Hipposideridae and Rhynonycteridae (Koopman, 1993, 1994; McKenna & Bell, 1997; Simmons, 1998; Simmons & Geisler, 1998; Teeling et al., 2002; Hand & Kirsch, 2003; Hand & Archer, 2005). However, about 30 years ago, the clade Hipposideridae + Rhinonycteridae was recognised again at the family level (Corbet & Hill, 1992; Bates & Harrison, 1997; Bogdanowicz & Owen, 1998; Hand & Kirsch, 1998). Later Rhinonycteridae was elevated to the family level based on the molecular data (Foley et al., 2015). The current taxonomic arrangement of the family Rhinolophidae distinguishes six subgenera and 15 species groups based on molecular and morphological data (Guillén-Servent et al., 2003; Zhou et al., 2009). Two subgenera, *Phyllorhina* and *Rhinolophus*, are exclusively distributed in the Afrotropic and Palaearctic regions (Csorba et al., 2003), placing them at the centre of focus in this thesis. The subgenus *Phyllorhina* is distributed predominantly across the Palaearctic, encompassing western Europe, the Mediterranean, and the Middle East. It is only found marginally in Afrotropics in north-eastern Africa (Csorba et al., 2003; Gaisler, 2013; Burgin, 2019; Bendjeddou et al., 2022). Currently, the subgenus comprises only one species group (*hipposideros* group) and it includes a single species *R. hipposideros* (Csorba et al., 2003; Simmons, 2005; Burgin, 2019). The subgenus *Rhinolophus*, also known as the Afro-Palaearctic *Rhinolophus* clade, is much more diversified and includes seven distinct species groups (Csorba et al., 2003; Demos et al., 2019). Among these groups, the focus of this thesis is primarily on the *ferrumequinum* and *fumigatus* species groups. Up to ten species are recognised within each of these two species groups (Csorba et al., 2003; Demos et al., 2019).

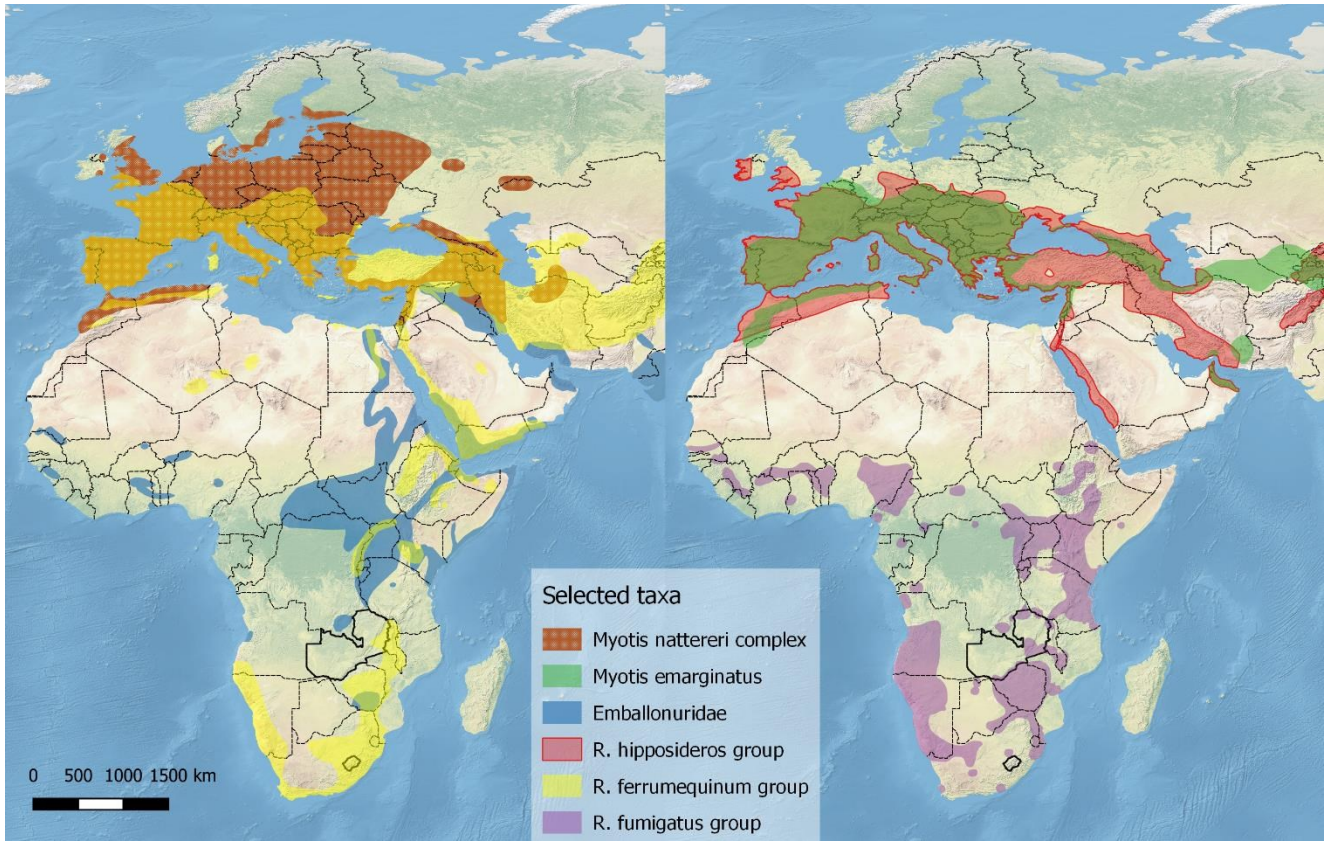

Fig. 3 The selected bat taxa in the western Old World. The distribution ranges of the entire studied bat species/groups are denoted by a specific colour. The solid line highlights the borders of Lesotho of Zambia that are countries of interest in the Paper 7 and 8. The distribution ranges were modified after iucnredlist.org, mammaldiversity.org, and mol.org.

The ambiguities in the bat phylogeny

The resolution of the bat phylogeny remains incomplete despite extensive efforts (Agnarsson et al., 2011; Shi & Raboski, 2015; Amador et al., 2018). For example, the family Myzopodidae could potentially be placed within any of the superfamilies in the suborder Yangochiroptera, or alternatively may be sister to all these yangochiropteran superfamilies (See Fig. 2; Hoofer et al., 2003; Van Den Bussche and Hoofer, 2004; Eick et al., 2005; Teeling et al., 2005; Miller-Butterworth et al., 2007; Agnarsson et al., 2011; Meredith et al., 2011; Amador et al., 2018). Another example we addressed in our studies concerns the unresolved intrafamilial relationships within the family Rhinolophidae (Guillen Servént et al., 2003; Foley et al., 2015; Dool et al., 2016; Demos et al., 2019). However, the largest number of unresolved relationships persist at both inter- and intraspecific levels, with ongoing descriptions of new genera and species (Goodman et al., 2012; Benda et al., 2016; Hutterer et al., 2019; Görföl et al., 2020; Monadjem et al., 2021). Due to this reason, I focused in this thesis on those taxa that were either not revised with the molecular methods, or the sampling of previous molecular studies was not complete (Fig. 2). Additionally, I added the data for the populations uncovered by the previous studies.

Within the family Vespertilionidae, we studied two species/species complexes that also inhabit the Czech Republic – *Myotis nattereri* and *M. emarginatus*. The *Myotis nattereri* complex forms one of the largest complexes of closely related bat species in Europe, which are morphologically hardly

distinguishable. Before genetic methods were used, the complex was believed to consist only of *M. nattereri* from Europe and North Africa, and the larger sister species *M. schaubi* from the Caucasus region (Horáček & Hanák, 1984; Horáček et al., 2000). Thanks to genetic studies, the number of species within the complex has increased to eight (Razgour et al., 2023). As a results, the distribution range of *M. nattereri* s.str. is now limited to the temperate zone of Europe and the Balkans (Fig. 3). The primary objective of this study was to fill gaps in previous sampling, with a particular focus on the Middle East. On the contrary, *Myotis emarginatus* is a bat species with a wide distribution range, spanning from Europe and North Africa through the Mediterranean to the Middle East and Central Asia (Fig. 3). Although many subspecies have been recognised, especially in the Asian part of its range, most samples collected in previous studies originated from Europe (Horáček et al. 2000; Dietz & Pir, 2023). Thus, the objective of this study was to conduct a comprehensive sampling of *M. emarginatus* across its entire range, including Asian samples, for the first time in genetic analysis. In subsequent study, morphologic data from across the entire distribution were explored, and the results were combined with the molecular results to propose integrative outcomes of relationships among the populations of *M. emarginatus*.

Of the family Emballonuridae, our study focused on three species that can be found in the Palaearctic region, namely *Coleura afra*, *Taphozous nudiventris*, and *T. perforatus* (Fig. 3). These bats have been extensively studied morphologically, resulting in many morphotypes and subspecies. Nonetheless, molecular studies within the Emballonuridae family, especially in the Old World, have been rather uncommon. The genera *Coleura* and *Paremballonura* have been the most thoroughly studied genera in the western part of the Old World. This is particularly true for the species that inhabit the islands of the western Indian Ocean (such as Madagascar, Pemba, Seychelles) (Goodman et al., 2006, 2012; Ruedi et al., 2012; Vallo et al., 2018). Therefore, the aim of this study was to use molecular methods to revise the relationships within the Palaearctic region and contribute to the taxonomy of the family Emballonuridae.

Conversely, the family Rhinolophidae has been thoroughly studied using both molecular and morphologic methods (e.g., Bogdanowicz & Owen, 1992; Csorba et al., 2003; Zhou et al., 2009; Stoffberg et al., 2010; Dool et al., 2016). We targeted three species groups – *hipposideros*, *ferrumequinum*, and *fumigatus* – which have been extensively examined (Guillén Servent et al., 2003; Stoffberg et al., 2010; Dool et al., 2016). Our studies involved sampling previously omitted population and analysing the most comprehensive dataset available to examine the entire species groups. The *hipposideros* group was considered to contain a sole species, the lesser horseshoe bat (*Rhinolophus hipposideros*). This widely distributed species inhabits areas ranging from the British Isles and North Africa to the Middle East and Central Asia (Fig. 3). A previous comprehensive genetic study, which covered species' entire range, indicated relatively low genetic diversity and likely positions of glacial refugia. Our study expanded on previous findings by introducing new samples from small, previously unstudied populations in Oman, Tajikistan, and Ethiopia. The goal was to compare these populations with others and revise their relationships, even though some subspecies from these areas have been previously described and synonymised. The *ferrumequinum* and *fumigatus* groups are part of the Afro-Palaearctic clade, with the two groups being in a sister position (Maree and Grant, 1997; Stoffberg et al., 2010; Benda & Vallo, 2012; Dool et al., 2016; Demos et al., 2019). Currently, four species are recognised within the *ferrumequinum* group, which have an extensive distribution across the southern Palaearctic and eastern Afrotropic regions (Fig.

3): *R. bocharicus*, *R. clivosus*, *R. ferrumequinum*, and *R. nippon* (Bogdanowicz, 1992; Csorba et al., 2003; Burgin, 2019). Although the species within this group have been extensively investigated using molecular methods (Benda & Vallo, 2012; Stoffberg et al., 2012; Bailey et al., 2016; Dool et al., 2016; Demos et al., 2019), the group itself has not been studied as a whole, and the relationships between the species remain unresolved. Therefore, this study investigated the phylogeny of the *ferrumequinum* group and intraspecific variations using a genetic approach. The horseshoe bats display similar morphological traits across groups due to convergence of the phenotypes of species from similar habitats. As a result, one species from southern Africa was reassigned from the *ferrumequinum* group to the *fumigatus* group (Jacobs et al., 2013). This finding, along with others, suggests a close morphological affinity between the *ferrumequinum* and *fumigatus* groups. Therefore, while examining another population from southern Africa, we compared the only *Rhinolophus* species from Lesotho (Fig. 3), identified as *R. clivosus* (Lynch & Watson, 1990; Lynch, 1994; Taylor, 2005; Monadjem et al., 2010, 2020b; Benda & Vallo, 2012), with species from both the *ferrumequinum* and *fumigatus* groups. The aim of this study was to contribute to the knowledge of *Rhinolophus* species found in southern Africa.

Finally, Zambia is a country located in southern Africa (Fig. 3), primarily covered by woodland savannas. The bat fauna of this region has been quite extensively studied (Ansell, 1978; Monadjem et al., 2020b), with the most recent taxonomic compendium reporting the presence of 73 bat species (Monadjem et al., 2020b). However, the diverse nature of cryptic species makes morphologic identification challenging, even in this country. Additionally, many bats have not had their distribution ranges accurately mapped due to incomplete exploration of their distribution ranges (Razgour et al., 2016). Our objective is to compile all Zambian bats present in the collections of National Museum in Prague (NMP) using both morphologic and molecular identification.

Aims of the study

This PhD thesis aimed to investigate the phylogenetic relationships within selected lesser-known or understudied bat groups in the western part of the Old World. Its primary objective was to shed light on the systematics of these groups. The thesis can be divided into three key points:

1) provide new insights into the intraspecific variation of the chosen bat species and reconstruct the interspecific relationships among the species groups. This was accomplished especially by extensive sampling across the largest possible part of the distribution ranges of these species/species groups.

2) contribute to resolving phylogenetic relationships within higher taxa, including clades, genera, and families. A comprehensive analysis of generated and publicly available data was conducted to determine the placement of the taxa under investigation within the higher taxa phylogeny.

3) express the taxonomic implications of the selected bat taxa by analysing the outcomes of points 1) and 2). We combined of all the analyses done in the research to produce taxonomic synthesis, demonstrating the validity of the recognised subspecies, species, and higher taxa.

To achieve these points, I employed molecular methods while my colleagues conducted the morphologic analyses and, on one occasion, echolocation data analysis. For the molecular methods, I extracted the bats' DNA from range of collections, but primarily from the National Museum in Prague. Typically, I obtained sequences of both mitochondrial and nuclear markers and assembled them with publicly available sequences from the GenBank. Next, the phylogenetic analyses, namely Maximum likelihood and Bayesian inference, were conducted to reconstruct the phylogeny. Subsequently, the sequences were utilised to estimate the time divergence and species delimitation. For the description of morphological patterns in particular populations, cranial and dental measurements along with forearm length were recorded as standardised dimensions referring to body size. In Paper 5, the analysis of the echolocation data was performed. The oscillograms, power spectra, and spectrograms were assessed to compare echolocation calls among the various populations.

Summary of publications

This PhD thesis is a cumulative work consisting of 8 manuscripts (6 published and 2 submitted). I am the first author of 4 studies and the second author of 4 studies. Details of my contribution to each work are in accordance with the CRediT authorship statement (Brand et al., 2015).

Paper 1

Uvizl M. & Benda P. (2021). Diversity and distribution of the *Myotis nattereri* complex (Chiroptera: Vespertilionidae) in the Middle East: filling the gaps. *Mammalian Biology*, 101, 963–977. https://doi.org/10.1007/s42991-021-00143-0

Contribution of MU: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – Review & Editing, Visualization

The *Myotis nattereri* complex forms one of the largest complexes of closely related bat species, which are morphologically difficult to distinguish. Before genetic methods were used, it was thought that *M. nattereri* was a single species distributed in Europe, the Middle East, Central Asia, and the Far East, including Japan (Tate, 1941; Ellerman & Morrison-Scott, 1951; Kuzâkin, 1965; Corbet, 1978). Within this range, *M. nattereri* was distributed across two centre areas – the first from western Europe to central Asia, and the second in eastern Asia. Approximately 40 years ago, bats from eastern Asia were differentiated into a distinct species (*M. bombinus*) through morphologic analysis, which led to a restriction of *M. nattereri*'s geographical range to encompass only Europe, the Middle East, and Central Asia (Fig. 3). The revision divided *M. nattereri* into two sister species based on body size: smaller *M. nattereri* from Europe, Middle East, and Central Asia, and larger *M. schaubi* from the Caucasus region (Horáček & Hanák, 1984; Horáček et al., 2000). The partition was further refined using molecular methods to demonstrate high cryptic diversity within this species. Currently, the species complex comprises up to eight recognised species, which restricts the distribution range of *M. nattereri* s.str. being limited to the temperate zone of Europe and from the Balkans to western Anatolia (Salicini et al., 2013; Çoraman et al., 2019; Razgour et al., 2023). The other species in the complex are *M. crypticus*, *M. escalerai*, *M. zenatius*, *M. nustrale*, *M. hoveli*, *M. tschuliensis*, and *M. schaubi* (Ibáñez et al., 2006; Mayer et al., 2007; García-Mudarra et al., 2009; Salicini et al., 2011, 2013; Puechmaille et al., 2012, 2023; Juste et al., 2018; Çoraman et al., 2019; Razgour et al., 2023). Most of the molecular studies have focused on European populations, with only a limited number of them examining samples from the Middle East and/or Central Asia (Çoraman et al., 2019; Smirnov et al., 2020; Kruskop & Solovyeva, 2021). The remaining studies investigated the morphology of Asian *M. nattereri* populations (Harrison, 1964; Horáček & Hanák, 1984; Benda et al., 2006, 2007, 2010, 2011, 2012).

The primary objective of this study was to fill the gaps in the knowledge regarding populations within the *M. nattereri* species complex in the Middle East. To achieve this, we conducted molecular and morphometric analyses. As the gene *ND1* was the most frequently used mitochondrial marker in previous studies of this species complex (Ruedi and Mayer 2001; Ibáñez et al. 2006; Mayer et al. 2007; García-Mudarra et al. 2009; Salicini et al. 2011; Juste et al. 2018; Çoraman et al. 2019; Kruskop & Solovyeva 2021), we have incorporated its sequences into our dataset. The study utilised skull craniodental measurements and forearm length (LAt) as a standard dimension for body size in a comparative morphometric analysis.

The study revealed that the taxonomic affiliations within the populations of *M. nattereri* species complex in most of the Middle East were established, leading to improved species distribution limits. Additionally, the presence of four genetic lineages in the region was confirmed. The lineages belong to two clades and three primarily size-defined morphotypes. These lineages indicate the presence of four distinct species: *M. nattereri* s.str., *M. hoveli*, *M. tschuliensis*, and *M. schaubi*. *Myotis nattereri* s.str. inhabits regions stretching eastwards as far as western Anatolia and Samos, *M. hoveli* is distributed in the eastern Mediterranean including Cyprus. *Myotis tschuliensis* is found in a region that extends from Crimea to Turkmenistan, while larger *M. schaubi* is confined to a limited geographical area in Armenia and Iran. Overall, the research provided insight into the relationships among *M. nattereri* species complex in the Middle East and has resulted in a more precise definition of the distribution boundaries of each species.

Paper 2

Uvizl M. & Benda P. (2021). Intraspecific variation of *Myotis emarginatus* (Chiroptera: Vespertilionidae) inferred from mitochondrial and nuclear genetic markers. *Acta Chiropterologica*, 23(2), 285–300. https://doi.org/10.3161/15081109ACC2021.23.2.002

Contribution of MU: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – Original Draft, Visualization

Myotis emarginatus is a bat species with a broad distribution extending from Europe and North Africa through the Mediterranean to the Middle East and Central Asia (Fig. 3; Horáček et al. 2000; Dietz & Pir, 2023). Despite its biogeographic origins in the Palaearctic region (Ruedi & Mayer, 2001), *M. emarginatus* belong to the subgenus *Chrysopteron*. This subgenus comprises all African *Myotis* species and parti-coloured species from eastern Asia (Stadelman et al., 2004; Agnarsson et al., 2011; Ruedi et al., 2013; Csorba et al., 2014; Patterson et al., 2019). The subgenus has been frequently employed in phylogenetic studies, but the inter-specific relationships have yet to be satisfactorily resolved. Four subspecies of *M. emarginatus* have been recognised across its wide distribution range (Koopman, 1994; Dietz & Pir, 2023). The nominotypical subspecies can be found from western Europe to eastern Mediterranean, while the other three subspecies, namely *M. e. desertorum*, *M. e. turcomanicus*, and *M. e. kuzyakini*, are present in western Asia, including the Middle East, the Caucasus, Arabian Peninsula, and Central Asia. Currently, there was a lack of molecular research on the relationships among populations and putative subspecies of *M. emarginatus* (Dietz & Pir, 2023). Additionally, when samples of *M. emarginatus* were used in some molecular study regarding all *Myotis* species, they were collected solely in the European part of its distribution (Ruedi & Mayer, 2001; Stadelman et al., 2004; Ibáñez et al., 2006; Mayer et al., 2007; García-Mudarra et al., 2009; Ruedi et al., 2013, Patterson et al., 2019).

Thus, we have gathered samples from almost the entire geographical range of *M. emarginatus* to conduct a comprehensive sampling, which includes of the genetic analysis of Asian samples for the first time. Our aim was to obtain a better understanding of this species and its range. We have obtained over 150 samples and sequenced two mitochondrial and three nuclear markers. The sequences were employed in a phylogenetic analysis to reconstruct the phylogenetic trees and haplotype network. These were done to revise the relationships among the populations of *M. emarginatus*, its relationship with related species, and to compare it with morphologic analysis of Benda et al. (2006).

The results of the phylogenetic analyses confirmed that *M. emarginatus* is a polymorphic species belonging to the African clade of the genus *Myotis*. The phylogenetic trees revealed the existence of two mitochondrial lineages and one nuclear lineage of *M. emarginatus*. The two mitochondrial lineages were separated by a vast 400km distribution gap extending between the eastern Mediterranean and eastern Middle East. This spatial arrangement supports the earlier morphological diversification into two distinct morphotypes – one found in Europe, North Africa, and the eastern Mediterranean, and the other in the Middle East and Central Asia. The latter two areas are the centres of further division within the Asian mitochondrial lineage. Nevertheless, a single nuclear lineage suggested that *M. emarginatus* is a widely distributed species. Overall, the geographical variation of *M. emarginatus* was limited, yet it corresponded to two subspecies within its species rank: *M. e. emarginatus* found from Europe to the Levant, and *M. e. desertorum* in the eastern Middle East to Central Asia.

Paper 3

Benda P. & **Uvizl M**. (2021). Taxonomic revision of *Myotis emarginatus*: detailed morphometric analysis and final evaluation of the evidence (Chiroptera: Vespertilionidae). *Lynx, n. s.*, 52, 25–54. https://doi.org/10.37520/lynx.2021.003

Contribution of MU: Investigation, Data curation, Writing – Review & Editing

This study builds on a genetic study of *Myotis emarginatus* (Paper 2) conducted across its entire distribution range, which spans from Europe and North Africa to the Middle East and Central Asia (Fig. 3; Horáček et al., 2000). Molecular analysis recognised only two subspecies in this area, but morphological analysis described up to four subspecies (Koopman, 1994; Dietz & Pir, 2023). Nevertheless, the most comprehensive morphologic studies of around 300 samples and 12 analysed craniodental characters have led to the identification of only two subspecies: one from Europe (*M. e. emarginatus*) and one from Asia (*M. e. desertorum*) (Benda et al., 2006; Benda & Gaisler, 2015). These results agreed with those of molecular study.

The aim of this study was to gather *M. emarginatus* samples from its most complete part of its distribution and conduct a synthesis of morphologic data with the previous molecular results. Almost 500 specimens, including the type material of all four subspecies, were subject to morphologic examination of more than 20 mainly craniodental dimensions.

The subsequent statistical analysis uncovered the existence of up to four distinct morphotypes: 1) the first morphotype, comprising of a small specimen with a short rostrum and a high braincase, originating in Europe and North Africa; 2) the second morphotype, a medium-sized specimen with a long rostrum, originating in the eastern Mediterranean; 3) the third morphotype, a large specimen with a long and wide rostrum, originating in the southern Middle East; and 4) the fourth morphotype, a large specimen with a narrow rostrum, originating in Crimea and extending to Central Asia. Upon combining these results with the available genetic data, it is apparent that the first two morphotypes belong to the European subspecies *M. e. emarginatus*, while the third morphotype is comprised of *M. e. desertorum*, distributed in the Middle East including southern Iran, Oman, and Afghanistan. In addition, the fourth morphotype is likely to represent the third subspecies *M. e. turcomanicus*, that inhabits the north-eastern parts of the Middle East, Crimea, the Caucasus, and West Turkestan. The remaining populations to investigate are located in the south-western Arabian Peninsula. Although not extensive, the geographic variation of *M. emarginatus* is consistent with the division

into two subspecies based on the molecular analysis and three subspecies based on the morphologic analysis.

Paper 4

Uvizl M., Šmíd J., Aghová T., Kotyková Varadínová Z., & Benda P. (2019). Molecular phylogeny and systematics of the sheath-tailed bats from the Middle East (Emballonuridae: *Taphozous* and *Coleura*). *Acta Chiropterologica*, 21(1), 23–34.
https://doi.org/10.3161/15081109ACC2019.21.1.002

Contribution of MU: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – Original Draft, Visualization

The family of sheath-tailed bats (Emballonuridae) constitutes a considerable part of the bat fauna of the Middle East, which is situated at the crossroad of three biogeographic regions – the Palaearctic, Oriental, and Afrotropical regions. Additionally, the Middle East is located within the arid zone that stretches from the west of northern Africa to the Indian peninsula, resulting in the coexistence of fauna from all three regions and enhancing the area's biodiversity. The family Emballonuridae has a circumtropical distribution and comprises two subfamilies (Simmons, 2005). The subfamily Taphozoinae is limited to tropical areas of the Old World, while the subfamily Emballonurinae is further divided into two tribes, Emballonurini in the Old World and Diclidurini in the New World (Robbins & Sarich, 1988; Griffiths & Smith, 1991; Koopman, 1994; McKenna & Bell, 1997; Simmons, 2005 Lim et al., 2008; Ruedi et al., 2012). Molecular studies within the Emballonuridae family have been rare. Only four papers have investigated the phylogeny of the Old World species (Goodman et al., 2006, 2012; Ruedi et al., 2012; Vallo et al., 2018), while other studies have analysed only one or two samples of these species (Lim et al., 2008; Wei et al., 2008; Çoraman et al., 2013; Maganga et al., 2014). Furthermore, the phylogeny of the subfamily Taphozoinae was not explored using molecular methods, while most studies examined only one species per study (Lim et al., 2008; Wei et al., 2008; Ruedi et al., 2012; Çoraman et al., 2013; Maganga et al., 2014). This study is focused on the sheath-tailed bats belonging to the family Emballonuridae present in the area – namely *Coleura afra*, *Taphozous nudiventris*, and *T. perforatus* (Fig. 3; Ellerman & Morrison-Scott, 1951; Harrison & Bates, 1991; Horáček et al., 2000). These bats were studied solely through morphologic analysis, which resulted in the detection of various morphotypes and subspecies. Nevertheless, the relationships between the populations of Palaearctic emballonurid species and the statuses of the described subspecies remain unresolved.

Therefore, the objective of this study was to use molecular methods to investigate the relationships among the emballonurid bats within the Palaearctic region, contributing to the taxonomy of the family. We used more than 100 samples from four species, and we generated sequences of three mitochondrial and five nuclear markers. These sequences were employed in phylogenetic analyses for the construction of phylogenetic trees and haplotype networks. Additionally, they were used for the estimation of the divergence time of species in the family Emballonuridae.

The genetic analysis has provided novel insights into the taxonomic structure of Palaearctic Emballonuridae populations, shedding light on their phylogenetic affinities. The phylogenetic trees demonstrated that *Coleura afra* populations found in southern Arabia and along the Red Sea coast

of Africa are distinct from other *Coleura* populations present in Africa and the Indian Ocean islands. The discovery confirmed the existence of a new species, resulting in the elevation of *C. gallarum* from the subspecific level. This increased the number of *Coleura* species to four. In the case of *T. nudiventris*, evidence of two mitochondrial lineages suggested that larger bats found in Mesopotamia were separate from smaller bats found in southern Arabia. However, the analysis of nuclear genes did not provide support for this separation, indicating only one nuclear lineage. Based on these findings, we suggested recognising two subspecies of *T. nudiventris* in the Middle East. The smaller bat belongs to the nominotypical subspecies, while the larger bats from Mesopotamia are classified as *T. n. magnus*. The existence of any other subspecies was not proven, therefore the proposed subspecies *T. n. zayidi* was considered to be a junior synonym of the nominotypical subspecies. The genetic structure of *T. perforatus* suggested limited genetic diversity in the area, revealing the existence of one subspecies, namely the nominotypical subspecies, of *T. perforatus* in the Middle East. These findings demonstrated that subspecies *T. p. haedinus*, previously believed to occur in Palaearctic, is confined to eastern Africa because our data cannot confirm its validity. In addition to previous results, our study does not support *Liponycteris* as a distinct subgenus of *Taphozous* as it was paraphyletic due the position of *T. nudiventris* within the genus *Taphozous*. Overall, this study revised the relationships among the populations of Palaearctic emballonurids and identified a newly recognised species of bat.

Paper 5

Benda P., **Uvizl M.**, Vallo P., Rieter A., & Uhrin M. (2022). A revision of the *Rhinolophus hipposideros* group (Chiroptera: Rhinolophidae) with definition of an additional species from the Middle East. *Acta Chiropterologica*, 24(2), 269–298.
https://doi.org/10.3161/15081109ACC2022.24.2.001

Contribution of MU: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – Review & Editing, Visualization

The lesser horseshoe bat, *Rhinolophus hipposideros*, is widely distributed species that inhabits areas ranging from the British Isles through northern and north-western Africa, to the Middle East and Central Asia (Fig. 3; Csorba et al., 2003; Burgin, 2019). It belongs to the *hipposideros* group, a distinct and monotypic clade within the family Rhinolophidae, also known as a subgenus *Phyllorhina* (Guillén Servent et al., 2003). Both morphologic and molecular evidence support this assertion, although the relationships with other *Rhinolophus* species groups remain unresolved (Bogdanowicz, 1992; Guillén Servent et al., 2003; Stoffberg et al., 2010; Foley et al., 2015; Dool et al., 2016). Several methods, including molecular methods (Kůs, 2008; Dool et al., 2013; Shahabi et al., 2019), were employed to investigate the intraspecific variability of *R. hipposideros*. The study, which gathered a comprehensive dataset consisting of both mitochondrial and nuclear markers from nearly whole distribution range, indicated a relatively low genetic diversity and probable locations of glacial refugia (Dool et al., 2013). Nevertheless, the study did not include certain peripheral populations in north-western Africa and the Middle East. Certain proposed subspecies were identified in these areas (Felten et al., 1977; Csorba et al., 2003; Burgin, 2019). The previous genetic study covered the subspecies described in Europe and Africa with type localities in Germany, England, Corsica, and Morocco) (Dool et al., 2013). However, the remaining two subspecies,

R. h. minimus and *R. h. midas* described in Eritrea and southern Iran, respectively, have not been examined using genetic methods (Csorba et al., 2003; Burgin, 2019).

The goal of this study was to compare these populations of *R. hipposideros* to all other populations and revise the relationships. To determine phylogenetic patterns, we conducted a morphological analysis on over 270 museum specimens in order to establish the population positions within their entire distribution range. Additionally, we conducted a molecular genetic comparison on a geographically representative subset of almost 100 specimens. The study utilised one mitochondrial and five nuclear markers to conduct molecular phylogenetic analyses. This facilitated the construction of phylogenetic trees, genetic delimitation of species, and estimation of divergence times. Additionally, we compared echolocation data from various parts within the species range.

Our study was built on previous research by incorporating new samples from small populations in Oman, Tajikistan, and Ethiopia. These populations exhibit morphological similarities to bats found throughout the rest of the distribution range. Nevertheless, the analysis of genetic markers and echolocation traits provided evidence that the lesser horseshoe bats found in Oman constituted a distinct yet closely related species to *R. hipposideros*. Further morphological research has revealed that bats in Oman possess the same traits as those initially described in *R. midas* from Iran, which was later synonymised with *R. hipposideros*. Consequently, we proposed to revive the name *R. midas*. Hence, the *hipposideros* group now consists of two distinct species, *R. hipposideros* and *R. midas*. *Rhinolophus hipposideros* is widespread in south-western Eurasia, as well as north-western and north-eastern Africa, whereas *R. midas* has a limited distribution around the Strait of Hormuz and Gulf of Oman. Furthermore, *R. hipposideros* s.str. can be subdivided into two subspecies: *R. h. hipposideros* in the Maghreb and in Europe and *R. h. minimus* in Crimea, the Caucasus, the Middle East, and north-eastern Africa. Besides genetic traits, these subspecies differ from each other in karyotype: *R. h. minimus* was found to have 2n = 58, whereas *R. h. hipposideros* had 2n = 54–56 (Zima et al., 1992; Zima, 2004; Volleth et al., 2013; Arslan and Zima, 2014; Kacprzyk et al., 2016). Nevertheless, no significant morphological differences were observed between the two subspecies of *R. hipposideros*. As a result, this study identified *R. midas* from eastern Oman and southern Iran as a sister species to *R. hipposideros*, reviving its name. Together, these two species comprise a distinct clade referred to as the *hipposideros* group, one of major clades within the *Rhinolophus* genus named *Phyllorhina*, whose relationships remained unresolved.

Paper 6

Uvizl, M., Kotyková Varadínová, Z., & Benda, P. Phylogenetic relationships among horseshoe bats within the *Rhinolophus ferrumequinum* group (Mammalia, Chiroptera). *Under review in Zoologica Scripta.*

Contribution of MU: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – Original Draft, Visualization

The horseshoe bats related to *Rhinolophus ferrumequinum* constitute a distinctive *ferrumequinum* group. Recently, there were four species recognised within this group, namely *R. bocharicus*, *R. clivosus*, *R. ferrumequinum*, and *R. nippon* (Bogdanowicz, 1992; Csorba et al., 2003; Burgin, 2019), with vast distributions across the southern Palaearctic and eastern Afrotropic regions (Fig. 3; Csorba et al., 2003; Burgin, 2019). This group is part of the Afro-Palaearctic clade of the *Rhinolophus* genus, also known as subgenus *Rhinolophus*. It belongs to one of seven other species

groups within this clade (Horáček et al., 2000; Dool et al., 2016). The four species shared a close morphological resemblance and *R. bocharicus* and *R. nippon* were initially considered subspecies of either *R. ferrumequinum* or *R. clivosus*, and some other species were thought to belong to *ferrumequinum* group. However, based on molecular data, these species have been reclassified into other Afro-Palaearctic groups (e.g., Zhou et al., 2009; Demos et al., 2019). Of the group's species, *R. bocharicus* has the most restricted geographical range, being mainly limited to Central Asia (Csorba et al., 2003; Benda & Gaisler, 2015). Although molecular studies confirmed its classification as a distinct species, its exact phylogenetic position within the *ferrumequinum* group remains unclear (Bailey et al., 2016). Currently, the species has been considered to be monotypic (Simmons, 2005; Burgin, 2019). Conversely, *R. clivosus* has a wide distribution, ranging from the Levant and northern Africa, through the Arabian Peninsula, to southern Africa (Burgin, 2019). The species has been extensively studied with molecular methods (Benda & Vallo, 2012; Stoffberg et al., 2012; Dool et al., 2016; Demos et al., 2019). It has also been suggested that the species is polytypic (Koopman, 1994; Csorba et al., 2003; Simmons, 2005; Benda & Vallo, 2012; Burgin, 2019). *Rhinolophus ferrumequinum* is a well-studied species found in the area from western Europe and northern Africa to India (Burgin, 2019). Multiple molecular studies looked into the intra-specific relationships within this species (Flanders et al., 2009, 2011; Stoffberg et al., 2010; Benda & Vallo, 2012; Dool et al., 2016; Demos et al., 2019), leading to the recognition of *R. ferrumequinum* as a monotypic species (Benda et al., 2012; Burgin, 2019). The fourth species, *R. nippon*, was once thought to be a subspecies of *R. ferrumequinum* found in eastern Asia, however, recent molecular studies have identified it as a distinct monotypic species with a range from southern China to Japan (Flanders et al., 2009, 2011; Koh et al., 2014; Burgin, 2019). While this group has been thoroughly examined using molecular methods (Benda & Vallo, 2012; Stoffberg et al., 2012; Bailey et al., 2016; Dool et al., 2016; Demos et al., 2019), none of these studies analysed all four species collectivelly.

Therefore, our aim was to revise all the species together to determine the relationships among the populations within the *ferrumequinum* group. The phylogenetic relationships and intraspecific variations were investigated using a genetic approach. One mitochondrial marker and five nuclear markers of almost 180 samples of three species were sequenced, and available sequences of remaining *R. nippon* as well as outgroups were supplemented from the GenBank. This enabled the construction of phylogenetic trees and network, the genetic delimitation of species, and the estimation of divergence times.

The study results revealed five major lineages within the *ferrumequinum* group, instead of the presently recognised four. A new species has, thus, been identified in addition to the previously identified four species within the *ferrumequinum* group. As the first lineages, *Rhinolophus bocharicus* formed a distinct monotypic species. The position of this species within the *ferrumequinum* group varied according to the marker used. The second and third lineages were formed from *R. clivosus* samples. The second lineage was identified in northern Africa, Levant, the Arabian Peninsula, and Socotra, while the third lineage was located in eastern and southern Africa, ranging from Ethiopia to South Africa. The differences between these two major groups led to the suggestion that these groups represent two distinct species. *Rhinolophus clivosus* was described in Arabia, leading to this name being used for populations within the second lineage. Regarding the third lineage, the prior name available for this species is *R. acrotis*. Furthermore, both *R. clivosus* and *R. acrotis* were further divided into two subspecies each. The fourth and fifth lineages, comprising *R. ferrumequinum* and *R. nippon*, respectively, were found to be monotypic. Moreover, the findings demonstrated that *R. clivosus* from northern Africa and the Levant underwent historical

introgression, which led to the replacement of its mtDNA with that of *R. ferrumequinum*. Overall, this study has presented a new *Rhinolophus* species and has expanded the total number of species in the *ferrumequinum* group to five, contradicting recent opinions (Burgin, 2019). The interspecific relationships within the *ferrumequinum* group remained unresolved as there is discrepancy between the phylogenetic trees generated from the mitochondrial and nuclear data, resulting in different topologies.

Paper 7

Benda, P., **Uvizl, M.**, Eiseb, S., & Avenant, N. On the systematic position of the horseshoe bats (Mammalia: Chiroptera) from Lesotho. *Under review in Mammalia.*

Contribution of MU: Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – Review & Editing, Visualization

The horseshoe bats of the genus *Rhinolophus* includes several species groups whose representatives may exhibit similar morphological traits due to convergence of the phenotypes of species from similar habitats (e.g., Csorba et al., 2003; Benda & Vallo, 2012; Jacobs et al., 2013). Traditionally, the single *Rhinolophus* species found in Lesotho has been attributed to *R. clivosus* s.l., a member of the *ferrumequinum* group known to inhabit African deserts and savannas (Lynch & Watson, 1990; Lynch, 1994; Taylor, 2005; Monadjem et al., 2010, 2020b; Benda & Vallo, 2012). However, recent taxonomic revisions have led to the description of three new species (Taylor et al., 2012) and the reassignment of one species from southern Africa from the *ferrumequinum* group to the *fumigatus* group (Jacobs et al., 2013). These findings suggest a close morphological affinity between the *ferrumequinum* and *fumigatus* groups, with the two groups being in a sister position (Maree and Grant, 1997; Stoffberg et al., 2010; Benda & Vallo, 2012; Dool et al., 2016; Demos et al., 2019).

In this study, we conducted genetic and morphologic analyses to determine the position of horseshoe bats from Lesotho within the *ferrumequinum* group. While examining another population from southern Africa, we compared the Lesotho *Rhinolophus* species with species from the *fumigatus* group due to their close relatedness to the *ferrumequinum* group. Our aim was to contribute to the knowledge of *Rhinolophus* species found in southern Africa using morphometric comparison of cranial and dental measurements, body size, and conducting genetic analysis of mitochondrial and nuclear markers.

The genetic results indicated that the Lesotho bats belong to the *fumigatus* group instead of the *ferrumequinum* group, where they were previously classified as part of the *R. clivosus* s.l. However, the mitochondrial and nuclear analyses produced two distinct phylogenetic trees. In the mitochondrial tree, the Lesotho bats shared haplotypes with the South African lineage of *R. damarensis*, while the sequences of Namibian *R. damarensis* were divided into two separate lineages. In contrast, the Lesotho bats and one South African bat formed a distinct lineage from a single *R. damarensis* lineage within the *fumigatus* group in the nuclear tree. Morphologically, they are only minimally distinguishable from other species in the *fumigatus* and *ferrumequinum* groups. The morphologic analysis provided evidence of their independence from *R. damarensis.* Therefore, while there has been historical introgression of mtDNA from *R. damarensis* to the Lesotho bats, the nuclear results suggested that the Lesotho horseshoe bats may be identified as a distinct species of horseshoe bats from Lesotho and South Africa. This bat species inhabits the mountainous areas of Lesotho and has also been detected at two sites in South Africa. No available name for this horseshoe

bat species can be found in the synonymy of the genus *Rhinolophus* (Allen, 1939; Ellerman et al., 1953; Roberts, 1954; Csorba et al., 2003; Simmons, 2005; Monadjem et al., 2020b). Therefore, this new *Rhinolophus* species from Lesotho and South Africa, was described under a new name, which extended the diversity of this genus in southern Africa (e.g., Taylor et al., 2012, 2018).

Paper 8

Benda P., **Uvizl M.**, Šklíba J., Mazoch V., & Červený J. (2022). African bats in the collection of the National Museum, Prague (Chiroptera). I. Bats from Zambia. *Lynx, n. s.*, 53, 291–332. https://doi.org/10.37520/lynx.2022.021

Contribution of MU: Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – Review & Editing

The genetic identification of bats is crucial because of their cryptic diversity (e.g., Mayer and von Helverson, 2001; Clare, 2011). Many animal species, including bats, have not had their distribution ranges accurately mapped due to incomplete exploration of their distribution ranges (Razgour et al., 2016). This knowledge gap even affects Zambia, a southern African country mainly covered by woodland savannas. Compared to other African countries, the bat fauna of this country is relatively well-known. According to the most recent taxonomic compendium, 73 bat species have been reported in Zambia (Ansell, 1978; Monadjem et al., 2020b). The National Museum in Prague (NMP) houses a small collection of bats from Zambia, which can expand our knowledge of Zambian bats.

The objective of our study was to catalogue all Zambian bats held in the collections of National Museum in Prague, in the context of the most recent and comprehensive compendium of bats of Zambia and surrounding countries by Monadjem et al. (2020b). Morphologic assessments were conducted on all bats, with molecular identification required for numerous species and/or species groups that were not identifiable by morphometric analysis, such as hipposiderids or pipistrelle-like vespertilionids.

The NMP collection of Zambian bats comprises 139 specimens from 32 species across eight families. The assessment confirmed the presence of two additional species in the Zambian fauna, *Afropipistrellus grandidieri* and *Neoromicia somalica*, and provided molecular evidence for the presence of *Miniopterus natalensis* s.str. in the country. In addition, the collection of bats from new locations enabled a more precise determination of the distribution ranges of some other species. Overall, the number of known bat species in Zambia has increased to 76, with 42.1% of them being housed within the NMP collection. This significantly contributes to the knowledge of the distribution and physical traits of the bat fauna.

General summary

This thesis comprised eight studies with common goals, and the results have provided new insights into the bat phylogeny. The primary aim was to investigate the diversity present within bat groups that have not yet been revised using molecular methods or those for which not all populations were previously included in the studies. The objective of this thesis was to fill the gaps and complement the knowledge of species affiliation and distribution of taxa in the *Myotis nattereri* complex in the western Palaearctic range using molecular and morphometric approaches (**Paper 1**). Then, we conducted a thorough analysis of genetic data from numerous Asian populations of *Myotis emarginatus* and compared them extensively with populations in Europe and North Africa. In addition, we conducted a detailed morphologic comparison of numerous populations, including ones from Asia, and tried to reconstruct interspecific relationships within the African *Myotis* clade (**Paper 2**, **Paper 3**). Next, we performed a multi-locus genetic analysis using data from Middle Eastern populations of three species of Emballonuridae family to ascertain their phylogenetic positions, determine intraspecific differentiation levels, and contribute to the group's taxonomy (**Paper 4**). Our analysis of genetic and morphological characteristics of representative specimens revealed unexpected diversity within the *Rhinolophus hipposideros* group (**Paper 5**). Then, we produced multi-locus genetic data for the *R. ferrumequinum* group to explore phylogenetic relationships among and within the species in this group (**Paper 6**). We also conducted similar analyses of comprehensive genetic data to study the only *Rhinolophus* population in Lesotho. Our results displayed a distinct separation of this population within the *R. fumigatus* group (**Paper 7**). Finally, the molecular and morphologic analyses helped to sort the Zambia bat variation found in NMP collections (**Paper 8**).

The aim of the Paper 1 was to fill the gaps in knowledge reagrding the distribution range of the *Myotis nattereri* species complex in the Middle East. The study revealed that *M. nattereri* s.str. is distributed as far east as western Turkey, *M. hoveli* is found in the eastern Mediterranean including Cyprus, *M. tschuliensis* inhabits a region stretching from Crimea to Turkmenistan, and larger *M. schaubi* is confined to a small area in Armenia and Iran. Overall, the research provided insight into the relationships among the *M. nattereri* species complex in the Middle East.

The findings of the Paper 2 and 3 confirmed that *M. emarginatus* is a polymorphic species within the African clade of the genus *Myotis*. The phylogenetic tree revealed two mitochondrial lineages separated by a 400km gap in distribution between the eastern Mediterranean and eastern Middle East. This arrangement supported the previous morphological diversification into two distinct morphotypes – one from Europe, North Africa, and the eastern Mediterranean, and the other from the Middle East and Central Asia. However, one nuclear lineage suggests that *M. emarginatus* is a single, broadly distributed species. The subsequent statistical analysis identified up to four distinct morphotypes: the first from Europe and North Africa, the second from the eastern Mediterranean, the third from the southern Middle East, and the fourth from Crimea to Central Asia. When combined with available genetic data, it was revealed that the fourth morphotype may represent the third subspecies within *M. emarginatus*. Together, the subspecies of *M. emarginatus* are as follows: *M. e. emarginatus* is distributed in the European and African Mediterranean, including islands, as well as in western and central Europe and the Levant. *Myotis e. desertorum* is found in the south-eastern part of the Middle East, including southern Iran, Oman, and Afghanistan; while *M. e. turcomanicus* inhabits the north-eastern parts of the Middle East, Crimea, the Caucasus, and West Turkestan.

The Paper 4 presented study on the emballonurid bats from the Middle East, which was the first to use genetic methods on this group of bats. The genetic analysis showed that populations of *Coleura afra* found in southern Arabia are distinct from other *C. afra* populations present in Africa, indicating the presence of a separate species – *C. gallarum*. In *T. nudiventris*, two mitochondrial lineages support the separation of larger bats from Mesopotamia and smaller bats from southern Arabia. However, the analysis of nuclear genes did not support this separation, indicating only one nuclear lineage. Based on this outcome, we suggested recognising two subspecies of *T. nudiventris* in the Middle East. The genetic structure of *T. perforatus* indicates low genetic variation across the area, demonstrating the presence of a single subspecies of *T. perforatus* in the Middle East. Moreover, our results do not support the subgenus *Liponycteris* as a distinct unit within the genus *Taphozous*.

The taxonomic arrangement of the *Rhinolophus hipposideros* group presented in the Paper 5 differed significantly from recent views. However, the analysis of genetic markers and echolocation traits provided evidence that the lesser horseshoe bats from Oman were a distinct yet closely related species to *R. hipposideros*. Further morphological research has revealed that these bats shared the same traits as those originally described for *R. midas* from Iran, which was later synonymised with *R. hipposideros*. In this study, we revived the name *R. midas*, inhabiting a small area in eastern Oman and southern Iran, as a sister species to *R. hipposideros*. Together, these two species form a distinct clade known as the *hipposideros* group, which is one of the major clades within the *Rhinolophus* genus. In addition, *R. hipposideros* s.str. can be divided into two subspecies: *R. h. hipposideros* in the Maghreb and Europe and *R. h. minimus* in Crimea, Caucasus, the Middle East, and north-eastern Africa. This division was supported by genetic and karyotypic data.

In the Paper 6, we presented a revision of the inter- and intraspecific relationships among the horseshoe bats in the *ferrumequinum* group. Our study revealed five major lineages within the *ferrumequinum* group, instead of the four currently recognised. We also identified a new species in addition to the previously identified four species within the *ferrumequinum* group. The analysis of nuclear data revealed two lineages within *R. clivosus*. The first nuclear lineage was found in northern Africa, Levant, the Arabian Peninsula, and Socotra, while the second lineage was found in eastern and southern Africa, spanning from Ethiopia to South Africa. The first lineage belongs to R. *clivosus* s. str., for the second lineage, the prior name available is *R. acrotis*. This would increase the total number of species in the *ferrumequinum* group to five. The results also revealed that *R. clivosus* experienced historic introgression from northern Africa and the Levant, leading to the replacement of its mtDNA with that of *R. ferrumequinum*. Nonetheless, the interspecific relationships within the *ferrumequinum* group could not be resolved due to variable results depending on the marker used.

The Paper 7 presented a novel perspective on the phylogenetic position and relationships of Lesotho horseshoe bats. Traditionally, these populations were considered part of *R. clivosus* s.l. species rank in the *ferrumequinum* group. Nevertheless, the genetic analysis of mitochondrial and nuclear markers conducted in this study revealed that the Lesotho bats belong to the *fumigatus* group instead of the *ferrumequinum* group. The mitochondrial data revealed that the Lesotho bats were grouped with the South African lineage of R. *damarensis*, while the sequences of Namibian *R. damarensis* resulted in two separate lineages. In contrast, the Lesotho bats and one South African bat formed a single, distinct lineage within the *fumigatus* group in the nuclear tree. The morphologic analysis also supported their independence from *R. damarensis.* Therefore, while there has been historical introgression of mtDNA from *R. damarensis* to the Lesotho bats, the nuclear results indicate that the Lesotho horseshoe bats may be identified as a distinct species of horseshoe bats

from the mountainous areas of Lesotho. This species has also been detected at two sites in South Africa. The discovery of this new species further increases the species diversity within the genus *Rhinolophus.*

Finally, the Paper 8 presented the NMP collection of Zambian bats that significantly contributed to the knowledge of both the distribution and physical traits of this bat fauna. The collection included 32 species, two of which are new to the Zambian bat fauna, and one species whose occurrence was genetically confirmed. The collection of bats from new locations allowed for a more precise determination of the distribution ranges of some other species. As a result, the number of known bat species in Zambia has increased to 76 species.

Altogether, these publications have made a contribution to our understanding of the phylogeny, taxonomy, and distribution of bats in the western part of the Old World. Consequently, two species have been raised from the subspecific level, one species has been recognised as a previously synonymised name, and one species has been newly described. Conversely, several subspecies that were previously recognised as subspecies are now considered to be junior synonyms. Therefore, these studies hold potential for further research on the evolution and systematics of bats, particularly in the groups such as Emballonuridae and Rhinolophidae, where the findings suggested additional unresolved relationships.

Taxonomic implications

This subchapter lists all the taxonomic implications made in the publications of this thesis, including the species for which our publications are referenced in the Mammal Diversity Database:

1) *Myotis hoveli*, *M. nattereri*, and *M. tschuliensis*. The Paper 1 supported the validity of *M. hoveli* and *M. tschuliensis* as separate species and demonstrated their position in relation to *M. nattereri* s.str. Additionally, the research expanded the knowledge on the distribution of these three species.

2) *Myotis emarginatus* and its subspecific diversity. Originally, four subspecies were recognised within *M. emarginatus*. Molecular revision in the Paper 2 supported the validity of two of them, *M. e. emarginatus* and *M. e. desertorum*. The morphologic synthesis in the Paper 3 also brought the evidence for the validity of a third subspecies, *M. e. turcomanicus*. Additionally, the fourth original subspecies, *M. e. kuzyakini* was considered a junior synonym of *M. e. turcomanicus*.

3) *Coleura afra* and *C. gallarum*. *Coleura gallarum* was currently considered a subspecies or synonym of *C. afra*. However, the results of the Paper 4 brought evidence that the populations of *C. afra* from the Arabian Peninsula and north-western Africa significantly differ from *C. afra* population from the remaining part of its distribution, based on molecular and morphologic analyses. Therefore, the former populations were suggested to recognise as a separate and fourth *Coleura* species, *C. gallarum*.

4) *Taphozous nudivetris* and *T. perforatus*. Several subspecies were recognised within these two species, but only three and two subspecies, respectively, were known to inhabit the Palaearctic region. Our results suggested that in the case of *T. nudiventris*, there are only two valid subspecies, *T. n. nudiventris* and *T. n. magnus*. Furthermore, in the Paper 4, we proposed that the third subspecies, *T. n. zayidi*, is a synonym of the nominotypical subspecies.

The study of *T. perforatus* revealed that only lineage of the nominotypical subspecies is present in the Palaearctic region. The second subspecies, *T. p. haedinus*, which was originally thought to be restricted to this region, is not limited to it. It is therefore difficult to comment on the validity of this subspecies, being only that it is not distributed in the Palaearctic region.

5) *Liponycteris*. This subgenus of *Taphozous* has traditionally been distinguished from subgenus *Taphozous* based on the morphologic differences. Moreover, it comprised two species, *T. hamiltoni* and *T. nudiventris*. Our results in the Paper 4 indicated that subgenus *Liponycteris* is paraphyletic to subgenus *Taphozous* when *T. nudiventris* is nested among other *Taphozous* species. Hence, *Liponycteris* may now only consist of *T. hamiltoni* or it may be a synonym of subgenus *Taphozous*.

6) *Rhinolophus hipposideros* and *R. midas*. Several subspecies were described and later synonymised within *R. hipposideros.* Currently, this species was considered monotypic, although distinct population groups were recognised based on the morphologic, karyotypic, and molecular analyses. A study in the Paper 5 revealed a deep split between populations found in Oman and those distributed elsewhere in the distribution. Based on morphologic and echolocation data, we suggested elevating the Omani population to the specific level using the available name *R. midas.* Its type locality lies just across the Strait of Hormuz in Iran. Furthermore, our findings indicated that *R. hipposideros* s.str. is not monotypic, but instead consist of two subspecies*, R. h. hipposideros* and *R. h. minimus*.

7) *Rhinolophus acrotis* and *R. clivosus*. *Rhinolophus clivosus* was previously regarded as a widely distributed species, with numerous subspecies described within its distribution range. However, a molecular revision in the Paper 6 has established that the species can be geographically divided into four mitochondrial lineages, whilst only two nuclear lineages exist. The significant genetic distance between these two *Rhinolophus* lineages justified the proportions of *R. clivosus* s.l. being split into two distinct species, *R. clivosus* s.str and *R. acrotis*. The observed distance was comparable to that between other *Rhinolophus* species. Additionally, further subdivision into *R. c. clivosus* and *R. c. socotranus*, and *R. a. acrotis* and *R. a. augur* was hinted at by mitochondrial lineages.

8) *Rhinolophus bocharicus*, *R. ferrumequinum* and *R. nippon*. These three species were subjected to the molecular revision in the Paper 6. The positions of *R. bocharicus* and *R. nippon* were previously described as sometimes being subspecies of *R. ferrumequinum*. However, our study demonstrated that all three species are valid and belong to the *ferrumequinum* group, along with *R. acrotis* and *R. clivosus*. Intra-specifically, all three species in question showed rather low diversity and thus, they were considered monotypic.

9) *Rhinolophus XXX sp. nov.* from Lesotho. Another population of *Rhinolophus* found in Lesotho was previously thought to be *R. clivosus* (=*R. acrotis*) belonging to *ferrumequinum* group. Nonetheless, the molecular analysis in the Paper 7, showed a strong mitochondrial affinity to *R. damarens*is, and in the nuclear tree, it formed separate clade within *fumigatus* group. Hence, this lineage was identified as a new species distributed in Lesotho and South Africa. Note that the name of this species will be revealed after the journal review.

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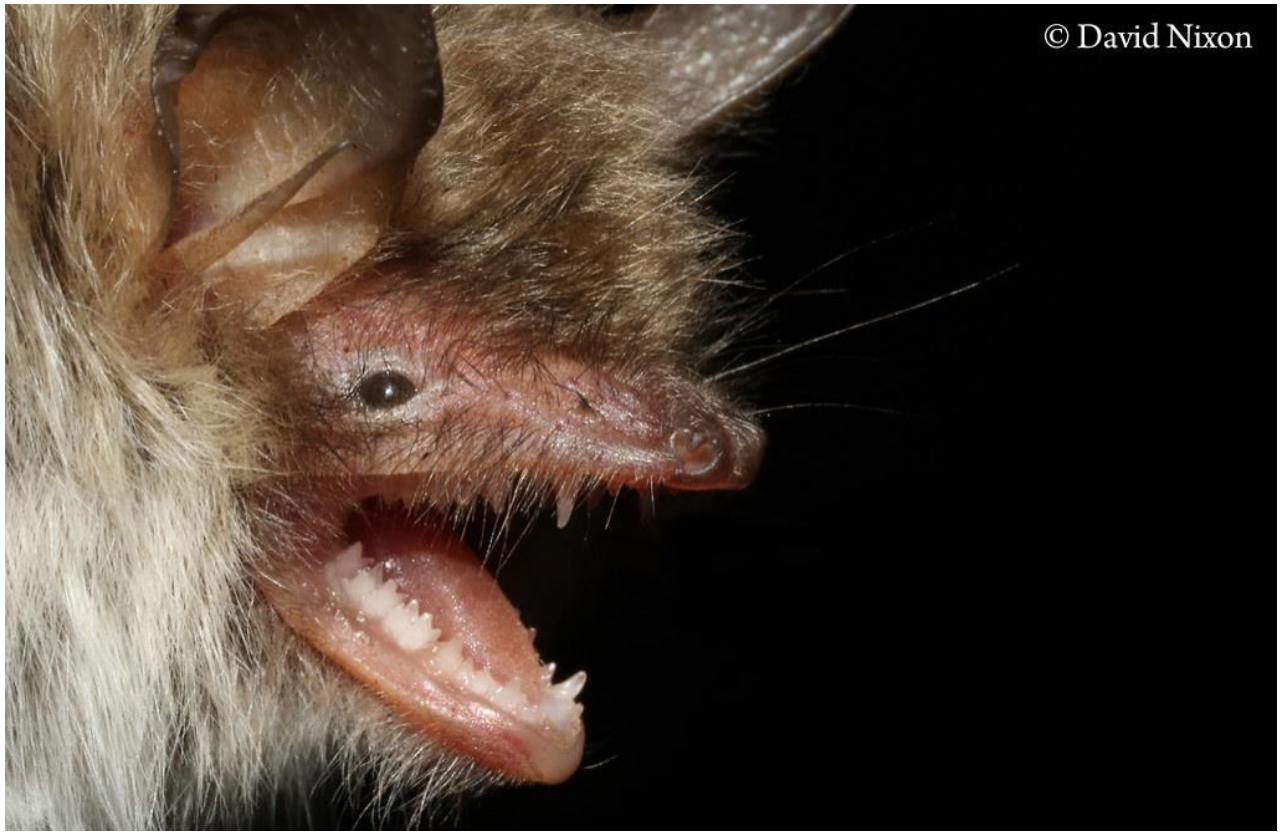
Publications

Paper 1: *Myotis nattereri* complex in the Middle East

Uvizl M. & Benda P. (2021). Diversity and distribution of the *Myotis nattereri* complex (Chiroptera: Vespertilionidae) in the Middle East: filling the gaps. *Mammalian Biology*. 101, 963–977.



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ORIGINAL ARTICLE

Diversity and distribution of the *Myotis nattereri* complex (Chiroptera: Vespertilionidae) in the Middle East: filling the gaps

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Abstract
Myotis nattereri represents a species complex that recently underwent taxonomic changes. Based on morphological evidence, two species were regarded to occur in the western Palaearctic; *M. nattereri* in Europe, Maghreb, Middle East, and Turkmenistan, and *M. schaubi* limited to Armenia and north-western Iran. Within *M. nattereri* sensu lato, several cryptic species were recently revealed using the morphological and molecular genetic approaches (*M. escalerai*, *M. zenatius*, *M. crypticus*, *M. tschuliensis*, *M. hoveli*), restricting *M. nattereri* s.str. to the temperate zone of Europe and the Balkans. Our aim was to complement the knowledge of diversity and distribution of the *M. nattereri* complex in the Middle East with help of molecular genetic (mitochondrial ND1 gene) and morphometric analyses. In this region, four genetic lineages of the complex belonging to two clades and three primarily size-defined morphotypes were confirmed in accordance with the previous studies. This mosaic represents four species, *M. nattereri* s.str., *M. hoveli*, *M. tschuliensis*, and *M. schaubi*, and all these species were demostrated to occur in allo- or parapatry to each other. *Myotis nattereri* s.str. was found only in western Anatolia and in the Aegean island of Samos. The occurrence *Myotis hoveli* was shown in the Levantine range of this species complex (Jordan, Israel, Syria, Lebanon, Cyprus, southern Anatolia) and also in the mountainous areas of eastern Turkey and northern Iraq. The range of *Myotis tschuliensis* represents a belt stretching from Crimea, via the Caucasus, Transcaucasia and northern Iran, to Turkmenistan. *Myotis schaubi* was confirmed only in its very restricted range in Iran and Armenia.

Keywords Bats · Molecular phylogeny · Morphometry · Palaearctic region · Systematics

Introduction

The taxonomy of the genus *Myotis* Kaup, 1829 underwent numerous changes in the recent years, the traditional intrageneric positions of many species were challenged and a number of species were newly described (see e.g., Ruedi et al. 2013, 2015, 2021; Csorba et al. 2014; Patterson et al. 2019; etc.). Among others, such changes concerned also the Palaearctic species complex of *Myotis nattereri* (Kuhl, 1817). For a long time, this complex was regarded as a single polytypic species *M. nattereri*, distributed in two well separated regions, in the Far East including Japan, and in the western Palaearctic, including Europe, the Maghreb, and the Middle East up to southern Turkmenistan (Tate 1941; Ellerman and Morrison-Scott 1951; Kuzâkin 1965; Corbet 1978; etc.). Based on detailed morphologic examination of mate-rial from the whole range and including also fossil find-ings, Horáček and Hanák (1984) revised the intraspecific taxonomy of *M. nattereri* s.l. and suggested to distinguish three separate species instead of one in this complex; *M. bombinus* Thomas, 1906 in the Far East, *M. schaubi* Kor-mos 1934 in the southern Caucasus and northern Iran, and *M. nattereri* in a large part of Europe, parts of the Middle East, and in the western Maghreb. The latter two species were reported to occur in sympatry in the southern Cauca-sus region, where populations of *M. nattereri* were referred to a separate subspecies, *M. n. tschuliensis* Kuzâkin, 1935, a form described from the Kopetdagh Mts. of Turkmenistan. The remaining populations of *M. nattereri*, occurring in the European, African and Levantine parts of the species range

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were regarded as the nominotypical subspecies, *M. n. nattereri*. This taxonomic arrangement was generally accepted by subsequent authors (Pavlinov and Rossolimo 1987; Koopman 1993, 1994; Borisenko and Pavlinov 1995; Horáček et al. 2000; Topál 2001; Simmons 2005; Benda et al. 2006; Aulagnier et al. 2008; Grimmberger and Rudloff 2009; etc.). Moreover, it was fully congruent with results of first molecular genetic analyses of the genus *Myotis* (Ruedi and Mayer 2001; Kawai et al. 2003).

However, additional genetic studies based on widely sampled material that started in the 2000s gradually discovered deep cryptic diversity in the populations referred to *M. nattereri* by Horáček and Hanák (1984), indicating that this taxon certainly comprised more than one species. Jones et al. (2006) reported a new mitochondrial lineage from north-eastern Turkey (Sarıkamış), which was sister to *M. schaubi* and not to *M. nattereri* of Europe; the authors tentatively named it *M. n. tschuliensis*, but suggested a possible species rank for it. Ibáñez et al. (2006) demonstrated the presence of further two separate cryptic lineages within the *Myotis nattereri* complex (hereafter also the *nattereri* complex) in the Iberian peninsula, deeply diverged from each other and from *M. nattereri* s.str. from central Europe. One of the Iberian lineages, widespread across the peninsula, was co-dentified with the name *M. escalerai* Cabrera, 1904 by Ibáñez et al. (2006), while the other lineage, found only in northern Spain, remained unnamed. Mayer et al. (2007) revealed a cryptic lineage in the Alps of Italy and Austria, deeply diverged from *M. nattereri* s.str. from northern part of central Europe. In a next step, García-Mudarra et al. (2009) confirmed the unity of the lineage from northern Spain with that from the Alps and also discovered an additional lineage within the *nattereri* complex, occurring in the western Maghreb. Finally, one more cryptic lineage of the complex was described from Corsica by Puechmaille et al. (2012).

In total, four new genetic lineages were discovered in the central and western Mediterranean, while in the Balkans, only *M. nattereri* s.str. was found, similarly as in the temperate parts of Europe (see also Salicini et al. 2011). Interestingly, most of these lineages occur in allo- or parapatry, nevertheless, they are referred to cryptic species due to significant genetic distances among them. These species were, except for the two named forms, *M. nattereri* and *M. escalerai*, for several years tentatively treated as *Myotis* sp. A, B, and C. Later on, the former two unnamed *Myotis* spp. were described as new species, *M. crypticus* Ruedi, Ibáñez, Salicini, Juste et Puechmaille, 2018 (sp. A) and *M. zenatius* Ibáñez, Juste, Salicini, Puechmaille et Ruedi, 2018 (sp. B) by Juste et al. (2018), while *Myotis* sp. C still remains formally undescribed. The final taxonomic division of the *nattereri* complex in Europe and North Africa could be summarised as follows: *M. nattereri* occurs in most of the temperate zone of Europe and in the Balkans, *M. escalerai* in Iberia, Balearic Islands, and southernmost France, *M. crypticus* in the Alps, Italian peninsula, southern France, and northern Spain, *M. zenatius* in the western Maghreb (Morocco and Algeria), and *Myotis* sp. C in Corsica only (Puechmaille et al. 2012; Juste et al. 2018). On the other hand, the taxonomic situation of the *nattereri* complex in the eastern part of its West-Palaearctic range, in the Middle East (here understood as including also the Caucasus region, Crimea, and Turkmenistan), was for a long time understudied with the help of molecular genetics.

While the differentiations among the lineages/species in the western part of the complex distribution were issued primarily from genetic differences that were followed by a search for some physical characters (see e.g. Puechmaille et al. 2012) to help the identification (besides the geographical afiliation of a lineage, where it is clear), in the eastern populations, the differentation among the taxa was given primarily by their morphometric traits. Bats of the *nattereri* complex are represented by three morphotypes related to body size within the Middle East (Horáček and Hanák 1984; Benda et al. 2006); the small-sized morphotype occurs in the west, in the western and southern coasts of Turkey, Cyprus, and in the Levant [greatest length of skull (LCr) 15.1–16.7 mm]; the medium-sized morphotype occurs in eastern Turkey, northern Iraq, Greater Caucasus Mts., Transcaucasia, northern Iran, and southern Turkmenistan (LCr 15.6–16.7 mm); and the large-sized morphotype in Armenia and north-western Iran (LCr 16.8–17.5 mm); for details see Benda et al. (2006, 2007, 2010, 2011, 2012). These three morphotypes were evaluated as three taxa, the small-sized morphotype as *M. n. nattereri*, the medium-sized morphotype as *M. n. tschuliensis*, and the large-sized morphotype as *M. schaubi* (Horáček and Hanák 1984). From the range of the small-sized morphotype, a new subspecies was decribed as *M. nattereri hoveli* Harrison 1964 from near Jerusalem, Israel (Harrison 1964). However, detailed morphological comparisons did not substantiate such taxonomic distinction (Horáček and Hanák 1984; Benda et al. 2006).

Until recently, minimum samples of the Middle Eastern populations were examined genetically: only two bats of *M. schaubi* from Iran by Ruedi and Mayer (2001) and Salicini et al. (2011), one individual assigned to *M. n. tschuliensis* from north-eastern Turkey by Jones et al. (2006), three specimens from southern Turkey assigned to *M. schaubi* by Çoraman et al. (2013) for obscure reasons, and one bat from Syria by Ruedi et al. (2013). Based on findings by Jones et al. (2006), Benda et al. (2006, 2011, 2012) suggested to consider the medium-sized morphotype as a separate species, *M. tschuliensis*. Additionally, Salicini et al. (2011) first noted the discrepancy between the nuclear and mitochondrial topologies within the *nattereri* complex in the position of *M. schaubi*, relative to the positions of four west-Mediterranean taxa, *M. nattereri*, *M. escalerai*, *M. crypticus*, and *M.*

zenatius. Such discrepancies were later discovered in more populations of the complex.

The taxonomic situation changed recently, when a broad-scale revision of the phylogenetic relationships within the *nattereri* complex in the western Palaearctic was published by Çoraman et al. (2019). Results of this revision confirmed the previous delimitations of the lineages in Europe and Africa as described above and summarised by Salicini et al. (2011), Puechmaille et al. (2012), and Juste et al. (2018). However, besides these five western lineages, Çoraman et al. (2019) defined three other lineages that occur in the Middle East, which they interpreted as full species; one in Israel and southern Turkey, another in Transcaucasia and Crimea, and the last one in the southern Caucasus and north-western Iran. The latter lineage found in Iran is clearly assigned to *M. schaubi*, and the Israeli-Turkish lineage was named *M. hoveli*, because this name was described from Israel (Harrison 1964; Çoraman et al. 2019). The Caucasian-Crimean lineage was afiliated to *M. tschuliensis*, but only tentatively, since the material from the type locality of this name (Çüli, Turkmenistan) was not examined in this study. Additionally, the populations from western Turkey were identified by Çoraman et al. (2019) as *M. nattereri* s.str.

In summary, three body-size morphotypes occurring in the Middle East correspond to three main lineages; the large-sized morphotype to *M. schaubi*, the medium-sized morphotype to *M. tschuliensis* (sensu Çoraman et al. 2019), and the small-sized morphotype from the Levantine samples with *M. hoveli*. Surprisingly, the bats of the small-sized morphotype from western Turkey represent *M. nattereri* s.str., while the bats of the small-sized morphotype from Crimea belong to the *M. tschuliensis* lineage.

These taxonomic changes were later complemented by Kruskop and Solovyeva (2021), who confirmed conspecificity of the medium-sized form from Transcaucasia and true *M. tschuliensis* from Turkmenistan, and by Smirnov et al. (2020), who assigned to this lineage also the samples from the east of the Russian Caucasus (Daghestan). Nevertheless, in the map of the Middle East, some populations of unknown afiliation still remain, despite the fact that the phylogenetic relationships within the whole *nattereri* complex are now quite well-studied.

Therefore, we aimed here to complement the knowledge of the *nattereri* complex in the Middle East in its broader sense. For this purpose, we generated mitochondrial sequences from available museum specimens originating in this region and also gathered morphometric data from these specimens. Our aims were to identify taxonomic afiliation of particular populations with help of molecular genetic and/or morphometric approaches, and where possible, to determine geographical limits of particular phylogroups, e.g. the separation level in the insular Cypriot populations or geographical limits of the taxa occurring in the region.

Materials and methods

Molecular genetic analysis

We used muscle tissues of 68 museum specimens of the *Myotis nattereri* complex housed in the collection of the National Museum, Prague, Czech Republic (NMP) that were collected in various parts of the Middle East, including Iran, Syria, Jordan, Lebanon, and Cyprus, and biopsy samples taken from three individuals handled in the eastern Aegean island of Samos, Greece (Table S1). Of them, 52 specimens provided a DNA product.

The genomic DNA was extracted from alcohol-preserved tissue samples using Geneaid Genomic DNA Mini Kit. We targeted complete mitochondrial gene for NADH dehydrogenase subunit 1 (ND1), since it was used most frequently in previous studies dealing with bats of the *nattereri* complex (Ruedi and Mayer 2001; Ibáñez et al. 2006; Mayer et al. 2007; García-Mudarra et al. 2009; Salicini et al. 2011; Juste et al. 2018; Çoraman et al. 2019; Kruskop and Solovyeva 2021). The gene was amplified with the primers ER65 (5′-CCTCGATGTTGGATCAGG-3′) and ER66 (5′-GTATGGGCCCGATAGCTT-3; Dietz et al. 2016). The PCR amplifications were treated as in Dietz et al. (2016). The PCR products were Sanger-sequenced from both sides using the PCR primers by Macrogen, Inc. (Amsterdam, the Netherlands). Our dataset was supplemented with 85 ND1 sequences of the *nattereri* complex from the GenBank. As outgroups, the ND1 sequences of *Myotis emarginatus* (Geoffroy, 1806), *M. bechsteinii* (Kuhl, 1817), *Nyctalus plancyi* (Gerbe, 1880), and *Pipistrellus nathusii* (von Keyserling et Blasius, 1839) were included (Table S1).

Sequences were edited and aligned using the MAFFT plugin (Katoh and Standley 2013) in Geneious 11.0.5 (https://www.geneious.com), subsequently manually edited and trimmed using Gblocks (Castresana 2000). Ambiguous positions or missing data were coded with ‘N’. Indels were treated as missing data. Sequences were translated to aminoacids to check for the presence of stop codons, which would indicate pseudogenes have been amplified. The total length of the alignment was 650 bp.

Phylogenetic analysis of the dataset was run using Bayesian inference (BI) and maximum likelihood (ML). The appropriate nucleotide substitution model was selected based on the Bayesian information criterion (BIC) using ModelFinder (Kalyaanamoorthy et al. 2017). We used MrBayes v3.2.6 (Ronquist and Huelsenbeck 2003) to run the BI analysis. We ran two independent chains for 20 million generations with trees sampled every 1000 generations. All other parameters were set to default. Stationarity and convergence of the runs were

inspected in Tracer v1.6 (Rambaut et al. 2014) and the value of the average standard deviations of the split frequencies that were lower than 0.01. The burn-in fraction was left as the default at 25% of sampled trees. Thus, from the 20,000 produced trees, the initial 5000 ones were discarded. A majority-rule consensus tree was produced from the post-burn-in trees with posterior probability (PP) values embedded. The BI analyses were run through CIPRES Science Gateway (Miller et al. 2010). Then, we inferred the maximum likelihood tree using the partition model in IQ-TREE (Chernomor et al. 2016; Nguyen et al. 2015). Node support was performed by ultrafast bootstrap (UFBoot; Hoang et al. 2017) with 1000 bootstrap and 1000 topology replicates. To verify robustness of the ML tree, the branch support was evaluated using SH-like approximate likelihood ratio test (SH-aLRT; Guindon et al. 2010) and a Bayesian-like transformation of aLRT (aBayes; Anisimova et al. 2011). SH-aLRT was performed with 1000 replications. aBayes branch support was used instead of Bayesian posterior probabilites because aBayes is more conservative, more robust to model violation and moreover exhibits the best power (Anisimova et al. 2011). The ML, SH-aLRT and aBayes analysis were run on IQtree web server (Trifinopoulos et al. 2016). The branches were considered supported with values > 80 for SH-aLRT, > 0.95 for aBayes and > 95 for ML.

We inferred haplotype networks for the ND1 gene using the same dataset as for phylogenetic reconstruction but shortened to 380 bp. We used shorter alignment because GenBank sequences were shorter than the origial sequences. The networks were estimated by a Median-joining network analysis (Bandelt et al. 1999) using the software PopART v.1.07 (http://popart.otago.ac.nz). Genetic distances were estimated using the Tamura-Nei model (Tamura and Nei 1993) in MEGA7 (Kumar et al. 2016).

Morphometric analysis

For comparative morphometric analysis, we used skull craniodental measurements and the forearm length (LAt) as a standard dimension referring to the body size. The skulls were measured in a standard way using mechanical calliper with accuracy to 0.02 mm; horizontal dental dimensions were taken on cingulum margins of teeth. The examined museum materials as well as other comparative materials are given in the List S1. We evaluated eleven craniodental dimensions in each skull (see Abbreviations below) including several indices that described the skull shape. Statistical analyses were performed using the Statistica 6.0 software.

Abbreviations

Measurements LAt = forearm length; LCr = greatest length of skull (excluding incisors); LCb = condylobasal length (excluding incisors); LaZ = zygomatic width; LaI = width of interorbital constriction; LaN = neurocranium width; ANc = neurocranium height; CC = rostral width between the labial margins of upper canines; M^3M^3 = rostral width between the labial margins of third upper molars (M^3); CM^3 = length of upper tooth-row between the mesial margin of canine and distal margin of molar (M^3); LMd = condylar length of mandible (excluding incisors); CM_3 = length of lower tooth-row between the mesial margin of canine and distal margin of third molar (M_3).

Collection acronyms BMNH = Natural History Museum, London, United Kingdom; CUP = Department of Zoology, Charles University, Prague, Czech Republic; HNHM = Hungarian Natural History Museum, Budapest, Hungary; ISEA = Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Krakow, Poland; NMP = National Museum (Natural History), Prague, Czech Republic; NMW = Natural History Museum, Vienna, Austria; SMF = Senckenberg Museum and Research Institute, Frankfurt am Main, Germany; TAU = Tel Aviv University, Tel Aviv, Israel; WIC = Willy Issel Collection, Stuttgart, Germany; ZMMU = Zoological Museum, Moscow State University, Moscow, Russia.

Others Alc = alcoholic preparation; M = mean; max., min. = dimension range margins; S = skull; SD = standard deviation; Sk = stuffed skin (balg); Sn = skeleton; ♀ = female; ♂ = male.

Results

Molecular genetic analysis

In our study, we sequenced the ND1 gene from 52 specimens of *Myotis nattereri* complex which were pruned to 21 unique haplotypes. Together with the previously published sequences and outgroups, the complete matrix contained 90 sequences of 650 bp.

ModelFinder selected the TPM2u + F + I + G4 model as the best-fit model. Under this model, the ML and BI analyses produced well-supported topologies, very similar to each other. Both topologies were equally supported and we chose to show the ML tree (Fig. 1).

The phylogenetic tree showed four main clades among the examined samples of the *nattereri* complex. The first main clade [A in Fig. 1] included *M. nattereri* s.str. within a wide range of published haplotypes from Europe (France and Ireland to Greece and central Ukraine) and the western part of Asian Turkey (Anatolia). We enriched this clade

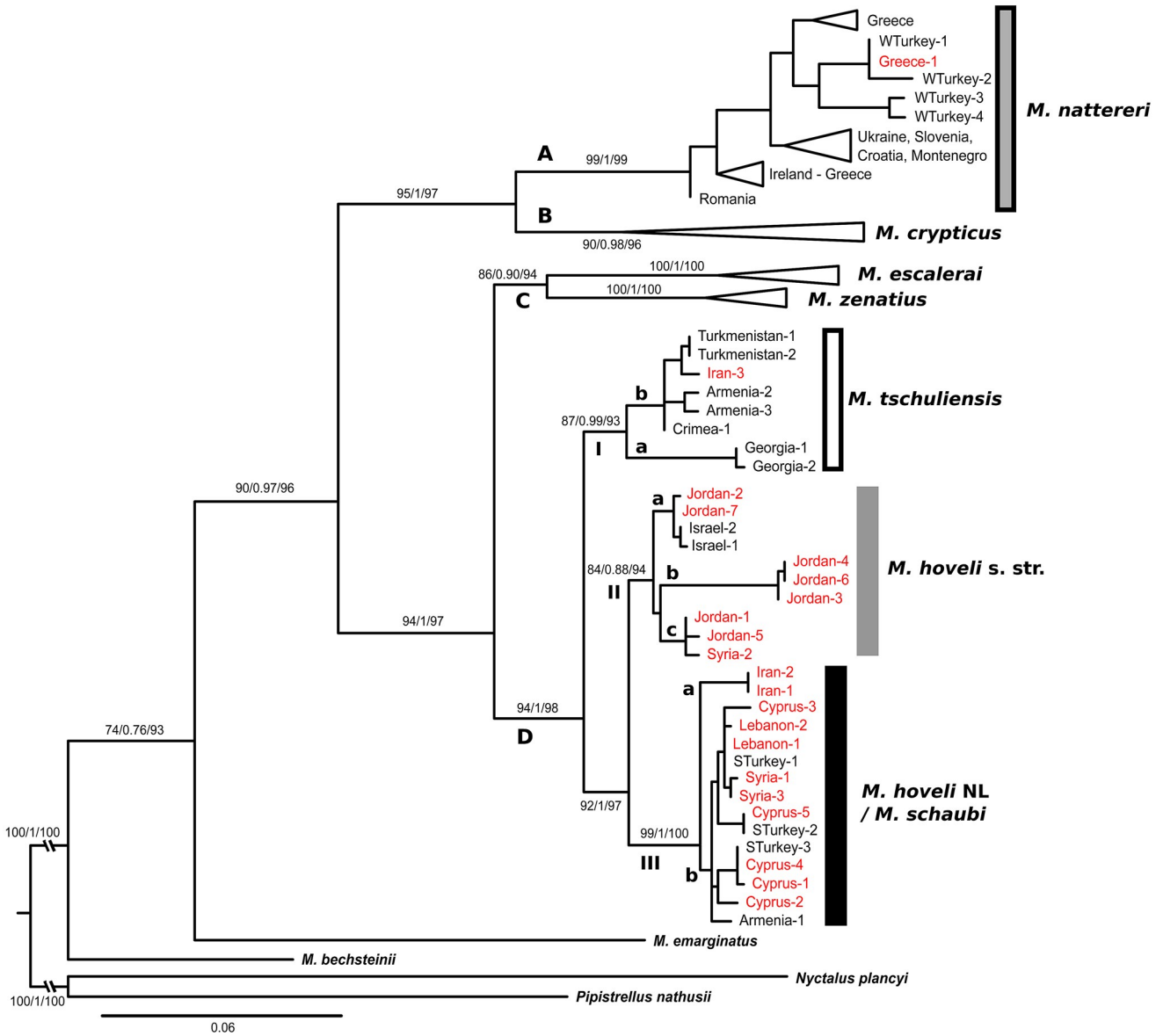

Fig. 1 Maximum-likelihood tree showing phylogenetic relationships among haplotypes of *Myotis nattereri* complex based on sequences (650 bp) of the mitochondrial ND1 gene. Branch support (SH-aLRT/aBayes/ML) is given at the nodes

with samples from the eastern Aegean island of Samos. The second clade [B] is sister to the previous one and included *M. crypticus*; this clade was subdivided into two subclades (not shown in Fig. 1) containing published haplotypes from Spain, Italy, Slovenia, and Austria. The two mentioned main clades [A + B] were sister to the rest of the samples of the *nattereri* complex that was again divided in other two other main clades [C + D]. The clade C was split in two groups, representing two sister species *M. escalerai* and *M. zenatius* from Spain and Morroco, respectively.

The last main clade [D], the most diversified one, contained three major groups [I–III], some of them receiving a slightly lesser support than inner groups of the previous clades (Fig. 1). The group I was in a basal position relative to the remaing groups II and III. It was formed by the samples of *M. tschuliensis* with two subgroups, one [I a] including the published haplotypes from Georgia, while the other subgroup [I b] contained the published haplotypes from Crimea, Armenia, and Turkmenistan, and new samples (in one haplotype) from northern Iran. The second group [II] of the last main clade [D] included the published haplotypes of *M. hoveli* from Israel and new samples from Jordan and southern Syria arranged in three subgroups [II a–c]. The first subgroup [II a] was composed of two published haplotypes from Israel and new samples from two north-westernmost localities known from Jordan (Kufranja Cave and Zubiya

Cave). The second subgroup [II b] contained haplotypes from new samples originating from isolated range in mountains of southern Jordan (Ash Shawbak Castle and Dhana Reserve). The third subgroup [II c] was composed of three haplotypes from new samples originating from Iraq Al Amir Cave near Amman, west-central Jordan, and from southernmost Syria (Talsh'hab).

The last group [III] of the clade D was in a sister position to the group II, and was diversified into two subgroups [a + b], even though this group was overall quite shallow concerning the branch lengths. The first subgroup [III a] was formed by the north-western Iranian samples, morphologically fitting *M. schaubi* (the large-sized morphotype, see below). The second subgroup [III b] contained a combination of bats belonging to the small-sized and the large-sized morphotypes, respectively. It comprised a mixture of new samples of the small-sized morphotype from Cyprus, Lebanon, and Syria, and published haplotypes of the small-sized morphotype from southern Turkey and of the large-sized morphotype from Armenia.

The relationships of haplotype groups/subgroups are illustrated by the percent values of genetic distances estimated for the examined gene (Table 1). While the mean p-distances among clades range between 6.96 and 13.29% (Tamura-Nei distances 7.50–15.53%), the mean distances among the groups of the clade D range between 3.71 and 5.72% (3.69–6.06%). The haplotype relationships of the clade D are also shown in a haplotype network (Fig. 2), where the sequences were shortened (380 bp) to include as much as possible published haplotypes.

In summary, representatives of two clades [A + D] of the *nattereri* complex were found in the Middle East. Bats of the clade A (*Myotis nattereri* s.str.) were found in western Anatolia and in the adjacent island of Samos. Bats of the clade D were found in the majority of the Middle Eastern part of the distribution range of the complex. The group I of this clade was found in areas creating a belt stretching from Crimea, via Transcaucasia and northern Iran, to southern Turkmenistan. The group II was revealed only in the southern Levant (south of ca. 33°10'N), in Israel, Jordan, and southernmost Syria. The group III represented two morphotypes from two separate regions of the Middle East, a small-sized bat from the northern Levant, including Lebanon, western Syria, southern Anatolia, and Cyprus, and a large-sized bat from north-western Iran and Armenia.

Morphometric comparison

The comparison of metric characters of bats of *Myotis nattereri* complex from the Middle East confirmed the existence of three morphotypes defined by the body and skull size. The large-sized morphotype (forarm length [LAt] 41.3–44.1 mm, mean [M] 42.8 mm; greatest length of skull [LCr] 16.8–17.5 mm, M 17.2 mm; Table 2) comprises the samples from north-western Iran and two specimens from Armenia. However, the small- and medium-sized morphotypes broadly overlap in their respective dimensions, but not with the large-sized morphotype (Fig. 3). Within the small- and medium-sized morphotypes, the morphotype affiliation of particular population is better defined by the mean values (see Table 2), since differences between dimension ranges are less distinctive. To the small-sized morphotype could be assigned the samples from western Anatolia (M LAt 40.3 mm, M LCr 15.9 mm), southern and eastern Anatolia (M LAt 40.2 mm, M LCr 15.9 mm), Cyprus (M LAt 40.4 mm, M LCr 15.9 mm), northern Levant (M LAt 40.5 mm, M LCr 15.8 mm), southern Levant (M LAt 40.1 mm, M LCr 15.9 mm), and Crimea (M LAt 39.1 mm, M LCr 15.4 mm), as well as from comparative samples from the Balkans (M LAt 40.2 mm, M LCr 15.7 mm; n = 32). The Crimean bats were on average the smallest representatives among the compared population samples. The medium-sized

Table 1 Mean percent values of the uncorrected p-distances (above diagonal) and Tamura-Nei distances (below diagonal) in the ND1 gene among populations of *Myotis nattereri* complex; the intrapopulation values are on the diagonal (bold typed)

		A	B	C	C	D I	D IIa	D IIb	D IIc	D IIIa	D IIIb
A	*M. nattereri*	**1.60***	8.96	12.52	10.82	12.67	10.97	11.28	10.82	11.28	11.75
B	*M. crypticus*	9.89	**3.25**	13.29	10.66	12.83	11.44	12.06	11.13	12.06	11.75
C	*M. escalerai*	14.48	15.53	**2.32**	7.42	8.50	8.19	8.35	8.35	9.43	9.43
C	*M. zenatius*	12.26	12.05	8.06	**2.15**	6.96	7.73	8.50	7.57	8.19	8.66
D I	*M. tschuliensis*	14.67	14.88	9.34	7.50	**2.16**	4.17	5.72	4.33	5.10	5.41
D IIa	*M. hoveli* s.str.	12.36	12.98	8.94	8.39	4.35	**0.28**	3.09	1.08	3.71	3.71
D IIb	*M. hoveli* s.str.	12.75	13.79	9.11	9.31	6.06	3.19	**0.10**	3.25	5.26	5.26
D IIIc	*M. hoveli* s.str.	12.19	12.60	9.14	8.22	4.52	1.09	3.36	**0.41**	3.55	3.55
D IIIa	*M. hoveli* NL / *M. schaubi*	12.82	13.83	10.46	8.96	5.37	3.85	5.55	3.69	**0.00**	2.16
D IIIb	*M. hoveli* NL / *M. schaubi*	13.41	13.40	10.47	9.52	5.72	3.86	5.56	3.69	2.21	**1.14**

See Fig. 1 for identification of the clade/group/subgroup. *Variation value only for the Greek-Turkish haplogroup

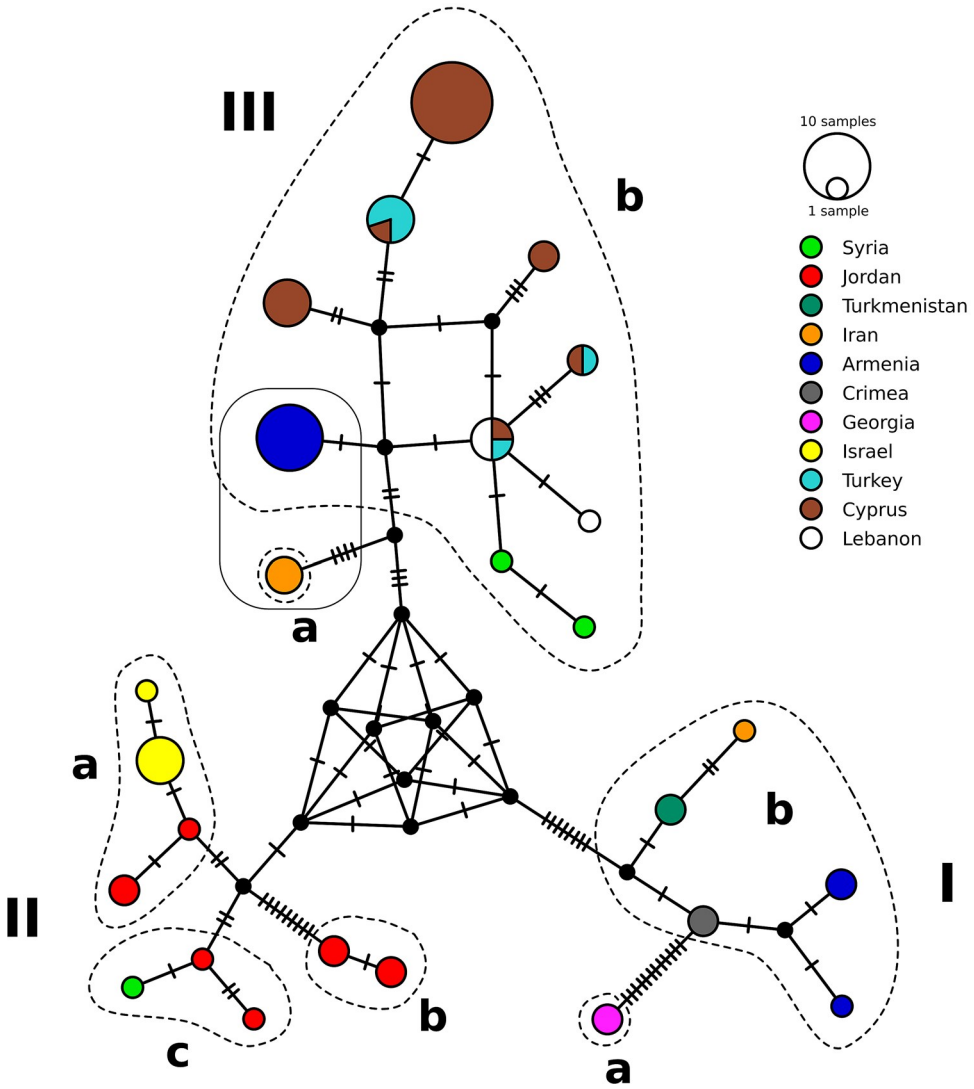

Fig. 2 Median-joining haplotype network of the clade D of *Myotis nattereri* complex based on 380 bp of the ND1 gene (see text for details). Circle sizes are proportional to the number of individuals with the particular haplotype. Mutation steps are shown as dashes across the branches, missing haplotypes are shown as small black circles

morphotype was found in the samples from Transcaucasia (M LAt 41.0 mm, M LCr 16.1 mm) and from the border region of northern Iran and southern Turkmenistan (M LAt 41.0 mm, M LCr 16.1 mm).

Besides the body and skull size, the samples differed also in skull shape, namely for rostrum and neurocranium. While in the most populations (small-sized morphotype from the W and S Middle East and Balkans, plus the large-sized morphotype from NW Iran and Armenia), the rostrum is relatively short and broad (M CM^3/LCb < 0.42 and CC/CM^3 > 0.65), in the populations from Crimea, Transcaucasia and Turkmenistan, the rostrum is relatively short and broad (M CM^3/LCb > 0.42 and CC/CM^3 < 0.64; Table 2); these two groups essentially do not overlap (Fig. 4). Additionally, bats of the medium- and large-sized morphotypes showed relatively low neurocranium (M ANc/LCr < 0.351), while bats of the small-sized morphotype showed relatively high neurocranium (M ANc/LCr > 0.353; Table 2).

The medium- and large-sized morphotypes occur in parapatry in Transcaucasia and can be regarded as parts of a size continuum comprising one large group instead of two (Fig. 3). However, these two groups could be distinguished based on the relative width of the interorbital constriction of skull, which decreases with the increasing of absolute skull size (Table 2). While the smallest representative of the large-sized morphotype (LCr 16.81 mm) showed relatively wider interorbital constriction (LaI 3.98 mm, LaI/LCr 0.237), the largest bat of the medium-sized morphotype (LCr 16.62 mm) had this dimension absolutely and relatively very narrow (LaI 3.49 mm, LaI/LCr 0.209), the narrowest from both sets of samples from Transcaucasia and all compared bats (Table 2).

On the other hand, samples of small-sized morphotype from western and southern Turkey and from the Levant including Cyprus, did not differ substantialy from each other in metric traits, including relative dimensions.

Table 2 Morphometric data on the examined sample of the *Myotis nattereri* complex from the Middle East

	n	*M*	min	max	SD	*n*	*M*	min	max	SD	*n*	*M*	min	max	SD
	W Turkey					S and E Turkey					Cyprus				
LAt	13	**40.29**	38.5	41.4	0.897	11	**40.20**	38.5	41.7	0.897	35	**40.41**	37.6	42.9	1.068
LCr	13	**15.87**	15.53	16.38	0.252	21	**15.94**	15.53	16.44	0.252	23	**15.92**	15.39	16.58	0.281
LCb	13	**14.83**	14.38	15.19	0.250	20	**14.88**	14.19	15.43	0.326	23	**14.89**	14.50	15.39	0.230
LaZ	12	**10.10**	9.81	10.57	0.243	20	**10.06**	9.66	11.02	0.300	22	**9.93**	9.58	10.31	0.166
LaI	13	**3.69**	3.43	3.97	0.170	21	**3.59**	3.33	3.93	0.138	23	**3.62**	3.43	3.84	0.109
LaN	13	**7.76**	7.38	8.06	0.195	21	**7.75**	7.38	8.31	0.204	23	**7.78**	7.51	8.12	0.146
ANc	13	**5.61**	5.43	5.86	0.133	18	**5.64**	5.42	6.28	0.193	23	**5.66**	5.38	5.86	0.110
CC	13	**3.99**	3.84	4.25	0.119	20	**4.02**	3.63	4.45	0.168	23	**4.03**	3.89	4.17	0.083
M^3M^3	13	**6.48**	6.27	6.68	0.148	21	**6.25**	5.75	6.92	0.232	23	**6.35**	6.14	6.57	0.108
CM^3	13	**6.14**	5.93	6.36	0.131	20	**6.08**	5.76	6.37	0.160	23	**6.16**	5.88	6.36	0.135
LMd	13	**11.46**	11.18	11.69	0.198	20	**11.61**	11.24	12.08	0.201	23	**11.44**	11.13	11.82	0.194
CM_3	12	**6.56**	6.41	6.83	0.118	19	**6.50**	6.31	6.74	0.126	23	**6.55**	6.33	6.76	0.129
CC/CM^3	13	**0.651**	0.623	0.679	0.015	20	**0.662**	0.619	0.706	0.023	23	**0.654**	0.619	0.687	0.019
CM^3/LCb	13	**0.414**	0.407	0.424	0.005	19	**0.408**	0.398	0.419	0.007	23	**0.413**	0.402	0.424	0.005
ANc/LCr	13	**0.355**	0.340	0.365	0.007	18	**0.354**	0.336	0.385	0.010	23	**0.355**	0.333	0.373	0.010
LaI/LCr	13	**0.233**	0.216	0.251	0.011	21	**0.225**	0.210	0.241	0.009	23	**0.227**	0.211	0.244	0.008
	N Levant					S Levant					NW Iran and Armenia				
LAt	22	**40.51**	38.5	42.4	1.090	19	**40.10**	38.9	41.4	0.654	7	**42.81**	41.3	44.1	0.953
LCr	17	**15.82**	15.16	16.42	0.312	23	**15.89**	15.23	16.64	0.297	12	**17.20**	16.81	17.50	0.246
LCb	17	**14.70**	14.15	15.08	0.252	24	**14.84**	14.05	15.52	0.279	12	**16.12**	15.67	16.50	0.215
LaZ	15	**9.99**	9.72	10.33	0.191	20	**10.03**	9.70	10.51	0.252	10	**10.71**	10.27	10.90	0.234
LaI	18	**3.58**	3.44	3.75	0.103	25	**3.69**	3.52	3.97	0.116	12	**4.08**	3.82	4.40	0.164
LaN	18	**7.80**	7.31	7.98	0.164	25	**7.91**	7.56	8.33	0.152	12	**8.40**	7.92	9.00	0.298
ANc	17	**5.63**	5.32	5.92	0.151	22	**5.77**	5.37	6.10	0.169	8	**6.05**	5.74	6.40	0.212
CC	17	**4.02**	3.54	4.33	0.188	24	**4.10**	3.90	4.35	0.128	8	**4.49**	4.27	4.70	0.120
M^3M^3	18	**6.26**	5.88	6.49	0.187	24	**6.29**	6.00	6.50	0.138	8	**7.06**	6.74	7.28	0.186
CM^3	17	**6.12**	5.82	6.41	0.128	25	**6.18**	5.88	6.44	0.125	12	**6.75**	6.60	6.93	0.123
LMd	18	**11.40**	11.08	11.74	0.177	25	**11.49**	11.04	12.07	0.252	12	**12.56**	12.23	12.90	0.212
CM_3	18	**6.54**	6.32	6.84	0.116	25	**6.53**	6.25	6.87	0.155	12	**7.19**	6.90	7.37	0.131
CC/CM^3	17	**0.657**	0.608	0.706	0.021	24	**0.663**	0.634	0.718	0.021	8	**0.664**	0.646	0.681	0.013
CM^3/LCb	16	**0.417**	0.407	0.430	0.007	24	**0.417**	0.408	0.427	0.004	12	**0.419**	0.405	0.430	0.007
ANc/LCr	16	**0.357**	0.345	0.371	0.007	21	**0.363**	0.341	0.381	0.010	8	**0.350**	0.333	0.370	0.013
LaI/LCr	17	**0.226**	0.217	0.240	0.007	23	**0.233**	0.224	0.241	0.005	12	**0.237**	0.221	0.253	0.010
	Crimea					Transcaucasia					N Iran and Turkmenistan				
LAt	15	**39.07**	37.2	40.7	1.189	5	**41.02**	39.2	41.9	1.096	6	**41.00**	40.3	42.0	0.672
LCr	11	**15.44**	15.09	15.88	0.283	7	**16.07**	15.62	16.62	0.335	8	**16.06**	15.68	16.28	0.174
LCb	11	**14.42**	14.14	14.82	0.268	6	**15.13**	14.72	15.58	0.297	8	**15.00**	14.62	15.28	0.208
LaZ	10	**9.72**	9.49	9.94	0.166	1		10.09			7	**9.92**	9.71	10.05	0.122
LaI	11	**3.70**	3.59	3.87	0.080	7	**3.62**	3.47	3.80	0.125	8	**3.56**	3.38	3.70	0.092
LaN	11	**7.66**	7.45	7.81	0.100	6	**7.88**	7.68	8.20	0.210	8	**7.73**	7.59	7.86	0.088
ANc	11	**5.53**	5.38	5.80	0.125	5	**5.57**	5.24	5.93	0.259	8	**5.61**	5.52	5.84	0.108
CC	11	**3.74**	3.64	3.87	0.086	5	**4.07**	3.98	4.13	0.060	8	**3.95**	3.82	4.11	0.091
M^3M^3	11	**6.16**	5.93	6.37	0.159	5	**6.52**	6.35	6.64	0.124	8	**6.45**	6.38	6.56	0.058
CM^3	11	**6.06**	5.88	6.23	0.117	7	**6.40**	6.29	6.59	0.115	8	**6.38**	6.28	6.48	0.060
LMd	11	**11.28**	10.94	11.64	0.234	7	**11.80**	11.60	12.07	0.190	8	**11.71**	11.49	11.98	0.188
CM_3	11	**6.48**	6.27	6.62	0.113	7	**6.77**	6.60	6.97	0.116	8	**6.76**	6.60	6.84	0.077
CC/CM^3	11	**0.617**	0.590	0.636	0.016	5	**0.637**	0.627	0.645	0.008	8	**0.618**	0.590	0.648	0.017
CM^3/LCb	11	**0.421**	0.415	0.429	0.004	6	**0.422**	0.414	0.427	0.004	8	**0.425**	0.418	0.430	0.004
ANc/LCr	11	**0.358**	0.346	0.374	0.010	5	**0.347**	0.335	0.357	0.009	8	**0.349**	0.343	0.362	0.007

Table 2 (continued)

	n	*M*	min	max	SD	*n*	*M*	min	max	SD	*n*	*M*	min	max	SD
LaI/LCr	11	**0.240**	0.235	0.250	0.005	7	**0.225**	0.209	0.236	0.009	8	**0.222**	0.210	0.232	0.007

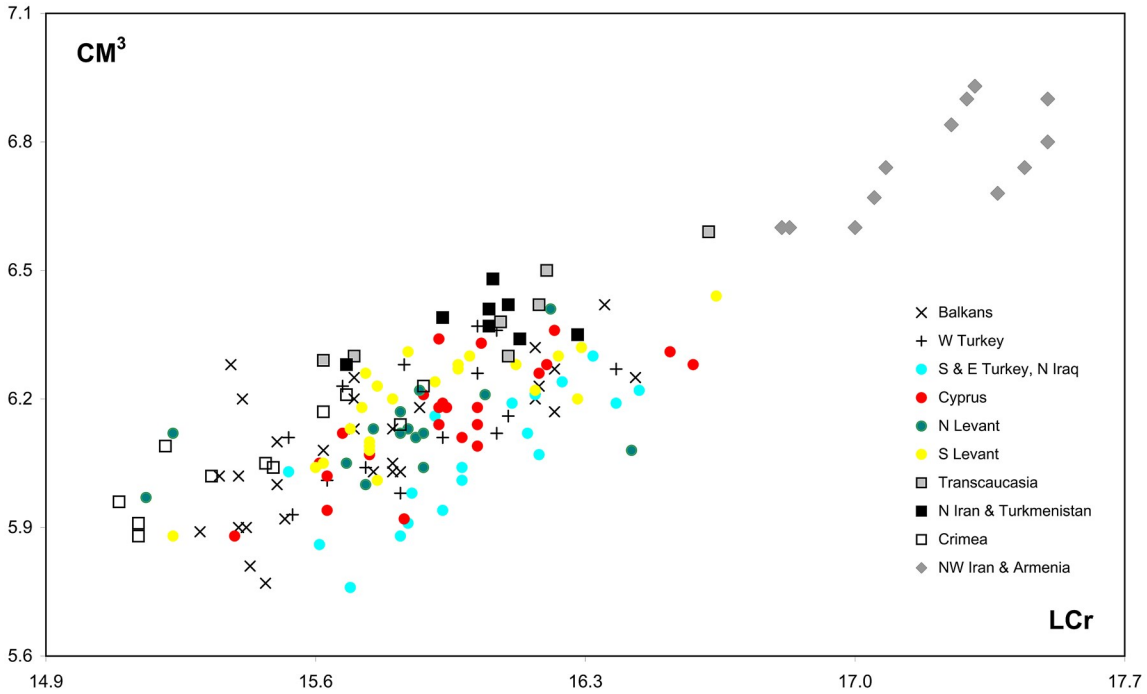

Fig. 3 Bivariate plot of skull dimensions of the compared samples of *Myotis nattereri* complex from the Middle East: greatest length of skull (LCr) against length of the upper tooth-row (CM^3); values in millimetres

Discussion

Our study aimed to fill the gaps and complement the knowledge of species afiliation and distribution of taxa in the *Myotis nattereri* complex in the Middle East, i.e. in the eastern part of its west-Palaearctic range. Basically, we tried to identify taxonomic afiliation of particular populations with help of molecular genetic and/or morphometric approaches.

We sequenced the mitochondrial ND1 gene from 52 specimens of the *nattereri* complex from various parts of the Middle East, including Iran, Syria, Lebanon, Jordan, Cyprus, and an east-Aegean island. The results of analysis of these sequences did not change significantly the picture suggested by Çoraman et al. (2019), but more precisely specified ranges of some lineages and sublineages. Generally, the resulting topologies were similar to those reported by previous authors (Ruedi et al. 2013; Juste et al. 2018; Çoraman et al. 2019; Kruskop and Solovyeva 2021; etc.). In the region, our results confirmed the occurrence of four species of the complex—in the sense of Çoraman et al. (2019)—belonging to two distinct clades.

The lineage corresponding to *Myotis nattereri* s.str., which occurs in most of temperate Europe and in the whole Balkan peninsula (see Smirnov et al. 2020), was found only in the westernmost part of the Middle East. Çoraman et al. (2019) reported it from three sites of western and southwestern Anatolia and our results demonstrated its presence in Samos, an island adjacent to the west-Anatolian coast (Fig. 5). Until now, bats of the *nattereri* complex were reported from two islands of Greece only, Corfu and Thassos (Niethammer 1962; Lane and Alivizatos 2006). Both these islands are rather densely forested and closely adjacent to the mainland, and the presence of *M. nattereri* s.str. is most probable on both islands, since only this lineage was found in the Balkans (Ibáñez et al. 2006; Çoraman et al. 2019). Findings from Samos indicate a third Greek island inhabited by this species. Hence, *M. nattereri* s.str. is able to colonise offshore islands when they are well structured geomorphologically and suficiently forested, like the three mentioned Greek islands are.

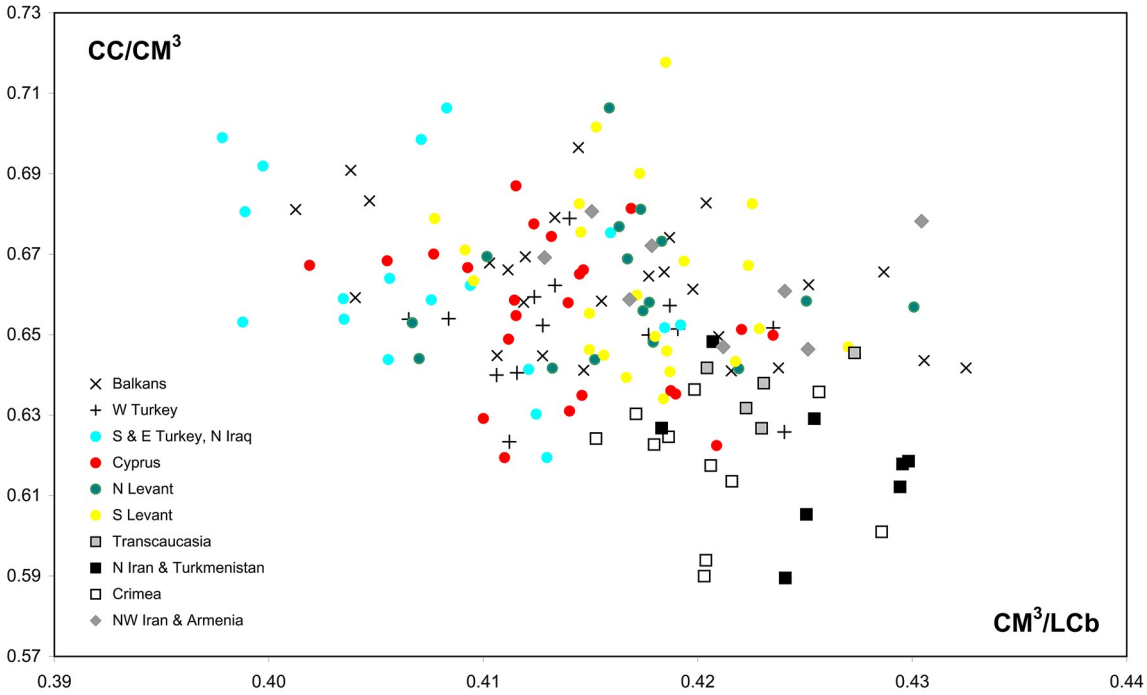

Fig. 4 Bivariate plot of relative skull dimensions of the compared samples of *Myotis nattereri* complex from the Middle East: relative length of rostrum (CM^3/LCb) against relative width of rostrum (CC/CM^3)

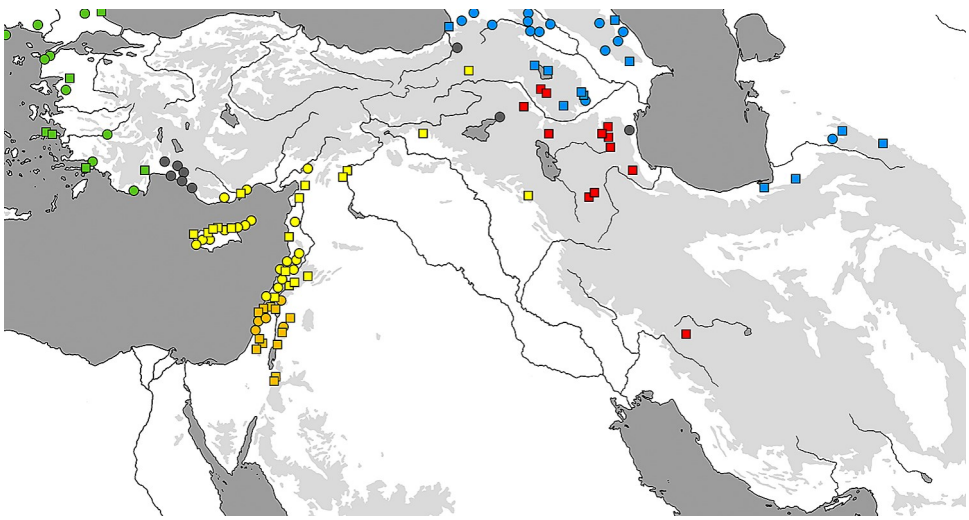

Fig. 5 Distribution of particular species of the *Myotis nattereri* complex in the Middle East and adjacent regions. Squares denote localities with taxonomic afiliation of the samples confirmed by the molecular genentic and/or morphologic evidences, circles denote localities of records unconfirmed by the analyses (the respective taxonomic afiliation is only expected). Colours denote specific taxa as follows: green—*M. nattereri* s.str., orange—*M. hoveli* s.str., yellow—*M. hoveli* NL, blue—*M. tschuliensis*, red—*M. schaubi*, grey—species afiliation unknown (two or more species possibilities)

According to Çoraman et al. (2019), *Myotis hoveli* is represented by two sublineages; *M. hoveli* s.str. (D II in Fig. 1) was reported from six sites covering most of the Mediterranean part of Israel (s.str. because the type locality of *M. hoveli* lies near Jerusalem, Israel). Our results further suggest that all analysed samples from Jordan and one Syrian specimen, originating from Talsh'hab, situated close to the Syrian-Jordanian border belong to this sublineage. Thus, *M. hoveli* s.str. is restricted to a limited territory of the southern Levant (Mediterranean habitats of Israel, western

Jordan, and south-western Syria; for details on distribution see Benda et al. 2006, 2010), while the rest of analysed Levantine samples belonged to a slightly divergent sister sublineage (D III). Thirteen bats of the *M. hoveli* s.str. sublineage from Israel beared two haplotypes (Çoraman et al. 2019), while the same number of specimens from Jordan and Syria produced eight different haplotypes organised into three haplogroups (Figs. 1, 2; D II a–c). This unusual diversity suggests an isolated and rather long evolutionary history of this sublineage in the eastern part of the Levantine Rift Valley. Therefore, this region could acted as a source area for colonisation of the Israeli part of the range of *M. hoveli* s.str.

Another sublineage of *M. hoveli* (D III), sister to *M. hoveli* s.str., was reported by Çoraman et al. (2019) from southern Turkey, from a belt comprising the provinces of Mersin, Hatay, Gaziantep, and Şanlıurfa. This mitchondrial sublineage was found to be shared by bats from two sites in Armenia. However, the latter bats differ from the Turkish ones morphologically as they represent the large-sized morphotype (while Turkish bats are of the small- to medium-sized morphotype) and mainly, they pertain to a different lineage according to nuclear genome (Çoraman et al. 2019). Therefore, these large Armenian bats were confirmed as a separate species, *M. schaubi*, as previously suggested by Horáček and Hanák (1984), see below for details. However, the bats of this sublineage from Turkey, which belong to the small-sized morphotype, were assigned to the *schaubi* lineage too (Çoraman et al. 2013, 2019). Here, we tentatively named these populations/sublineage as *M. hoveli* NL (northern Levant) for clarity. These bats were found by our analysis in Cyprus (six haplotypes from 25 specimens, incl. one haplotype that was shared with the Lebanese populations), Lebanon (two haplotypes from three bats), and western Syria (two haplotypes from four bats).

The geographical limits of both sublineages of *M. hoveli*, the south-Levantine *M. hoveli* s.str. and north-Levantine *M. hoveli* NL, are shared in southern Lebanon. While Çoraman et al. (2019) reported the former sublineage from three sites in northernmost Israel, our records of the latter sublineage come from sites in southern part of Lebanon (Benda et al. 2016), including Beaufort Castle, situated some 25 km north of the Israeli localities. Such a division of two genetic sublineages runing through the southern part of Lebanon has an analogy with the phylogeographical divisions of *Myotis emarginatus* in the Levant (Uvizl and Benda 2021). In the latter bat species, two Levantine sublineages are present in Lebanon and their division line goes across mountains of southern and central parts of the country. Without doubt, the territory of southern Lebanon represents a biogeographical border as well, where limits of distribution ranges of many species are situated, among bats e.g., of *Rhinopoma microphyllum* (Brünnich, 1782), *Myotis mystacinus* (Kuhl, 1817), *Eptesicus anatolicus* Felten, 1971, and *Plecotus macrobullaris* Kuzâkin 1965 (for details see Benda et al. 2016).

The western limits of *M. hoveli* is not possible to specify with certainty at the moment, since only few bats from south-western Turkey were examined with help of molecular genetic tools. The genetic analysis remains the only way to distinguish between *M. hoveli* and *M. nattereri*, both occurring in this part of Turkey. The analysis of nuclear data from Turkey (Çoraman et al. 2019) indicates that these species are well differentiated and do not interbreed, after all, these species are members of two separate lineages of the genus *Myotis* (Ruedi et al. 2013). On the other hand, the morphologic comparisons did not bring good results concerning separation of these species (Benda et al. 2006; Çoraman et al. 2019; this study). The geographical border between ranges of these two species (unless they live in sympatry there) lies in the approximatelly 350 km wide belt along the Levantine Sea coast between Termessos (Antalya Prov.) and Silifke (Mersin Prov.). Even in this region, *M. nattereri* s.l. was demostrated to be a common faunal element (von Helversen 1989; Benda and Horáček 1998; Fig. 5).

The only island population of *M. hoveli* from Cyprus demostrated very close genetic similarity to the mainland Levantine populations, both Syrian-Lebanese and south-Anatolian ones (Fig. 2); the genetic distances between the Cypriot and mainland bats lie in the range of 0–10 substitutions within the whole ND1 sequence (mean p-distance 1.14%; Table 1). On the one hand, such finding could suggest a concurrence of a long-time radiation within the island conditions with a recent exchange/s of the genome between the Cypriot and mainland populations or even continuous gene flow across the Levantine Sea. This problem could be solved if a higher number of samples is sequenced from the mainland areas that surround Cyprus (the Cypriot populations seem to be sampled in a suficient volume, see List S1 and Benda et al. 2007, 2018). Without doubts, *M. hoveli* from Cyprus does not represent a unique evolutionary unit and is a part of the north-Levantine sublineage.

The identification of eastern limits of the distribution range of *M. hoveli* represents one of the unexpected results of this study. The eastern margins of the known continous range of *M. hoveli* in the Levant are positioned at the westernmost extension of the Euphrates in southern Turkey (Karataş and Sachanowicz 2008; Çoraman et al. 2019; Fig. 5). The bats recorded more to the east, in northern Iraq and in north-eastern Turkey, were referred to *M. nattereri tschuliensis* by Horáček and Hanák (1984) and Benda et al. (2006), and a peculiar position of the Iraqi specimens within *M. nattereri* s.l. was noted already by Rzebik-Kowalska et al. (1978). Three examined specimens of these populations, one from Geli Ali Beg in the Arbil Province, northern Iraq (ISEA 5148), another from Sarıkamış in the Kars Province, northeastern Turkey (NMP 90568), and another from the

Birklin Cave in the Diyarbakır Province, south-eastern Turkey (NMW 34374), are members of the medium-sized morphotype according to their skull size (LCr 16.38 mm [Geli Ali Beg], 16.32 mm [Sarıkamış], 16.8 mm [Birklin Cave]). Their plain skull dimensions fit with the dimensions of *M. hoveli* and *M. tschuliensis* (Fig. 3), while their skull shapes positioned all three bats into variation range of *M. hoveli*, but not *M. tschuliensis* (Fig. 4). Comparing to other samples, the relative length of rostrum in these specimens is small (CM^3/LCb 0.406, 0.408, 0.407) and the relative width of rostrum is large (CC/CM^3 0.664, 0.706, 0.698), these values fall exactly within the dimension ranges of *M. hoveli*, while completely outside the ranges of *M. tschuliensis* (Table 2). The identification of the Sarıkamış specimen as *M. hoveli* correspond to the results of genetic analysis; the cytochrome *b* gene sequence extracted from this bat (Jones et al. 2006) was used by Ruedi et al. (2013) and Smirnov et al. (2020). These authors found it in a sister position to a sequence of *M. schaubi* from Iran, a result corroborating our results and results of Çoraman et al. (2019) concerning phylogenetic position of *M. hoveli*.

The presence of *M. hoveli* in the central parts of the Middle East better helps to understand the sharing of a part of the genome by *M. hoveli* and *M. schaubi*. Based on the evaluation of data from mitochondrial and nuclear markers, Çoraman et al. (2019) suggested to explain this sharing by a past introgression of the mitochondrial genes from one species into other. Since the original local Levantine populations are most probably represented by *M. hoveli* s.str. from the southern Levant, the introgression was thus probably directed from *M. schaubi*. It is better to imagine, since this bat is much larger than *M. hoveli* and mainly, is not known to possess other genetic lineages than the D III (Fig. 1). The introgression occurred when now separated lineages were in contact and this contact perhaps underwent in the Armenian Highlands (eastern Turkey, northern Iraq, north-western Iran), a region where the distribution ranges of both species almost meet also nowadays (Fig. 5). In the past, these ranges could overlap and the mitochontrial gene interchange could be under way, although the nuclear genomes of both species remained untouched by this former sympatric or parapatric occurrence. Since the north- and south-Levantine sublineages of *M. hoveli* (NL and s.str.) were separated (and could be until now), the mitochondrial genome of *M. schaubi* did not affect the south-Levantine sublineage (*M. hoveli* s.str.).

These two species, *M. hoveli* and *M. schaubi*, are the only species of the *nattereri* complex in the Middle East that share their mitochondrial genome, although similar past introgression between other species of the complex was described also from the western Mediterranean, in *M. nattereri* and *M. crypticus* (Çoraman et al. 2019). However, albeit the differention and clear identification of *M. hoveli* and *M. schaubi* (sublineage D III) is not possible using mitochondrial markers, it could be made with help of nuclear markers (see Çoraman et al. 2019), but more easily with help of morphological examination as these species represent two distinct size morphotypes (Fig. 3, Table 2). While in *M. hoveli*, the largest skull length is smaller than 16.7 mm, in *M. schaubi* smallest skull length is larger than 16.8 mm.

In contrast to all other species of the *nattereri* complex, *Myotis schaubi* is a species well identifiable based on metric characters (clearly distinguishable not only from *M. hoveli*; see Fig. 2), although based on the analysis of mitochondrial markers, such clear differentiation is not possible (see above). Since *M. schaubi* was first described as a fossil species from the Upper Pliocene of Hungary (Kormos 1934), Çoraman et al. (2019) suggested to name its recent populations as *M. araxenus* Dal', 1947. This name was created for the Armenian populations of the complex (originally as *M. nattereri araxenus*, see Dal' 1947) and considered a subspecies of *M. schaubi* (Horáček and Hanák 1984; Koopman 1993, 1994; Borisenko and Pavlinov 1995; Simmons 2005; etc.). Since Çoraman et al. (2019) did not bring a relevant support for their conclusion, we maintain the name *M. schaubi* in use, similarly as Smirnov et al. (2020) and Kruskop and Solovyeva (2021) already did. The distribution range of this bat was well defined previously (Horáček and Hanák 1984; Benda et al. 2012); the species occurs in a limited range covering the north-western section of Iran and southern Armenia. This range lies—according to the current knowledge—in parapatry to other species of the complex, *M. hoveli* and *M. tschuliensis* (Benda et al. 2012; Çoraman et al. 2019; Fig. 5). This parapatry or even allopatry to other taxa supports the concept of the past contacts and mitochondrial gene flow between *M. schaubi* and *M. hoveli*, rather than a continuing gene exchange. If the occurrence of both species is currently separated, the exchange of the genomes is not possible and the phylogenetic positions of all species is stable.

The last species of the *nattereri* complex that occurs in the Middle East, *M. tschuliensis*, could be distinguished from other species of the complex based on the analysis of genetic markers (Çoraman et al. 2019; Smirnov et al. 2020; Kruskop and Solovyeva 2021; this study) as well as the morphometric comparison (Smirnov et al. 2020; Kruskop and Solovyeva 2021; this study). The skull shape differentiates this bat from all other species of the complex in the region. This species is represented by two mitochondrial sublineages, one occurring in Georgia and another in a belt stretching from Crimea, via Transcaucasia (Armenia) to northern Iran and southern Turkmenistan (Çoraman et al. 2019; Kruskop and Solovyeva 2021; this study). Which sublineage occurs in Daghestan (Russia) cannot be determined for the moment, since Smirnov et al. (2020) used a different marker in their analysis. However, these

two sublineages most probably live in sympatry in Transcaucasia (see Fig. 5) and thus, this division has certainly no taxonomic meaning. Nevertheless, only an additional research could help to evaluate whether the distinct Georgian sublineage represents a real unique product of evolution or a phantom without an evolutionary history. Besides the mentioned countries, *M. tschuliensis* was found also in Azerbaijan (Horáček and Hanák 1984; Benda et al. 2011). Despite the genetic uniformity within one sublineage, this species exists as two size morphotypes, small-sized in Crimea and medium-sized in the rest of its range. The range of *M. tschuliensis*, spread between Crimea and Kopetdagh Mts. resembles again one of the phylogroups of *M. emarginatus*, its subspecies *M. e. turcomanicus* having an identical range, which, however, continues more to the east, up to to Kirghizstan and Tajikistan (Benda et al. 2006; Uvizl and Benda 2021).

In summary, we proposed to fill areas of uncertainties in the Middle Eastern distribution range of the complex of *Myotis nattereri*. The taxonomic afiliations of the populations occurring in most of these areas were really filled and the distribution limits of particular species are now better defined. However, some questions concerning this topic still remain open. More detailed sampling is needed mainly for the revision of populations in the centre of the region, where the state borders of Turkey, Iran, Armenia, and Azerbaijan meet and where also the ranges of three species of the *nattereri* complex meet. Such sampling could help to understand the mutual range limits of these species and/or their possible sympatric occurrence. More profound sampling in Transcaucasia could elucidate the mutual phylogenetic positions and geographical extents of two lineages found in *M. tschuliensis*. Last but not least, a detailed sampling is needed also in Turkey, to define more precisely the geographical limits of *M. nattereri* and *M. hoveli*, respectively, and to define geographical and phylogenetic limits of the latter species in the eastern part of the country. In easternmost Turkey and adjacent regions, very detailed sampling and analyses of nuclear markers are necessary to proof whether the three species occuring the central parts of the Middle East really do not interbreed. However, all these details could well follow the results of our present study.

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Declarations

Conflict of interest The authors declare that there is no conflict of interest.

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Intraspecific variation of *Myotis emarginatus* (Chiroptera: Vespertilionidae) inferred from mitochondrial and nuclear genetic markers

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Geoffroy's bat, *Myotis emarginatus*, is the only species of the African clade of the genus *Myotis* distributed in the south-western part of the Palaearctic. Due to its extensive distribution range, stretching across several ecologic zones from the European and African Mediterranean, Central Europe, through the Levant and Caucasus to West Turkestan and south-eastern Middle East, this bat is traditionally considered to be a variable and polytypic species. While one subspecies was recognized in Europe and North Africa, up to four subspecies were reported from the Asian part of the species range. Nevertheless, the systematic positions of different populations and the validity of particular taxa remained unclear. Our aim was to revise the phylogenetic status of *M. emarginatus* and, for the first time, genetically analyse samples from the Asian part of its range to provide new insight into its intraspecific variation. We analysed sequences of two mitochondrial and three nuclear markers from more than 130 samples from all parts of the species range, together with sequences from other species from the African clade of the genus *Myotis*. According to the previous morphometric results of body and skull dimensions, *M. emarginatus* can be divided into two groups of populations: the small-sized and more variable bats of Europe, the Maghreb and Levant; and the large-sized bats of the rest of the Asian range. This division was well supported by mitochondrial genes, which separated two main lineages within the species: the western lineage from Europe, the Maghreb and Levant; and the eastern lineage from the eastern Middle East and West Turkestan. Both mitochondrial lineages were further divided into two sublineages: the western lineage to sublineages from the Holy Land and the rest of the Mediterranean range; and the eastern lineage to sublineages from Oman and southern Iran, and northern Iran and West Turkestan. In contrast, the nuclear genes reconstructed only one lineage through the whole distribution range, suggesting *M. emarginatus* to be a monophyletic species. Nevertheless, on the basis of previously described geographical variability in morphology and the newly described mitochondrial variation, we recognize two subspecies within *M. emarginatus*: small-sized *M. e. emarginatus* distributed in the Mediterranean, western and central Europe and Levant; and large-sized *M. e. desertorum* in the eastern Middle East, from Oman to West Turkestan.

Key words: mtDNA, morphology, systematics, Western Palaearctic, nuclear DNA, molecular phylogenetics

Introduction

Geoffroy's bat, *Myotis emarginatus* (Geoffroy, 1806), has a Mediterranean distribution (Fig. 1) over the south-western part of the Palaearctic (Corbet, 1978; Koopman, 1994; Horáček *et al*., 2000): in the Maghreb from Morocco to Tunisia; in Europe from Portugal and the Netherlands, over central and southern Europe to the Balkans, Crimea and Caucasus region, including many Mediterranean islands (Topál, 2001; Dietz *et al*., 2007); and in Asia from the Levant, Asia Minor and Iraq to southeastern Kazakhstan, southern Kirghizstan and eastern Afghanistan (Rybin *et al*., 1989; Habilov, 1992; Benda *et al*., 2006, 2012; Benda and Gaisler, 2015; Al-Sheikhly *et al*., 2016). In the south, it occupies isolated range patches in north-eastern Oman, and in western Saudi Arabia and Yemen; in the latter area, it reaches the Afrotropical region (Harrison, 1977; Gaucher, 1995; Al-Jumaily, 2003).

Myotis emarginatus represents the only Palaearctic species of the African clade of the genus *Myotis* (Stadelmann *et al*., 2004; Ruedi *et al*., 2013; Patterson *et al*., 2019) that otherwise comprises nine Afrotropical species — *M. anjouanensis* Dorst, 1960, *M. bocagii* (Peters, 1870), *M. dieteri* Happold, 2005, *M. goudoti* (Smith, 1834), *M. morrisi* Hill, 1971, *M. nimbaensis* Simmons, Flanders, Fils, Parker, Suter, Bamba, Douno, Keita, Morales et Frick, 2021, *M. scotti* Thomas, 1927, *M. tricolor* (Temminck, 1832), and *M. welwitschii* (Gray, 1866), and it includes also six Oriental *Myotis* species — *M. bartelsi* Jentink, 1910, *M. formosus* (Hodgson, 1835), *M. hermani* Thomas, 1923, *M. rufoniger*

(Tomes, 1858), *M. rufopictus* (Waterhouse, 1845) and *M. weberi* (Jentink, 1890) (Stadelmann *et al*., 2004; Csorba *et al*., 2014; Simmons *et al.*, 2021).

Seven Afrotropical and two Oriental species of the African *Myotis* clade have already been used in genetic studies (Stadelmann *et al*., 2004; Ruedi *et al*., 2013, Csorba *et al*., 2014; Patterson *et al*., 2019) but the relationships among species within the clade have not been satisfactorily resolved so far. The only statistically supported relations are the sister positions of *M. rufoniger* (East Asia) with *M. welwitschii* (sub-Saharan Africa) and of *M. anjouanensis* (Comoros) with *M. goudoti* (Madagascar); *M. bocagii* (sub-Saharan Africa) could be the closest relative to the latter pair. There is no doubt that *M. emarginatus* is the most frequently used species of the African *Myotis* clade in genetic analyses (Ruedi and Mayer, 2001; Stadelmann *et al*., 2004; Ibáñez *et al*., 2006; Mayer *et al*., 2007; García-Mudarra *et al*., 2009; Ruedi *et al*., 2013, Patterson *et al*., 2019) but no study has yet focused on its intraspecific variability in detail or on resolving a phylogenetic picture for this species.

With its broad range, stretching over a wide longitudinal belt across several ecological and biogeographical zones, *M. emaginatus* is traditionally considered to be a variable and polytypic species (Ognev, 1928; Ellerman and Morrison-Scott, 1951; Corbet, 1978; Koopman, 1994; Horáček *et al*., 2000; Topál, 2001; Simmons, 2005; Dietz *et al*., 2007). Up to four subspecies are recognized: *M. e. emarginatus* (Geoffroy, 1806) in the Mediterranean Basin and adjacent areas of Europe, the Maghreb and Levant; *M. e. desertorum* (Dobson in Blanford, 1875) in the south-eastern part of the Middle East; *M. e. turcomanicus* Bobrinskoy, 1925 in the western part of West Turkestan; and *M. e. kuzyakini* Rossolimo and Pavlinov, 1979 in the eastern part of West Turkestan. These opinions on geographical variation are based mostly on comparisons of colour morphs and/or metric data of an insufficient number of specimens (see Topál, 2001).

Benda *et al*. (2006) examined more than 300 museum specimens from most parts of the species range; they suggested that the intensity of the colouration tinges is probably linked to the humidity level of the local habitat. The pale individuals are reported to occur in the lowland semi-arid regions of West Turkestan and Iran, whereas the individuals found in arboreal habitats of the Mediterranean (including Anatolia and the Levant) are mainly dark reddish-brown or orange-brown. Hence, they concluded that the colouration represents a character adaptive to the local environment, with little or no

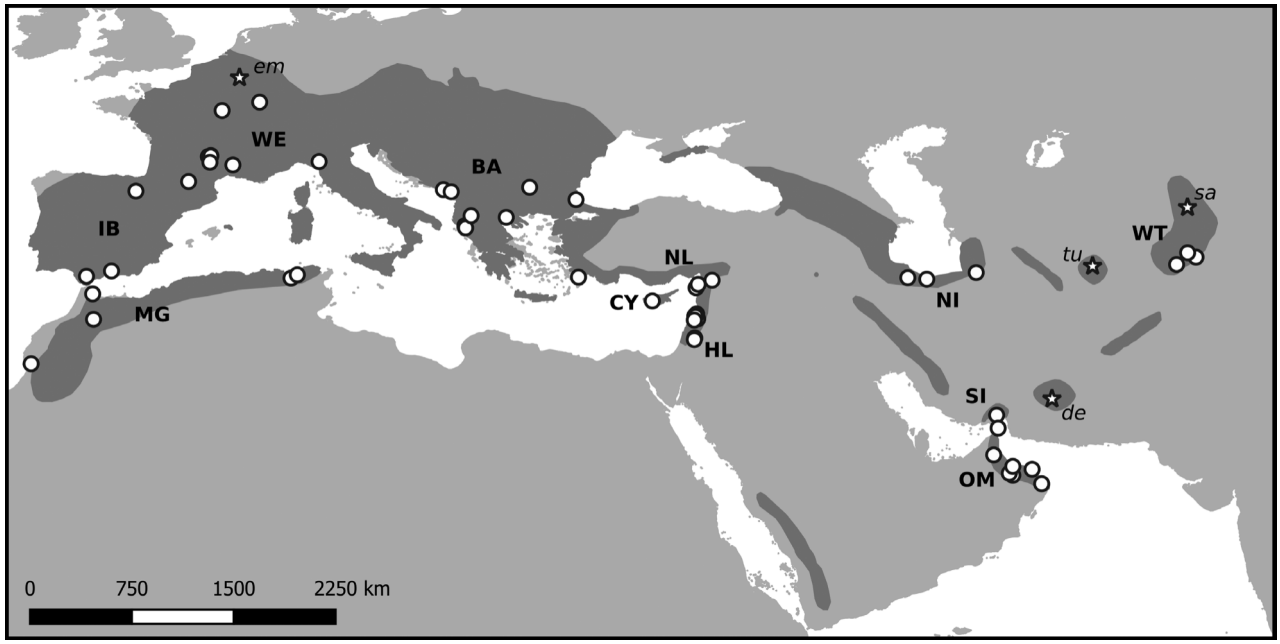

FIG. 1. Map of the distribution range of *M. emarginatus* (dark grey; as defined by Horáček *et al.*, 2000; Benda *et al*., 2006, 2010, 2012; etc.) and the localities of origin and grouping of the examined samples. The circles with asterisks indicate type localities of examined type specimens in the morphometric analysis by Benda *et al*. (2006): de = *desertorum* Dobson, 1875, em = *emarginatus* Geoffroy, 1806, sa = *saturatus* Kuzâkin, 1934 (= *kuzyakini* Rossolimo and Pavlinov, 1979), tu = *turcomanicus* Bobrinskoy, 1925. The capital letters denote geographical groupings of specimens: BA = Balkans, HL = Holy Land, IB = Iberia, MG = Maghreb, NI = North Iran, NL = North Levant, OM = Oman, SI = South Iran, WE = West Europe, WT = West Turkestan

reflection in the phylogenetic relationships within the species as a whole.

On the other hand, Benda *et al*. (2006) evaluated the geographical variation in *M. emarginatus* by morphometric analysis of the above mentioned large set of museum specimens. Their results revealed three basic groups of size morphotypes within the distribution range of the species, which the authors assigned to two subspecies. The large-sized morphotype comprised all populations of the eastern parts of the species range (Crimea, Caucasus, Iran, Afghanistan, West Turkestan) and was identified under the prior name from the region, *M. e. desertorum*. The remaining two morphotypes, including the small-sized morphotype of populations from southwestern and central Europe and the northern part of the Balkans, as well as the medium-sized morphotype originating from two separate regions, the Maghreb and eastern Mediterranean (southern Balkans to the Levant), were considered to represent the nominotypical subspecies.

The available results of intraspecific genetic analyses of mitochondrial genes (Ibáñez *et al*., 2006; Mayer *et al*., 2007; García-Mudarra *et al*., 2009) covered only the Mediterranean and Central European parts of the range of *M. emarginatus*; the samples from Morocco, Iberia, Belgium, Germany, Greece and Israel were found to represent a single clade with very low divergence. This is in accordance with traditional opinion that the whole Mediterranean area, including the southern parts of Central Europe, is inhabited by only one taxon: the nominotypical subspecies. However, the systematic position of populations in the eastern parts of the distribution range of *M. emarginatus* remains unclear, as well as the mutual relations of the Mediterranean and Asian populations.

Therefore, the intraspecific status of *M. emarginatus* in its whole distribution range appears to be unresolved and poses a challenge for detailed revision. For this purpose, we generated a geographically representative multilocus genetic dataset based on both mitochondrial and nuclear markers and applied genetic analysis to: (1) provide new insights into the intraspecific variation of *M. emarginatus*; (2) present a taxonomic synthesis of the intraspecific variation of *M. emarginatus* based on combined results of the new genetic analysis and the morphometric analysis by Benda *et al*. (2006); and (3) contribute to resolving phylogenetic relationships within the African clade of the genus *Myotis*.

Materials and Methods

Sampling

In the genetic analyses, we used tissue samples of 87 specimens of *M. emarginatus* to extract DNA from the collection of the National Museum Prague (NMP), Czech Republic, and 13 tissue samples of this bat kindly provided by Sébastien J. Puechmaille (University of Montpellier, France), including two samples from the National Museum of Natural History, Paris, France. Twelve additional sequences of *M. emarginatus* were downloaded from the GenBank. We supplemented this dataset with GenBank sequences of species from the African clade of the genus *Myotis*: five sequences of *M. anjouanensis*, 21 sequences of *M. bocagii*, three sequences of *M. formosus*, 17 sequences of *M. goudoti*, two sequences of *M. nimbaensis*, eleven sequences of *M. tricolor* together with 16 newly sequenced specimens from the NMP collection, three sequences of *M. rufoniger*, one sequence of *M. scotti* and twelve sequences of *M. welwitschii*. As an outgroup, we added 26 GenBank sequences of six species from the different *Myotis* clades (Stadelmann *et al*., 2004; Ruedi *et al.*, 2013; Patterson *et al.*, 2019) and five sequences of two species from the family Emballonuridae (for details, see Appendix).

DNA Extraction and Sequencing

Genomic DNA was extracted from alcohol-preserved tissue samples using the Geneaid Genomic DNA Mini Kit. We targeted two mitochondrial markers (mtDNA), namely 1103 bp of cytochrome-*b* gene (*Cyt-b*) and 515 bp of *D-loop* of the control region (*D-loop*), and three nuclear markers (nDNA), namely 683 bp of the recombination activating gene 2 (*Rag2*), 518 bp of acyl-coenzyme A oxidase 2 intron (*ACOX2*) and 344 bp of the signal transducer and activator of transcription 5A intron (*STAT5A*). We sequenced both strands for all sequences. We used primers that have been specifically designed for the order Chiroptera and provided good amplification in previous studies (see Puechmaille *et al*., 2011; Dool *et al*., 2016; Patterson *et al*., 2019; Uvizl *et al.*, 2019). For the primer names, their sequences and annealing temperatures, see Supplementary Table S1.

Sequences were edited and aligned using the MAFFT plugin (Katoh and Standley, 2013) in Geneious 11.0.5 (https://www.geneious.com) and subsequently manually edited and trimmed using Gblocks (Castresana, 2000). Heterozygous positions in the nDNA markers were coded with the IUPAC codes and ambiguous positions or missing data were coded with 'N'. Indels were treated as gaps. Sequences of protein-coding markers were translated to amino acids to check for the presence of stop codons, which would indicate that pseudogenes have been amplified. The two final multilocus datasets were formed according to the mode of inheritance of the markers: mitochondrial and nuclear. The mitochondrial dataset contained *Cyt-b* and *D-loop* sequences of total length 1618 bp and the nuclear dataset contained *Rag2*, *ACOX* and *STAT* sequences of total length 1546 bp. The datasets were partitioned by gene. Furthermore, we generated gene trees for individual markers to assess whether the markers provide a congruent phylogenetic signal.

Phylogenetic Reconstructions

Phylogenetic analyses of both datasets were run using Bayesian inference (BI) and maximum likelihood (ML). The

appropriate nucleotide substitution model for each partition was selected based on the Bayesian information criterion using ModelFinder (Supplementary Table S2; Kalyaanamoorthy *et al.*, 2017). We used MrBayes v3.2.6 (Ronquist and Huelsenbeck, 2003) to run the BI analysis. Appropriate substitution models were specified for each partition and all parameters were unlinked across partitions. We ran two independent runs for 20 million generations, with trees sampled every 1000 generations. All other parameters were set to default. Stationarity and convergence of the runs were inspected in Tracer v1.6 (Rambaut *et al*., 2014) and the values of the average standard deviations of the split frequencies were lower than 0.01. The burn-in fraction was left as the default at 25% of sampled trees. Thus, from the 20,000 trees produced, 5000 were discarded. A majority-rule consensus tree was produced from the post-burnin trees with posterior probability values embedded. The BI analyses were run through CIPRES Science Gateway (Miller *et al*., 2010). We inferred the ML tree using the partition model in IQ-TREE (Nguyen *et al.*, 2015; Chernomor *et al.*, 2016). Searching for the best-scoring ML was performed by ultrafast bootstrap (UFBoot; Hoang *et al.*, 2018), with 1000 bootstrap and 1000 topology replicates. To verify the robustness of the ML tree, branch support was evaluated using the SH-like approximate likelihood ratio test (SH-aLRT; Guindon *et al.*, 2010) and a Bayesian-like transformation of aLRT (aBayes; Anisimova *et al.*, 2011). The SH-aLRT was performed with 1000 replications. aBayes branch support was used instead of Bayesian posterior probabilities because aBayes is more conservative, more robust to model violation and exhibits the best power (Anisimova *et al.*, 2011). The ML, SH-aLRT and aBayes analyses were run on the IQtree web server (Trifinopoulos *et al.*, 2016).

We inferred haplotype networks for *Cyt-b* using two datasets formed according to the length of sequences. Dataset HNshort contained 104 sequences of length 703 bp and dataset HNlong contained 93 sequences of length 1024 bp. We used two datasets because valuable GenBank sequences from North Africa and western Europe were considerably shorter by more than 300 bp than the rest of the sequences. The networks were estimated by median-joining network analysis (Bandelt *et al.*, 1999) using PopART v.1.07 software (http://popart.otago.ac.nz). Genetic distances were conducted using the Tamura-Nei model (Tamura and Nei, 1993) in MEGA7 (Kumar *et al.*, 2016).

Terminology

The geographical terms used to denote the origin of the examined material (plus acronyms used in the figures; see Fig. 1) are as follows: Balkans (BA) = Albania, Bulgaria, Greece, Montenegro and Turkish Thrace; Cyprus (CY) = Cyprus; Holy Land (HL) = Jordan and southern Lebanon; Iberia (IB) = Spain; Levant = North Levant plus Holy Land (NL+HL); Maghreb (MG) = Morocco and Tunisia; Mediterranean (MT) = Balkans plus Maghreb plus West Europe (BA+MG+WE); North Iran (NI) = northern Iran; North Levant (NL) = Greek Dodecaneses, northern Lebanon and Syria; Oman (OM) = Oman; South Iran (SI) = southern Iran; West Europe (WE) = France and Italy; West Turkestan (WT) = Tajikistan.

Results

The resulting mitochondrial dataset comprised 161 *Cyt-b* and 121 *D-loop* sequences that were pruned to 95 unique haplotypes. The nuclear dataset comprised 104 *ACOX*, 107 *STAT* and 75 *Rag2* sequences that were pruned to 57 haplotypes. *Cyt-b* sequences contained 408 parsimony informative positions (36.99% of total length) and this marker contributed most to the genetic differentiation within the African *Myotis* clade. *D-loop* sequences with 177 parsimony informative positions (21.26% of total length) provided the second highest contribution to the genetic variation. Nuclear markers provided much less genetic differentiation due to the slower mutation rate: *ACOX*, *STAT* and *Rag2* sequences contained 28 (5.41%), 26 (7.54%) and eight (1.17% of total length) parsimony informative positions, respectively. For particular substitution models of mitochondrial and nuclear trees, see Supplementary Table S2. The phylogenetic trees obtained by both ML and BI analyses of the concatenated datasets showed slightly different topologies; however, the variant nodes were not supported by either BI or ML. Furthermore, the intraspecific divergences of *M. emarginatus* in both mitochondrial and nuclear trees were very shallow. The trees showed higher ML bootstrap support and therefore we present the ML trees here (Fig. 2A and 2B). The resolution of the gene trees was in accordance with genetic variation. The *Cyt-b* and *D-loop* gene trees looked almost identical to their combined tree. The *ACOX* gene tree showed relatively well-resolved topology for the African *Myotis* clade, the *STAT* gene tree showed polytomy in the clade and *Rag2* showed really low diversity among the species. For more details, see Supplementary Figs S2–S6.

In the tree based on the mitochondrial dataset (Fig. 2A), *M. emarginatus* formed a well-supported monophyletic unit, as did all other *Myotis* species of the African clade and the African clade as a whole. However, the relationships between the species remained unresolved due to low support of the connecting nodes. The only supported connecting nodes were those joining together *M. tricolor* and *M. nimbaensis*, *M. welwitschii* and *M. rufoniger*, *M. bocagii* and *M. scotti*, and *M. goudoti* and *M. anjouanensis*. In addition, *M. emarginatus* (see below), *M. tricolor*, *M. welwitschii* and *M. bocagii* were further divided into well-supported intraspecific subgroups. *Myotis tricolor* was split into four subgroups (from: South Africa and Lesotho; South Africa only; Ethiopia; Kenya), *M. welwitschii* was divided into two subgroups (from: Kenya and Uganda; Malawi and Tanzania) and *M. bocagii* formed three subgroups (from: Senegal, Ghana, DR Congo and Kenya; Kenya and Tanzania).

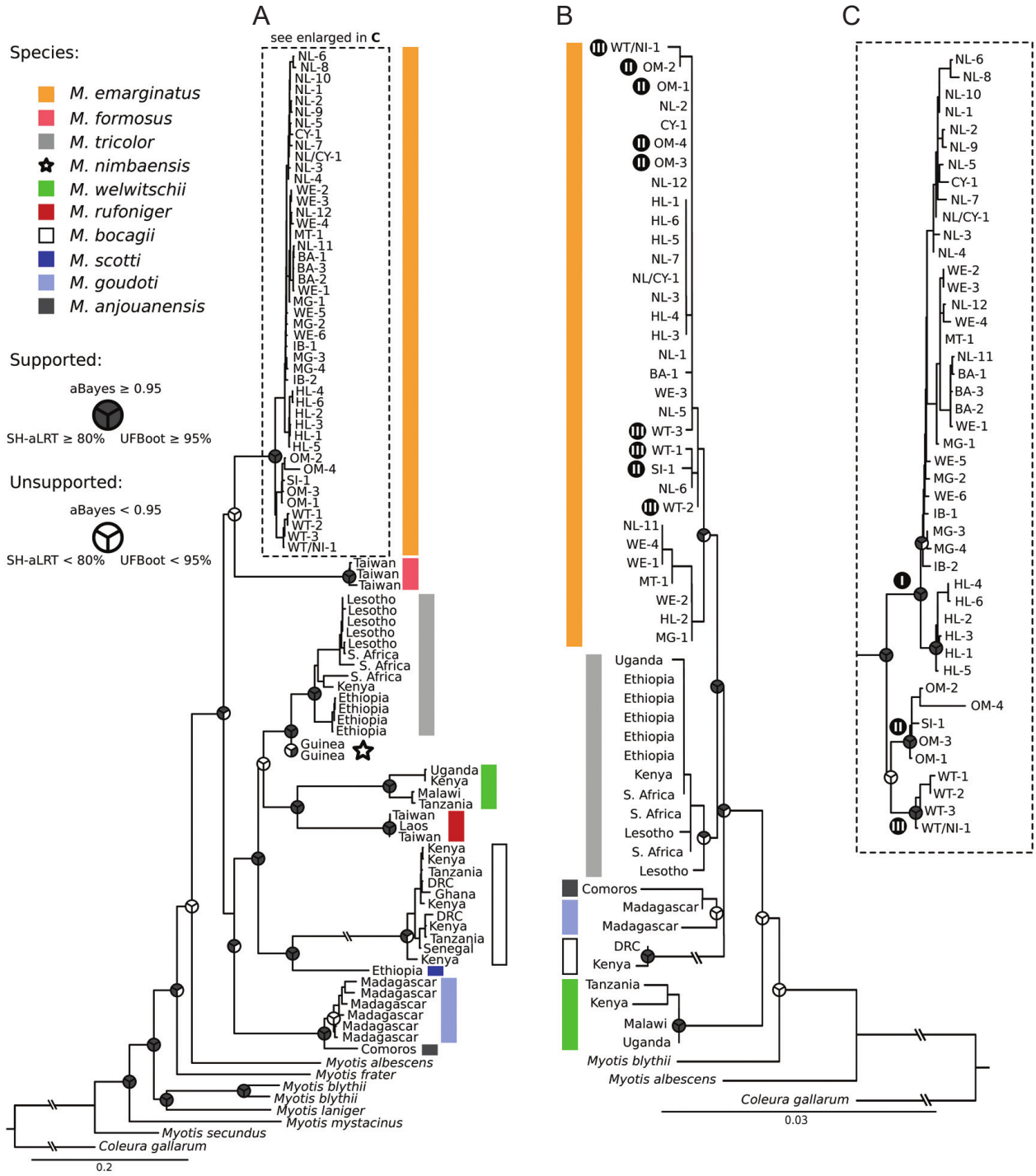

FIG. 2. Maximum likelihood tree of reconstructed phylogenetic relations of *M. emarginatus* with selected species from the African clade of genus *Myotis* based on (A) mitochondrial dataset and (B) nuclear dataset with (C) enlarged part of mitochondrial tree focused only on *M. emarginatus*. Branch support values are shown by pie charts on the nodes. Roman numerals refer to the group delimitation used in text

In *M. emarginatus*, the mitochondrial tree (Fig. 2C) diversified into three subgroups (I–III). Subgroup I comprised all haplotypes from Europe (including the Balkans), Levant (including the Holy Land and Cyprus) and the Maghreb. The other two subgroups (II, III) comprised sequences from the Asian part of the *M. emarginatus* range: subgroup II from Oman and southern Iran; subgroup III from northern Iran and Tajikistan. Subgroup I showed low diversification with genetic distances 0.18–0.73% within the subgroup. The genetic distances between subgroups I and II and subgroups I and III were

TABLE 1. Percent values of interspecific Tamura-Nei distances of *Cyt-b* among mitochondrial subgroups of *M. emarginatus* (below diagonal). The diagonal corresponds to the within-group genetic divergence estimated for *Cyt-b* in each subgroup. See Fig. 1 for identification of subgroups

Mitochondrial subgroup	A	B	C	D	E
A (IB+WE+WB+BA+MG) %	0.27				
B (NL+CY) %	0.18–0.55	0.26			
C (HL) %	0.37–0.64	0.46–0.82	0.13		
D (OM+SI) %	1.47–1.60	1.57–1.85	1.84–2.08	0.22	
E (NI+WT) %	1.57–1.66	1.67–2.04	2.04–2.13	0.93–1.12	0.13

1.47–2.08% and 1.57–2.22%, respectively (Table 1). However, the support of connecting nodes within *M. emarginatus* was low, thus the precise relationship between the subgroups is not yet clear.

The haplotype networks inferred from two *Cyt-b* datasets of *M. emarginatus* further showed the inner branching within this species (Fig. 3). The centre of the haplotype network calculated from the HNshort dataset (703 bp of *Cyt-b*, containing also the published sequences — Fig. 3A) was formed by sequences from 46 specimens originating from western Europe, the Maghreb, Balkans, North Levant and Cyprus. From this central haplotype, 22 other haplotypes shared by sequences of one to six specimens from Europe, the Maghreb and North Levant were separated by differences of one to three substitutions. Furthermore, two Omani and one south-Iranian haplotype (containing sequences from seven and one specimens, respectively) from the subgroup II were distant by ten substitutions from the central haplotype. Finally, a single haplotype containing sequences of 20 samples of the subgroup III from northern Iran and Tajikistan was distant by nine substitutions from the closest Omani haplotype and by 18 substitutions from the central haplotype.

In the haplotype network calculated from the HNlong dataset (1024 bp of *Cyt-b* — Fig. 3B), the haplotype containing sequences of most specimens was formed by 19 haplotypes from western Europe, the Maghreb and the Balkans (haplotype MT-1). Five European and Maghrebian haplotypes, containing one or two sequences, respectively, were divided

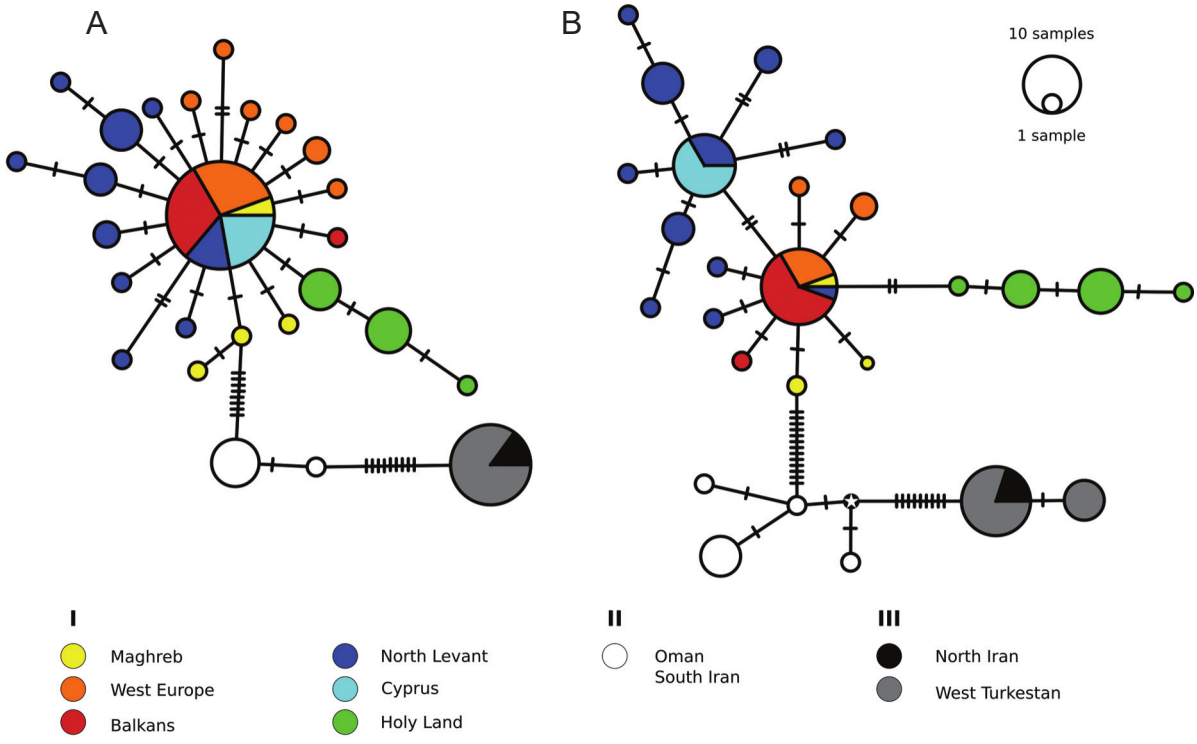

FIG. 3. Median haplotype network of shorter (A) and longer (B) *Cyt-b* sequences of *M. emarginatus*. Coloured circles and circle sectors represent different geographic populations. Black circle with white star represents hypothetical haplotypes connecting those represented by samples. Hatch marks denote base-pair substitutions

from the central Euro-Mediterranean haplotype by only one substitution. The Levantine branch was formed by the main haplotype (haplotype NL+CY-1) sharing sequences of twelve specimens and seven other haplotypes containing one to five sequences from North Levant that differed by one to three substitutions from this main haplotype. Specimens from the Greek island of Symi (geographically belonging to the North Levant) joined the Euro-Mediterranean subgroup I. Four haplotypes containing one to six specimens from the Holy Land differed from the central Euro-Mediterranean haplotype by two to five substitutions. One to five Omani and South Iranian sequences from the subgroup II formed four haplotypes that were 13–15 substitutions distant from the central Euro-Mediterranean haplotype. Finally, the sequences from the subgroup III formed two haplotypes shared by three sequences from northern Iran and twelve from Tajikistan, and five sequences solely from Tajikistan, respectively. These two haplotypes were distant by 14–15 substitutions from the closest Omani haplotype and by 27–28 substitutions from central Euro-Mediterranean haplotype MT-1.

The geographical limits of the network branches (sublineages) from the North Levant and Holy Land run through central Lebanon (Fig. 4). Samples of the North Levantine and Holy Land sublineages were collected in mountain localities of Lebanon (1120–1255 and 1170–1420 m a.s.l., respectively). In contrast, specimens of the North Levantine sublineage in Syria and Turkey were collected from lowland sites (6–542 m a.s.l.) and those of the Holy Land sublineage in Jordan from the uplands (741–812 m a.s.l.).

The tree based on the nuclear dataset (Fig. 2B) showed *M. emarginatus* as well as the rest of the *Myotis* species from the African clade as monophyletic units. One exception is *M. anjouanensis*, which formed an inner branch of *M. goudoti*, making the latter species paraphyletic. The nuclear tree showed only moderate support for the African clade, with an unsupported position of *M. welwitschii* outside the African clade. The relationships between particular species of the clade were again unresolved due to low support of the connecting nodes. Low intraspecific variation, and thus no population structure, has been found in *M. emarginatus* as well as in the other species from the African clade. Hence, *M. emarginatus*, as well as *M. tricolor*, *M. welwitschii* and *M. bocagii*, were represented by a single nuclear lineage each over its whole distribution range.

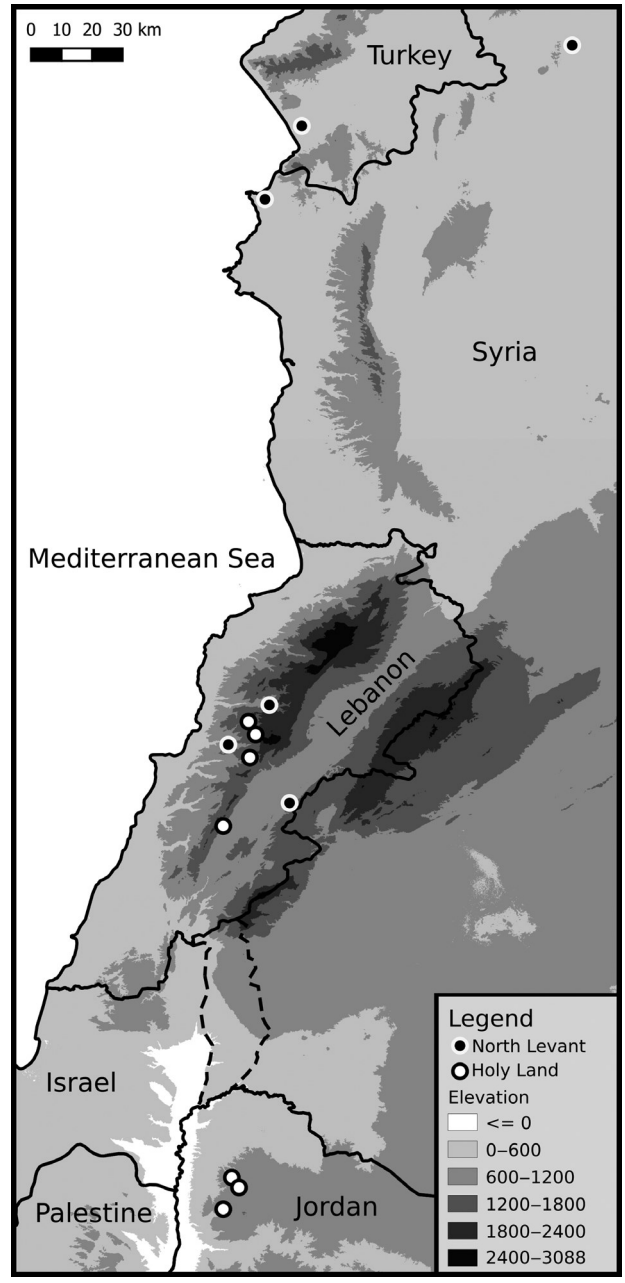

FIG. 4. Map of the Levant. Circles denote localities of samples used in molecular analysis, differences in colour indicate mitochondrial subgroup: black circles with white margin mark the subgroup B from North Levant, white circles with black margin mark the subgroup C from Holy Land

DISCUSSION

The intraspecific classification of geographical variation in *M. emarginatus* was suggested several times and the number of taxonomic units within this species varied between two and four. While the populations of Europe and North Africa were considered to belong to only one subspecies, two to four subspecies were reported from the Asian part of the

species range (Ognev, 1928; Ellerman and Morrison-Scott, 1951; Corbet, 1978; Koopman, 1994; Horáček *et al*., 2000; Topál, 2001; Simmons, 2005; Dietz *et al*., 2007). This inconsistency indicates that the Asian populations are crucial in understanding the intraspecific relationships in the species. For the first time, we generated and analysed genetic data from most of the Asian populations of the species and compared them with broadly sampled European and North African populations. We tried to reconstruct the interspecific relationships of *M. emarginatus* within the African *Myotis* clade by employing newly generated sequences.

Our analyses uncovered the existence of at least three mitochondrial lineages and, interestingly, only one nuclear lineage within *M. emarginatus*. Taking a closer look, the phylogenetic tree (Fig. 2B) revealed two nuclear lineages. However, these two lineages do not match with the mtDNA lineages. Moreover, the nuclear division is without branch support, possibly because three nuclear markers are not enough to resolve the relationships within the African *Myotis* clade (see Morales *et al*., 2019). The available results of the morphometric examination of an extensive set of specimens (Benda *et al*., 2006) showed two main, geographically exclusive morphotypes in this species, according to differences in the body and skull sizes. The small- to medium-sized bats occurred in Europe, the Maghreb and Levant, including Cyprus; the large bats were distributed from the Caucasus region, including the Crimea, through Iran to West Turkestan and Afghanistan. The morphological differences seemed to be in the cline but there is a 400–600 km wide gap between the ranges of both morphotypes. Therefore, isolation by distance could lead to divergence within *M. emarginatus* and these two separately sized morphotypes, occurring in two separate ranges, were assigned to two separate subspecies: *M. e. emarginatus* and *M. e. desertorum* (Benda *et al*., 2006).

The molecular genetic analysis results for the two mitochondrial markers of *M. emarginatus* revealed the following three lineages within this species rank: (I) Circum-Mediterranean (European, Maghrebian and Levantine) lineage, (II) South Iranian and Omani lineage; and (III) North Iranian and West Turkestani lineage. The relatively low values of genetic distances among these lineages are consistent with intraspecific variability. Furthermore, the lineages almost conform with the above-defined morphotypes/taxa. While the Circum-Mediterranean lineage corresponds to the small-sized morphotype, two mitochondrial lineages were demonstrated in the eastern part of the Middle East. On the phylogenetic tree (Fig. 2A, C), these two Asian lineages seemed to be sisters, although their connecting node has no branch support and thus their exact position remains unclear. The close proximity was confirmed by low genetic diversity between the two Asian lineages (ca. 1% of Tamura-Nei distance on *Cyt-b*).

Surprisingly, the results of genetic analysis of the multilocus nuclear dataset revealed only one lineage from the whole species range. This finding is in contrast to findings in other *Myotis* species complexes: *M. nattereri* or *M. mystacinus* were originally similarly distributed but recently were divided into several species using genetic analysis (von Helversen *et al*., 2001; Ibáñez *et al*., 2006; Mayer *et al*., 2007; Juste *et al*., 2018). On the other hand, our results are in accord with the variation found in other similarly distributed species, such as *Rhinolophus hipposideros* (Dool *et al*., 2013), *Pipistrellus pipistrellus* (Boston *et al*., 2014), or *Tadarida teniotis* (Amorim *et al*., 2020). Also *M. tricolor* showed similar divisions in the mitochondrial data and uniformity in the nuclear evidence. Therefore, our nuclear dataset suggests that *M. emarginatus* is uniform over the whole range from West Europe to West Turkestan, and thus does not support any distinct units. However, the combination of size morphotypes defined by Benda *et al*. (2006) and the three mitochondrial lineages defined in the present analysis suggests at least two natural phylogenetic units: all western populations, comprising the small- and medium-sized morphotypes and mitochondrial lineage I from Europe, North Africa and the Levant; and Asian populations, comprising the large-sized morphotype and mitochondrial lineages II and III from Oman through Iran to West Turkestan. The existence of two phylogenetic units is supported by the geographical distances when the gap between populations is 600 km wide and also by the genetic distances, which are between 1.47% and 2.13% for *Cyt-b*. The closest Asian localities to western populations are from northern Iraq and Caucasus, with a gap of more than 400 km. Those populations were not genetically analysed and samples from Iraq were not available even for morphological evaluation. However, due to the close geographical proximity to Asian populations, as well as the morphological proximity of Caucasian samples, the Iraqi and Caucasian populations were assigned to Asian populations. Thus, we suggest that the two phylogenetically and geographically detached units could be regarded as two subspecies of

M. emarginatus despite the absence of measurable nuclear differences.

This arrangement unifies the populations of *M. emarginatus* over the whole Mediterranean and most parts of the European range into one unit: the western bats. The morphological variation in this unit, examplified by variations in body and skull size (Benda *et al*., 2006), is probably related to the highly heterogeneous environment, ranging from dry scrublands at the southern limits of the occurrence range (Morocco, Israel, Jordan) to the deciduous or mixed forests of the central latitudes of Europe (Netherlands, Germany, Czech Republic, Poland). In contrast, little variation in genetic diversity was revealed when the distance among mtDNA haplotypes within this unit was only 0.18–0.82%. Taking a closer look (Figs. 2C, 3B), western bats could be divided into two subgroups according to branch support: those from Europe, Maghreb and North Levant; and those from the Holy Land. The star-like shapes in haplotype networks (Fig. 3) reconstructed from *Cyt-b* datasets suggest a fast and recent colonization of the whole Mediterranean and continental European range, possibly from a Levantine refugium where populations seem most diversified, to create two separate sublineages: the North Levantine and the Holy Land. Nevertheless, further taxonomic subdivision is not warranted as, metrically, the bats from northern and southern parts of the Levant represent a single group (Benda *et al.*, 2006). These two sublineages could be based on historical divergence: outside Lebanon the bats of the Holy Land occur in the upland areas whereas the North Levantine sublineage was detected mostly in the lowlands (Benda *et al*., 2006, 2010, 2016; Fig. 4). These distinct environments are perhaps occupied by bats from isolated refugia that were situated in separate parts of the geographically diverse landscape of the Levant. Now, bats from both refugia/sublineages might be in secondary contact in the Lebanon mountains. Overall, the arrangement of the European, African and Levantine populations into one unit corresponds to the opinions of many previous authors, who regarded these populations as one common taxon: the nominotypical subspecies (Ellerman and Morrison-Scott, 1951; Corbet, 1978; Harrison and Bates, 1991; Koopman, 1994; Topál, 2001; Karataş and Özgül, 2003; Benda *et al*., 2006; Dietz *et al*., 2007; Mayer *et al*., 2007; Albayrak, 2015).

The populations of *M. emarginatus* of the Asian range (excluding the Levant) form a second phylogenetic unit: the eastern bats. These populations include the largest representatives of the species and occur in very arid areas from northern Iraq, Iran and north-eastern Oman through eastern Afghanistan to the southern and eastern parts of West Turkestan, as well as in Caucasus (Fig. 1); this part of the species range is geographically separated from the European and Levantine populations. The earlier name corresponding to the distribution range of this unit is *Vespertilio desertorum* Dobson, 1875 (Ellerman and Morrison-Scott, 1951; Simmons, 2005), originating from Jalk, Baluchistan, south-eastern Iran (Blanford, 1875). Thus, the name *M. e. desertorum* (Dobson, 1875) is regarded here as the valid name of the unit.

Recent molecular studies placed *M. emarginatus* into the African clade of the genus *Myotis*, even though it is essentially a Palaearctic species (Stadelmann *et al.*, 2004, 2007; Patterson *et al.*, 2019). These studies considered *M. emarginatus* as a sister species to the sub-Saharan *M. tricolor*, but with poor phylogenetic support. Even the topology within the African *Myotis* clade differs, except for its species content (Stadelmann *et al.*, 2004, 2007; Patterson *et al.*, 2019), a result that is also concordant with the present reconstructions (Fig. 2). Surprisingly, advances in molecular markers, such as ultraconserved elements, have not yet helped to fully solve the topology (Morales *et al.*, 2019) and perhaps genome analysis is needed to solve the position of *M. emarginatus* within the African clade of the genus *Myotis* and to reconstruct interspecific relationships within the African clade in general.

In summary, the results of our analysis confirmed *M. emarginatus* to be a polymorphic species belonging to the African clade of the genus *Myotis*. Although the geographical variation of *M. emarginatus* is not extensive and the differences originated in relatively late events, its extent and geographical scale correspond to the divisions of two rather well-supported subspecies within its species rank: *M. e. emarginatus* (Geoffroy, 1806) distributed in the European and African Mediterranean, including islands, in western and central Europe, and in the Levant, including Cyprus; and *M. e. desertorum* (Dobson, 1875) in the eastern part of the Middle East, including Oman, Iran, West Turkestan and most likely also Afghanistan as well as the Caucasus. Although rather extensive, our coverage of the distribution range of *M. emarginatus* was not complete, namely in its Asian part. Thus, the phylogenetic and taxonomic positions of some *M. emarginatus* populations still remain unclear. This is valid especially for populations living in the south-western part of Arabia, where neither morphometric nor

genetic data are available. For a complete taxonomic revision of the species rank of *M. emarginatus*, a more complete synthesis of the geographical coverage and both genetic and profound morphological evidence are required.

Supplementary Information

Contents. Supplementary Tables: Table S1. Names, sequences and annealing temperatures of primers used in this study; Table S2. Substitution models as identified by ModelFinder for the different partitions used in MrBayes and IQTREE, respectively; Supplementary Figures: Fig. S1. Maximum likelihood tree of the reconstructed phylogenetic relations of *M. emarginatus* with selected species from the African clade of the genus *Myotis* based on the concatenated dataset of all markers; Fig. S2. Maximum likelihood tree of the reconstructed phylogenetic relations of *M. emarginatus* with species from the African clade of the genus *Myotis* based on *Cyt-b*; Fig. S3. Maximum likelihood tree of the reconstructed phylogenetic relations of *M. emarginatus* with available species from the African clade of the genus *Myotis* based on *D-loop*; Fig. S4. Maximum likelihood tree of the reconstructed phylogenetic relations of *M. emarginatus* with available species from the African clade of the genus *Myotis* based on *ACOX*; Fig. S5. Maximum likelihood tree of the reconstructed phylogenetic relations of *M. emarginatus* with available species from the African clade of the genus *Myotis* based on *STAT*; Fig. S6. Maximum likelihood tree of the reconstructed phylogenetic relations of *M. emarginatus* with available species from the African clade of the genus *Myotis* based on *Rag2*. Supplementary Information is available exclusively on BioOne.

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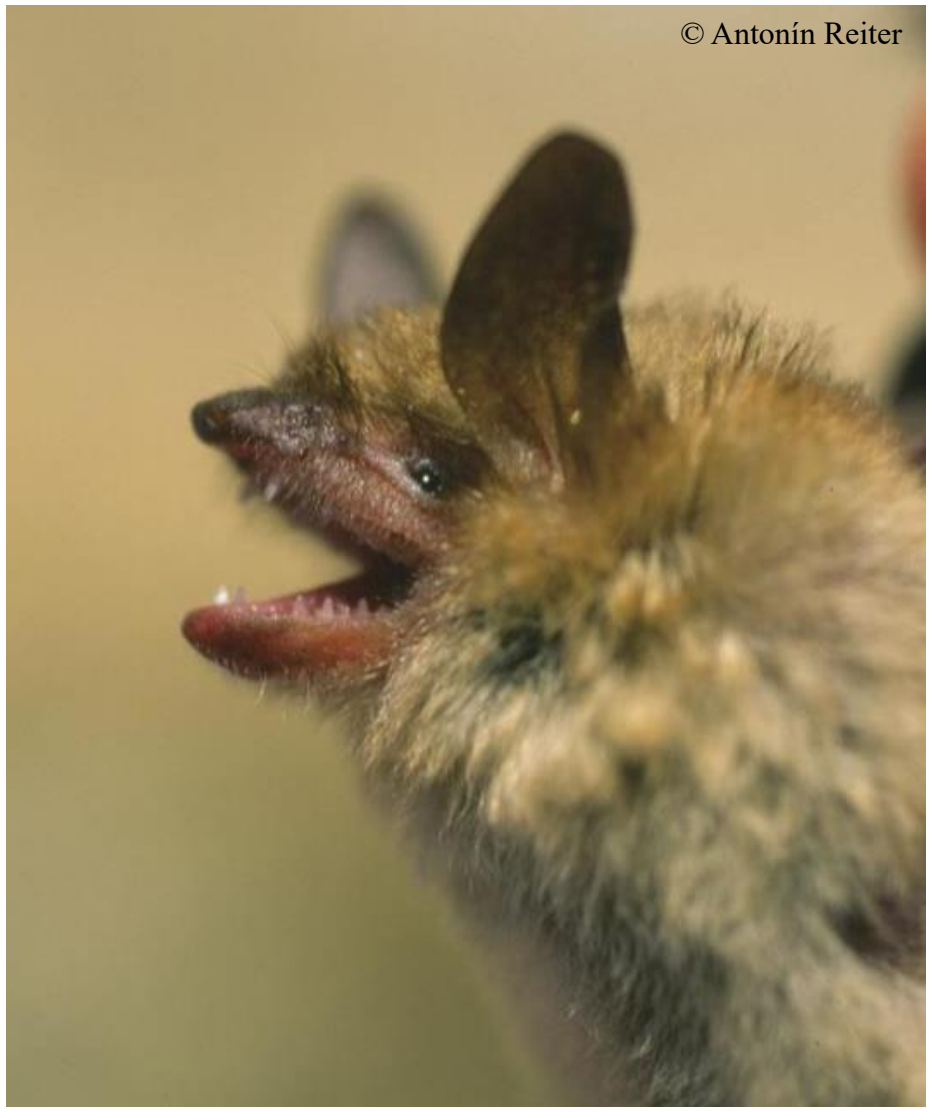

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Taxonomic revision of *Myotis emarginatus*: detailed morphometric analysis and final evaluation of the evidence (Chiroptera: Vespertilionidae)

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Abstract. The Geoffroy's bat, *Myotis emarginatus*, is the only species distributed in the Palaearctic belonging to the African clade of the genus *Myotis*. It occurs extensively across several ecologic zones of Europe, north-western Africa, and western and central Asia, and hence it was considered to be a polytypic species. Only one subspecies was reported from Europe and North Africa, up to four subspecies were recognised in Asia. However, the validity of particular taxa as well as the systematic positions of different populations remained ambiguous. Here we present a revision of the intraspecific phylogenetic structure of *M. emarginatus* based on combination of the available results of a molecular genetic analysis with the results of a thorough morphologic examination of an extensive specimen set from almost the whole range of its distribution. The previously described geographic variability in the mitochondrial markers demonstrated grouping of haplotypes of *M. emarginatus* into three main lineages that occur in (1) the Mediterranean Basin (including central Europe, the Maghreb and Levant), (2) Oman and south-eastern Iran, and (3) northern Iran and West Turkestan. The morphologic comparison uncovered the existence of four main, geographically exclusive morphotypes in *M. emarginatus*, concerning the body, skull and tooth sizes, and skull and tooth shapes: (1) rather small bats with short rostrum and high braincase, occurring in Europe and north-western Africa; (2) rather medium-sized bats with long rostrum and short braincase from the Levant including Cyprus; (3) large bats with wide and long rostrum from the south-eastern parts of the Middle East, including Oman, south-eastern Iran and eastern Afghanistan, and (4) large bats with narrow and short rostrum, occurring in Crimea, the Caucasus region, and West Turkestan. As a synthesis of the results of both approaches, we suggest to recognise three subspecies within the *Myotis emarginatus* species rank – *M. e. emarginatus* (Geoffroy, 1806) distributed in the Mediterranean, central and western Europe, north-western Africa, and in the Levant; *M. e. desertorum* (Dobson, 1875) in the south-eastern Middle East, including southern Iran, Oman, and Afghanistan; and *M. e. turcomanicus* Bobrinskoj, 1925 in the Caucasus region, Crimea, Transcaucasia, and West Turkestan.

Key words. *Myotis emarginatus*, morphology, morphometry, phylogenetics, taxonomy.

INTRODUCTION

The Geoffroy's bat, *Myotis emarginatus* (Geoffroy, 1806), is a bat with the Mediterranean type of distribution, occuring in the south-western part of the Palaearctic (Corbet 1978, Koopman 1994, Horáček et al. 2000). It represents the only species of the African clade of the genus *Myotis* (sometimes referred to the subgenus *Chrysopteron* Jentink, 1910), substantially distributed

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in the Palaearctic (Stadelmann et al. 2004, Ruedi et al. 2013, Morales et al. 2019). This bat occurs in the Maghreb from Morocco to Tunisia, in Europe from Portugal and the Netherlands through central and southern Europe to the Balkans, Crimea and Caucasus region, including some Mediterranean islands (Topál 2001, Dietz et al. 2007); and discontinuously in Asia from the Levant, Asia Minor and Iraq to south-eastern Kazakhstan, southern Kirghizstan, and eastern Afghanistan (Rybin et al. 1989, Habilov 1992, Benda et al. 2006, 2012, Benda & Gaisler 2015, Al-Sheikhly et al. 2016). However, this species also almost reaches the Afro-tropical region in Arabia, its range continues patchily to north-eastern Oman and to western Saudi Arabia and Yemen (Harrison 1977, Gaucher 1995, Al-Jumaily 2003).

In this broad range (Fig. 1), stretching over a wide longitudinal belt across several ecologic and biogeographic zones, *M. emaginatus* was traditionally considered a polytypic species (Ognev 1928, Ellerman & Morrison-Scott 1951, Corbet 1978, Koopman 1994, Horáček et al. 2000, Topál 2001, Simmons 2005, Dietz et al. 2007). Up to four subspecies have been recognised; viz. *M. e. emarginatus* (Geoffroy, 1806) in the Mediterranean Basin and adjacent areas of Europe, the Maghreb and Levant (terra typica [t.t.]: Charlemont [Givet, Champagne-Ardenne, France]; Geoffroy-Saint-Hilaire 1806: 198); *M. e. desertorum* (Dobson in Blanford, 1875) in the south-eastern part of the Middle East (t.t.: Jálk, Balúchistán [Iran]; Blanford 1875: 309); *M. e. turcomanicus* Bobrinskoj, 1925 in the western part of West Turkestan (t.t.: Moorghab [= Murgab] River Valley, Turkmen-Kala, West Turkestan [Turkmenistan]; Bobrinskoj 1925: 359); and *M. e. kuzyakini* Rossolimo et Pavlinov, 1979 in the eastern part of West Turkestan (t.t.: Taškent [Uzbekistan]; Kuzâkin 1934: 320). However, the opinions on geographic variation in this bat are based mostly on the comparisons of colour morphs and/or of metric data of an insufficient number of specimens (see Topál 2001).

Despite this relatively simple geographic division, the history of the intraspecific taxonomic classification of *M. emarginatus* is not straightforward. While the opinions concerning the taxonomic arrangement of the Mediterranean and European populations of this bat are rather consistent and assign these populations more or less constantly to the nominotypical subspecies (see e.g. Ognev 1928, Ellerman & Morrison-Scott 1951, Harrison 1964, Corbet 1978, Gaisler 1983, Harrison & Bates 1991, Kowalski & Rzebik-Kowalska 1991, Koopman 1994, Horáček et al. 2000, Topál 2001, Karataş & Özgül 2003, Albayrak 2015, etc.), the opinions on the subspecific variation within the Asian part of the species range are more intricate. Considering the latter populations, four names have been created in the rank of *M. emarginatus*, while only one of them was synonymised early after its description.

The name *M. lanaceus* (t.t.: Shastun, Dizak, Baluchistan [Iran]; Thomas 1920: 933), originally created as *lanceus* and corrected to the current form by Wroughton (1920), is now considered to be a junior synonym of the name *Vespertilio desertorum* Dobson, 1875 as these names were described from the sites within a distance of only 50 km and the colouration and body size of the representatives are reported to be almost identical (Ognev 1928, Ellerman & Morrison-Scott 1951, DeBlase 1980).

The taxon *desertorum* was originally considered to be a separate species distributed from eastern Transcaucasia to Central Asia and Iran (Dobson 1878, Satunin 1896, 1914, Bianki 1917, Thomas 1920). On the other hand, Bobrinskoj (1925) and Ognev (1927) regarded the West Turkestani populations of *M. emarginatus* to belong to *M. e. turcomanicus*, differing from the nominotypical and Iranian forms by ear morphology and pelage colouration. However, Ognеff & Heptner (1928) and Ognev (1928) very early synonymised the latter name with *M. e. desertorum*, which they considered to occur in West Turkestan and Iran, and besides that, they

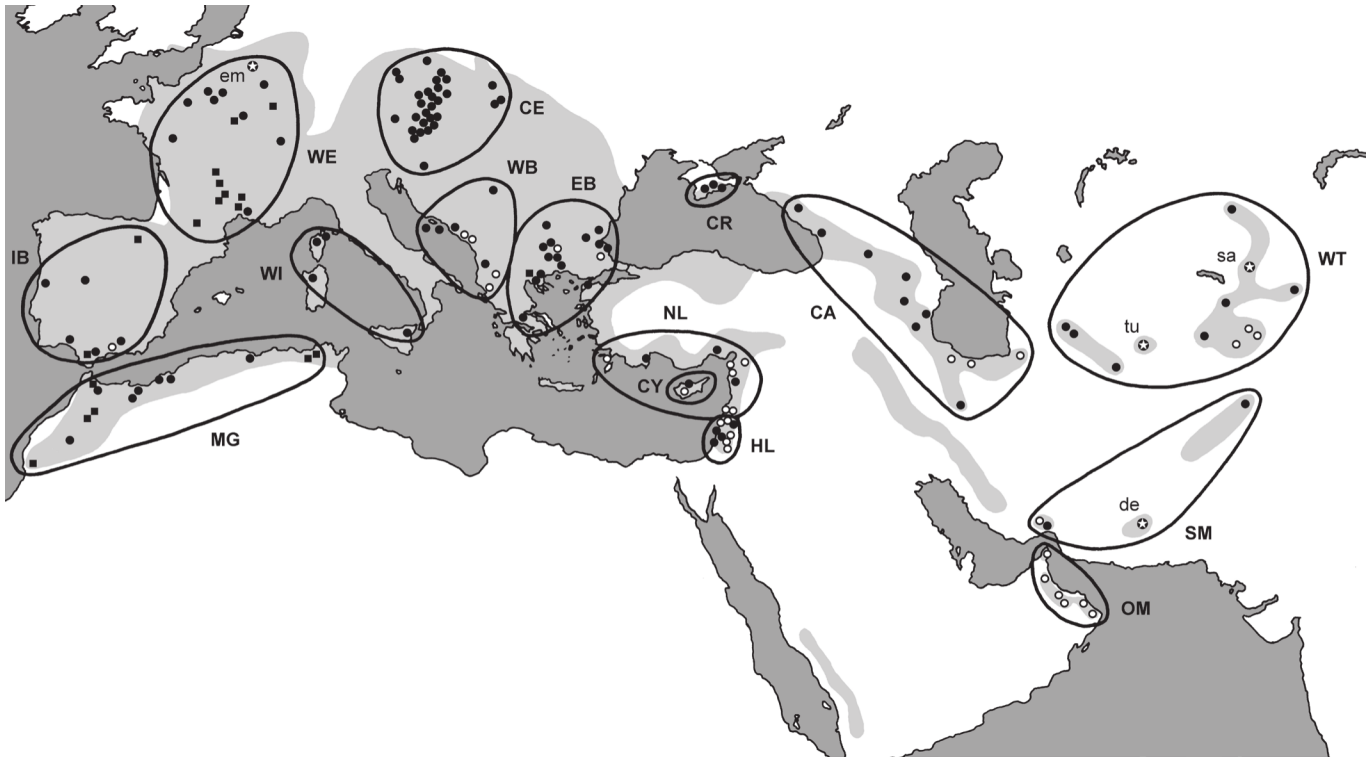


Fig. 1. Map of the distribution range of *Myotis emarginatus* (pale grey; after numerous sources) and the localities of origin and grouping of the examined samples; full circles indicate the samples examined in the present morphologic comparison, open circles indicate the samples examined both in the present morphologic comparison and in the molecular genetic analysis by Uvizl & Benda (2021a), and full squares the samples used only in the genetic analysis. The circles with asterisk indicate type localities of the examined type specimens: de = *desertorum* Dobson, 1875, em = *emarginatus* Geoffroy, 1806, sa = *saturatus* Kuzâkin, 1934 (= *kuzyakini* Rossolimo et Pavlinov, 1979), tu = *turcomanicus* Bobrinskoj, 1925. The ovals denote geografic groupings of specimens: CA = Caucasus, CE = Central Europe, CR = Crimea, CY = Cyprus, EB = East Balkans, HL = Holy Land, IB = Iberia, MG = Maghreb, NL = North Levant, OM = Oman, SM = SE Middle East, WB = West Balkans, WE = West Europe, WI = West Islands, WT = West Turkestan.

recognised only *M. e. emarginatus* within the remaining species range (Crimea, Transcaucasia, Europe, North Africa). However, Kuzâkin (1934, 1935) defined a third Asian subspecies from the eastern part of West Turkestan, *M. e. saturatus* Kuzâkin, 1934 [nec *M. yumanensis saturatus* Miller, 1897; replaced with *M. e. kuzyakini* as a nomen novum, see Rossolimo & Pavlinov 1979: 13], being darker coloured than other Asian taxa, while he regarded the form *turcomanicus* just a synonym of *M. e. desertorum*, following Ognev (1928).

Ellerman & Morrison-Scott (1951) suggested all three Asian forms as valid subspecies, *desertorum*, *turcomanicus*, and *saturatus* [= *kuzyakini*], besides the nominotypical subspecies. Strelkov (1963) distinguished only two subspecies within the area of the former Soviet Union, the darker *M. e. emarginatus* in Europe and the Caucasus region, and the paler *M. e. desertorum* in West Turkestan. Since the latter name is the prior synonym in the non-European part of the distribution range, Strelkov's (1963) taxonomic division applies to the whole species range (perhaps with the exceptions of North Africa and the Levant). Afterwards, Kuzâkin (1965) and Strelkov (1981) presented a three subspecies concept in *M. emarginatus* in its former Soviet range; it comprised a relatively dark and small-sized *M. e. emarginatus* in Europe, North Africa, Crimea, and Transcaucasia, a relatively pale and small-sized *M. e. desertorum* in the deserts of Iran and southern Turkmenistan, and a relatively dark and large *M. e. saturatus* [= *kuzyakini*] in eastern West Turkestan. Corbet (1978) adopted this conception for the whole range of the species, i.e. he assigned also the populations of North Africa to *M. e. emarginatus* along with those of Europe, but the status of the Levantine bats remained unspecified.

DeBlase (1980) determined even two to three colour forms in the territory of Iran and co-identified them with separate subspecies; he restricted the pale *M. e. desertorum* to southeastern Iran only; in southwestern, western and northern parts of the country he found the dark *M. e. emarginatus*, and a possible third unnamed form with a very reddish pelage in the Caspian region. Additionally, Harrison & Bates (1991), who first evaluated *M. emarginatus* samples from Oman, identified these bats as *M. e. desertorum*.

As a result, Koopman (1994) summarised the intraspecific taxonomy of *M. emarginatus* with four subspecies, *M. e. emarginatus* in Europe, northwestern Africa and south-western Asia, *M. e. desertorum* in Oman to Afghanistan, *M. e. turcomanicus* in Turkmenistan to Afghanistan [!], and *M. e. saturatus* [= *kuzyakini*] in Uzbekistan. Horáček et al. (2000) adopted this arrangement, although they did not specify distribution of the particular subspecies. On the other hand, Simmons (2005) recognised only three subspecies in *M. emarginatus*, i.e. the above-mentioned content by Koopman (1994) except *kuzyakini*, and considered the Uzbekistani populations a part of the nominotypical form from Europe.

These opinions could be summarised as follows; the authors who evaluated only individual samples or small sample series of *M. emarginatus* reported significant differences in the pelage colouration and the different colour morphs assigned to separate taxa, while the authors who evaluated large sets of *M. emarginatus* from different types of habitats, found a mosaic of colouration morphs (see also Topál 2001). Therefore, Benda et al. (2006) who compared a large set of specimens from most parts of the species range, suggested the intensity of colourations tinges to be most probably linked to humidity level of the particular habitat; the pale individuals were reported to occur in the lowland semi-arid regions of West Turkestan and Iran, while individuals found in the arboreal habitats of the Mediterranean (including parts of western Asia) were dark reddish- or orange-brown coloured. Thus, they concluded that the pelage colouration represents a varying character adaptive to the local environment conditions with a low or no reflection in the phylogenetic relations within the species as a whole.

Additionally, Benda et al. (2006) were the only who tried to evaluate the geographic variation in *M. emarginatus* with the help of a morphometric analysis of a large set of museum specimens. Their results showed three basic groups of size morphotypes within the species distribution range, which were referred by these authors to two subspecies. The large-sized morphotype contained all the populations of the eastern parts of the species range (Crimea, Caucasus, Iran, Afghanistan, West Turkestan) and was identified under the prior name originating from the region, *M. e. desertorum*. The other two morphotypes, the small-sized morphotype of populations from south-western and central Europe and the northern part of the Balkans as well as the medium-sized morphotype originating from two separate regions, the Maghreb and eastern Mediterranean (southern Balkans to the Levant), were considered to represent the nominotypical subspecies. This view of two subspecies in *M. emarginatus* has been recently adopted by López-Baucells (2019).

Several initial molecular genetic analyses of the mitochondrial sequences of *M. emarginatus* (Ibáñez et al. 2006, Mayer et al. 2007, García-Mudarra et al. 2009) covered only the Mediterranean Basin and Central Europe. The samples from Morocco, Iberia, Belgium, Germany, Greece, and Israel formed a single clade with very low divergences – according to indirect indications by the respective authors, about less than 1% of genetic distances. This was in concert with

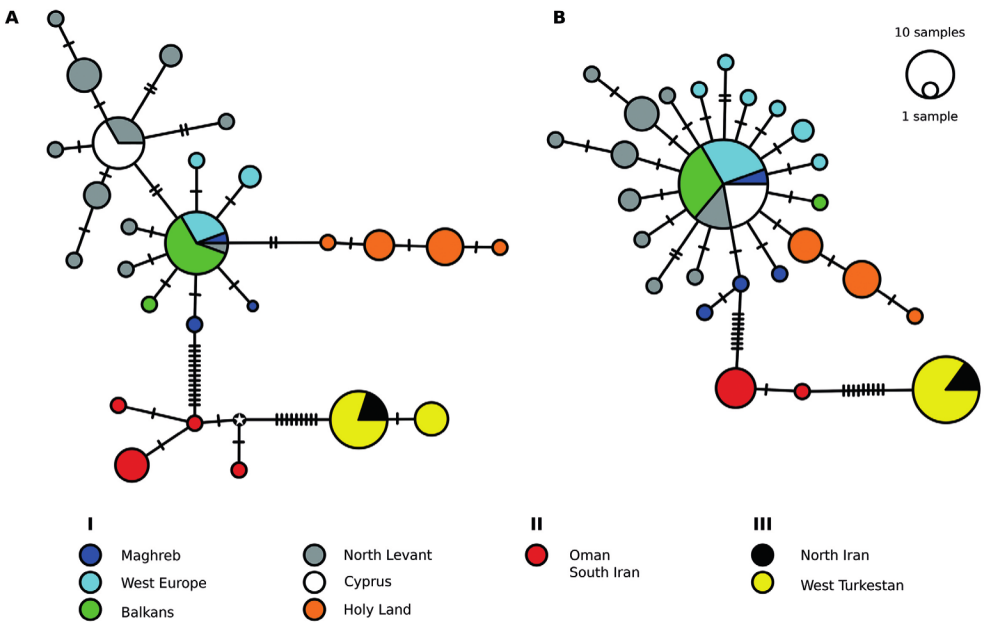


Fig. 2. Median haplotype networks computed from sequences of the mitochondrial gene for cytochrome *b* of *Myotis emarginatus* (after Uvizl & Benda 2021a, slightly modified); A – a network from the sequences of length 1024 bp (n=93), B – a network from the sequences of length 703 bp (n=104). Coloured circles and circle sectors represent different geographic populations. Black circle with a white star represents hypothetical haplotype connecting those represented by samples, hatch marks denote base pair substitutions.

the traditional opinions that the Mediterranean Basin and Central Europe are inhabited by only one taxon, i.e. the nominotypical subspecies (see above). Neverthelles, the systematic position of the remaining populations of *M. emarginatus* remained unclear and the same applied to the mutual relations of the Euro-Mediterranean and Asian populations of this bat.

As a next step, we performed a thorough molecular genetic analysis of more than a hundred of samples of *M. emarginatus* from most of its distribution range, from Morocco and France to Oman and Tajikistan (see Fig. 1), using both mitochondrial and nuclear markers (Uvizl & Benda 2021a). While the results of analysis of the mitochondrial genome (D-loop of the control region and the cytochrome *b* gene) revealed existence of three haplotype groups (Fig. 2), the nuclear markers (recombination activating gene 2, acyl-coenzyme A oxidase 2 intron, and signal transducer and activator of transcription 5A intron) did not show any inner arrangement of the haplotypes and all sequences belonged to one well supported common lineage. The geographic arrangement of the mitochondrial haplogroups corresponded with three regions, (1) Europe and the Mediterranean Basin, (2) Oman and south-eastern Iran, and (3) northern Iran and West Turkestan; the Euro-Mediterranean lineage was again diversified into three sublineages, Euro-Maghrebian, North Levantine, and South Levantine (Holy Land), see Fig. 2. The interpretation of these results followed the preliminary results of the morphometric analysis by Benda et al. (2006) and suggested to accept two basic phylogroups within the species, the western and eastern ones, corresponding with two subspecies sensu Benda et al. (2006), *M. e. emarginatus* in the west and *M. e. desertorum* in the east of the species range.

However, these conclusions were based on a morphologic comparison that used only a geographically limited set of samples (some 320 specimens) and mainly, only a basic set of analysed variables (12 craniodental dimensions). Therefore, the intraspecific status of particular populations of *M. emarginatus* in the whole species cannot be considered resolved. The results of the profound molecular genetic analyses must be interpreted in the light of results of a profound morphologic analysis. Thus, we conducted a fine morphologic examination of a set of almost five hundred specimens from Europe, North Africa, Middle East, Caucasus region and West Turkestan, i.e. from all parts of the distribution range of this bat (except for south-western Arabia; see Fig. 1).

The results of this morphologic analysis along with the results of the molecular genetic analysis (Uvizl & Benda 2021a) enabled us to make a sufficiently supported synthesis of the intraspecific arrangement of *M. emarginatus*, and its taxonomic formulation.

MATERIAL AND METHODS

Analysis

In the morphologic analysis, museum material of more than 460 specimens of *Myotis emarginatus* from the majority of its distribution range was used, see Fig. 1 and Appendix for the origin of the specimens examined. Primarily cranial and dental data were used for the analyses, see Abbreviations and terminology for the dimensions taken. In the comparison, the type material of the names *emarginatus* Geoffroy, 1806, *desertorum* Dobson, 1875, *turcomanicus* Bobrinskoj, 1925, and *saturatus* Kuzâkin, 1934, was evaluated.

The specimens were measured in a standard way using mechanical or optical calipers. Statistical analyses were performed using the Statistica 6.0 software. We performed also a stepwise discriminant function analysis as a test of an importance of particular dimensions for the intraspecific variation. Statistically significant parameters most affecting the morphologic variation were selected and employed in a subsequent canonical analysis.

Abbreviations and terminology

Dimensions. LAt = forearm length (incl. wrist); – LCr = greatest length of skull; – LCb = condylobasal length; – LaZ = zygomatic width; – LaI = width of interorbital constriction; – LaInf = rostral width between infraorbital foramens; – LaN = neurocranium width; – ANc = neurocranium height; – CC = rostral width between upper canines (incl.); – M^3M^3 = rostral width between 3rd upper molars (incl.); – CM^3 = length of upper tooth-row between canine and 3rd molar (incl.); – CP^4 = length of upper tooth-row between canine and 3rd premolar (incl.); – P^2P^3 = length of upper tooth-row between 1st and 2nd premolars (incl.); – LP^3 = mesio-distal crown length of upper 2nd premolar (P^3); – LP^4 = mesio-distal crown length of upper 3rd premolar (P^4); – LaP^4 = palato-labial crown width of upper 3rd premolar (P^4); – M^1M^3 = length of upper molar-row (incl.); – LaM^1 = palato-labial crown width of upper 1st molar; – LaM^2 = palato-labial crown width of upper 2nd molar; – LaM^3 = palato-labial crown width of upper 3rd molar; – LMd = condylar length of mandible; – ACo = height of coronoid process; – CM_3 = length of lower tooth-row between canine and 3rd molar (incl.).

Collections. BMNH = Natural History Museum, London, United Kingdom; – EBD = Doñana Biological Station, Seville, Spain; – ISEA = Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Cracow, Poland; – IVB = Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno, Czech Republic; – JOC = Ján Obuch Collection, Blažovce, Slovakia; – MHNG = Natural History Museum, Geneva, Switzerland; – MNHN = National Museum of Natural History, Paris, Fran-ce; – MSNG = Civil Museum of Natural History Giacomo Doria, Genoa, Italy; – MUB = Department of Zoology, Masaryk University, Brno, Czech Republic; – NMP = National Museum (Natural History), Prague, Czech Republic; – NMW = Natural History Museum, Vienna, Austria; – SMF = Research Institute and Museum Senckenberg, Frankfurt am Main, Germany; – TAU = Tel Aviv University, Tel Aviv, Israel; – VMO = Regional Museum, Olomouc, Czech Republic; – ZFMK = Zoological Institute and Museum Alexander Koenig, Bonn, Germany; – ZIN = Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia; – ZMH = Zoological Museum, University of Hamburg, Hamburg, Germany; – ZMMU = Zoological Museum of the Moscow State University, Moscow, Russia; – ZZZ = Zoological Collection, Faculty of Science, University of Zagreb, Zagreb, Croatia.

Other abbreviations. A = alcoholic specimen; – B = skin (balg); – ♀ = female; – ♂ = male; – M = mean; – max, min = range margins; – S = skull; – SD = standard deviation; – Sk = skeleton.

Geographic terms (considering origin of the examined material; in parentheses acronyms used in text and Fig. 1): Balkans (BA) = West Balkans plus East Balkans (WB+EB); – Caucasus (CA) = Azerbaijan, northern Iran, Georgia, and Russian Caucasus; – Central Europe (CE) = Austria, northern Croatia, Czech Republic, and Slovakia; – Crimea (CR) = Crimea (Ukraine); – East Balkans (EB) = Bulgaria, Greece, and Turkish Thrace; – Holy Land (HL) = Israel, Jordan, and southern Lebanon; – Iberia (IB) = Portugal and Spain; – Levant = North Levant plus Holy Land (NL+HL); – Maghreb (MG) = Algeria, Morocco, and Tunisia; – North Levant (NL) = Greek Dodecaneses, northern Lebanon, Syria, and southern Turkey; – Oman (OM) = Oman; – SE Middle East (SM) = Afghanistan and south-eastern Iran; – West Balkans (WB) = Albania, southern Croatia, Montenegro, and Serbia; – West Europe (WE) = France and Switzerland; – West-Central Europe (WCE) = Iberia, West Europe, West Islands, plus Central Europe (IB+WE+WI+CE); – West Islands (WI) = Corsica, Sardinia, and Sicily; – West Turkestan (WT) = north-eastern Iran, Kazakhstan, Kirghizstan, Tajikistan, Turkmenistan, and Uzbekistan.

RESULTS

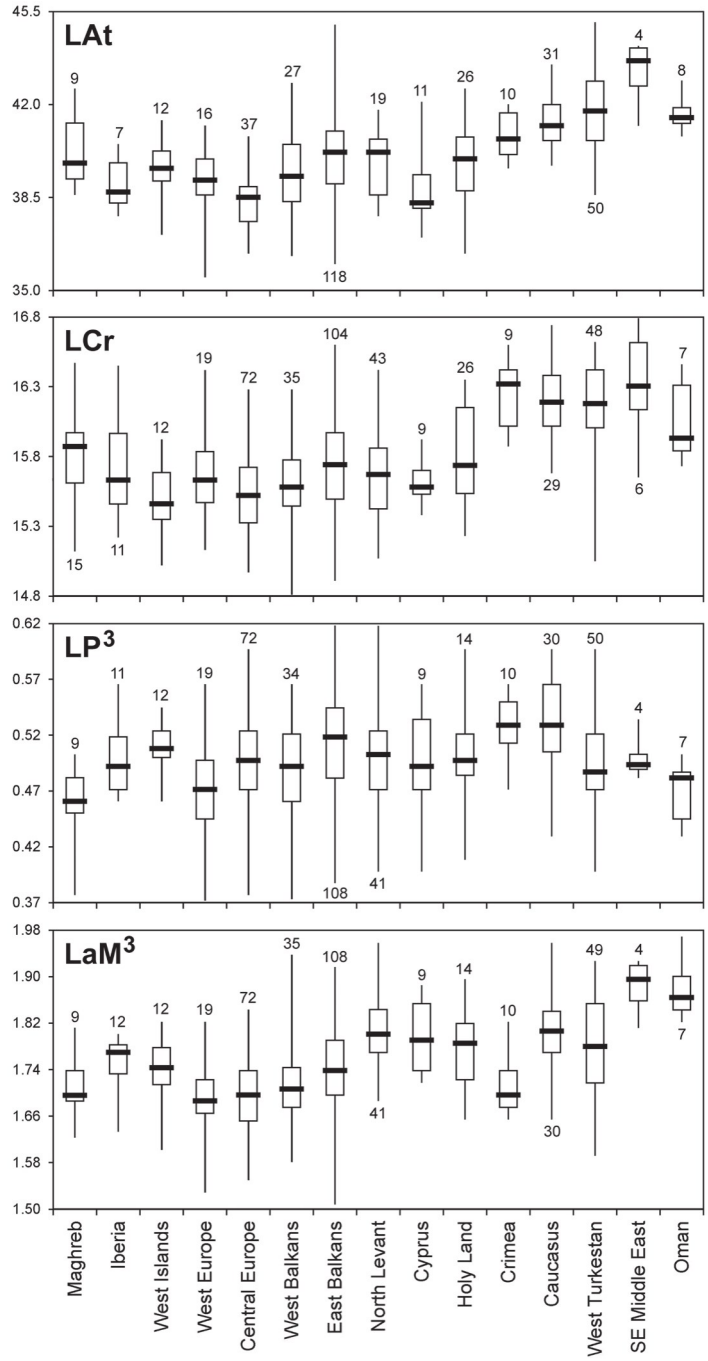
The comparison of morphometric characters of particular population sets of *Myotis emarginatus* samples documented an extreme variation in body, skull, and tooth sizes as well as in skull and tooth shapes. In most dimensions, both in their absolute and relative values, the ranges found in the most numerous sets (n>25) overlapped with or exceeded the ranges of the less numerous sets. However, metric trends in the particular population sample sets were well

Table 1. Morphometric data on the examined sets of specimens. For the geographic delimitations of particular sets see Fig. 1, for dimension abbreviations see Abbreviations and terminology; PC dim = PCA results computed from skull dimensions, PC ind = PCA results from skull indices

variable	Maghreb (MG)					Iberia (IB)					West Islands (WI)				
	n	**M**	min	max	SD	n	**M**	min	max	SD	n	**M**	min	max	SD
LAt	9	**40.34**	38.6	42.6	1.482	7	**39.03**	37.8	40.5	1.044	12	**39.54**	37.1	41.4	1.105
LCr	15	**15.78**	15.12	16.47	0.349	11	**15.71**	15.22	16.45	0.388	12	**15.51**	15.02	15.92	0.254
LCb	15	**14.83**	14.17	15.57	0.323	11	**14.82**	14.17	15.37	0.363	12	**14.57**	13.88	14.97	0.298
LaZ	15	**9.88**	9.57	10.18	0.194	11	**9.76**	9.12	10.20	0.316	8	**9.60**	9.12	10.02	0.274
LaI	15	**3.58**	3.45	3.73	0.077	11	**3.50**	3.37	3.67	0.094	12	**3.47**	3.32	3.63	0.105
LaInf	15	**3.84**	3.67	4.00	0.097	12	**3.73**	3.34	3.92	0.175	12	**3.75**	3.47	3.95	0.149
LaN	15	**7.42**	7.20	7.60	0.127	12	**7.27**	6.88	7.60	0.182	12	**7.21**	7.08	7.35	0.092
ANc	15	**5.87**	5.58	6.14	0.142	12	**5.81**	5.38	6.17	0.215	12	**5.72**	5.48	5.98	0.127
CC	15	**4.03**	3.81	4.22	0.127	10	**3.88**	3.35	4.17	0.285	11	**3.90**	3.57	4.18	0.198
M^3M^3	15	**6.23**	5.83	6.47	0.176	12	**6.00**	5.60	6.28	0.204	12	**6.05**	5.67	6.33	0.209
CM^3	15	**6.32**	5.93	6.54	0.157	11	**6.28**	5.98	6.50	0.156	12	**6.24**	5.86	6.42	0.148
CP^4	15	**3.09**	2.89	3.34	0.124	11	**3.19**	3.03	3.34	0.088	12	**3.18**	3.00	3.34	0.087
P^2P^3	15	**1.03**	0.91	1.13	0.063	11	**1.06**	1.01	1.19	0.057	11	**1.08**	1.02	1.11	0.035
LP^3	9	**0.46**	0.38	0.50	0.037	11	**0.50**	0.46	0.57	0.033	12	**0.51**	0.46	0.54	0.023
LP^4	15	**1.22**	1.07	1.30	0.066	12	**1.21**	1.15	1.30	0.044	12	**1.24**	1.11	1.32	0.060
LaP^4	15	**1.39**	1.32	1.45	0.034	12	**1.35**	1.29	1.40	0.032	12	**1.36**	1.27	1.42	0.045
M^1M^3	15	**3.56**	3.39	3.87	0.130	12	**3.54**	3.34	3.66	0.094	12	**3.48**	3.26	3.63	0.100
LaM^1	9	**1.56**	1.48	1.68	0.066	12	**1.58**	1.52	1.65	0.040	12	**1.55**	1.45	1.61	0.057
LaM^2	9	**1.81**	1.76	1.91	0.051	12	**1.83**	1.76	1.96	0.055	12	**1.80**	1.65	1.86	0.055
LaM^3	9	**1.71**	1.62	1.81	0.055	12	**1.75**	1.63	1.80	0.059	12	**1.74**	1.60	1.82	0.060
LMd	15	**11.65**	11.17	12.15	0.266	11	**11.56**	11.22	11.85	0.223	12	**11.46**	10.95	11.92	0.243
ACo	15	**3.45**	3.18	3.57	0.110	11	**3.36**	2.92	3.52	0.203	12	**3.37**	3.18	3.58	0.112
CM_3	15	**6.74**	6.44	6.97	0.145	11	**6.66**	6.42	6.88	0.150	12	**6.66**	6.23	6.95	0.204
CC/CM^3	15	**0.637**	0.605	0.663	0.018	10	**0.616**	0.584	0.654	0.041	11	**0.626**	0.597	0.663	0.021
CM^3/LCr	15	**0.400**	0.383	0.408	0.006	10	**0.399**	0.387	0.407	0.006	12	**0.402**	0.390	0.412	0.006
LaInf/LCr	15	**0.244**	0.238	0.250	0.004	11	**0.237**	0.219	0.255	0.010	12	**0.242**	0.230	0.255	0.008
LaN/LCr	15	**0.470**	0.456	0.483	0.008	11	**0.463**	0.452	0.489	0.011	12	**0.465**	0.458	0.478	0.007
ANc/LaN	15	**0.791**	0.772	0.811	0.013	12	**0.798**	0.766	0.830	0.019	12	**0.793**	0.765	0.822	0.017
ANc/LCr	15	**0.372**	0.359	0.389	0.009	11	**0.371**	0.353	0.380	0.009	12	**0.369**	0.360	0.378	0.006
P^2P^3/LCr	15	**0.065**	0.057	0.070	0.004	11	**0.068**	0.064	0.075	0.003	11	**0.070**	0.065	0.073	0.003
P^2P^3/CM^3	15	**0.162**	0.147	0.176	0.008	11	**0.169**	0.158	0.184	0.008	11	**0.173**	0.161	0.188	0.009
LP^3/P^2P^3	9	**0.445**	0.400	0.478	0.028	11	**0.468**	0.436	0.485	0.016	11	**0.475**	0.434	0.505	0.021
LaM^3/M^1M^3	9	**0.485**	0.436	0.511	0.024	12	**0.494**	0.460	0.522	0.015	12	**0.501**	0.484	0.521	0.010
LaM^3/LaM^1	9	**1.096**	0.994	1.152	0.047	12	**1.104**	1.061	1.152	0.029	12	**1.124**	1.065	1.181	0.034
PC1 dim	15	**0.295**	-0.990	1.779	0.730	12	**0.576**	-0.568	1.969	0.851	12	**0.678**	-0.102	2.531	0.766
PC2 dim	15	**-0.686**	-1.582	0.370	0.640	12	**0.168**	-0.985	1.656	0.831	12	**0.363**	-0.635	1.142	0.557
PC1 ind	15	**0.668**	-0.722	3.055	0.887	12	**0.202**	-1.960	2.002	1.137	12	**-0.106**	-0.708	0.460	0.418
PC2 ind	15	**-0.240**	-1.644	0.896	0.629	12	**0.405**	-0.696	1.127	0.531	12	**0.605**	-0.530	2.107	0.771

→

Fig. 3. Univariate plots of compared samples of *Myotis emarginatus* (bold horizontal lines = medians, boxes = upper and lower quartiles, lines = ranges); examples of the plain dimensions.


LAt
45.5
42.0
38.5
35.0
9
7
12
16
37
27
118
19
11
26
10
31
50
4
8
LCr
16.8
16.3
15.8
15.3
14.8
15
11
12
19
72
35
104
43
9
26
9
29
48
6
7
LP3
0.62
0.57
0.52
0.47
0.42
0.37
9
11
12
19
72
34
108
41
9
14
10
30
50
4
7
LaM3
1.98
1.90
1.82
1.74
1.66
1.58
1.50
9
12
12
19
72
35
108
41
9
14
10
30
49
4
7
Maghreb
Iberia
West Islands
West Europe
Central Europe
West Balkans
East Balkans
North Levant
Cyprus
Holy Land
Crimea
Caucasus
West Turkestan
SE Middle East
Oman

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detectable from the comparison of the basic statistical values (mean/median, upper and lower quartiles; Figs. 3, 4, Table 1). The metric characters clustered the population sets into several groups; particular characters, such as body and skull size, rostrum and braincase shape, size of teeth, were compared separately (the ranges in parentheses below are delimited by the lower and upper quartile values, respectively, giving the best picture of the metric trend of a set within the whole species variation).

Based on the body and skull plain dimensions, best characterised by the forearm length (LAt) and largest length of skull (LCr), four size categories could be separated among *M. emarginatus* sample sets, see Fig. 3 and Table 1. The smallest body and skull dimensions were typical for the samples from West-Central Europe (IB+WE+WI+CE; LAt 37.6–40.3 mm; LCr 15.3–16.0 mm) and from Cyprus (LAt 38.1–39.4 mm; LCr 15.5–15.7 mm); medium-sized bodies and skulls were typical for the samples from the Maghreb (LAt 39.2–41.3 mm; LCr 15.6–16.0 mm), Balkans (WB+EB; LAt 38.3–41.0 mm; LCr 15.4–16.0 mm), and Levant (LAt 38.7–40.8 mm; LCr 15.4–16.2 mm); medium-sized bodies but large skulls were found in bats from Crimea (LAt 40.1–41.7 mm; LCr 16.0–16.5 mm) and the Caucasus (LAt 40.6–42.0 mm; LCr 16.0–16.4 mm); and large body and skull dimensions were typical for bats from West Turkestan (LAt 40.6–42.9 mm; LCr 16.0–16.5 mm), SE Middle East (LAt 42.7–44.1 mm; LCr 16.1–16.7 mm), and from Oman (LAt 41.3–41.9 mm; LCr 15.8–16.4 mm).

The absolute length of rostrum in *M. emarginatus* conformed with the overall skull size in a prevailing number of the sample sets. The only exception was found in the small-sized bats from Cyprus where the length of rostrum is slightly bigger than a value equal to the skull length. However, six categories appeared when the shape of rostrum was evaluated, i.e. its relative length and relative width (see Fig. 4). The most common category was the relatively medium--long and relatively narrow rostrum; such shape was found in the samples from the Maghreb (CM^3/LCr 0.398–0.404; LaInf/LCr 0.240–0.247), Balkans (CM^3/LCr 0.396–0.408; LaInf/LCr 0.236–0.251), Crimea (CM^3/LCr 0.404–0.408; LaInf/LCr 0.238–0.247), Caucasus (CM^3/LCr 0.399–0.406; LaInf/LCr 0.238–0.249), and West Turkestan (CM^3/LCr 0.399–0.408; LaInf/LCr 0.236–0.252). In the samples from the Levant, the rostrum was relatively medium-long to long but narrow (CM^3/LCr 0.403–0.412; LaInf/LCr 0.236–0.248), while in the Cypriot bats the rostrum was relatively medium-long to long but wide (CM^3/LCr 0.403–0.410; LaInf/LCr 0.245–0.254). The bats from West-Central Europe had a relatively short and narrow rostrum (CM^3/LCr 0.392–0.405; LaInf/LCr 0.235–0.249). The large bats from the SE Middle East and Oman showed a relatively very long rostrum, but while in the Omani bats it was relatively medium-wide to narrow (CM^3/LCr 0.412–0.415; LaInf/LCr 0.239–0.250), in the SE Middle Eastern samples the rostrum was relatively wide (CM^3/LCr 0.410–0.414; LaInf/LCr 0.243–0.258).

The shape of braincase showed extreme variability among the compared sample sets of *M. emarginatus*; eight shape types could be defined among the compared sample sets (Fig. 4). A relatively wide and high braincase was found in the bats from the Maghreb (LaN/LCr 0.464–0.474; ANc/LCr 0.366–0.376) and Balkans (LaN/LCr 0.461–0.477; ANc/LCr 0.360–0.377), while a relatively wide and low braincase in the Cypriot bats (LaN/LCr 0.466–0.475; ANc/LCr 0.354–0.368). A relatively medium-wide and high braincase was documented in the West-Central European bats (LaN/LCr 0.454–0.476; ANc/LCr 0.364–0.378), while a relatively medium-wide and low braincase in the bats from West Turkestan (LaN/LCr 0.454–0.470; ANc/LCr 0.352–0.362). A relatively medium-wide and medium-high braincase were shown by the samples from the Levant (LaN/LCr 0.458–0.475; ANc/LCr 0.355–0.370) and Caucasus (LaN/LCr 0.457–0.468; ANc/LCr 0.358–0.367). A relatively narrow and high braincase was found in

Table 1. (continued)

variable	West Europe (WE)					Central Europe (CE)					West Balkans (WB)				
	n	**M**	min	max	SD	n	**M**	min	max	SD	n	**M**	min	max	SD
LAt	16	**39.00**	35.5	41.2	1.554	37	**38.32**	36.4	40.8	1.160	27	**39.41**	36.3	42.8	1.465
LCr	19	**15.72**	15.13	16.42	0.375	72	**15.52**	14.97	16.28	0.274	35	**15.57**	14.81	16.28	0.344
LCb	19	**14.78**	14.27	15.38	0.335	69	**14.63**	13.83	15.51	0.306	35	**14.64**	13.75	15.34	0.322
LaZ	15	**9.77**	9.53	10.07	0.169	67	**9.57**	8.87	10.31	0.231	34	**9.65**	8.72	10.19	0.273
LaI	19	**3.63**	3.42	3.79	0.109	72	**3.52**	3.17	3.85	0.124	35	**3.51**	3.26	3.93	0.127
LaInf	19	**3.83**	3.65	4.08	0.102	72	**3.71**	3.43	4.02	0.114	35	**3.77**	3.47	4.12	0.169
LaN	19	**7.39**	7.13	7.67	0.141	72	**7.32**	6.94	7.91	0.148	35	**7.32**	6.91	7.93	0.216
ANc	19	**5.86**	5.71	6.06	0.106	72	**5.70**	5.36	5.98	0.139	35	**5.78**	5.37	6.12	0.161
CC	19	**3.99**	3.51	4.25	0.153	72	**3.93**	3.58	4.18	0.130	35	**3.96**	3.58	4.22	0.134
M^3M^3	19	**6.15**	5.93	6.48	0.147	72	**6.07**	5.68	6.33	0.152	34	**6.07**	5.71	6.53	0.204
CM^3	19	**6.23**	6.00	6.62	0.157	72	**6.19**	5.80	6.48	0.136	35	**6.24**	5.70	6.57	0.169
CP^4	19	**2.99**	2.82	3.29	0.119	72	**3.17**	2.84	3.42	0.121	35	**3.18**	2.89	3.50	0.137
P^2P^3	19	**1.02**	0.90	1.11	0.064	72	**1.05**	0.90	1.28	0.075	34	**1.05**	0.90	1.16	0.064
LP^3	19	**0.47**	0.37	0.57	0.057	72	**0.49**	0.38	0.60	0.044	34	**0.49**	0.37	0.57	0.047
LP^4	19	**1.17**	1.04	1.27	0.059	72	**1.24**	1.03	1.36	0.064	35	**1.23**	1.10	1.35	0.071
LaP^4	19	**1.32**	1.21	1.51	0.071	72	**1.33**	1.18	1.81	0.072	35	**1.32**	1.18	1.42	0.053
M^1M^3	19	**3.41**	3.21	3.66	0.113	72	**3.50**	3.24	3.79	0.124	35	**3.49**	3.29	3.79	0.111
LaM^1	19	**1.53**	1.41	1.70	0.076	72	**1.59**	1.42	1.86	0.069	35	**1.58**	1.45	1.70	0.058
LaM^2	19	**1.77**	1.68	1.93	0.070	72	**1.82**	1.65	1.98	0.069	35	**1.81**	1.66	1.95	0.067
LaM^3	19	**1.69**	1.53	1.82	0.067	72	**1.69**	1.55	1.84	0.074	35	**1.71**	1.58	1.94	0.069
LMd	19	**11.53**	11.05	11.93	0.240	72	**11.46**	10.78	12.08	0.235	35	**11.52**	10.82	12.02	0.289
ACo	18	**3.39**	3.18	3.55	0.092	72	**3.39**	2.94	3.83	0.135	35	**3.43**	3.17	3.71	0.120
CM_3	19	**6.67**	6.42	7.22	0.190	72	**6.64**	6.25	7.05	0.165	35	**6.64**	6.12	6.90	0.167
CC/CM^3	19	**0.640**	0.554	0.663	0.026	72	**0.635**	0.592	0.670	0.021	35	**0.634**	0.597	0.656	0.016
CM^3/LCr	19	**0.396**	0.388	0.405	0.005	72	**0.399**	0.385	0.414	0.006	35	**0.401**	0.386	0.412	0.006
LaInf/LCr	19	**0.244**	0.233	0.260	0.008	72	**0.239**	0.226	0.260	0.007	35	**0.242**	0.226	0.263	0.009
LaN/LCr	19	**0.470**	0.443	0.500	0.013	72	**0.472**	0.453	0.515	0.011	35	**0.470**	0.442	0.509	0.013
ANc/LaN	19	**0.793**	0.773	0.821	0.017	72	**0.779**	0.725	0.827	0.022	35	**0.790**	0.682	0.829	0.026
ANc/LCr	19	**0.373**	0.363	0.391	0.009	72	**0.368**	0.349	0.381	0.008	35	**0.371**	0.347	0.386	0.010
P^2P^3/LCr	19	**0.065**	0.059	0.070	0.004	72	**0.067**	0.059	0.096	0.005	34	**0.068**	0.058	0.074	0.004
P^2P^3/CM^3	19	**0.164**	0.147	0.178	0.009	72	**0.169**	0.147	0.192	0.012	34	**0.169**	0.142	0.184	0.010
LP^3/P^2P^3	19	**0.461**	0.301	0.527	0.050	72	**0.474**	0.348	0.576	0.039	34	**0.462**	0.395	0.535	0.032
LaM^3/M^1M^3	19	**0.497**	0.469	0.517	0.012	72	**0.482**	0.410	0.508	0.016	35	**0.490**	0.462	0.511	0.012
LaM^3/LaM^1	19	**1.112**	1.007	1.228	0.057	72	**1.065**	0.901	1.161	0.047	35	**1.084**	1.019	1.186	0.034
PC1 dim	19	**-0.790**	1.200	-1.758	0.775	72	**-0.774**	1.005	-2.465	0.687	35	**-0.647**	1.136	-1.935	0.761
PC2 dim	19	**-1.140**	-2.504	-0.124	0.616	72	**0.138**	-1.711	2.114	0.780	35	**-0.011**	-1.875	1.362	0.994
PC1 ind	19	**1.363**	-0.184	2.348	0.661	72	**0.266**	-3.437	2.554	0.896	35	**0.159**	-1.558	2.451	0.910
PC2 ind	19	**-0.187**	1.691	-1.789	0.906	72	**-0.426**	1.341	-2.498	0.910	35	**-0.309**	2.149	-1.754	0.823

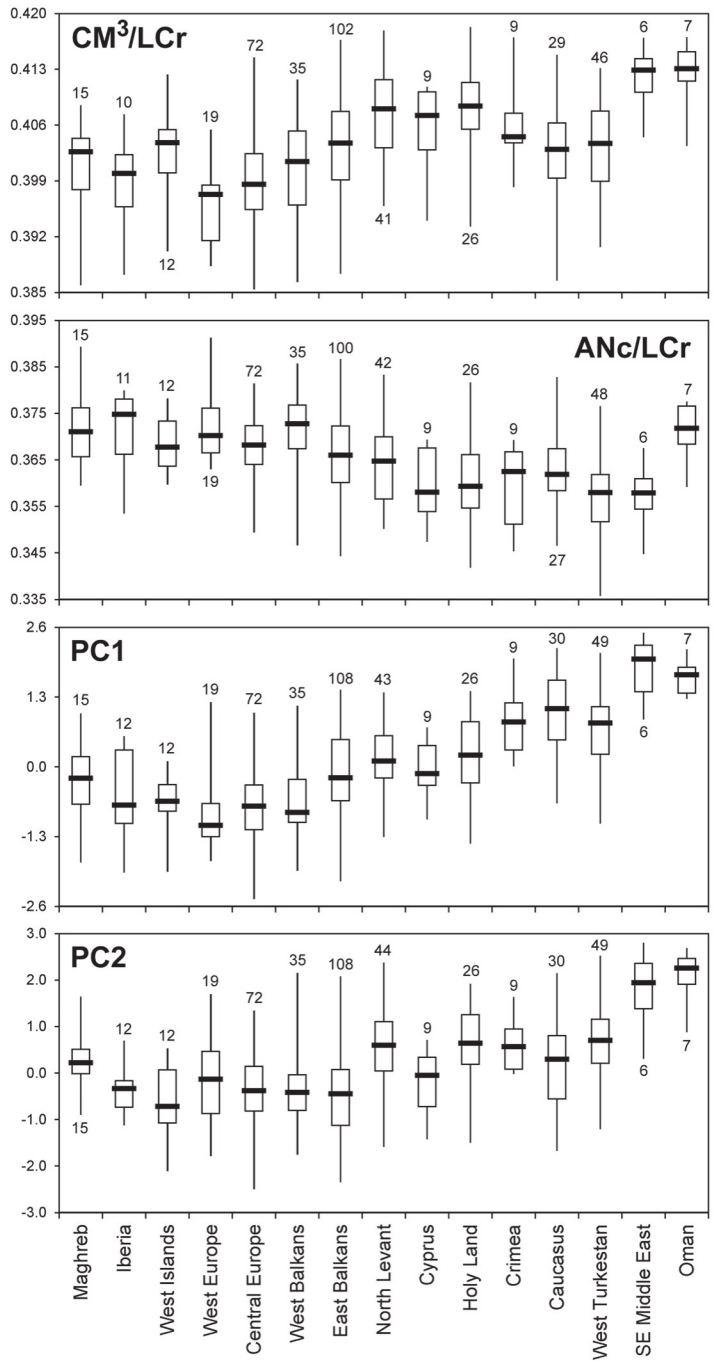
the Omani bats (LaN/LCr 0.456–0.458; ANc/LCr 0.368–0.377), relatively narrow and medium--high braincase in the bats from Crimea (LaN/LCr 0.447–0.455; ANc/LCr 0.351–0.367), and relatively narrow and low braincase in the bats of the SE Middle East (LaN/LCr 0.450–0.467; ANc/LCr 0.354–0.361).

Table 1. (continued)

variable	East Balkans (EB)					North Levant (NL)					Cyprus (CY)				
	n	**M**	min	max	SD	n	**M**	min	max	SD	n	**M**	min	max	SD
LAt	118	**40.08**	36.0	45.0	1.340	28	**39.81**	37.8	41.8	1.238	11	**38.88**	37.0	42.1	1.537
LCr	104	**15.73**	14.91	16.60	0.364	43	**15.66**	15.07	16.42	0.308	9	**15.62**	15.38	15.92	0.169
LCb	103	**14.91**	14.07	15.95	0.377	43	**14.74**	14.18	15.37	0.260	9	**14.66**	14.28	15.08	0.252
LaZ	95	**9.72**	9.20	10.25	0.244	37	**9.63**	9.24	10.02	0.189	9	**9.61**	9.34	10.02	0.195
LaI	110	**3.55**	3.35	3.90	0.112	43	**3.58**	3.29	3.79	0.104	9	**3.54**	3.43	3.64	0.075
LaInf	108	**3.86**	3.53	4.30	0.142	43	**3.77**	3.52	4.07	0.118	9	**3.89**	3.76	4.04	0.089
LaN	109	**7.36**	7.03	7.75	0.143	42	**7.30**	6.79	7.79	0.173	9	**7.31**	7.17	7.49	0.112
ANc	103	**5.75**	5.38	6.05	0.154	42	**5.70**	5.44	6.12	0.135	9	**5.61**	5.48	5.77	0.095
CC	106	**4.00**	3.53	4.35	0.152	42	**3.95**	3.79	4.17	0.085	9	**4.05**	3.86	4.22	0.128
M^3M^3	110	**6.16**	5.62	6.87	0.193	43	**6.15**	5.87	6.46	0.134	9	**6.06**	5.88	6.33	0.149
CM^3	110	**6.35**	5.88	6.75	0.164	43	**6.37**	5.95	6.62	0.161	9	**6.34**	6.06	6.54	0.129
CP^4	107	**3.26**	3.00	3.58	0.128	41	**3.30**	2.95	3.63	0.131	9	**3.31**	3.21	3.42	0.072
P^2P^3	108	**1.09**	0.93	1.21	0.063	41	**1.03**	0.90	1.14	0.095	9	**1.10**	0.97	1.19	0.060
LP^3	108	**0.52**	0.39	0.62	0.042	41	**0.50**	0.40	0.62	0.048	9	**0.49**	0.40	0.57	0.053
LP^4	108	**1.25**	1.07	1.43	0.074	41	**1.27**	1.15	1.42	0.054	9	**1.31**	1.24	1.42	0.053
LaP^4	108	**1.35**	1.19	1.78	0.067	41	**1.41**	1.24	1.73	0.091	9	**1.37**	1.30	1.42	0.048
M^1M^3	106	**3.56**	3.26	3.76	0.893	41	**3.66**	3.26	3.82	0.106	9	**3.63**	3.50	3.71	0.084
LaM^1	108	**1.60**	1.40	1.77	0.077	39	**1.69**	1.59	1.82	0.064	9	**1.68**	1.61	1.76	0.056
LaM^2	108	**1.84**	1.70	1.99	0.065	41	**1.90**	1.74	2.06	0.066	9	**1.89**	1.84	1.98	0.045
LaM^3	108	**1.74**	1.51	1.92	0.067	41	**1.81**	1.69	1.96	0.075	9	**1.79**	1.72	1.88	0.067
LMd	107	**11.71**	10.83	12.52	0.330	43	**11.65**	11.14	12.15	0.225	9	**11.46**	11.26	11.79	0.180
ACo	106	**3.48**	3.11	3.80	0.130	43	**3.49**	3.28	3.70	0.103	9	**3.50**	3.32	3.68	0.118
CM_3	107	**6.76**	6.22	7.17	0.200	43	**6.79**	6.39	7.17	0.152	9	**6.68**	6.44	6.89	0.119
CC/CM^3	106	**0.629**	0.572	0.671	0.018	42	**0.621**	0.582	0.673	0.018	9	**0.639**	0.612	0.668	0.017
CM^3/LCr	104	**0.404**	0.387	0.417	0.006	43	**0.407**	0.396	0.418	0.007	9	**0.406**	0.394	0.411	0.005
LaInf/LCr	104	**0.245**	0.228	0.266	0.008	43	**0.241**	0.228	0.260	0.008	9	**0.249**	0.242	0.257	0.006
LaN/LCr	104	**0.468**	0.447	0.491	0.009	42	**0.467**	0.445	0.503	0.011	9	**0.468**	0.450	0.480	0.009
ANc/LaN	102	**0.782**	0.741	0.830	0.020	42	**0.781**	0.725	0.846	0.022	9	**0.767**	0.748	0.791	0.013
ANc/LCr	100	**0.366**	0.344	0.387	0.009	42	**0.364**	0.350	0.383	0.009	9	**0.359**	0.347	0.369	0.008
P^2P^3/LCr	104	**0.069**	0.059	0.077	0.004	41	**0.066**	0.056	0.075	0.004	9	**0.070**	0.062	0.075	0.004
P^2P^3/CM^3	108	**0.172**	0.146	0.190	0.010	41	**0.162**	0.139	0.186	0.015	9	**0.173**	0.153	0.183	0.010
LP^3/P^2P^3	108	**0.473**	0.398	0.538	0.029	41	**0.482**	0.410	0.570	0.039	9	**0.447**	0.398	0.514	0.035
LaM^3/M M	108	**0.490**	0.384	0.520	0.019	41	**0.492**	0.367	0.555	0.027	9	**0.495**	0.476	0.512	0.013
LaM^3/LaM^1	108	**1.093**	0.982	1.266	0.045	39	**1.068**	0.777	1.186	0.060	9	**1.070**	1.000	1.118	0.033
PC1 dim	108	**-0.061**	1.433	-2.131	0.786	44	**0.146**	1.381	-1.306	0.802	9	**-0.025**	0.733	-0.983	0.526
PC2 dim	108	**-0.055**	-3.332	2.116	1.135	44	**0.750**	-0.869	2.371	0.654	9	**1.059**	0.082	1.894	0.579
PC1 ind	108	**-0.224**	-2.790	2.518	0.964	44	**-0.522**	-2.090	1.994	0.963	9	**-0.704**	-1.637	0.692	0.710
PC2 ind	108	**-0.480**	2.075	-2.347	0.848	44	**0.437**	2.374	-1.587	0.974	9	**-0.337**	0.709	-1.425	0.782

→

Fig. 4. Univariate plots of compared samples of *Myotis emarginatus* (see Fig. 3 for explanations); examples of relative and statistical dimensions (for details concerning the PC analysis results see text).


CM3/LCr
ANc/LCr
PC1
PC2
Maghreb
Iberia
West Islands
West Europe
Central Europe
West Balkans
East Balkans
North Levant
Cyprus
Holy Land
Crimea
Caucasus
West Turkestan
SE Middle East
Oman

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Molar size was evaluated in *M. emarginatus* through the comparison of the upper molar-rows, i.e. the mesio-distal lengths of all three molars (M^1M^3), and the palato-labial width of particular upper molars (LaM^1, LaM^2, LaM^3); five basic categories were found concerning these characters (see also Fig. 3). Small upper molars including small M^3 were found in the bats from the Maghreb (M^1M^3 3.47–3.63 mm; LaM^3 1.68–1.74 mm), West-Central Europe (M^1M^3 3.34–3.61 mm; LaM^3 1.65–1.79 mm), and the Balkans (M^1M^3 3.42–3.61 mm; LaM^3 1.67–1.80 mm). Medium-sized upper molars including medium-sized M^3 showed the bats from the Levant (M^1M^3 3.61–3.76 mm; LaM^3 1.72–1.85 mm) and Cyprus (M^1M^3 3.55–3.71 mm; LaM^3 1.73–1.86 mm), while medium-sized upper molars with small M^3 were observed in the Crimean bats (M^1M^3 3.56–3.61 mm; LaM^3 1.67–1.74 mm). Large upper molars including large M^3 were found in the bats from the SE Middle East (M^1M^3 3.80–3.86 mm; LaM^3 1.85–1.92 mm) and in the Omani bats (M^1M^3 3.76–3.86 mm; LaM^3 1.84–1.91 mm). Large upper molars but only medium-sized M^3 were documented in the bats from the Caucasus (M^1M^3 3.68–3.78 mm; LaM^3 1.77–1.84 mm) and West Turkestan (M^1M^3 3.53–3.82 mm; LaM^3 1.71–1.86 mm). Shape differences or marked differences in the relative size of particular molars between the compared sample sets were not found.

The size of the small upper premolars ($P^{2–3}$) was in an intermediate position (P^2P^3/LCr 0.062–0.070) in the majority of *M. emarginatus* sample sets, only three exceptions were found. The bats from the Mediterranean islands showed relatively very large-sized rows of the small upper premolars, both from Cyprus (P^2P^3/LCr 0.069–0.072) and West Islands (P^2P^3/LCr 0.068–0.072). On the other hand, the Omani bats showed relatively small-sized upper small premolars (P^2P^3/LCr 0.059–0.060). The smallest tooth examined, the second upper premolar (P^3), was found to be the smallest (in absolute values) in the bats from the Maghreb (LP^3 0.45–0.49 mm), West Europe (LP^3 0.44–0.50 mm), and from Oman (LP^3 0.44–0.49 mm), while the largest in the bats from Crimea (LP^3 0.51–0.55 mm) and Caucasus (LP^3 0.50–0.57 mm). In other sample sets, this premolar was found to be medium-sized (LP^3 0.46–0.54 mm; Fig. 3).

To summarise the above observations, four basic groups of populations could be sorted out within the distribution range of *M. emarginatus*, based on the absolute and relative metric characters. Moreover, some populations, creating isolated islands within the range (both in sea islands and geographically separated areas), showed again more separated positions in certain aspects. (1) The group of samples from the western and central parts of the Mediterranean Basin, i.e. between the Maghreb and Iberia in the west and the Balkans in the east, including Central Europe (MG+IB+WI+WE+CE+WB+EB), represents small or medium-sized bats, with a relatively rather short and narrow rostrum, wide and high braincase, small molars and small to medium sized premolars; among these samples, the bats from West Islands are the absolutely smallest in the skull and molar sizes and braincase width, and largest in the length of the premolar-row. (2) The group of samples from the Levant, including Cyprus (NL+CY+HL), represents small or medium-sized bats, but with a relatively long and narrow rostrum, and wide and low braincase, medium-sized molars, and small to medium-sized premolars; within this group, the Cypriot samples show a difference in skull size and rostrum shape, being the smallest in body and skull size, largest in rostrum width and the small premolar-row. (3) The group of samples from Crimea, Caucasus and West Turkestan (CR+CA+WT), represents large bats, with a relatively narrow and medium-long rostrum, narrow and low braincase, small or medium-sized molars, and relatively rather small premolars; among these samples, the bats from Crimea differed by a relatively very narrow braincase and small third upper molars. (4) The group of samples from Oman, south-eastern Iran and Afghanistan (OM+SM) represents large bats, with

Table 1. (continued)

variable	Holy Land (HL)					Crimea (CR)					Caucasus (CA)				
	n	**M**	min	max	SD	n	**M**	min	max	SD	n	**M**	min	max	SD
LAt	26	**39.73**	36.4	42.6	1.323	10	**40.81**	39.6	42.0	0.889	31	**41.34**	39.7	43.5	0.964
LCr	26	**15.79**	15.23	16.35	0.356	9	**16.24**	15.87	16.60	0.244	29	**16.19**	15.68	16.74	0.287
LCb	26	**14.87**	14.18	15.47	0.325	9	**15.21**	14.73	15.75	0.296	28	**15.27**	14.75	16.06	0.278
LaZ	25	**9.66**	9.19	9.89	0.171	10	**9.82**	9.50	10.08	0.184	26	**9.94**	9.47	10.28	0.241
LaI	26	**3.56**	3.42	3.94	0.121	10	**3.60**	3.48	3.80	0.105	30	**3.70**	3.43	3.93	0.129
LaInf	26	**3.84**	3.68	4.05	0.095	10	**3.92**	3.78	4.10	0.117	30	**3.95**	3.74	4.08	0.104
LaN	26	**7.36**	7.12	7.72	0.135	10	**7.32**	7.10	7.48	0.141	29	**7.48**	7.08	7.74	0.170
ANc	26	**5.70**	5.41	6.05	0.189	9	**5.83**	5.67	6.03	0.104	27	**5.88**	5.59	6.14	0.151
CC	25	**4.02**	3.80	4.25	0.118	10	**4.15**	4.02	4.28	0.105	30	**4.11**	3.85	4.32	0.133
M^3M^3	26	**6.23**	5.87	6.45	0.159	10	**6.29**	6.15	6.48	0.134	30	**6.36**	6.02	6.63	0.164
CM^3	26	**6.44**	6.11	6.68	0.141	10	**6.57**	6.42	6.78	0.111	31	**6.52**	6.18	6.76	0.131
CP^4	13	**3.28**	3.00	3.45	0.125	10	**3.21**	3.08	3.32	0.090	30	**3.35**	3.03	3.53	0.113
P^2P^3	14	**1.05**	0.95	1.19	0.078	10	**1.06**	0.98	1.18	0.065	30	**1.07**	0.91	1.19	0.067
LP^3	14	**0.50**	0.41	0.60	0.047	10	**0.53**	0.47	0.57	0.031	30	**0.53**	0.43	0.60	0.041
LP^4	14	**1.25**	1.15	1.33	0.060	10	**1.25**	1.17	1.32	0.046	30	**1.36**	1.16	1.48	0.110
LaP^4	14	**1.37**	1.29	1.43	0.044	10	**1.37**	1.30	1.42	0.047	30	**1.45**	1.30	1.53	0.054
M^1M^3	14	**3.68**	3.47	3.84	0.097	10	**3.58**	3.42	3.68	0.084	30	**3.72**	3.39	3.87	0.105
LaM^1	14	**1.67**	1.56	1.77	0.058	10	**1.63**	1.55	1.70	0.042	30	**1.72**	1.54	1.80	0.059
LaM^2	14	**1.89**	1.83	1.95	0.039	10	**1.85**	1.74	1.94	0.061	30	**1.95**	1.78	2.04	0.053
LaM^3	14	**1.78**	1.65	1.90	0.068	10	**1.72**	1.65	1.82	0.055	30	**1.81**	1.65	1.96	0.092
LMd	26	**11.72**	11.03	12.20	0.310	9	**11.97**	11.64	12.45	0.301	31	**12.03**	11.62	12.35	0.178
ACo	26	**3.50**	3.13	3.75	0.144	9	**3.67**	3.40	3.98	0.168	29	**3.58**	2.76	3.81	0.197
CM_3	26	**6.85**	6.53	7.18	0.174	10	**7.02**	6.85	7.20	0.118	31	**6.97**	6.61	7.23	0.125
CC/CM^3	25	**0.624**	0.573	0.656	0.020	10	**0.632**	0.608	0.649	0.012	30	**0.631**	0.588	0.680	0.023
CM^3/LCr	26	**0.408**	0.393	0.418	0.005	9	**0.406**	0.398	0.417	0.005	29	**0.403**	0.386	0.415	0.007
LaInf/LCr	26	**0.243**	0.229	0.258	0.008	9	**0.241**	0.234	0.252	0.006	29	**0.244**	0.229	0.256	0.007
LaN/LCr	26	**0.466**	0.443	0.486	0.011	9	**0.451**	0.443	0.460	0.006	29	**0.462**	0.430	0.482	0.011
ANc/LaN	26	**0.775**	0.728	0.813	0.022	9	**0.795**	0.765	0.820	0.018	27	**0.788**	0.749	0.834	0.020
ANc/LCr	26	**0.361**	0.342	0.382	0.010	9	**0.359**	0.345	0.369	0.009	27	**0.363**	0.347	0.383	0.008
P^2P^3/LCr	14	**0.067**	0.061	0.076	0.003	9	**0.066**	0.061	0.074	0.004	29	**0.066**	0.056	0.073	0.004
P^2P^3/CM^3	14	**0.163**	0.149	0.183	0.011	10	**0.162**	0.153	0.177	0.008	30	**0.164**	0.141	0.182	0.010
LP^3/P^2P^3	14	**0.477**	0.415	0.564	0.041	10	**0.495**	0.445	0.537	0.026	30	**0.496**	0.406	0.644	0.043
LaM^3/M^1M^3	14	**0.483**	0.462	0.500	0.013	10	**0.480**	0.468	0.498	0.010	30	**0.483**	0.376	0.515	0.024
LaM^3/LaM^1	14	**1.064**	0.952	1.154	0.051	10	**1.055**	1.032	1.082	0.022	30	**1.044**	0.811	1.136	0.056
PC1 dim	26	**0.304**	1.406	-1.433	0.694	9	**0.765**	2.013	0.009	0.668	30	**1.279**	2.212	-0.679	0.578
PC2 dim	26	**0.286**	-1.338	1.436	0.711	9	**-1.118**	-2.303	-0.481	0.560	30	**0.307**	-1.834	1.742	0.847
PC1 ind	26	**-0.713**	-3.334	1.084	0.902	9	**-0.001**	-1.810	1.393	0.827	30	**-0.095**	-1.761	2.183	1.017
PC2 ind	26	**0.595**	1.919	-1.503	0.766	9	**0.616**	1.632	-0.017	0.597	30	**0.186**	2.145	-1.671	0.953

a relatively very long and medium-wide to very wide rostrum, narrow braincase, large molars, and small premolars; these two population sets differ from each other by the relative height of braincase, being small in the SE Middle Eastern bats but large in the Omani bats, and by the relative length of the premolar-row (P^2P^3/LCr), being medium-large in the former sample set, but very small (smallest among all examined samples) in the Omani bats.

Table 1. (continued)

variable	West Turkestan (WT)					SE Middle East (SM)					Oman (OM)				
	n	**M**	min	max	SD	n	**M**	min	max	SD	n	**M**	min	max	SD
LAt	50	**41.75**	38.6	45.1	1.553	4	**43.18**	41.2	44.2	1.391	8	**41.63**	40.8	42.9	0.643
LCr	48	**16.17**	15.05	16.62	0.338	6	**16.31**	15.65	16.79	0.416	7	**16.06**	15.73	16.46	0.295
LCb	48	**15.23**	14.13	15.89	0.325	6	**15.42**	14.85	15.83	0.340	7	**15.16**	14.62	15.69	0.347
LaZ	34	**9.85**	9.60	10.17	0.158	6	**9.96**	9.64	10.17	0.209	7	**9.89**	9.62	10.33	0.266
LaI	50	**3.61**	3.37	3.85	0.110	7	**3.71**	3.54	3.88	0.114	7	**3.73**	3.58	3.87	0.098
LaInf	49	**3.93**	3.58	4.32	0.149	7	**4.04**	3.82	4.35	0.208	7	**3.94**	3.75	4.20	0.146
LaN	48	**7.48**	7.10	7.72	0.148	7	**7.51**	7.32	7.72	0.121	7	**7.33**	7.21	7.53	0.118
ANc	48	**5.77**	5.42	6.15	0.161	6	**5.83**	5.65	6.17	0.184	7	**5.96**	5.66	6.12	0.157
CC	47	**4.07**	3.75	4.26	0.120	7	**4.27**	4.07	4.58	0.171	6	**4.08**	3.94	4.23	0.112
M^3M^3	48	**6.30**	5.85	6.60	0.150	7	**6.48**	6.02	6.75	0.257	7	**6.36**	6.16	6.49	0.139
CM^3	48	**6.53**	6.12	6.83	0.148	7	**6.70**	6.47	6.94	0.176	7	**6.62**	6.51	6.76	0.091
CP^4	49	**3.21**	2.92	3.50	0.142	4	**3.31**	3.26	3.37	0.054	7	**3.36**	3.24	3.50	0.084
P^2P^3	49	**1.04**	0.87	1.24	0.070	4	**1.03**	0.88	1.11	0.106	7	**0.96**	0.93	1.05	0.040
LP^3	50	**0.49**	0.40	0.60	0.043	4	**0.50**	0.48	0.53	0.023	7	**0.47**	0.43	0.50	0.028
LP^4	50	**1.23**	1.09	1.42	0.072	4	**1.34**	1.29	1.38	0.040	7	**1.31**	1.23	1.37	0.047
LaP^4	50	**1.40**	1.24	1.58	0.067	4	**1.51**	1.49	1.54	0.025	7	**1.46**	1.41	1.51	0.044
M^1M^3	49	**3.64**	3.39	3.92	0.085	4	**3.82**	3.74	3.89	0.066	7	**3.82**	3.74	3.95	0.074
LaM^1	50	**1.65**	1.51	1.87	0.093	4	**1.75**	1.72	1.79	0.031	7	**1.73**	1.64	1.80	0.057
LaM^2	50	**1.91**	1.64	2.47	0.108	4	**2.02**	1.97	2.07	0.048	7	**1.99**	1.90	2.06	0.058
LaM^3	49	**1.78**	1.59	1.93	0.089	4	**1.88**	1.81	1.93	0.052	7	**1.88**	1.82	1.97	0.067
LMd	52	**11.94**	11.23	12.41	0.277	7	**12.05**	11.54	12.38	0.327	7	**11.98**	11.60	12.28	0.254
ACo	49	**3.55**	3.35	3.87	0.121	7	**3.69**	3.52	3.87	0.127	7	**3.75**	3.63	3.97	0.113
CM_3	50	**6.97**	6.45	7.32	0.165	7	**7.13**	6.78	7.37	0.243	7	**6.98**	6.82	7.14	0.120
CC/CM^3	46	**0.625**	0.588	0.665	0.017	7	**0.636**	0.616	0.662	0.018	6	**0.618**	0.601	0.629	0.012
CM^3/LCr	46	**0.403**	0.391	0.413	0.006	6	**0.412**	0.404	0.417	0.004	7	**0.412**	0.403	0.417	0.004
LaInf/LCr	48	**0.243**	0.227	0.265	0.010	6	**0.248**	0.229	0.259	0.013	7	**0.245**	0.235	0.258	0.008
LaN/LCr	48	**0.462**	0.434	0.488	0.010	6	**0.461**	0.448	0.473	0.010	7	**0.457**	0.451	0.460	0.003
ANc/LaN	48	**0.772**	0.739	0.835	0.020	6	**0.779**	0.754	0.820	0.022	7	**0.813**	0.785	0.828	0.015
ANc/LCr	48	**0.357**	0.336	0.377	0.009	6	**0.357**	0.345	0.367	0.008	7	**0.371**	0.359	0.378	0.007
P^2P^3/LCr	48	**0.064**	0.053	0.074	0.004	4	**0.063**	0.056	0.069	0.004	7	**0.060**	0.059	0.064	0.002
P^2P^3/CM^3	48	**0.159**	0.133	0.181	0.010	4	**0.156**	0.136	0.170	0.015	7	**0.145**	0.141	0.158	0.006
LP^3/P^2P^3	49	**0.477**	0.396	0.545	0.031	4	**0.491**	0.448	0.560	0.048	7	**0.486**	0.461	0.527	0.026
LaM^3/M^1M^3	49	**0.489**	0.438	0.518	0.016	4	**0.493**	0.471	0.513	0.017	7	**0.493**	0.480	0.512	0.010
LaM^3/LaM^1	49	**1.080**	0.966	1.153	0.040	4	**1.075**	1.047	1.108	0.029	7	**1.089**	1.053	1.153	0.040
PC1 dim	49	**0.765**	2.119	-1.061	0.843	6	**1.817**	2.495	0.881	0.751	7	**1.633**	2.191	1.264	0.582
PC2 dim	49	**-0.589**	-2.583	2.028	1.059	6	**0.154**	-1.301	1.443	0.902	7	**0.307**	-1.112	1.343	0.726
PC1 ind	49	**0.313**	-2.015	2.606	1.053	6	**-0.069**	-0.514	0.452	0.413	7	**-0.284**	-0.840	0.581	0.482
PC2 ind	49	**0.690**	2.522	-1.209	0.767	6	**1.560**	2.801	-0.301	1.018	7	**2.179**	2.689	0.877	0.655

The results of the principal component analysis well described the size and shape trends in the west-east scope of the geographic range of *M. emarginatus* (Fig. 4). The results of the analysis employing 15 skull plain dimensions most important for description of the inter-population differences (LCr, LCb, CC, M^3M^3, CM^3, CP^4, P^2P^3, LP^4, LaP^4, LaM^1, LaM^2, LaM^3, LMd, ACo, CM_3) showed a trend of (geographically interrupted) cline increasing of the skull

size from the western Mediterranean in the west to the south-eastern part of the Middle East in the east. The 1st component of these results (covering 49.25% of the total variance) followed the size differences and demonstrated the West and Central European samples to be smallest among the sample sets, while the samples from Oman and SE Middle East to be the largest, with the Levantine (incl. Cypriot) samples to be medium-sized and the Crimean, Caucasian, and Turkestani bats being intermediate in size between the latter two groups (Fig. 4). Similarly, the results of the analysis employing seven relative dimensions selected as most important in the inter-population differences (CC/CM^3, CM^3/LCr, CP^4/LCr, P^2P^3/LCr, LaM^3/M^1M^3, LaM^3/LaM^1) sorted out groups following the differences given mostly by shapes of the skull and/or teeth. The 2nd component of these results (covering 24.63% of the total variance) separated three main population groups (Fig. 4); (1) bats with a relatively long and wide rostrum, large molars and small premolars from SE Middle East and Oman, (2) bats with a relatively long and narrow rostrum, medium-sized molars and premolars from the Levant (excl. Cyprus), Crimea, Caucasus, and West Turkestan, and (3) bats with a relatively short and narrow rostrum, small molars and premolars from the Mediterranean Basin (excl. the Levant) and Central Europe.

The results of the cluster analysis (UPGMA, Euclidean distances) computed from the percentages of the mean metric differences in morphometric traits between particular sample sets, employing all collected 23 plain dimensions of the body, skull and teeth, and 13 relative dimensions of the skull and teeth (see Table 1), showed very similar divisions of the geographic sets of *M. emarginatus*, as they were observed from the empiric morphometric comparisons and described above (Fig. 5). The geographic content of the species was splitted into two main groups, Asian and Euro-Mediterranenan; the Asian group was again divided into two groups,

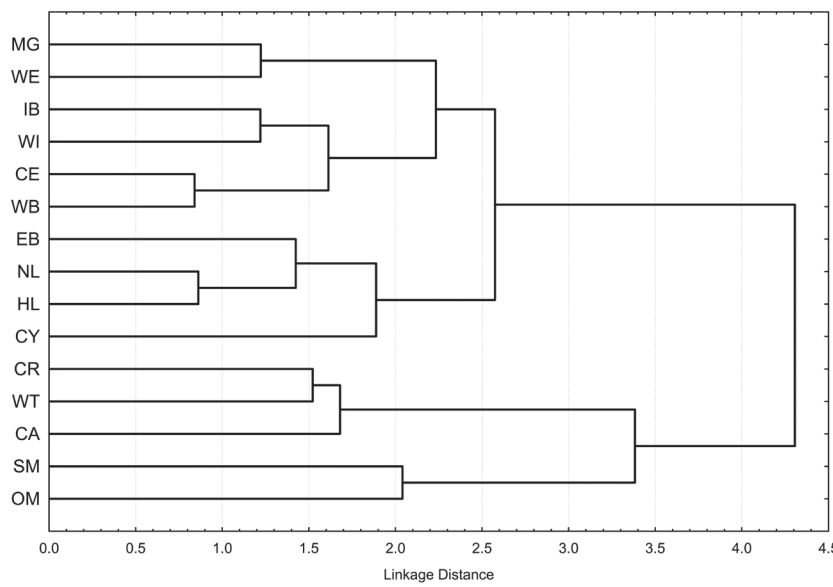

Fig. 5. Similarities among geographic sample sets of *Myotis emarginatus* based on the results of cluster analysis (UPGMA) of the mean values of 23 plain dimensions and 13 relative dimensions.

the northern populations (Crimea, Caucasus, West Turkestan) and south-eastern populations (Oman, south-eastern Middle East), and the Euro-Mediterranean group also in two subgroups, the East-Mediterranen populations (East Balkans, North Levant, Holy Land, Cyprus) and the West-Mediterranean and Central European populations (Maghreb, Iberia, West Europe, West Islands, Central Europe, West Balkans).

DISCUSSION

As reviewed in detail above, the intraspecific classification in *Myotis emarginatus* was proposed several times and the number of taxonomic units within this species varied between two and four. While the populations of the broad area of the European and African Mediterranean and of Central Europe were considered to belong to only one subspecies, one to four subspecies were reported from the Asian part of the species range. This divergence implies that the Asian populations play a crucial role in understanding of phylogenetic relations within the species. Our analysis covered most of the Mediterranean range of the species and for the first time, the comparison included a majority of the Asian populations of *M. emarginatus*. Only the samples from the north-eastern margin of Mesopotamia and from south-western Arabia were unavailable for examination and their position within the species phylogeny was not evaluated.

Our analysis uncovered the existence of four main, geographically exclusive morphotypes in *M. emarginatus*, concerning the body, skull and tooth sizes, and skull and tooth shapes: (1) rather small bats with a short rostrum and high braincase, occurring in Europe and north-western Africa; (2) rather medium-sized bats with a long rostrum and short braincase from the Levant including Cyprus; (3) large bats with a wide and long rostrum from the south-eastern parts of the Middle East, including Oman, south-eastern Iran and eastern Afghanistan, and (4) large bats with a narrow rostrum, occurring in Crimea, the Caucasus region, and West Turkestan. Such a shift of metric characteristics within the south-west Palaearctic range, with smallest bats in the west and largest bats in the east, was previously observed in several other bat taxa (see e.g. Bogdanowicz 1990, Benda & Horáček 1995, Benda & Gvoždík 2010) and thus, in *M. emarginatus* it could be considered just a common pattern present in bats in general. However, unlike the body and skull size, the variation in skull shape did not show the cline shift, and moreover, the "shift" in the metric traits in *M. emarginatus* is not continual, i.e. it does not represent a typical cline, found in some other *Myotis* species of the western Palaearctic (cf. Bogdanowicz 1990, Benda & Horáček 1995).

Additionally, the results of the mitochondrial genetic analysis of *M. emarginatus* performed by Uvizl & Benda (2021a) revealed an existence of three main lineages within the species rank; (1) Euro-Mediterranean lineage, (2) South Iranian and Omani lineage, and (3) Caucasian and West Turkestani lineage. The Euro-Mediterranean lineage was again divided into three sublineages, Euro-Maghrebian, North Levantine, and South Levantine (Holy Land). The lineages well conform in the geographic delimitations to the above defined morphotypes, with an exception of the Levantine morphotype, which belongs to two sublineages, North Levantine and South Levantine (Holy Land), respectively. Both these East-Mediterranean sublineages, although well separated geographically, were neither supported statistically nor by significant genetic distances among the populations, which were only shallow (Uvizl & Benda 2021a).

Thus, the morphotypes and genetic lineages could be integrated into natural phylogenetic units as follows: (1) western bats, comprising the small- and medium-sized morphotypes of the bats from Europe, North Africa and the Levant, and the lineages from the Mediterranean, North

Levant and Holy Land; (2) south-eastern bats, comprising the large sized morphotype from the south-eastern parts of the Middle East and the lineage from southern Iran and Oman; and (3) north-eastern bats, comprising the large-sized morphotype from Crimea, Caucasus, and West Turkestan, and the lineage from the Causcasus region and West Turkestan. Since the differences between these units are formally quantified by the genetic distances of the cytochrome *b* gene in the range of 0.93–2.13% (Uvizl & Benda 2021a), these three phylogenetically and geographically separated units could be regarded as subspecies of *M. emarginatus*.

This conception modifies the conclusions presented by Uvizl & Benda (2021a) concerning the Asian populations of *M. emarginatus*. These conclusions were based on the results of a profound molecular genetic analysis in a combination with results of the preliminary metric comparison by Benda et al. (2006). The latter comparison differentiated two basic size categories among the samples of *M. emarginatus*, small western bats and large eastern bats, and this rather superficial view was supported by the basic separation of the mitochondrial sequences and with the biogeographic data – the western and eastern bats live in an apparent allopatry, being separated by a broad gap in the species occurrence in the central parts of the Middle East, some 400–600 km wide. However, the results presented here corroborate much better the three subspecies conception, which much better follows the results of analysis of the mitochondrial markers as well as the results of a fine morphologic analysis, covering besides the size traits also the skull shape and dental characters, and mainly, a much more representative set of samples from all substantial range parts. The biogeographic aspect could also be applied, since the two eastern lineages are well separated by desert or mountain areas (see below for more detailed comments), i.e., in a degree similar to the isolation of the western bats.

The conception of the unit of western bats intergrates the populations of *M. emarginatus* of the whole Mediterranean Basin and all parts of Europe (except Crimea and Cis-Caucasia) into one taxon (see also Uvizl & Benda 2021a). The rather high internal variation within this unit was confirmed by the results of both analyses, the morphologic and genetic comparisons, and is uncomparable to the diversity found in two other units. However, the latter two units cover much smaller numbers of samples, but mainly, much smaller geographic scopes than the unit of the western bats. The morphologic variation in the unit of western bats, evident in body and skull size as well as skull shape, is very probably related to the extremely variable enviroment range stretching from dry scrublands at the southern limits of the occurrence range (Morocco, Israel, Jordan), where the large-sized bats live, to rather humid forests of the range northern limits (Netherlands, Germany, Czech Republic, Poland), where the small-sized bats occur, and which also includes specific conditions in the Mediterranean islands (Corsica, Sardinia, Sicily, Cyprus) with morphologically extremely variable bats. On the other hand, the very shallow genetic variation among the mtDNA haplotypes within this unit (0.18–0.82%) supports the phylogenetic compactness of this unit (Uvizl & Benda 2021a).

The Levantine populations were separated into two distinct sublineages of the mitochondrial genes (Uvizl & Benda 2021a), North Levantine and Holy Land ones. However, these mitochondrial groups do no affect the morphologic grouping of these populations, bats of both lineages represent an identical morphotype. The genetic distance between these two sublineages is rather low, 0.46–0.82% (Uvizl & Benda 2021a), lower than between the lineages representing the three subspecies considered (see above). The border between the parapatric geographic ranges of these lineages present in the Levant suggests strong philopatry in females of these lineages that, however, has no effect in the real gene flow as showed by the morphometric data. The bats of the Holy Land lineage occur mainly in the upland Mediterranean habitats of the southern

Levant, while the North Levantine lineage was detected in a mosaic of lowlands and uplands of the northern Levant including Cyprus.

An almost identical geographic division into two phylogenetical groups was found in another bat occurring in the Levant, *Myotis hoveli* Harrison, 1964 (see Uvizl & Benda 2021b). This common phylogentic pattern suggest a past existence of isolated refugia temporarily dividing the biota of the Levant into northern and southern segments. Currently the bats descending from these refugia occur in close allopatry or even sympatry (see the maps by Uvizl & Benda 2021a, b) and the former refugial division has affected the local phylogenetic context of the two species (Çoraman et al. 2019, Uvizl & Benda 2021a). On the other hand, the territory of Lebanon, where the borderlines between the two lineages in both *Myotis* species occur, represents a biogeographic boundary as well, where the limits of distribution ranges of several bat species are situated (see Benda et al. 2016a).

The arrangement of the European, African and Levantine populations into one unit conforms to the opinions of numerous previous authors, who regarded these populations as one common taxon (Ellerman & Morrison-Scott 1951, Corbet 1978, Harrison & Bates 1991, Koopman 1994, Topál 2001, Karataş & Özgül 2003, Benda et al. 2006, Dietz et al. 2007, Mayer et al. 2007, Albayrak 2015, López-Baucells 2019). On the contrary, most of these opinions considered also the populations of Crimea and Caucasus to be a part of this Euro-Mediterranean taxon, but this conception is not supported by our results.

Five names are available from the range of the unit of western bats according to the previous reviews (Miller 1912, Ellerman & Morrison-Scott 1951, Corbet 1978, Simmons 2005); viz. *Vespertilio emarginatus* Geoffroy, 1806; *V. rufescens* Crespon, 1844; *V. ciliatus* Blasius, 1853; *Myotis ciliata budapestiensis* Margó, 1880; and *Vespertilio neglectus* Fatio, 1890. Since all these names were created based on the materials from western or central Europe (France, Germany, Switzerland, Hungary; see Miller 1912), i.e. from an area representing just a small segment of the range of the unit of western bats, they naturally constitute synonyms of the prior name, *V. emarginatus*. The latter name is thus also a name for the western subspecies, i.e. *M. emarginatus emarginatus* (Geoffroy, 1806).

The populations of *M. emarginatus* of the Asian range (excluding the Levant) belong to two phylogenetic units, the south-eastern bats and the north-eastern bats, well separated from each other by genetic and morphologic traits, but not in an enormous geographic distance. The south-eastern bats represent the largest representatives of the species, with a relatively very long and rather wide rostrum, thus morphologically most distant from other populations. According to the available records, this unit occurs in the most arid areas of the species range, in southern Iran, eastern Afghanistan and north-eastern Oman, and this part of the species range is geographically separated from the other range segments (Fig. 1). This unit perhaps includes also the populations of south-western Iran (see DeBlase 1980, Benda et al. 2012), and maybe also of south-western Arabia (cf. Gaucher 1995, Al-Jumaily 2003). Two names originate from the distribution range of this unit, *Vespertilio desertorum* Dobson, 1875, and *Myotis lanaceus* Thomas, 1920, both were based on the material collected in eastern Baluchistan, Iran, at sites situated some 50 km from each other (Jalk and Shastun). Although these forms were primarily considered to be separate species (Dobson 1878, Satunin 1896, 1914, Bianki 1917, Thomas 1920), now both names are considered synonyms of *M. emarginatus*, and the name *lanaceus* a junior synonym of the name *desertorum*. It is clear just from the comparison of the descriptions given by the authors of these names (see Blanford 1875: 309; and Thomas 1920: 933; unfortunately, the type specimen of *M. lanaceus* was not available for examination) as well as

from the critical opinions by relevant subsequent authors (Ognev 1928, Kuzâkin 1935, Lay 1967, DeBlase 1980). Hence, the name *M. emarginatus desertorum* (Dobson, 1875) apparently represents the valid name of the unit of the south-eastern bats, in accordance with many precedent authors (Ellerman & Morrison-Scott 1951, Etemad 1969, Corbet 1978, DeBlase 1980, Harrison & Bates 1991, Simmons 2005, Benda et al. 2006, 2012, Benda & Gaisler 2015, Uvizl & Benda 2021a).

The unit of north-eastern bats comprises populations of *M. emarginatus* living in the longitudinally very large belt of areas, stretching from Crimea, via the Greater Caucasus Mts., Transcaucasia, and the Caspian-Hyrcanian region of northern Iran, to the southern and eastern parts of West Turkestan, including southern Kirghizstan and south-eastern Kazakhstan. This range consists of a very diverse spectrum of environments, including very humid lowland forests of western Georgia and northern Iran, dry scrublands of mountains and upper plateaus of eastern Iran and southern Kirghizstan, mountain forests of the Caucasus, or arid lowland steppes of southern Turkmenistan and Tajikistan. Within this large and variable range, despite its ecologic diversity, *M. emarginatus* creates one stable morphotype of large bats with a rather short and narrow rostrum. Due to variable humidity conditions in this extensive range, a tinge variation in the pelage colouration was observed and reported by many authors (Kuzâkin 1965, Strelkov et al. 1978, DeBlase 1980); populations of arid habitats were reported to be pale yellowish-grey while bats of humid environments dark reddish-brown (see the review by Benda et al. 2006). This colour variation led the previous authors to division of the populations of the unit of north-eastern bats into up to three subspecies, *M. e. emarginatus* in Crimea and Caucasus, *M. e. desertorum* or *M. e. turcomanicus* in Turkmenistan and *M. e. saturatus / M. e. kuzyakini* in eastern Uzbekistan and adjacent areas (Kuzâkin 1934, 1965, Corbet 1978, DeBlase 1980, Strelkov 1981). However, as we previously concluded (Benda et al. 2006), the pelage colouration in *M. emarginatus* is a varying character, adaptive to habitat, and without a direct reflection in the species taxonomy. The evidence available from the present analyses supports this conclusion.

The interconnection of the *M. emarginatus* populations of Crimea and the Caucasus (including northern Iran) into one taxon is not surprising from the biogeographical perspective, such a relationship is known from other *Myotis* species (Benda et al. 2016b, Çoraman et al. 2019, 2020, Uvizl & Benda 2021b), although not universally (cf. Topál 1971, Strelkov 1972). The interconnection of the Crimean-Caucasian populations with the populations of West Turkestan is more interesting, considering the distinct ecologic conditions in these regions.

Two descriptions were made in the range of this unit, *M. emarginatus turcomanicus* Bobrinskoj, 1925 and *M. lanaceus saturatus* Kuzâkin, 1934, the latter name (being pre-occupied) with a replacement name *M. e. kuzyakini* Rossolimo et Pavlinov, 1979. Since the name created by Bobrinskoj (1925) has a priority over the latter two names, we consider *M. e. turcomanicus* to represent a valid name of the populations of the north-eastern bats and the remaining two names its junior synonyms.

The geographic distribution of the phylogenetic units / subspecies of *M. emarginatus* in western Asia – *emarginatus* in the south-west, *turcomanicus* in the north, and *desertorum* in the south-east – does not fully correspond with the opinions presented by previous authors reporting intraspecific divisions in this bat (Kuzâkin 1965, Corbet 1978, DeBlase 1980, Koopman 1994, Simmons 2005, Benda et al. 2006, 2012, López-Baucells 2019). The interpretation based on the present results is closest to the arrangement by Benda et al. (2006), who, however, joined the present units of north-eastern and south-eastern bats into one taxon based solely on the large

size of body and skull in these populations and also on the clear geographic separation between the western populations of the Mediterranean and Europe and the eastern populations of the eastern Middle East and the Caucasus region (and others living eastwards). However, results of the molecular genetic analysis supported the separation of the populations of the eastern Middle East into a northern unit (*turcomanus*) and southern unit (*desertorum*); *M. emarginatus* is the only species of the genus *Myotis* Kaup, 1829, distributed in the southern parts of the Middle East, out of the Mediterranean arboreal zone. These southern populations perhaps became geographically isolated during the past glaciation events and at present occurr only in limited areas, separated by mountains (Zagros, Hindu Kush) and deserts (Lut, Kavir) from the northern populations. This north-south division along the Iranian plateau and the Hindu Kush uplands is perhaps a more common phenomenon in bats, such a division was demonstrated also in *Rhinolophus hipposideros* (Borkhausen, 1797), a horseshoe bat species occurring in a similar range within the Middle East as *M. emarginatus* (see Shahabi et al. 2019). However, the geographic variation in bat populations occurring across the south-eastern Middle East remains insufficiently studied.

To be concluded, the results of our analysis confirmed *M. emarginatus* to be a polymorphic species. Although the geographic variation in this bat is not extensive, its extent and geographic scale conforms to the divisions of three subspecies within its species rank; *M. e. emarginatus* (Geoffroy, 1806) distributed in the European and African Mediterranean including islands, in western and central Europe, and in the Levant; *M. e. desertorum* (Dobson, 1875) in the south--eastern part of the Middle East, including southern Iran, Oman and Afghanistan; and *M. e. turcomanicus* Bobrinskoj, 1925 in the north-eastern parts of the Middle East, in Crimea and the Caucasus, and in West Turkestan.

SOUHRN

Taxonomická revise netopýra brvitého (*Myotis emarginatus*): podrobná morfometrická analysa a závěrečné vyhodnocení dostupných podkladů (Chiroptera: Vespertilionidae). Netopýr brvitý (*Myotis emarginatus*) je jediným druhem africké linie rodu *Myotis* (někdy uznávané jako podrod *Chrysopteron*) rozšířeným především v palearktické oblasti. Obývá široce několik ekologických zon Evropy, severozápadní Afriky a západní Asie a díky tomu byl vždy považován za polymorfní a polytypický druh. Zatímco v Evropě a v severní Africe byl vždy uznáván jen jeden poddruh tohoto netopýra, až čtyři poddruhy byly rozlišovány v Asii. Ovšem platnost jednotlivých taxonů stejně jako systematická posice jednotlivých populací zůstavaly nejasné – bylo tak nutno provést zevrubnou revisi vnitrodruhové fylogenetické struktury *Myotis emarginatus*, jejíž výsledky zde předkládáme. Revise je založena na kombinaci publikovaných výsledků molekulárně genetické analysy (Uvizl & Benda 2021a) s výsledky důkladného morfologického vyšetření rozsáhlého souboru jedinců z téměř celého areálu rozšíření druhu. Dříve popsaná geografická variabilita mitochondriálních markerů prokázala seskupení haplotypů *Myotis emarginatus* do tří hlavních linií, které se vyskytují allopatricky, (1) v Mediterranní zoně Evropy, Levanty a severní Afriky, (2) v jihovýchodní části Blízkého východu a (3) v severním Iranu a v Západním Turkestanu. Morfologické srovnání odhalilo existenci čtyř hlavních a geograficky vymezených morfotypů v rámci celého druhu, vymezených metrickými znaky tělesnými, lebečními a zubními, a fenetickými znaky lebečními a zubními: (1) spíše malí netopýři s krátkou obličejovou a vysokou mozkovou částí lebky, žijící v Evropě a severozápadní Africe, (2) středně velcí netopýři s dlouhou obličejovou a krátkou mozkovou částí lebky žijící v Levantě včetně Kypru, (3) velcí netopýři se širokou a dlouhou obličejovou částí lebky žijící v jihovýchodní části Blízkého východu, (4) velcí netopýři s úzkou obličejovou částí lebky, žijící na Krymu, Kavkaze a v Západním Turkestanu. Synthesou výsledků obou přístupů hodnocení geografické variability navrhujeme vymezit v ranku *Myotis emarginatus* tři poddruhy, rozšířené ve vzájemně isolovaných areálech: *Myotis emarginatus emarginatus* (Geoffroy, 1806), k němuž náležejí populace Mediterranní Evropy, severozápadní Afriky a Levanty, včetně středomořských ostrovů a střední a západní Evropy; *M. e. desertorum* (Dobson, 1875) rozšířený na jiho-

východě Blízkého východu, včetně Omanu, Afghanistanu a jihovýchodního Iranu (snad i v jihozápadním Iranu) a *M. e. turcomanicus* Bobrinskoj, 1925 rozšířený v pásů území táhnoucího se od Krymu, přes Velký Kavkaz, Zakavkazí, Hyrkánskou oblast i aridní části severního Iranu po Západní Turkestan.

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APPENDIX
List of specimens examined

Afghanistan: 2 ♀♀ (SMF 38916, 38917 [S+A]), Kabul, 18 July 1962, 8 May 1963, leg. D. Meyer-Oehme.

Albania: 1 ♀ (NMP 96605 [S+A]), Fshat, 1 July 2018, leg. P. Benda; – 1 ♀ (NMP 96497 [S+A]), Gjirokastër Castle, 2 July 2015, leg. P. Benda, F. Spitzenberger, M. Uhrin & E. Weiss; – 2 ♀♀ (NMP

96501, 96502 [S+A]), Krongj, Vris Stream, 3 July 2015, leg. P. Benda, F. Spitzenberger, M. Uhrin & E. Weiss; – 1 ♂ (NMP 96524 [S+A]), Selcë, Selcë River, 8 July 2015, leg. P. Benda, F. Spitzenberger, M. Uhrin & E. Weiss.

Algeria: 5 ♀♀ (MUB A050, 243, 244, 246, VMO 4717 [S+B]), Bejaïa, Aokas, 21 May 1981, 14 May 1982, leg. J. Gaisler; – 1 ♀ (ISEA 9617 [S+B]), Madagh, 10 April 1982, leg. K. Kowalski & B. Rzebik-Kowalska; – 3 ♂♂, 1 ♀, 1 ind. (ISEA 9606, 9614–9616, 9618 [S+B]), Sig, 21 December 1978, 2 and 30 July 1979, 29 October 1979, 4 December 1982, leg. K. Kowalski & B. Rzebik-Kowalska.

Austria: 1 ♂ (NMW 51071 [S+B]), Bad Fischau-Brunn, Niederösterreich, 23 January 1993, leg. E. Baar; – 1 ♂ (NMW 29424 [S+B]), Drachenhöhle Cave, Steiermark, 4 January 1976, leg. A. Mayer; – 1 ♀ (NMW 30430 [S+B]), Eggenburg, Niederösterreich, 19 March 1979, leg. H. J. Lauermann; – 1 ♀ (NMW 29988 [S+A]), Göttlesbrunn, Niederösterreich, 1905, leg. Schlereth; – 1 ♀ (NMW 51073 [S]), Hohenau an der Raab, Steiermark, 14 March 1993, leg. H. Polt; – 4 ♀♀ (NMW 9117–9119 [S+B], 9158 [S]), Kaltenleutgeben, Wien 25, 19 June 1955, 27 May 1963, leg. J. Vornatscher & K. Bauer; – 5 ♂♂, 2 ♀♀ (NMW 19982, 51072, 51152, 51426, 52209 [S+B], 36982, 51427 [S]), Kirchberg am Wechsel, Niederösterreich, 26 May 1965, 6 August 1975, 16 March 1985, 15 March 1986, 21 May 1993, 27 July 1994 & 12 August 1994, leg. A. Mayer & B. Freitag; – 1 ♂ (NMW 12030 [S+B]), Kleinzell, Lilienfeld, Niederösterreich, 4 December 1966, leg. H. Hartmann; – 1 ♂ (NMW 30431 [S+B]), Laab im Walde, Niederösterreich, 23 June 1979, leg. A. Mayer; – 2 ♀♀ (NMW 52322, 53498 [S]), Laussa, Oberösterreich, 27 July 1991, 3 August 1996, leg. J. Blumenschein & E. Weiss; – 1 ♀ (NMW B4794 [S]), Laxenburg, Niederösterreich, 20 August 1930, collector unlisted; – 1 ♂ (NMW 52323 [S]), Naas, Steiermark, 7 January 1996, leg. A. Mayer; – 1 ♀ (NMW 15709 [S+B]), Peggau, Graz-Umgebung, Steiermark, 20 January 1973, leg. A. Mayer; – 1 ♀ (NMW 53496 [S]), Pöttsching, Mattersburg, Burgenland, 14 July 1988, leg. A. Baar; – 1 ♂ (NMW 29425 [S+B]), Pyhra, Niederösterreich, 25 June 1977, leg. O. Moog; – 1 ♀ (NMW 30432 [S+B]), Scheiblingkirchen-Thernberg, Niederösterreich, 16 May 1981, leg. H. Rasch.

Azerbaijan: 1 ♂ (NMP 91691 [S+B]), Boyuk Taglar, 26 September 1967, leg. I. Rahmatulina; – 12 ♀♀ (NMP 91395, 91397–91404 [S+A], 91396 [A], 91393, 91394 [S+B]), Mingəçevir, 25–26 June 1984, leg. V. Hanák; – 2 ♀♀ (ZIN 77341, 77342 [S+B]), Lenkoran', čajnaâ fabrika No. 1, 10 July 1984, leg. I. Rahmatulina.

Bosnia and Herzegovina: 3 ♂♂ (NMP 96810, 96811 [S+A], 96812 [A]), Zavala, Bjelušica pećina Cave, 29 August 1977, leg. J. Červený & J. Kučera.

Bulgaria: 7 ♂♂ (NMP 38560, 38561, 38563, 47/72C, 47/72F, 47/72G, 47/72I [S+B]), Âgodina, 2 August 1971, leg. J. Červený, I. Horáček, A. Taušl & D. Vítek; – 3 ♂♂ (NMP 50317–50319 [S+A]), Âgodi-na, Imamova dupka Cave, 15 August 1978, leg. P. Donát, J. Flegr, J. Janda & V. Vohralík; – 3 ♂♂ (NMP 50314–50316 [S+A]), Âgodina, Sančeva dupka Cave, 15 August 1978, leg. P. Donát, J. Flegr, J. Janda & V. Vohralík; – 9 ♀♀ (NMP 50360–50368 [A]), Arkutino, 13 May 1983, leg. D. Král & D. Scholz; – 1 ♂ (NMP 38562 [S+B]), Gela, 31 July 1971, leg. J. Červený, I. Horáček, A. Taušl & D. Vítek; – 5 inds. (NMP 50326–50330 [S+A]), Golâm Kamăk, 13 July 1979, leg. D. Holečková, P. Donát, I. Horáček, J. Jirouš & V. Vohralík; – 1 ♂, 6 ♀♀ (NMP 50222, 50231–50233 [S+A], 50207, 50223, 50303 [A]), Karlukovo, 3 July 1976, 12 June & 4 June 1977, 9 August 1978, leg. V. Bejček, M. Braniš, P. Donát, J. Flegr, V. Hanák, I. Horáček, K. Hůrka, J. Janda, J. Jirouš, J. Škopek, V. Švihla, P. Vašák & V. Vohralík; – 1 ♀ (NMP 50325 [S+A]), Komunari, 12 July 1979, leg. D. Holečková, P. Donát, I. Horáček, J. Jirouš & V. Vohralík; – 6 ♂♂ (NMP 50339–50344 [S+A]), Orehovo, 30 August 1980, leg. D. Holečková, J. Jirouš, H. Prágerová & V. Vohralík; – 2 ♀♀ (NMP 50205 [S+A], IVB 11/35 [S+B]), Peŝera, Snežânka Cave, 19 September 1962, leg. J. Gaisler; – 1 ♀ (IVB 38/1603 [S+B]), Peŝera, Ušatovi dupki, 8 August 1967, leg. J. Gaisler; – 1 ♀ (NMP 47/72/C96 [S+B]), Primorsko, 17 August 1971, leg. J. Červený, I. Horáček, A. Taušl & D. Vítek; – 31 ♀♀ (NMP 49147, 49149, 49153–49161, 49163, 49164 [S+B], 50183–50190, 50192–50197 [S+A], 50278, 50281, 50285, 50286 [A]), Sliven, Zmeevi dupki Cave, 25 May 1957, 15 July 1975, leg. V. Hanák & J. Červený; – 2 ♂♂ (NMP 50150, 50151 [S+B]), Velingrad, Lepenica peŝera Cave, 9 July 1981, leg. J. Flousek, R. Fuchs & V. Vohralík.

Croatia: 1 ♂, 1 ♀ (ZZZ 3056, 3057 [S+B]), Korčula, Postrana, 29 July 1969, leg. B. Đulić; – 2 ♀♀ (ZZZ 3052, 3053 [S+B]), Pupnat, Korčula, 28 July 1969, leg. B. Đulić; – 1 ♀ (ZZZ 3076 [S+B]), Vis, 30 July 1969, leg. B. Đulić; – 2 ♂♂, 2 ♀♀ (ZZZ 307, 344, 349, 350 [S]), Zagreb, Veternica Cave, date & collector unlisted; – 1 ♀, 3 inds. (ZZZ 352, 3102, 10568, 10571 [S]), Croatia, site, date & collector unlisted.

Cyprus: 1 ♂ (NMP 97118 [S+A]), Malatya spring, 18 May 2018, leg. P. Benda & M. Uhrin; – 6 ♂♂, 3 ♀♀ (NMP 90400, 90401, 90931–90935, 91264 [S+A], 90936 [A]), Troodos Forest, 4.5 km SW of Kakopetria, 29 March, 11 April & 13 October 2005, 27 July 2006, leg. P. Benda, I. Horáček, P. Hulva & R. Lučan.

Czech Republic: 1 ♂ (NMP 24/69 [S+B]), Dobrošov, 20 March 1969, leg. M. Anděra & P. Zbytovský; – 2 ♀♀ (NMP 58/59, 61/59 [S]), Javoříčko, Javoříčské jeskyně Cave, 27 January 1959, leg. V. Hanák; – 15 ♀♀ (NMP 831/59, 833/59, 834/59, 836/59, 837/59, 157/62, 158/62, 161/62–163/62 [S+B], 832/59, 835/59, 841/59, 159/62, 160/62 [S]), Jevišovice, 24 July 1959, 14 June 1962, leg. V. Hanák & K. Hůrka; – 1 ♀ (NMP 127/62 [S+B]), Karlštejn, Gaislerova štola Mine, 23 March 1962, leg. V. Hanák; – 14 ♀♀, 2 inds. (NMP 194/65, 195/65 [S+B], 183/65–193/65, 197/65, 200/65, 201/65 [S]), Lednice, 13 June 1965, leg. V. Hanák; – 1 ♂, 1 ♀ (NMP 119/63 [S+B], 120/63 [B]), Mikulov, Na Turoldě Cave, 4 March 1963, leg. J. Gaisler & V. Hanák; – 1 ♂ (NMP 350/58 [S]), Mníšek pod Brdy, 8 March 1958, leg. J. Sklenář; – 1 ♀ (NMP 350/59 [S+B]), Sloup, Sloupské jeskyně Cave, 1 February 1959, leg. V. Hanák; – 2 ♂♂ (NMP 228/59, 229/59 [S]), Šternberk, 30 January 1959, leg. V. Hanák; – 1 ♀ (NMP v12151 [S]), Šumperk, date & collector unlisted; – 1 ♂ (NMP 24B/61 [S]), Velehrad, 10 February 1961, leg. V. Hanák; – 1 ♂ (NMP zn22 [S]), Vranov nad Dyjí, 31 July 1957, leg. V. Hanák.

France: 1 ♀ (ZFMK 97.110 [S+B]), Aigues-Mortes, Gard d'France, 27 June 1958, leg. C. König; – 2 ♂♂ (MNHN 1997-317, 1997-318 [S]), Buré d'Orval, Meurthe-et-Moselle, April 1931, leg. H. Heim de Balsac; – 1 ♂, 3 ♀♀ (MNHN 1963-865, 1963-866, 1984-89, 1984-90 [S+A]), Cachan, Val-de-Marne, 31 March 1946, 26 January 1947, 31 March 1948, leg. J. Balazuc & J. de Bauffremont; – 1 ♀ (SMF 19365 [S+B]), Cap Corse, Brando, Corsica, 27 September 1953, leg. H. Kahmann; – 1 ind. (MNHN 1997-1947 [B]; paratype of *Vespertilio emarginatus* Geoffroy, 1806), Charlemont, date unlisted, leg. Colonel Geoffroy; – 3 ♀♀ (SMF 19171–19173 [S+B]), Les Baux, Grotte des Fées Cave, Bouches-du-Rhone, 20 June 1958, leg. C. König; – 2 ♀♀ (MNHN 1998-955, 1998-956 [S+B]), Les Riceys, Grotte de Frolle Cave, Aube, 13 March 1981, leg. J. Cuisin; – 1 ♂ (MNHN 1963-868 [S+B]), Mériel, Setoise, 9 February 1947, leg. J. Balazuc; – 1 ♂, 2 ♀♀ (MNHN 1984-116–1984-118 [S+A]), Mortagne, Orne, date & collector unlisted; – 1 ♀ (SMF 50429 [S+B]), Rapale, Corsica, 28 August 1976, leg. H. E. Back; – 1 ♀ (SMF 19366 [S+B]), Sorio, Corsica, 3 July 1956, leg. H. Kahmann; – 1 ♀ (MHNG 1255.06b [S+A]), Tourtenay, Deux-Sèvres, 23 December 1951, leg. F. Chanudet; – 1 ♀ (MNHN 1963-867 [S+A]), Varreddes, Seine-et-Marne, 13 February 1944, leg. J. Balazuc.

Georgia: 7 ♀♀ (NMP 91528, 91536, 91541, 91547, 91550–91552 [S+B]), Džal, 14 July 1964, leg. V. Hanák; – 2 ♀♀ (NMP 91508, 91510 [S+B]), Svetichoveli, Mcheta, 11 July 1964, leg. V. Hanák; – 2 ♀♀ (ZMMU S84000, S84001 [S+B]), Georgia (undef.), 29 and 31 August 1939, leg. A. Kuzâkin.

Greece: 3 ♂♂ (NMP 96624, 96625 [S+A], 96626 [A]), Ampeli, Kourkouniotis, Symi, 23 August 2012, leg. P. Benda; – 29 ♀♀ (NMW 31360–31369, 31371–31373, 31376, 31378, 31379, 31382 [S+A], 31370, 31374, 31375, 31377, 31380, 31381, 31383–31386 [S+B], 31359 [S]), Fledermaushöhle Cave, Petralona, 3 June 1977, leg. J. Wirth; – 2 ♂♂ (NMW 35454, 35455 [S+B]), Korykische Grotte (Sarandavli Tropfsteinhöhle) Cave, ESE of Delphi, 7 August 1979, leg. A. Baar & W. Baar; – 8 ♀♀ (NMW 45753–45760 [S+A]), Bunker-Stollen an Abzweigung Stavros an Straße Rendina–Asprovalta, 12 July 1979, leg. U. Passauer; – 1 ♀ (NMP 48630 [S+B]), Xanthi, Kosynthos River, 17 June 1989, leg. R. Chaloupka, V. Hanák & V. Vohralík.

Iran: 1 ♂ (NMP 90856 [S+A]), Ali Abad, 28 June 2006, leg. P. Benda & A. Reiter; – 1 ♂ (BMNH 77.828 [S+B]), Azad-Khan Cave, Mahallet, date unlisted, leg. E. Etemad; – 1 ind. (JOC unnumbered [Sk]), Bazangan, 8 October 2002, leg. J. Obuch; – 1 ♀ (NMP 90765 [S+A]), Emamzadeh Mousa, Razmiyan, 12 May 2006, leg. P. Benda & A. Reiter; – 1 ♀ (NMP 48448 [S+A]), Gishan, Bandar Abbas, 19 April 2000, leg. P. Benda & A. Reiter; – 1 ♀ (NMP 48465 [S+A]), Isin, Bandar Abbas, 30 April 1977, leg.

B. Pražan; – 1 ♀, 3 inds. (BMNH 9.1.4.33, 74.11.21.29, 74.11.21.30 [S], MSNG 44541 [A]; incl. the syntype series of *Vespertilio desertorum* Dobson, 1875), Jalk, Baluchestan, date unlisted, leg. Royal Army Medical College & W. J. Blanford; – 1 ♀ (NMP 90884 [S+A]), Pul, Chalus, 1 June 2006, leg. P. Benda & A. Reiter; – 1 ♀ (NMP 94106 [S+Sk]), Qutur Su, 29 September 2011, leg. M. Andreas, P. Benda, A. Reiter & M. Uhrin.

Israel: 1 ♀ (TAU M6570 [S+B]), Hazorea, 14 April 1975, leg. D. Makin; – 6 ♀♀, 3 inds. (NMW 33850, TAU M2474–M2476, M2481, M6547, M6864, M6865 [S+B], TAU M2858 [S]), Nahal Oren, Etsba Cave, Mt. Carmel, 24 April 1960, 6 May 1962, 14 April 1975, 5 April 1976, leg. D. Harrison & D. Makin; – 1 ♂ (TAU M6373 [S+B]), Maagan Mikhael, 10 June 1974, leg. D. Makin.

Italy: 8 ♀♀ (SMF 17165–17167, 17173–17177 [S+A]), Lingulaglossa, Grotta Corruccio Cave, Catania, Sicilia, 10 July 1955, leg. K. Klemmer & H. E. Krampitz; – 1 ♂ (SMF 10836 [S+A]), Sassari, Grotta del Inferno Cave, Sardinia, 22 March 1951, leg. H. Felten, Frich & Müller.

Jordan: 1 ♀ (NMP 92523 [S+A]), Arjan, 25 May 2009, leg. P. Benda & A. Reiter; – 4 ♀♀ (NMP 92554–92557 [S+A]), Kufranja, Iraq Al Wahaj Cave, 26 May 2009, leg. P. Benda & A. Reiter; – 3 ♀♀ (NMP 92520, 92521 [S+A], 92522 [A]), Zubiya, Zubiya Cave, 24 May 2009, leg. P. Benda & A. Reiter.

Kazakhstan: 12 ♀♀ (ZIN 62160–62171 [S+A]), Eastern slopes of the Karatau Mts., ur. Altyntau, Suzakskij Dist., 2 July 1975, leg. P. Strelkov; – 1 ♀ (ZMMU S83999 [S+B]), Kazakhstan (undef.), 10 June 1944, leg. O. Bogdanov.

Kirghizstan: 1 ♀ (SMF 77779 [S+A]), Sasik Ungur, 30 May 1990, leg. J. Červený.

Lebanon: 2 ♀♀ (NMP 93554, 93555 [S+A]), Aanjar Cave, 5 June 2010, leg. P. Benda & M. Uhrin; – 1 ♀ (NMP 91893 [S+A]), Afqa Cave, 17 January 2008, leg. P. Benda, I. Horáček, R. Lučan & M. Uhrin; – 2 ♂♂ (NMP 93574, 93575 [S+A]), El Jaouz Cave, Khirbet Qanafar, 9 June 2010, leg. P. Benda & M. Uhrin; – 1 ♂ (NMP 93544 [S+A]), Faraya, pond, 2 June 2010, leg. P. Benda & M. Uhrin; – 2 ♂♂ (NMP 93540, 93541 [S+A]), Faraya, Raymond Cave, 2 June 2010, leg. P. Benda & M. Uhrin; – 2 ♂♂ (NMP 95793, 95794 [S+A]), Jezzine, Pont El Khalass, 23 June 2006, leg. I. Horáček, P. Hulva, R. Lučan & P. Němec; – 2 ♂♂ (NMP 93562 [S+A], 93563 [A]), Majdal Tarshish, Qattine Aazar Chasm, 7 June 2010, leg. P. Benda & M. Uhrin; – 1 ♀ (NMP 91758 [S+A]), Marjaba, mine, 19 January 2007, leg. P. Benda, R. Černý, I. Horáček & R. Lučan.

Montenegro: 2 ♂♂, 2 ♀♀ (NMP 90213–90216 [S+A]), Rijeka Crnojevića, Rijeka Crnojevića River, 1 August 2002, leg. P. Benda; – 1 ♂, 1 ♀ (NMP 90206, 90207 [S+A]), Risan, Sopot Cave, 31 July 2002, leg. P. Benda.

Morocco: 1 ♀ (MHNG 1492.87 [S]), 145 km ENE of Marrakech, Grotte du Caïd Cave, Aïd Mehommed, 5 June 1978, leg. P. Strinati; – 1 ♀ (MNHN 1985-1564 [S+A]), Berkane, Grotte de Tazarine Cave, 1955, leg. A. Brosset; – 2 ♀♀ (ZFMK 61.217, 61.218 [S+B]), Taforalt, 30 April 1961, leg. H. Roer.

Oman: 1 ♀ (NMP 93772 [S+A]), Al Hoota Cave, 8 April 2011, leg. P. Benda, A. Reiter & M. Uhrin; – 1 ♂ (NMP 93819 [S+A]), Al Khudhayrah, 10 April 2011, leg. P. Benda, A. Reiter & M. Uhrin; – 1 ♂ (NMP 93788 [S+A]), Misfah, 9 April 2011, leg. P. Benda, A. Reiter & M. Uhrin; – 1 ♂ (NMP 93996 [S+A]), Sal Alah, Birkat Khaldiyah, 13 March 2012, leg. P. Benda, A. Reiter & M. Uhrin; – 2 ♀♀ (NMP 93753 [S+A], 93754 [A]), Sawt, 6 April 2011, leg. P. Benda, A. Reiter & M. Uhrin; – 1 ♀ (NMP 93794 [S+A]), Tanuf, Ain Ghubrat Cave, 10 April 2011, leg. P. Benda, A. Reiter & M. Uhrin; – 1 ♀ (NMP 93735 [S+A]), Tayma, 3 April 2011, leg. P. Benda, A. Reiter & M. Uhrin.

Portugal: 1 ♀ (SMF 18066 [S+A]), Coimbra, Bordalao, 13 July 1928, leg. M. M. da Gama.

Russia: 1 ♂ (ZMMU S21535 [S+B]), Kavkazskij zapovednik Reserve, Majkopskij Dist., 27 August 1932, collector unlisted.

Serbia: 8 ♀♀, 1 ind. (ZIN 35058–35061, 35422, 48086, ZMMU S43760, S43761 [S+B], NMW 9367 [S]), Beograd, Topčider, 31 May 1936, 11 May, 15 May and 24 May 1942, 6 June 1946, 15 August 1949, leg. V. Martino, E. Martino, Ž. Adamovič & A. Petrova.

Slovakia: 1 ♂ (NMP 24/61 [B]), Drienovec, Drienovecká jaskyňa Cave, 18 February, 1961, leg. V. Hanák; – 1 ♂ (NMP 171/58 [S]), Hačava, Hačavská jaskyňa Cave, 7 February 1958, leg. V. Hanák; – 1 ♂ (NMP 14/74 [S+B]), Haligovce, Aksamitka Cave, 28 July 1972, leg. I. Horáček; – 1 ♂ (NMP j4 [S]), Jihoslovenský kras [Slovakian Karst Mts.], 6–12 December 1956, leg. V. Hanák.

Spain: 1 ♂ (EBD 15538 [S+B]), Casa Dos Guejigales, Ronda, Málaga, Sra. de las Wieres, 19 June 1987, collector unlisted; – 1 ♂ (SMF 21481 [S+B]), Linares de Riofrio, Salamanca, 30 August 1962, leg. H. Grünn; – 1 ♂, 1 ♀ (EBD 9925, 9926 [S+B]), Cueva del-Negro Cave, Monte Carbonal, Huétor de Santillán, Granada, 21 August 1963, collector unlisted; – 1 ♂ (EBD 9927 [S+B]), Palacio de Doñana, Huelva, 27 July 1966, collector unlisted; – 5 ♀♀ (EBD 9606, 9684, 9686, 9701, 9707 [S]), Pantano de los Bermejales, 4 June & 26 July 1983, collector unlisted; – 1 ♂ (EBD 15428 [S+B]), Perezoso de Camilla, 4 km N of Lisero de la Hana del Ravel, 4 July 1987, collector unlisted.

Syria: 1 ♀, 1 ind. (TAU M8427, M9440 [S+B]), Mount Hermon, 27 April 1988, 20 June 1995, leg. E. Erez, D. Makin & B. Shalmon; – 16 inds. (NMP 90326–90341 [S]), Qala'at Salah Ad Din, 13 October 2004, leg. R. Lučan; – 8 ♀♀ (NMP 48939–48946 [S+A]), Qala'at Samaan, 3 June 2001, M. Andreas, A. Reiter & D. Weinfurtová; – 1 ♀ (NMP 47927 [S+A]), Ras Al Bassit, 18 May 1995, leg. P. Benda.

Switzerland: 1 ♂ (MHNG 967.95 [S]), Doubs, Grotte du Moron Cave, 16 November 1946, leg. V. Aelen.

Tajikistan: 1 ♂ (NMP 95754 [S+A]), Kalkot, Ar Arak Cave, 17 May 2016, leg. P. Benda, A. Reiter & M. Uhrin; – 1 ♀ (NMP 95724 [S+A]), Kulob, 6 May 2016, leg. P. Benda, A. Reiter & M. Uhrin; – 15 ♀♀ (NMP 95714–95719, pb6164–pb6170 [S+A], 95720, pb6178 [A]), Levap, 5 May 2016, leg. P. Benda, A. Reiter & M. Uhrin; – 1 ♀, 1 ind. (ZMMU S94708, S94709 [S+B]), meždu k. Majkata i Amandara, 20 July 1959, leg. O. Bogdanov.

Turkey: 9 ♀♀ (NMP 47932, 47935–47939, 47941–47943 [S+A]), Çevlik, 20 May 1995, leg. P. Benda, J. Čiháková & J. Flegr; – 8 ♀♀ (NMW 13419, 13423, 13426–13431 [S+A]), Höhle S Kiyiköy (= Midye), Kirklareli, 2–3 June 1968, leg. K. Bauer & F. Spitzenberger; – 1 ♂ (NMW 34373 [S+B]), Olimpos, Antalya, 31 July 1984, leg. F. Spitzenberger; – 1 ♂ (NMP 47959 [S+B]), Safe suyu Cave, 1 September 1996, leg. M. Andreas, P. Benda & M. Uhrin; – 1 ♂ (NMP T93/33 [S+A]), Sarpdere, Dupnisa Cave, 16 October 1993, P. Benda & I. Horáček; – 1 ind. (ZMH 2836/S9185 [S+B]), Tarsus Adana, date unlisted, leg. K. Leonhardt; – 1 ♀ (NMW 11815 [S+B]), Burgruine 4 km SE Yalova, Canakkale, 31 May 1967, leg. K. Bauer, F. Spitzenberger, M. Ganso & L. Wald.

Turkmenistan: 3 ♀♀ (ZIN 54176, 56666, 59541 [S+B]), Bahardenskaâ peŝera Cave, 12 May 1965, 12 May 1967, 12 June 1970, leg. H. Babaev & P. Strelkov; – 2 ♀♀ (ZIN 54195, 57944 [S+B]), Svincovyj Rudnik, Kučitan'-Tau Mts., 31 May 1967, 18 May 1971, leg. P. Strelkov; – 1 ♂ (ZMMU S104386 [S+A]; holotype of *Myotis emarginatus turcomanicus* Bobrinskoj, 1925), Turkmen Kala, Murgab Valley, 11 June 1917, leg. S. T. Bil'kevič; – 1 ind. (ZMMU S29213 [S+B]), Verhne-Skobelevskij, Ašhabadskij Dist., 6 June 1925, leg. S. Ognev.

Ukraine, Crimea: 5 ♀♀ (ZIN 43963–43967 [S+A]), Buhta-Barahta, Karadag, 1 August 1960, leg. N. Filipova & A. Popov; – 1 ♀ (ZIN 44054 [S+B]), Karadag, 6 June 1960, leg. Dmitrieva; – 3 ♀♀ (ZIN 29329, 29330, ZMMU S28574 [S+B]), Karasu-Baši, 25 June 1938, leg. B. Popov; – 1 ♀ (ZIN 54335 [S+B]), Simferopol', June 1967, leg. P. Strelkov & A. Konstantinov.

Uzbekistan: 3 ♂♂, 2 ♀♀ (ZMMU S94707, ZIN 57299, 62511–62513 [S+B]), Taškent, Kara-Kamyš River, 15 May & 27 June 1946, 22 July 1953, leg. A. Andruško & O. Bogdanov; – 7 ♀♀, 1 ind. (ZMMU S6818, S29234–S29236, S94117, S94710, ZIN 57300, 59441 [S+B]; including the holotype of *Myotis lanaceus saturatus* Kuzâkin, 1934), Taškent, 15 June 1932, 7 July 1947, 8 May 1949, leg. R. Meklenburcev, O. Bogdanov & S. Ognev.

Paper 4: Molecular phylogeny of the Palaearctic sheath-tailed bats

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Molecular phylogeny and systematics of the sheath-tailed bats from the Middle East (Emballonuridae: *Taphozous* and *Coleura*)

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The family of sheath-tailed bats (Emballonuridae) constitutes a considerable part of the bat fauna of the Middle East. This region on the crossroad of three biogeographical realms represents the sole significant extension of the family range into the Palaearctic, otherwise the family is distributed mostly in the tropics. Three emballonurid species occur in the Middle East, *Coleura afra*, *Taphozous perforatus* and *T. nudiventris*, each with a number of morphology-based subspecies reported from the region. For this study, we assembled a dataset of more than hundred samples that covers the Middle Eastern parts of the ranges of the respective species. We generated sequences of up to three mitochondrial and five nuclear markers and reconstructed a time-calibrated phylogeny of the family to infer the evolutionary history of emballonurids in the Middle East and to revise their intra- and interspecific taxonomy. The populations of *Coleura* from southern Arabia and the Red Sea coast of Africa show a low genetic structure, although as a lineage are well separated from other *Coleura* populations of Africa and the Indian Ocean islands. We suggest this Afro-Arabian lineage to represent a separate taxon which could be regarded as a species of its own, *C. gallarum*. Similarly, low genetic structure across the study area we revealed in *T. perforatus*; this indicates that only one taxon of this bat is present in the Middle East and adjacent areas that should be co-identified with the nominotypical form. On the contrary, *T. nudiventris* presents two clearly separated clades; one of them comprises the nominotypical form of north-eastern Africa and southern Arabia, as well as the eastern Arabian populations assigned to *T. n. zayidi*, which is thus unjustified, and the latter name to be considered a junior synonym of *T. n. nudiventris*. On the other hand, the analysis did not resolve satisfactorily the phylogenetic position of the large body-sized Mesopotamian populations of *T. nudiventris*, which thus remains to be regarded as a subspecies *T. n. magnus*. Finally, the position of *Liponycteris* as a separate subgenus of *Taphozous* was not found to be justified, while the traditional divisions of the family into the subfamilies Taphozoinae and Emballonurinae and the latter into the tribes Emballonurini and Diclidurini were supported by the analysis results.

Key words: Chiroptera, mitochondrial DNA, molecular genetics, nuclear DNA, southern Palaearctic

Introduction

The sheath-tailed bats (family Emballonuridae Gervais, 1855) have a circumtropical distribution with two core areas in the Old World part of their range, the Afrotropical and Oriental-Australasian regions (Simmons, 2005), which are biogeographically connected by the Middle East (including north-eastern Africa, the Arabian Peninsula and Iran). The family comprises two subfamilies: the circumtropical Emballonurinae, which is further divided into two tribes (the New World Diclidurini Gray, 1866 and the Old World Emballonurini; Robbins and Sarich, 1988; Griffiths and Smith, 1991; McKenna and Bell, 1997; Lim *et al*., 2008; Ruedi *et al*., 2012), and the Old World Taphozoinae Jerdon, 1867, which includes the genera *Taphozous* Geoffroy, 1818, and *Saccolaimus* Temminck, 1838 (Koopman, 1994; Simmons, 2005).

The Middle East and areas adjoining to it are inhabited by both subfamilies. The Emballonurinae is represented by *Coleura afra* (Peters, 1852), Taphozoinae by *Taphozous perforatus* Geoffroy, 1818 and *T. nudiventris* Cretzschmar, 1830 (Ellerman and Morrison-Scott, 1951; Harrison and Bates, 1991; Horáček *et al*., 2000). The two *Taphozous* species have been recognized to belong to two different subgenera, the former to *Taphozous* s.str., and the latter to *Liponycteris* Thomas, 1922 (Simmons, 2005). *Liponycteris* differs from *Taphozous* in having an

occipital helmet on the skull, partly naked (unfurred) body and larger body size (Thomas, 1922; Rosevear, 1965).

Coleura afra is a sub-Saharan species with a small range that extends to the southernmost Arabian Peninsula (Fig. 1 — Hayman and Hill, 1971; Horáček *et al*., 2000; Benda *et al*., 2011, 2013; Vallo *et al.*, 2018). Based on the variation in pelage colouration and body size, three subspecies were recognised: *C. a. afra* (Peters, 1852), *C. a. gallarum* Thomas, 1915, and *C. a. nilosa* Thomas, 1915 (Koopman, 1975; Dunlop, 1997). Only *C. a. gallarum* has been reported to occur in the Middle East, namely in the southern part of the Arabian Peninsula. Populations from coastal Sudan, Djibouti and Somalia (where the type locality is found) have also been referred to this subspecies (Fig. 1 — Harrison, 1964*b*; Koopman, 1975; Pearch *et al*., 2001). The recognition of subspecies within *Coleura afra* has been questioned by some and as a result it is currently considered a monotypic species (Koopman, 1994; Simmons, 2005; Happold, 2013). However, recent phylogenetic studies found considerable morphological and genetic differences between populations across its range, which again raised the possibility of subspecies being recognized in *C. afra* (Goodman *et al.*, 2012; Vallo *et al.*, 2018).

Taphozous perforatus is widespread in Africa, ranging from Senegal in the west and Swaziland in the south up to Egypt. Its range extends further east through isolated populations in the Levant (Israel, Palestine, and Jordan), along the Arabian coast, southern Iran and Pakistan to India (Fig. 1 — Harrison and Bates, 1991; Koopman, 1994; Horáček *et al*., 2000). Altogether, seven subspecies have been described, mostly on the basis of different body size and pelage colouration (*T. p. perforatus* Geoffroy, 1818, *T. p. senegalensis* Desmarest, 1820, *T. p. haedinus* Thomas, 1915, *T. p. sudani* Thomas, 1915, *T. p. swirae* Harrison, 1958, and *T. p. rhodesiae* Harrison, 1964*a*), but only between three and six are recognized as valid (Kock, 1969; Hayman and Hill,

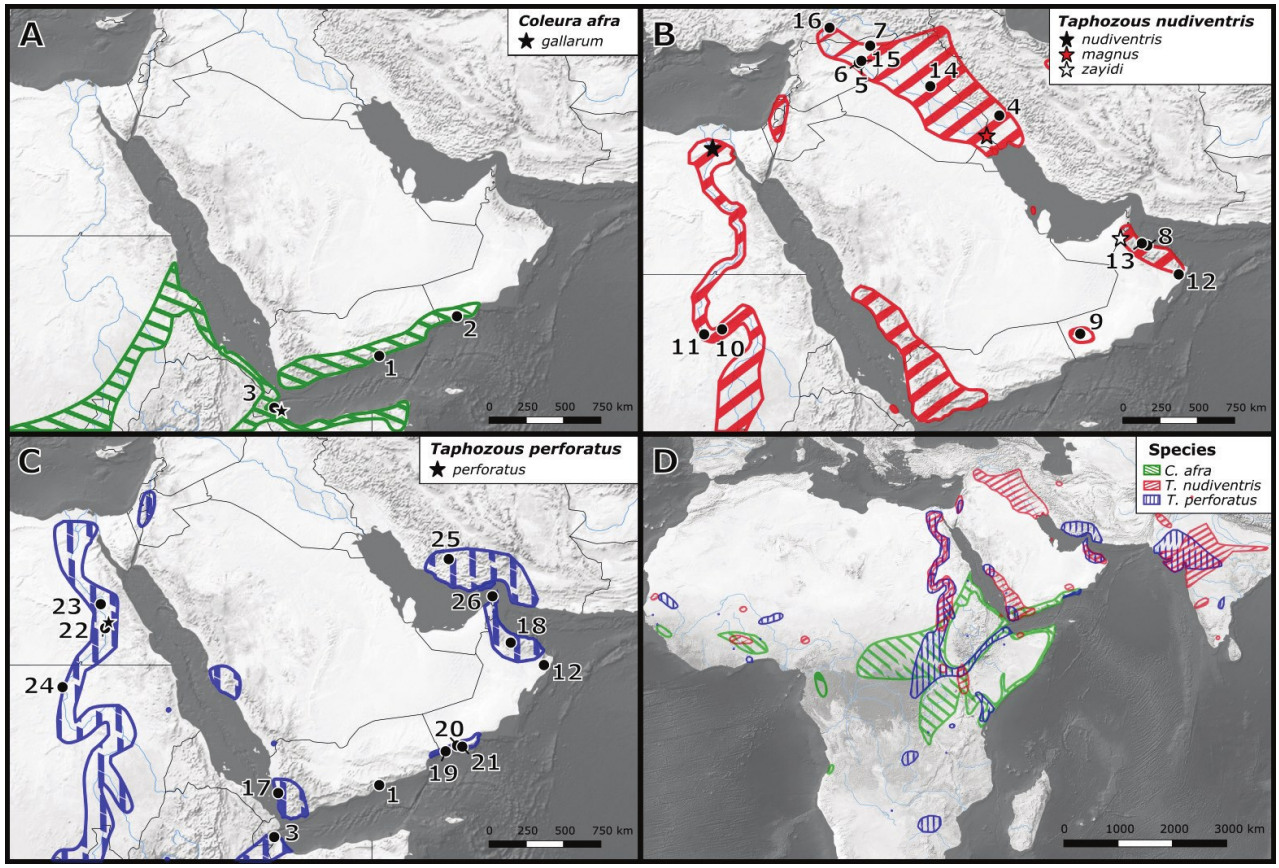

FIG. 1. The distributions of three species of Emballonuridae in the Middle East (A–C), and the whole distributions of these species (D) (modified after Harrison and Bates, 1991; Benda *et al*., 2012; Happold and Happold, 2013). Black dots indicate sample localities, their numbers correspond to those in Supplementary Table S1; stars denote type localities (see legends of particular species maps)

1971; Koopman, 1994; Simmons, 2005). Two of them occur in the Middle East. *Taphozous p. perforatus* ranges along the Nile in Egypt and Sudan, then through the Levant, north-eastern Oman, southern Iran, Pakistan and India. *Taphozous p. haedinus* is distributed in eastern Africa, south-western Arabia, and India (Fig. 1 — Thomas, 1915*b*; Ellerman and Morrison-Scott, 1951; Kock, 1969; Harrison and Bates, 1991; Koopman, 1994).

Taphozous nudiventris occurs in sub-Saharan Africa from Senegal to Tanzania and Somalia, along the river Nile to Egypt, then further east through the Middle East to India and Myanmar (Fig. 1 — Harrison and Bates, 1991). The Middle Eastern part of its range spans from Egypt and Sudan through the Levant and Mesopotamia (from south-eastern Turkey via Syria and Iraq to western Iran) to eastern Iran and southern Arabia (Fig. 1 — Harrison and Bates, 1991; Sachanowicz *et al*., 1999; Horáček *et al*., 2000). Five subspecies have been recognised on the basis of different body size, pelage colouration and extent, and the presence of a gular sac in some (*T. n. nudiventris* Cretzschmar, 1830, *T. n. kachhensis* Dobson, 1872 (in Stoliczka, 1872), *T. n. magnus* von Wettstein, 1913, *T. n. nudaster* Thomas, 1915, and *T. n. zayidi* Harrison, 1955; Simmons, 2005). Three of them occur in the Middle East and north-eastern Africa. *Taphozous. n. nudiventris* is found from Africa to the Levant and south-western Arabia (Harrison and Bates, 1991; Koopman, 1994; Horáček *et al*., 2000; Benda *et al*., 2012). *Taphozous n. magnus* is known from the Mesopotamia and Bahrain, and given its considerable body size difference from all other subspecies it has been suggested that species-level recognition may be warranted for this taxon (Benda *et al*., 2006; Benda and Gaisler, 2015). *Taphozous n. zayidi* is only known from north-eastern Oman and the eastern United Arab Emirates. The description was based on a distinct colour pattern (Harrison, 1955), its validity has however been questioned as the pelage coloration was not found to hold across the subspecies (Benda *et al*., 2006).

The intraspecific taxonomy and systematics of all three Middle Eastern emballonurids remains uncertain as the populations have only been examined morphologically (Thomas, 1915*a*, 1915*b*; Harrison, 1955, 1964*b*; Koopman, 1975; Harrison and Bates, 1991; Benda *et al*., 2006; Benda and Gaisler, 2015). As reviewed above, the validity of some of the taxa is doubted and the phylogenetic position or even taxonomic status of most populations is unknown. Therefore, a thorough revision using genetic tools is needed to elucidate these ambiguities. For this purpose, we generated a multilocus genetic dataset based on both mitochondrial and nuclear markers and applied molecular phylogenetic approaches to (1) provide new insights into the inter- and intraspecific variation in the emballonurid bats of the Middle East and adjoining regions; (2) reconstruct the phylogenetic relationships of the genus *Taphozous* and infer the placement of the Middle Eastern populations within the phylogeny of the genus; and (3) contribute to the taxonomy of the Middle Eastern populations of the family Emballonuridae.

Materials and Methods

Sampling

We used muscle tissue samples of 104 specimens of four emballonurid species (*Coleura afra*, *Taphozous nudiventris*, *T. perforatus* and *T. mauritianus* Geoffroy, 1818) from the collection of the National Museum, Prague, Czech Republic (NMP), and the Smithsonian Institution, Washington, D.C., USA (USNM), to extract DNA (see Supplementary Table S1). We supplemented this dataset with 54 sequences of related species deposited in GenBank. We added single sequences without voucher (museum) numbers only when they were used in other studies (see Supplementary Table S1). As the outgroup we used *Nycteris hispida* Geoffroy, 1818 from the family Nycteridae Van der Hoeven, 1855, a sister group to the Emballonuridae (Teeling *et al*., 2005).

DNA Extraction and Sequencing

The genomic DNA was extracted from the alcohol-preserved tissue samples using Geneaid Genomic DNA Mini Kit. We targeted three mitochondrial markers (mtDNA), including 1126 bp of cytochrome *b* (*Cyt-b*), 533 bp of 16S rRNA (*16S*) and 689 bp of D-loop of the control region (*D-loop*), and five nuclear markers (nDNA), consisting of 684 bp of the recombination activating gene 2 (*Rag2*), 479 bp of acyl-coenzyme A oxidase 2 intron (*ACOX*), 724 bp of COP9 signalsom subunit 7A intron (*COPS*), 527 bp of the signal transducer and activator of transcription 5A intron (*STAT*), and 618 bp of biglycan intron (*BGN*). We sequenced both strand for all sequences. We used primers that have been specifically designed for the order Chiroptera and provided good amplification in previous studies (see e.g., Puechmaille *et al*., 2011; Salicini *et al*., 2011; Thong *et al*., 2012; Dool *et al*., 2016). For the primer names, their sequences and annealing temperatures, see Supplementary Table S2. For the amplification of the *D-loop*, two reverse primers were used: HSC for *Taphozous perforatus* and H607 for *T. nudiventris* and *T. mauritianus*.

Phylogenetic Reconstructions

Sequences were edited and aligned using the MAFFT plugin (Katoh and Standley, 2013) in Geneious 8.1.6 (Kearse *et al*., 2012) and subsequently edited by eye. Heterozygous positions in the nDNA markers were coded with the IUPAC codes and ambiguous positions or missing data were coded with 'N'.

Indels were treated as gaps. Sequences of protein-coding markers were translated to aminoacids to check for the presence of stop codons, which would indicate pseudogenes have been amplified. Alleles of nuclear markers were estimated using PHASE (Flot, 2010) with the probability threshold set to 0.7. The dataset was split in two multilocus datasets according to the mode of inheritance of the markers. The first dataset contained only mitochondrial markers (124 samples) of a total length of 2350 bp. The second dataset contained phased nuclear markers (68 samples — 34 individuals) of a total length of 3038 bp. The two datasets were partitioned by gene, and the protein-coding genes (*Cyt-b*, *Rag2*) as well as the nuclear introns (*ACOX, COPS, STAT, BGN*) were further partitioned by codon position. The appropriate nucleotide substitution model for each partition was selected based on the Bayesian information criterion (*BIC*) using PartitionFinder v1.1.1 (Supplementary Table S3 — Lanfear *et al*., 2012, 2014).

Phylogenetic analyses of both datasets were run using Bayesian inference (BI) and maximum likelihood (ML). We used MrBayes v3.2.6 (Ronquist and Huelsenbeck, 2003) to run the BI analysis. Appropriate substitution models were specified for each partition and all parameters were unlinked across partitions. We ran two independent runs for 100 million generations with trees sampled every 1000 generations. All other parameters were set to default. Stationarity and convergence of the runs were inspected in Tracer v1.6 (Rambaut *et al*., 2014) and the value of the average standard deviations of the split frequencies that were lower than 0.01. The burn-in fraction was left as the default at 25% of sampled trees. Thus, from the 100,000 produced trees, 25,000 were discarded. A majority-rule consensus tree was produced from the post-burnin trees with posterior probability (*PP*) values embedded. ML analysis was conducted by RAxML (Stamatakis, 2014) with bootstrap values inferred using 1000 pseudoreplications.

We also performed a coalescent-based species-tree estimation using *BEAST (Heled and Drummond, 2010) implemented in the BEAST v1.8.4 (Drummond *et al*., 2012). We used both mtDNA and nDNA data, but only samples that had at least one nuclear marker sequenced were included. This dataset contained 92 samples (46 individuals) of a total length of 5391 bp. Each marker was considered one partition, and HKY model was used for all. Other *BEAST settings, except the calibration point and number of generations (500 million sampled every 50,000 generations), were as described bellow for the divergence time estimation analysis. Uncorrected *p*-distances between species/clades/groups were calculated for the *Cyt-b* in MEGA v7.0 (Kumar *et al.*, 2016).

Divergence Time Estimation

For the molecular dating analysis, we used our newly generated sequences of *Cyt-b*, 16S and *Rag2* for one individual of *Taphozous perforatus* and *T. mauritianus* and two individuals of *T. nudiventris* and *Coleura afra*. It is customary to use one sample per species in divergence dating analyses, but our results (see below) showed the presence of two well-differentiated lineages within *T. nudiventris* and *C. afra* and hence we used two individuals of each to time-calibrate these splits. We supplied the dataset with GenBank sequences of other emballonurid species, consisting of 24 species and the outgroup *Nycteris hispida* (Supplementary Table S4).

The analysis was set up in BEAUti and run in BEAST v1.8.4. We used uncorrelated relaxed molecular clocks (Drummond *et al.*, 2006) for all genes. As a calibration point, we employed the oldest known fossil of the family Emballonuridae, †*Tachypteron franzeni* Storch, Sigé and Habersetzer, 2002. It is known from the Middle Eocene deposits of Germany and its age is estimated at approximately 47 Million years ago (Ma; Storch *et al.*, 2002). We used a lognormal prior distribution for this calibration point (offset 47, mean 4.0) following Ruedi *et al.* (2012). Further, we used a birth-death model of evolution (Gernhard, 2008). BEAST was run twice for 50 million generations and parameters and trees were saved every 1000 generations. Tracer v1.6 was used to confirm adequate mixing of the MCMC chains and acceptable effective sample sizes (ESS > 200). LogCombiner was used for burn-in (25%) and merging trees files, TreeAnnotator was used for identifying the maximum clade credibility tree. All analyses were run through CIPRES Science Gateway (Miller *et al*., 2010).

Results

Phylogenetic Reconstructions

The mitochondrial dataset comprised 118 sequences of *Cyt-b*, 66 of *D-loop* and 19 of *16S*. The nuclear dataset comprised 60 phased sequences of *Rag2*, 44 of *ACOX*, 12 of *COPS*, 44 of *STAT*, and 16 of *BGN*. The phylogenetic trees obtained by both ML and BI analyses of the mitochondrial and nuclear datasets separately (Fig. 2) or combined (Supplementary Fig. S1) had almost identical topologies. All three analyses showed a clear separation between lineages containing the representatives of Emballonurinae and Taphozoinae.

The Emballonurinae represented by *Coleura afra* formed a separate and supported monophyletic clade in both the mitochondrial and nuclear tree. Haplotypes of southern Arabian and Djiboutian samples clustered together (Fig. 2). In the mtDNA-only tree they formed one of five haplogroups identified within this species. The other four haplogroups were formed by samples from Ghana, Gabon, mainland Tanzania and Kenya with Pemba Island. Intraspecific relationships among these haplogroups were not supported in either the ML or BI analysis. The five haplogroups were separated by substantial genetic distances, with the mean *p*-distance between these groups for *Cyt-b* being 3.6% (Table 1).

The mitochondrial trees showed that two genera — *Saccolaimus* and *Taphozous* — were clearly separated within the Taphozoinae. Species of the latter genus formed three supported groups: *T. mauritianus* and *T. longimanus* Hardwicke, 1825 (in Fig. 2 as Group I); the Australasian species *T. melanopogon* Temminck, 1841 together with the Middle Eastern *T. perforatus* (Group II) and *T. nudiventris* (Group III). Group I was sister to the other two

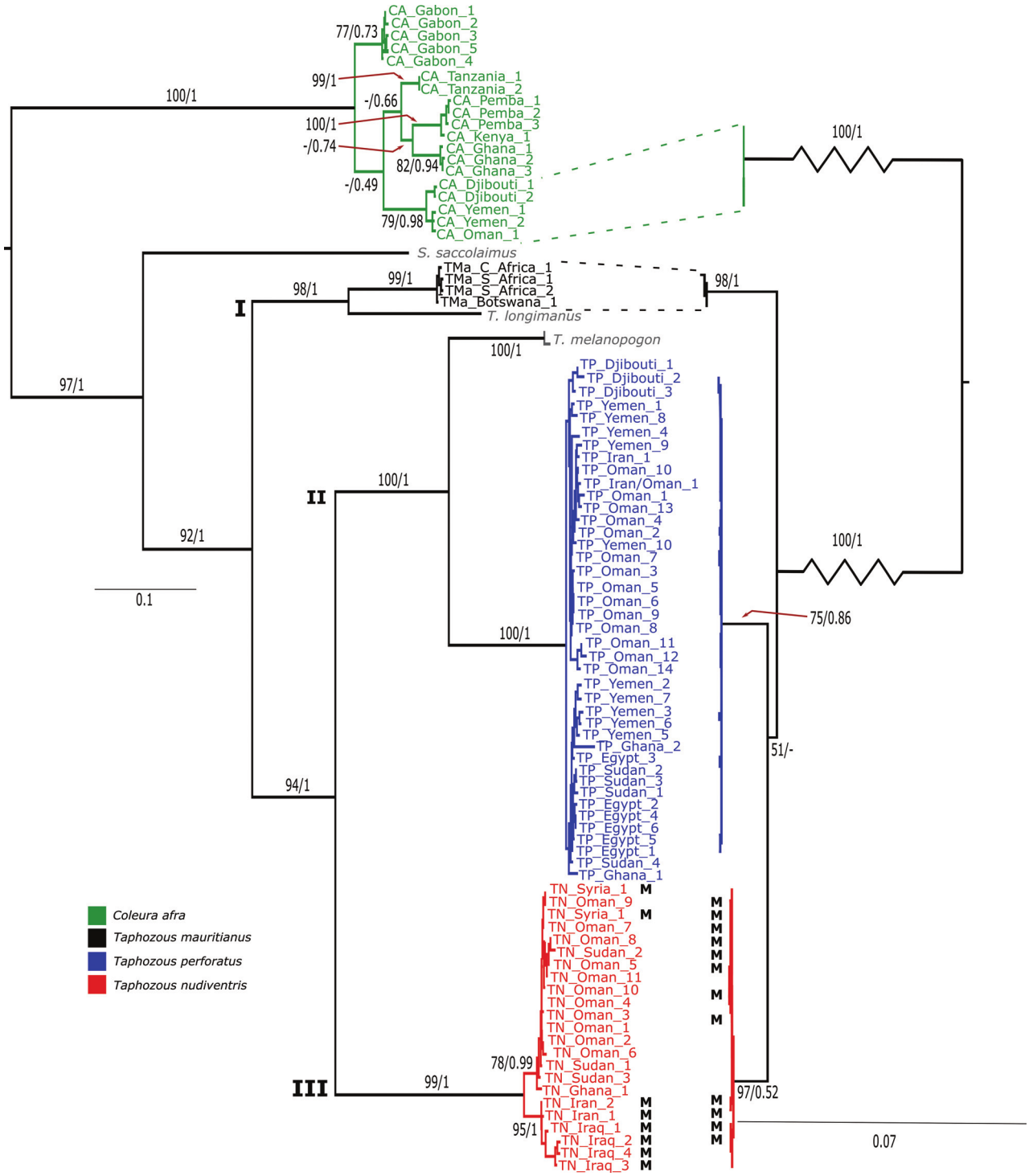

FIG. 2. Maximum likelihood tree of reconstructed phylogenetic relations of selected forms of the family Emballonuridae based on mitochondrial dataset (left tree) and Bayesian inference tree based on nuclear dataset (right tree). Bootstrap values and Posterior Probability values are shown above/below branches. Roman numerals refer to the group delimitation used in text. Bold 'M' at haplotype designations denote specimens of the large-sized morphotype, *T. n. magnus*. Color images are only available in electronic form

groups. *Taphozous perforatus* (Group II) had a low intraspecific diversity (mean *p*-distance for *Cyt-b* being 0.6%). *Taphozous nudiventris*, the only member of Group III, was divided into two well-supported lineages, one lineage included the Iranian, Iraqi and Syrian samples (in Fig. 2 as haplotypes TN_Iraq and TN_Iran; for details see Supplementary Table S1), and the other lineage was

Table 1. Percent values of *p*-distances on *Cyt-b* among the haplotype groups of *Coleura*

No.	Haplotype group	1	2	3	4	5	6
1	*C. kibomalandy*	–					
2	*C. seychellensis*	5.81	–				
3	Yemen + Djibouti	7.83	9.16	–			
4	Tanzania	8.17	9.43	5.10	–		
5	Pemba Isl. + Kenya	9.82	10.15	6.53	3.76	–	
6	Gabon	8.37	9.17	5.09	4.56	5.51	–
7	Ghana	8.79	9.64	5.86	3.66	4.01	5.14

composed of samples from southern Arabia and Sudan, as well as Syria and Turkey (the Turkish sample belongs to the haplotype TN_Syria_1). The mean genetic distance between the two lineages was 3.8% for *Cyt-b* (Table 2). The nuclear trees recovered all three species sampled for the nuclear markers as reciprocally monophyletic. All of them were well supported in the ML analysis, but only *T. mauritianus* was also supported by the BI. Also, the relationships between the three *Taphozous* species were not supported. All species showed low intraspecific variation.

Molecular Dating

The tree topology reconstructed in the calibrated analysis was supported in all nodes and corresponded to those of the ML and BI analyses (Fig. 3). Of the species we studied, *Coleura afra* diverged from its two sister species (*C. seychellensis* Peters, 1868 and *C. kibomalandy* Goodman *et al*., 2012) 6.3 Ma (95% highest posterior density [HPD]: 4.6–8.6 Ma) and the Djibouti-Arabian group diverged from the mainland Tanzanian group 3.8 Ma (95% HPD: 2.2–5.9 Ma). The *Taphozous* species began to diversify 22.7 Ma (95% HPD: 17.9–28.7 Ma). *Taphozous nudiventris* diverged from the other species 17.1 Ma (95% HPD: 12.9–21.7 Ma) and its two subgroups — the Mesopotamian and Afro-Arabian — diverged 2.7 Ma (95% HPD: 1.6–4.2 Ma). *Taphozous perforatus* separated from its sister species *T. melanopogon* 10.1 Ma (95% HPD: 7.0–13.6 Ma).

Discussion

In this study, we performed a multilocus genetic dataset covering Middle Eastern populations of the three species of sheath-tailed bats (Emballonuridae) to determine their phylogenetic positions, the level of intraspecific differentiations and to contribute to the taxonomy of the group. The topology and age estimates of our calibrated tree are in agreeemnt with those in Ruedi *et al*. (2012), which is not surprising considering that the same calibration point was employed. However, compared to their study, the results presented here are based on a more robust taxon and gene sampling (12 versus 25 species; two versus three markers). We have added new sequences of two *Taphozous* species and sequences of 11 species of other genera from GenBank. Further, we used a relaxed molecular clock, which we believe better captures the evolutionary rate of the markers used at this phylogenetic depth (Drummond *et al*., 2006).

Emballonurids form two main lineages that correspond to the traditionally recognised subfamilies, the circumtropical Emballonurinae and the Old World Taphozoinae (Koopman, 1994; Simmons, 2005). The Emballonurinae are divided into two main subclades that correspond to the Old World Emballonurini and the New World Diclidurini tribes

Table 2. Percent values of interspecific *p*-distances on *Cyt-b* among selected Emballonuridae taxa

No.	Taxon	1	2	3	4	5	6
1	*T. perforatus*	–					
2	*T. melanopogon*	12.5	–				
3	*T. nudiventris* others	17.7	16.7	–			
4	*T. nudiventris* Iran	17.0	16.6	3.8	–		
5	*T. mauritianus*	17.5	17.1	16.4	15.4	–	
6	*T. longimanus*	19.2	19.3	17.0	17.3	11.8	–
7	*C. afra*	23.8	23.0	21.4	22.2	23.5	22.7

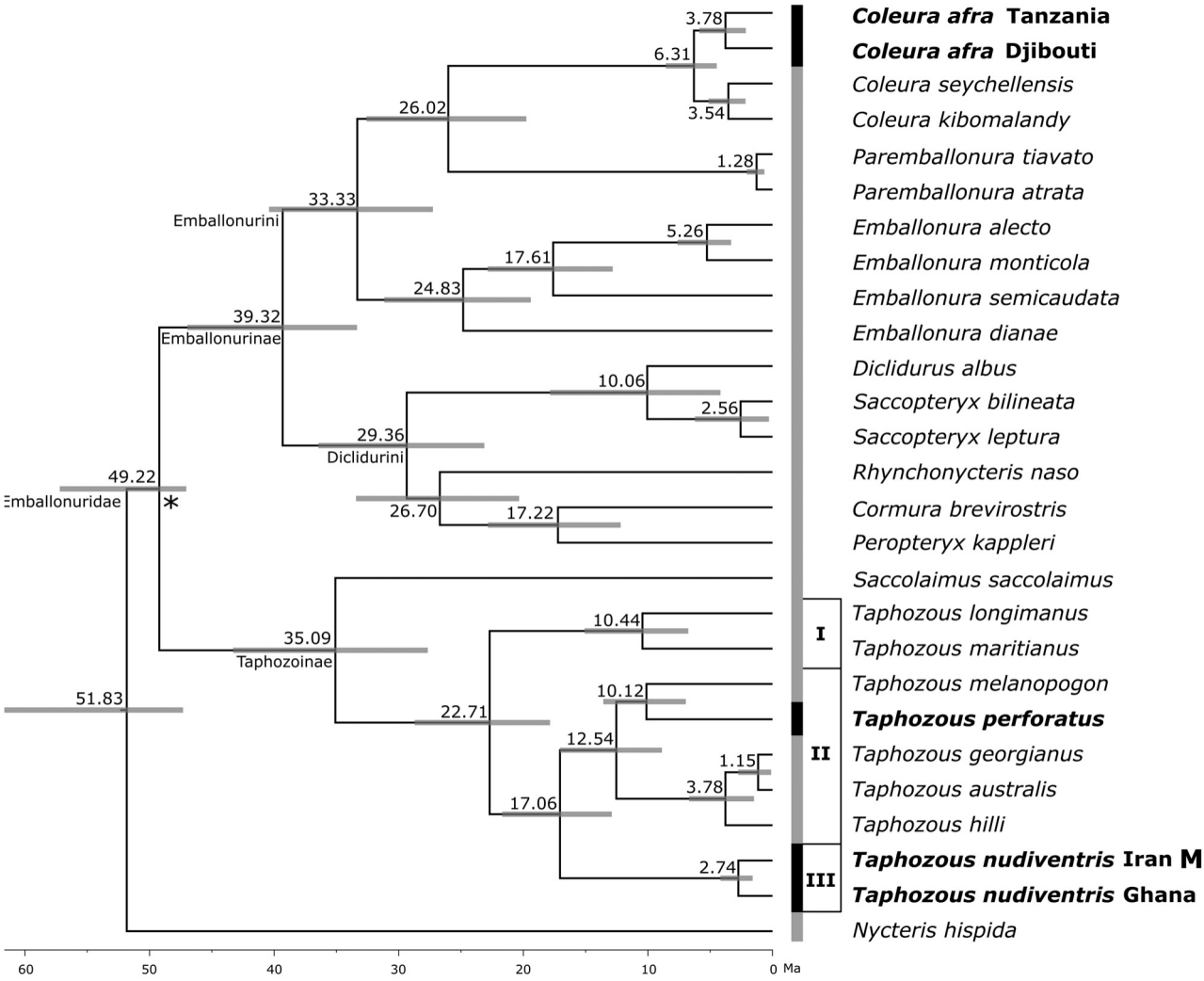

FIG. 3. Chronogram of the family Emballonuridae based on a Bayesian inference of combined mitochondrial (*Cyt-b* and *16S*) and nuclear (*Rag2*) markers. The numbers at nodes show mean divergence time estimates (Ma) and horizontal boxes 95% highest posterior density intervals of these estimates. Black are species with range in the Middle East, greyish are species from other regions. The node with (*) indicates the position of the calibration point. Roman numerals refer to group delimitation used in text. All nodes are supported ($PP \geq 0.95$). Bold 'M' denote specimens of the large-sized morphotype, *T. n. magnus*

(Robbins and Sarich, 1988; Griffiths and Smith, 1991; McKenna and Bell, 1997; Lim *et al*., 2008; Ruedi *et al*., 2012). According to our results, these subclades separated from each other in the Middle Eocene. Within the former tribe, the genera *Coleura* Peters, 1867 and *Paremballonura* Goodman *et al*., 2012 form an African clade (Goodman *et al*., 2012; Ruedi *et al*., 2012). The estimated age of separation of this clade is consistently placed in the Upper Oligocene (26.0 Ma in this study; 23.3 Ma in Ruedi *et al*., 2012). The crown diversification within *Coleura* occured in the Upper Miocene (6.3 Ma), when the ancestor of *C. seychellensis* and *C. kibomalandy* colonised Madagascar, while the ancestor of *C. afra* remained in the African mainland (Goodman *et al*., 2012). The *C. afra* populations from Djibouti and mainland Tanzania then separated at about the same time as when *C. seychellensis* separated from *C. kibomalandy* and supposedly colonised Seychelles (3.8 Ma and 3.5 Ma, respectively). Apparently, the diversification within *C. afra* corresponds in age with the diversification between other *Coleura* species, suggesting *C. afra* may represent a complex of several species rather than one highly variable species.

In the Middle East, *C. afra* occurs only in southern Arabia, along the Arabian Sea coast of southern Yemen and south-western Oman. The Arabian specimens included in our analyses originate from two localities separated by an air distance of almost 600 km, yet they show very low genetic variation. Moreover, both are genetically very similar to the

samples from Djibouti. The Arabian and Djiboutian populations thus appear to belong to one taxon.

Thomas (1915*a*) originally described the *Coleura* populations from this area as a separate species, *C. gallarum*, which he differentiated from *C. afra* by its smaller body size, smaller tooth size and more brownish pelage. Our findings of a considerable differentiation within *C. afra* are in concert with a recent study by Vallo *et al*. (2018), who found three morphotypes within this species. Among these morphotypes, the Yemeni-Sudanese one had the smallest body and skull sizes, and mainly extended rostrum. Between the other African populations of *Coleura*, genetic and morphological differences were found to be less pronounced; the populations of the southern Arabian Peninsula and coastal north-eastern Africa differed in these characters most markedly from the remaining populations of *C. afra* from Central and West Africa. Material from the type locality of *C. gallarum* (Zeyla, north-western Somalia — Thomas, 1915*a*) could unfortunately not be included in our study. However, the Djiboutian specimens included in our analyses originate less than 50 km from it and since there are no apparent biogeographic barriers between the two sites we presume they represent the same population. Despite the fact the intraspecific relationships of *C. afra* were not resolved and the lack of nuclear data from the non-Middle Eastern populations, the high level of genetic differentiation (both genetic and morphological) within *C. afra* indicates the presence of several evolutionary entities within this species. The Arabian-Djiboutian lineage is genetically the most divergent, with *Cyt-b p*-distances ranging from 5.0–6.3% (4.9–6.5% in Vallo *et al*. (2018) who used Kimura 2-parameter distances for the same gene). These distances alone are suggestive of the presence of more than one species (Baker and Bradley, 2006). Moreover, these values are of the same order as those between the insular species *C. seychellensis* and *C. kibomalandy*, which are considered full species (Goodman *et al*., 2012; Vallo *et al*., 2018), even though the difference between isolated insular versus continuous continental distribution have to be considered. Hence, we suggest the recognition of *C. gallarum* as a valid species distributed in southern Yemen, Oman, Djibouti, north-western Somalia, and eastern Sudan, as originally suggested by Thomas (1915*a*). This taxon thus represents a second species of the genus *Coleura* living in the continental Afrotropics, and the fourth species in this genus. Unfortunately, without a more continuous geographic sampling from the other parts of *C. afra* range and more data (including nuclear data), we cannot reach taxonomic conclusions about the other *C. afra* lineages.

In the subfamily Taphozoinae, the onset of *Taphozous* diversification dates to the Early Miocene when Group I, formed by the Afrotropical *T. mauritianus* and Oriental *T. longimanus*, separated from the remaining species of the genus. These two species then diverged from each other in the Late Miocene. Group II started to diversify in the Middle Miocene; it includes the Australian species *T. georgianus* and *T. australis*, which are closely related as confirmed by morphological data (Chimimba and Kitchener, 1991), and whose diversification was probably the most recent among extant *Taphozous* species (Middle Pleistocene; 1.2 Ma). The diversification of the widespread *T. perforatus* and *T. melanopogon* took place in the Late Miocene. Group III, which includes only *T. nudiventris*, split off from Group II in the Early Miocene.

Taphozous perforatus (Group II), a species that is broadly distributed across Africa and south-western Asia, belongs to a clade along with Australian and Oriental species. Intraspecifically, *T. perforatus* shows a low genetic diversity in both mitochondrial and nuclear datasets across all sampled Middle Eastern and African populations (*Cyt-b p*-distances 0.6%). Two subspecies of *T. perforatus* were reported to occur in the Middle East, *T. p. perforatus* and *T. p. haedinus*. The latter was distinguished on the basis of its dark colourations of the pelage and wing membranes (Thomas, 1915*b*; Ellerman and Morrison-Scott, 1951; Harrison, 1964*b*; Kock, 1969; Koopman, 1994). Based on our sampling of this species throughout the Middle East and northern Africa (Fig. 1) and the lack of genetic structuring among these samples, we conclude that the recognition of two subspecies within *T. perforatus* in this region is not justified. As our material included samples from near the type locality (Kom Ombo, Egypt; Kock, 1969), we propose that all populations in the Middle East and north-eastern Africa represent one taxon, *T. p. perforatus*. Since we did not examine the material of *T. p. haedinus* from the type locality (Chandler's Falls, Kenya; Thomas, 1915*a*) or any other East African localities, we do not make any conclusions regarding the status of this subspecies. The close relationship of *T. perforatus* to the Oriental species and the fact that its range stretches to the Indian subcontinent (Koopman, 1994) indicate that *T. perforatus* is likely of Asian origin and that the African and Middle Eastern parts of its range are a result of a rapid westward expansion

that is apparent from the lack of genetic structure across its populations.

The previous morphology-based systematic reconstructions of *Taphozous* placed *T. nudiventris* (Group III) aside from all other species in a distinct subgenus *Liponycteris* (together with *T. hamiltoni*; Thomas, 1922; Rosevear, 1965; Koopman, 1994; Horáček *et al*., 2000; Simmons, 2005). However, in most of our analyses, *T. nudiventris*, the type species of the subgenus, was reconstructed as an inner group of the genus that is a sister to Group II. Only the analyses of the nuclear dataset showed low support for this topology. Thus, the phylogenetic arrangement renders the subgenus *Taphozous* paraphyletic with respect to *Liponycteris*. Therefore, we suggest abandoning the subgeneric classification of *Taphozous* and the use of the subgenus *Liponycteris*.

Taphozous nudiventris experienced a relatively old intraspecific diversification in the Afro-Arabian transition region, where the Mesopotamian and Afro-Arabian populations separated in the Pliocene. This species shows two distinct size-differentiated morphotypes in the Middle East (Harrison and Bates, 1991; Benda *et al*., 2006; Benda and Gaisler, 2015). The small-sized morphotype occurs in the Levant, southern Arabian Peninsula, eastern Iran, Afghanistan, Pakistan, India and the entire African part of the species range, and contains two subspecies in the Middle East, *T. n. nudiventris* and *T. n. zayidi* (Harrison, 1955, 1964*b*; Harrison and Bates, 1991; Koopman, 1994; Horáček *et al*., 2000). The large-sized morphotype, generally ranked as a distinct subspecies *T. n. magnus*, ranges in Mesopotamia from Turkey to Iran and in Bahrain (Harrison and Bates, 1991; Sachanowicz *et al*., 1999; Benda and Gaisler, 2015). The genetic analysis of mitochondrial dataset revealed a shallow intraspecific split into two lineages in *T. nudiventris*; one lineage represented by samples from Syria, Turkey, southern Arabia, and Africa, the other from Syria, Iraq, and western Iran. The first lineage occurs in a region inhabited by the smaller morphotype, assigned to *T. n. nudiventris* (Nile Valley in the northern Sudan) and *T. n. zayidi* (north-eastern Oman). *Taphozous n. zayidi* was originally described and further treated as differing only by its greyish pelage, but this characteristic was questioned by Benda *et al*. (2006). Our results suggest that the populations belonging to *T. n. nudiventris* and *T. n. zayidi* form one evolutionary lineage. We thus suggest that the name *T. n. zayidi* Harrison, 1955 is a junior synonym of *T. n. nudiventris* Cretzschmar, 1830, since the separation of populations from Oman is not supported by neither genetic nor morphological evidence.

The large-bodied subspecies *T. n. magnus* was represented in our analyses by samples from Iran, Iraq, Turkey and Syria. Interestingly, these samples did not form a monophyletic lineage in either the mtDNA or nDNA trees. While samples from Iran and Iraq clustered together in a distinct lineage, samples from Turkey and Syria were scattered in the clade of the small-bodied Afro-Arabian individuals. The nuclear data revealed basically no within species structure/differentiation. This inconsistency of the morphological and genetic evidence might be a result of ongoing but incomplete merging of mtDNA and nDNA genetic pools between two previously isolated populations (i.e. morphotypes) that are now in contact in certain areas. Another plausible explanation is that the large body size was selected for by locally specific conditions and individuals attain a large size in the Mesopotamia regardless of their phylogenetic affinity. In that case, body size does not have taxonomic significance.

Future research should focus on a broader sampling that would include the type locality of the nominotypical subspecies of *T. nudiventris* (Giza, Egypt) as well as the Levant, where the small-bodied form occurs relatively close to the large-bodied Mesopotamian morphotype. Until then, the question of the intraspecific differentiation within *T. nudiventris* in the Middle East remains unanswered. Regardless, a taxonomic conclusion the data allow us to make is that the Mesopotamian populations do not represent a distinct species as previously suggested (Benda *et al*., 2006; Karataş and Sachanowicz, 2008).

In summary, the results of the genetic analysis presented herein bring new insights to the phylogenetic affinities and taxonomic arrangements of the Middle Eastern populations of the family Emballonuridae. The populations of *Coleura* of southern Arabia and the Red Sea coast of Africa represent a separate taxon, *C. gallarum*. Contrary to the previous views, the Middle Eastern populations of *T. perforatus* contain only one form as no subspecific variation was observed. The documented genetic variation in *T. nudiventris* suggests that populations considered as *T. n. zayidi* are best viewed as belonging to *T. n. nudiventris*. Whereas, the phylogenetic position of the Mesopotamian populations traditionally recognized as *T. n. magnus* remains elusive and needs further investigation. Finally, the position of *Liponycteris* as a separate subgenus of *Taphozous* was not supported.

Supplementary Information

Contents: Supplementary Fig. S1. Phylogenetic tree obtained by coalescent-based species-tree estimation from concatenated dataset of all markers. Posterior probability values are shown above/below branches. Supplementary Tables: Table S1. Original sequences and sequences from GenBank used in the molecular genetic analysis. Numbers in parentheses in the locality column correspond to locality numbers as shown in Fig. 1A–1C; Table S2. Names, sequences and annealing temperatures of primers used in this study. For the amplification of D-loop two reverse primers were used: HSC for *T. perforatus* and H607 for *T. nudiventris* and *T. mauritianus*; Table S3. Substitution models as identified by PartitionFinder for the different partitions. Numbers in parentheses denote codon position; Table S4. Species and their sequences used in the molecular dating analysis Supplementary Information is available exclusively on BioOne.

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Paper 5: Revision of the *Rhinolophus hipposideros* group

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A revision of the *Rhinolophus hipposideros* group (Chiroptera: Rhinolophidae) with definition of an additional species from the Middle East

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Initially, the *Rhinolophus hipposideros* group was defined by two morphological traits, the structure of the nose-leaf and the shape of basioccipital bone of the skull. Originally, it consisted of two species, *R. hipposideros* and *R. midas*, whereas currently it is considered to contain a single species, *R. hipposideros*, under whose rank both original species have been joined. The interpretation of geographic variability within the group has traditionally been based on variation in body and skull size, nose-leaf shape, and several selected skull and tooth characters. This approach resulted in delimitations of up to seven subspecies, mostly in the Mediterranean area, a conception introduced more than a hundred years ago and accepted by many authors till today. We investigated the phylogenetic relationships among populations of *R. hipposideros* with the help of molecular genetic, morphological, and acoustic examinations. Our analysis uncovered the existence of an unexpected diversity within the *R. hipposideros* group, challenging its current phylogenetic and taxonomic arrangements. The molecular genetic analysis of almost 100 samples and morphological examinations of about 300 specimens showed two main, geographically exclusive, phylogenetic lineages within the group, well delimited by molecular characteristics and possessing two distinct morphotypes and two distinct echotypes. These two lineages are isolated deep enough to be considered separate species. One of them, *R. hipposideros* s.str., is widespread over the south-western Eurasia and north-western and north-eastern Africa, and the other, *R. midas*, is distributed in a small range around the Strait of Hormuz and Gulf of Oman. The extensive range of *R. hipposideros* s.str. is inhabited at least by two subspecies, separated mainly by the genetic characters, whereas the morphological and echolocation traits do not distinguish the populations sufficiently. The western *R. h. hipposideros* occurs in the Maghreb and Europe west of the Dnieper River, Bosporus, and the Strait of Karpathos, and the eastern *R. h. minimus* lives east of this boundary, including the populations of Crimea, Caucasus, the Middle East, and north-eastern Africa (Sudan to Djibouti). The two subspecies also differ in karyotype, with 2n = 58 in *R. h. minimus* and 2n = 54–56 in *R. h. hipposideros*. The taxonomic position of the easternmost populations of *R. hipposideros* s.str. (West Turkestan, Afghanistan, Kashmir) remains unresolved and has to be investigated more elaborately and using a more extensive sample set.

Key words: molecular analysis, taxonomy, *Rhinolophus*, morphometrics, echolocation data

Introduction

The *Rhinolophus hipposideros* group is one of the numerous groups that divide the genus *Rhinolophus* Lacépède, 1799, the only genus of the chiropteran family Rhinolophidae. The group currently contains a single species, the lesser horseshoe bat, *Rhinolophus hipposideros* (André, 1797). Originally, it was defined by Andersen (1905) as the *Rhinolophus midas* group, comprising two species, *R. hipposideros* and *R. midas* Andersen, 1905. This definition of the group was based on a typical structure of the sella of the nose-leaf, bearing a very low and rounded off posterior connecting process, and an extremely narrow basioccipital bone of the skull, reported to be distinct in both characters from other groups of the genus *Rhinolophus*. Since Andersen (1918) joined the two species into one under the prior name *R. hipposideros*, this name was also transferred to the group name. The *R. hipposideros* group was then reported as a separate and monotypic unit within the genus by numerous followers,

despite the variable numbers and contents of other groups considered (Allen, 1939; Ellerman and Morrison-Scott, 1951; Koopman, 1994; Horáček *et al*., 2000; Csorba *et al*., 2003; Simmons, 2005; Burgin, 2019; etc.).

Besides Andersen's (1905) original definition made on the simple comparison of a few morphological characters, justification of the determination of the *R. hipposideros* group within the genus *Rhinolophus* was supported by the results of additional analyses of morphometric data by Bogdanowicz (1992) and genetic data by Guillén Servent *et al*. (2003), Stoffberg *et al*. (2010), Foley *et al*. (2015), and Dool *et al*. (2016). The basal and very separate position of this group within the genus *Rhinolophus* was stressed by Guillén Servent *et al*. (2003), who suggested delimiting it into the subgenus *Phyllorhina* Leach, 1816.

Because the group currently consists of a single species, *R. hipposideros*, its intraspecific variation also represents the only variation detectable in the group. This bat is a typical faunal element of the western Palaearctic (Fig. 1), where it occurs in a broad belt of the Mediterranean and temperate zones of Europe, North Africa, and western Asia (Csorba *et al*., 2003; Gaisler, 2013; Burgin, 2019; Bendjeddou *et al*., 2022); its distribution range comprises the Mediterranean Maghreb (Morocco to Tripolitania), southern, western and central Europe (from Portugal, Ireland, and Germany to western and southern Ukraine, as well as the Balkans), numerous Mediterranean islands; the Levant, including Sinai; Anatolia; Crimea; the Caucasus region; Iran; Afghanistan; Kashmir; and West Turkestan. Moreover, *R. hipposideros* also marginally extends to the Afrotropics; it occurs in south-western Arabia, Eritrea, Djibouti, Ethiopia, and Sudan (Fig. 1). Within this broad range, the bat is considered a polytypic species; up to seven subspecies have been defined and recognised (Andersen, 1918; Ellerman and Morrison-Scott, 1951; Koopman, 1994). Although several attempts to analyse the intraspecific structure of *R. hipposideros* have been made, this issue is still considered unresolved (see Burgin, 2019).

Based on the body size, structure of the infraorbital region of the skull, and the presence and

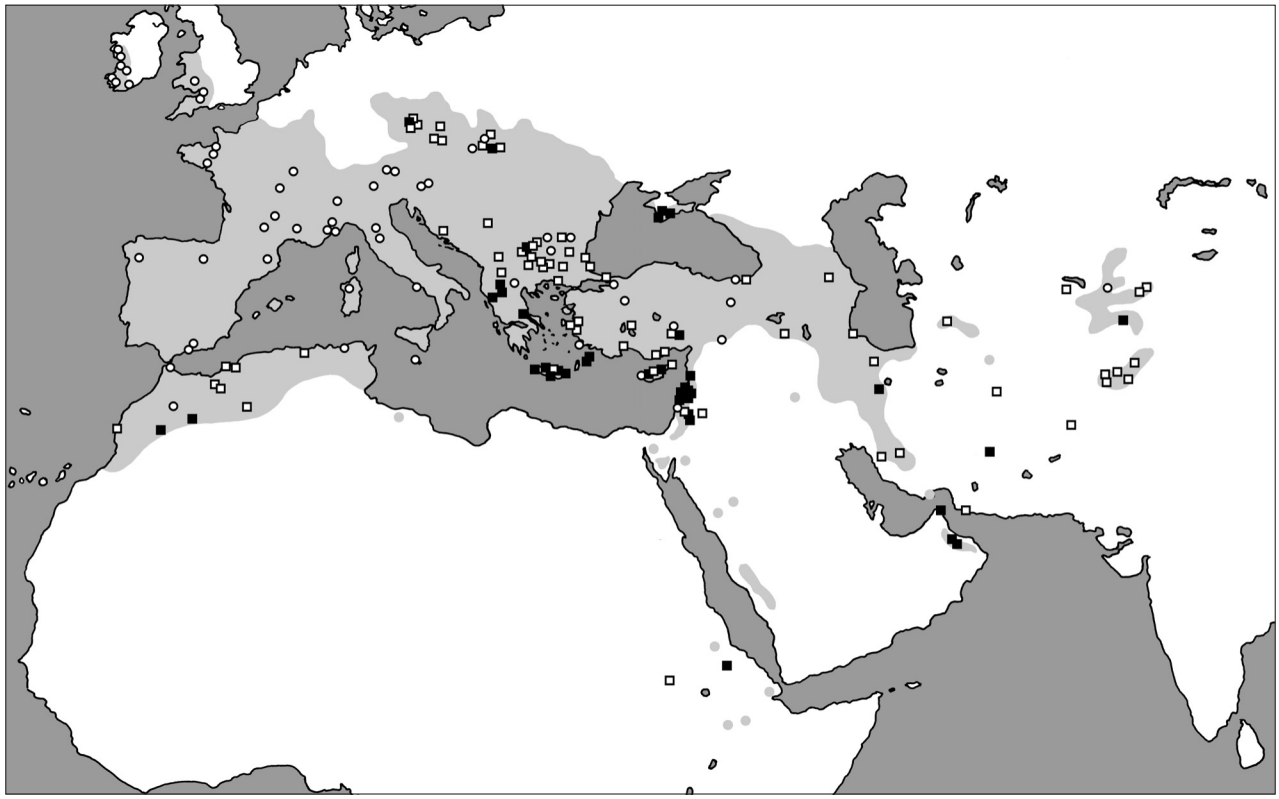

FIG. 1. Map of the distribution range of *Rhinolophus hipposideros* group (pale grey; after numerous sources) and the localities of origin and grouping of the examined samples (some symbols can denote more sites); full squares indicate the samples examined in both molecular genetic and morphological comparisons, open squares indicate the samples examined in the morphological comparison only, and open circles the samples used in molecular genetic analysis only. Grey dots denote isolated records out of the known distribution range

position of the small lower premolars, Andersen (1905, 1907, 1918) defined six subspecies in *R. hipposideros*, and these taxa have been listed as tentatively valid by various authors until present (see Ellerman and Morrison-Scott, 1951; Koopman, 1994; Horáček *et al*., 2000; Roer and Schober, 2001; Csorba *et al*., 2003; Simmons, 2005; Burgin, 2019). Csorba *et al*. (2003) and Burgin (2019) defined the distribution ranges of these subspecies as follows: *R. h. hipposideros* (André, 1797) [type locality (t.l.) Germany] in continental Europe north of the Alps, from the Netherlands to southern Ukraine; *R. h. minutus* (Montagu, 1808) [t.l. Wiltshire, England] in western Ireland and south-western Great Britain; *R. h. minimus* von Heuglin, 1861 [t.l. Kérén in den Bogosländern (= western Eritrea)] in Mediterranean Europe from Portugal to the Levant, including Sinai and the Mediterranean islands, and in western Arabia, southern Sudan, Eritrea, Djibouti, and central Ethiopia; *R. h. midas* Andersen, 1905 [t.l. Jask, Persian Gulf (southern Iran)] in western Asia, from the Caucasus, Transcaucasia, and northern Iraq, to southern Kazakhstan, western Kirghizstan, and Kashmir; *R. h. majori* Andersen, 1918 [t.l. Patrimonia, northern Corsica] in Corsica; and *R. h. escalerae* Andersen, 1918 [t.l. Mogador (= Essaouira), Ha-ha, Morocco] in the Mediterranean zone of north-western Africa.

With the exception of the latter two names, all of the above-mentioned forms were originally described as separate species that were, however, soon synonymised with *R. hipposideros* (Blasius, 1857; Peters, 1871; Dobson, 1876; Trouessart, 1879; Andersen 1904, 1918). Moreover, several authors have demonstrated the morphological inadequacies of these numerous subspecies and showed them to be difficult to identify, because the particular characters exhibit a variable occurrence in particular populations of the species (Miller, 1912; Grulich, 1949; Panouse, 1951; Saint Girons and Caubère, 1966; Felten *et al*., 1977; Palmeirim, 1990). Although such variability led to the description of additional taxa, both at the species and subspecies levels (currently invalid), see e.g., *R. h. alpinus* Koch, 1865 [t.l. the Alps], *R. phasma* Cabrera, 1904 [t.l. Madrid, Spain], *R. h. vespa* Laurent, 1937 [t.l. Korifla, Morocco], or *R. moravicus* Kostroň, 1943 [t.l. Ponikev and Kadeřín, Moravia (= Czech Republic)], the mosaic-like occurrence of traditional identification characters resulted in the abandonment of taxonomic division at small geographic scales (Corbet, 1978). The variability in various morphological characters was thus interpreted as an individual variation influenced by local environmental conditions rather than a result of phylogenetic separation (Saint Girons and Caubère, 1966; Palmeirim, 1990; Salinas-Ramos *et al*., 2021).

Felten *et al*. (1977) proposed the only revision of intraspecific taxonomy in *R. hipposideros*. Using an evaluation of the characters suggested by Andersen (1905, 1918) — body size, shape of rostrum, and size and position of premolars — Felten *et al*. (1977) delimited four population groups in the species and tentatively identified with the subspecies: *R. h. hipposideros* in Europe (including Corsica) and the Levant, *R. h. minimus* in north-eastern Africa and Crete, *R. h. midas* in the Middle East from north-eastern Turkey to Afghanistan, and an unnamed form in the islands of the central Mediterranean (Sicily, Pantelleria) and in western Turkey [Felten *et al*. (1977) did not evaluate some populations, e.g., those of North Africa, British Isles, or West Turkestan]. This geographic division of morphotypes in *R. hipposideros* was revised only to a small extent and only to certain populations; the examined specimens of the Middle Eastern populations were found to fit the morphotypes defined by Felten *et al*. (1977) — see Benda *et al*. (2006) and Benda and Gaisler (2015). However, bats from Crete did not fit the morphological criteria that Felten *et al*. (1977) gave for *R. h. minimus* when a large set of samples was examined (Benda *et al*., 2009). In general, subsequent authors did not follow the conclusions that Felten *et al*. (1977) suggested regarding intraspecific relationships in *R. hipposideros* and their taxonomic arrangement.

Another type of evidence of the geographic variability in *R. hipposideros* was found and widely documented in karyotype (Zima *et al*., 1992; Zima, 2004; Volleth *et al*., 2013; Arslan and Zima, 2014; Kacprzyk *et al*., 2016); three chromosome races were described in *R. hipposideros*, (1) the populations of 2n = 54 from Ireland, Spain, Germany, and Switzerland; (2) 2n = 56 from Italy, Greece, Bulgaria, Czech Republic, and Slovakia; and (3) 2n = 58 from Jordan, Syria, Turkey, and Iran. These chromosome races thus seem to be geographically well-defined forms, with one living in the western part of Europe, another in the eastern part of Europe, and a third in the Middle East.

Molecular genetic analyses focused on intraspecific variation in *R. hipposideros* (Kůs, 2008; Dool *et al*., 2013; Shahabi *et al*., 2019) have shown — in both mitochondrial and nuclear markers — a split of the species into two main lineages, the western one comprising the Maghrebian and European

populations (Maghreb, British Isles, central and southern Europe, Sardinia, Malta, and Crete) and the eastern one covering the Asian populations (Turkey, Cyprus, Levant, Iran, Tajikistan).

Burgin (2019) recently summarised the main message of the review presented above, although he did not propose a taxonomic synthesis revising the old intraspecific arrangement of *R. hipposideros* (suggested already by Andersen, 1905). However, the available data suggest this arrangement is untenable and the bat's intraspecific relationships need a profound revision. Thus, to identify the phylogenetic pattern in *R. hipposideros*, we carried out a morphological examination of a set of more than 270 museum specimens with the aim of defining the positions of particular populations from its whole distribution range. Simultaneously, we subjected a geographically representative subset of these specimens to a molecular genetic comparison. In addition, we compared the echolocation data from various parts of the species range. Results of these approaches are presented here, and we propose a revised view of the systematic relationships within the *R. hipposideros* group, including its taxonomic interpretation.

Nomeclatural Note

Although *R. hipposideros* ranks among the most common and most frequently mentioned bats of Europe and the western Palaearctic as well, the author and year of description of this species was confused for a long time. For almost 150 years, the creation of this name was attributed to J. M. Bechstein; initially to Bechstein (1801) (see e.g., Blasius, 1857; Kolenati, 1860; Koch, 1865; Peters, 1871; Dobson, 1876; Trouessart, 1879; Méhely, 1900; Cabrera, 1904), later to Bechstein (1800 [= 1799; see Benda and Mlíkovský, 2022]) (see e.g., Andersen, 1905; Miller, 1912; Ellerman and Morrison-Scott, 1951; Lay, 1967; Corbet, 1978; DeBlase, 1980; Qumsiyeh, 1985; Harrison and Bates, 1991; Horáček *et al*., 2000; Simmons, 2005; etc.). It was only recently that Tupinier (2001) and Kožurina (2006) pointed out that an older mention of this bat name was published by Borkhausen (1797), and the nomenclatural authority of this author over *R. hipposideros* has been nowadays accepted by numerous authors (see e.g. Benda *et al*., 2008, 2009, 2010, 2012, 2016; Kruskop, 2012; Lino *et al*., 2014; Benda and Gaisler, 2015; Downs *et al*., 2016; Burgin, 2019; Bendjeddou *et al*., 2022). However, Benda and Mlíkovský (2022) demonstrated that Borkhausen (1797) was not the oldest publication of the name *hipposideros*, while the available evidence shows that André (1797) published it earlier than Borkhausen (1797). Because the official publication dates for the purposes of zoological nomenclature are 19 April 1797 for André (1797), and 30 September 1797 for Borkhausen (1797), the former work takes priority over the latter and the author of the name *hipposideros* is André (1797).

Materials and Methods

Molecular Genetic Analysis

Sampling, amplification, and sequencing

In the molecular genetic analysis, we used muscle tissue samples of 92 specimens of *R. hipposideros* from the collection of the National Museum, Prague, Czech Republic (NMP) to extract DNA (Fig. 1 and Supplementary Table S1A). The genomic DNA was extracted from the alcohol-preserved tissue samples using Geneaid Genomic DNA Mini Kit. We targeted one mitochondrial marker (mtDNA), including 1,128 bp of the cytochrome *b* gene (*Cyt-b*) and five nuclear markers (nDNA), consisting of 536 bp of acyl-coenzyme A oxidase 2 intron (*ACOX*), 616 bp of biglycan intron (*BGN*), 741 bp of COP9 signalsom subunit 7A intron (*COPS*), 480 bp of the rogdi atypical leucine zipper (*ROGDI*), and 521 bp of the signal transducer and activator of transcription 5A intron (*STAT*). We sequenced both strands for all sequences. We used primers that have been specifically designed for the order Chiroptera and provided good amplification in previous studies (see, e.g., Puechmaille *et al*., 2011; Salicini *et al*., 2011; Thong *et al*., 2012; Dool *et al*., 2016).

We supplemented this dataset with 155 *Cyt-b* sequences from previous studies (Ibáñez *et al*., 2006; Li *et al*., 2006; García-Mudarra *et al*., 2009; Çoraman *et al*., 2013; Dool *et al*., 2013, 2016). As a multiple outgroup, we added 38 GenBank sequences of 28 other *Rhinolophus* species (Dool *et al*., 2016; Taylor *et al*., 2018) and sequences of three *Hipposideros* species from the sister family Hipposideridae (for details see Supplementary Table S1A). The largest possible set of shorter sequences of the *Cyt-b* gene of *R. hipposideros* (Supplementary Table S1B) from GenBank was used for the test of geographic grouping of particular mtDNA haplotypes. For the primer names, their sequences, and annealing temperatures, see Supplementary Table S2. The PCR products were Sanger-sequenced from both sides using the PCR primers by Macrogen, Inc. (Amsterdam, the Netherlands).

Phylogenetic reconstruction

Sequences were edited and aligned using the MAFFT plug-in (Katoh and Standley, 2013) in Geneious 11.0.5 (https://www.geneious.com), subsequently manually edited and trimmed using Gblocks (Castresana, 2000). Heterozygous positions in the nDNA markers were coded with IUPAC codes and ambiguous positions or missing data were coded with 'N'. Indels were treated as gaps. Sequences of protein-coding markers were translated to amino acids to check for the presence of stop codons, which would indicate that pseudogenes have been amplified. Alleles of nuclear markers were estimated using PHASE

(Flot, 2010) with the probability threshold set to 0.7. The two final multilocus datasets were made according to the mode of inheritance of the markers, mitochondrial and nuclear datasets. The mitochondrial dataset contained *Cyt-b* sequences of a total length of 1,128 bp. The nuclear dataset contained *ACOX*, *BGN*, *COPS*, *ROGDI*, and *STAT* sequences of a total length of 2,894 bp. The latter dataset was partitioned by gene.

Phylogenetic analyses of both datasets were run using Bayesian inference (BI) and maximum likelihood (ML). The appropriate nucleotide substitution model for each partition was selected based on the Bayesian information criterion (BIC) ModelFinder (Supplementary Table S3 — Kalyaanamoorthy *et al*., 2017). We used MrBayes v3.2.6 (Ronquist and Huelsenbeck, 2003) to run the BI analysis. Appropriate substitution models were specified for each partition and all parameters were unlinked across partitions. We ran two independent runs for 20 million generations with trees sampled every 1,000 generations. All other parameters were set to default. Stationarity and convergence of the runs were inspected in Tracer v1.6 (Rambaut *et al*., 2014) and the values of the average standard deviations of the split frequencies were lower than 0.01. The burn-in fraction was left as the default at 25% of sampled trees. Thus, from the 20,000 produced trees, 5,000 were discarded. A majority-rule consensus tree was produced from the post-burnin trees with posterior probability (*PP*) values embedded. The BI analyses were run through CIPRES Science Gateway (Miller *et al*., 2010). Then, we inferred the maximum-likelihood tree using the partition model in IQ-TREE (Nguyen *et al*., 2015; Chernomor *et al*., 2016). Searching for the best-scoring ML was performed by ultrafast bootstrap (UFBoot — Hoang *et al*., 2018) with 1,000 bootstrap and 1,000 topology replicates. To verify robustness of the ML tree the branch supports were evaluated using SH-like approximate likelihood ratio test (SH-aLRT — Guindon *et al*., 2010) and a Bayesian-like transformation of aLRT (aBayes — Anisimova *et al*., 2011). SH-aLRT was performed with 1,000 replications. aBayes branch support was used instead Bayesian posterior probabilities because aBayes is more conservative, more robust to model violation and moreover exhibits the more confident resolution (Anisimova *et al*., 2011). The ML, SH-aLRT and aBayes analysis were run on IQtree web server (Trifinopoulos *et al*., 2016). To see whether the single nuclear markers show the same or different topology we prepared the phylogenetic trees for each nuclear marker.

Species delimitation and divergence time estimation

For the species delimitation and molecular dating analyses, we used only pruned nuclear dataset employed in phylogenetic analyses constituted from phased sequences of *ACOX*, *BGN*, *COPS* and *STAT*. For *R. hipposideros*, we used sequences of only two individuals, one from each diverged lineage (see below) from Cyprus and Oman. Furthermore, the data set was truncated by species represented by less than three markers, and therefore the sequences of *R. landeri* and *R. pearsonii* were omitted.

The species delimitation was conducted by Bayesian phylogenetics and phylogeography (BPP v3; Rannala and Yang, 2003; Yang and Rannala, 2010). This analysis was carried out to evaluate the phylogenetic species boundaries. The species tree topology, which was reconstructed using only nuclear loci (see above), was used as a fixed guide tree (algorithm A10 — Rannala and Yang, 2003; Yang and Rannala, 2010). We replicated twice the runs for each of four different combinations of priors on divergence depth and effective population sizes (τ and θ, respectively — see Table 1 in Demos *et al*., 2019), as the probability of delimitation by BPP is sensitive to these two parameters (Leaché and Fujita, 2010; Yang and Rannala, 2010). Each replicate was conducted with either the reversible-jump Markov chain Monte Carlo algorithm 0 (with parameter e = 1) or 1 (with parameters a = 2, m = 1 — Yang and Rannala, 2010). All eight BPP analyses were then run with the default settings. Lineages were considered statistically supported when the generated delimitation posterior probabilities (*PP*) exceeded 0.95 under all four prior combinations.

The divergence time estimation was set up in BEAUti and run in BEAST v1.8.4. We followed the settings from Dool *et al*. (2016) and used strict molecular clocks and Yule speciation process (Yule, 1925; Gernhard, 2008) for all genes. The substitution model was taken from phylogenetic reconstructions (see above). As a calibration point, we employed the age of the root of the family Rhinolophidae which was estimated at 37 Ma (Stoffberg *et al*., 2010). For an alternative divergence time reconstruction, we also used a family root age of 16.92 Ma (Foley *et al*., 2015). We used a lognormal prior distribution for this calibration point. BEAST was run three times for 20 million generations and parameters and trees were saved every 1,000 generation. Tracer v1.6 was used to confirm adequate mixing of the MCMC chains and acceptable effective sample sizes (ESS > 200). LogCombiner was used for burn-in (25%) and merging trees files, TreeAnnotator was used for identifying the maximum clade credibility tree. All analyses were run through CIPRES Science Gateway (Miller *et al*., 2010).

Uncorrected *p*-distances between haplotypes were calculated for the *Cyt-b* in MEGA11 (Tamura *et al*., 2021). The bootstrap was performed with 1,000 replications.

Morphometric Comparison

For the comparative morphometric analysis and for the description of morphological trends in particular populations, we used cranial and dental measurements and the forearm length (LAt) as a standardised dimension referring to the body size. The skulls and teeth were measured using mechanical and optical callipers with accuracy to 0.02 mm and 0.01 mm, respectively; horizontal dental dimensions were taken on cingulum margins of teeth. The examined museum materials are given in Appendix I (see also Fig. 1). We evaluated 18 cranial and 19 dental dimensions (i.e., plain dimensions) in each skull (see the measurements taken below); the skull and tooth shapes were described with the help of relative dimensions (indices) calculated from the plain dimensions; nine cranial and 17 dental indices were used (see Supplementary Tables S5 and S6). In accordance with Felten's *et al*. (1977) findings, sexual dimorphism was not considered in the morphometric comparisons.

For the statistical evaluation and definition of trends in morphological characters, the examined museum specimens were grouped into six sample sets, with respect to the geographic origin of the samples and to the geographic separation of lineages shown by the molecular genetic analysis that preceded the morphological comparison. The compared sample sets were defined as follows (see Tables 2 and 3): Central Europe (CEU) — 55 samples from the Czech Republic and Slovakia; West Mediterranean (WMT) — 106 samples from Morocco, Algeria, Croatia, Serbia, Albania, Kosovo, North Macedonia, Bulgaria, and Greece (including Crete); East Mediterranean (EMT) — 83 samples from Syria, Crimea (Ukraine), Rhodes (Greece), Cyprus, Lebanon, Jordan, Turkey; Central Asia (CAS) — 25

samples from Iran, Azerbaijan, Turkmenistan, Uzbekistan, Kirghizstan, Tajikistan, and Afghanistan; Oman (OMA) — four samples from north-eastern Oman; north-eastern Africa (NEA) — two samples from Ethiopia and Sudan. Two type specimens examined (*R. midas* Andersen, 1905 and *R. h. escalerae* Andersen, 1918) were evaluated separately off the sets to avoid affecting the statistical results.

Statistical analyses were performed using Statistica 6.0 software. In the cluster analysis, the unweighted pair group method with arithmetic mean was employed (UPGMA; Euclidean distances); the analysis was used to calculate differences between the mean values of morphometric traits among the particular sets of samples, and it was employed separately for 27 plain and relative dimensions of the skull and for 36 plain and relative dimensions of the teeth, respectively. Stepwise discriminant function analysis was performed as a test of importance of particular dimensions and their indices for geographic variation; statistically significant parameters most affecting morphological variation were selected and employed in a subsequent canonical analysis that was used to test grouping or separation of population sample sets of similar or different morphotypes, respectively. Statistical significance of differences in skull measurements between groups were assessed using ANOVA (one-way analysis of variance).

The following measurements were taken: (1) External dimension — LAt = forearm length; (2) Cranial dimensions — LCr = greatest length of skull incl. praemaxillae; LOc = occipitocanine length; LCc = condylocanine length; LaZ = zygomatic width; LaI = width of interorbital constriction; LaInf = rostral width between infraorbital foramens; LaNc = neurocranium width; LaM = mastoidal width of skull; ANc = neurocranium height; LBT = largest horizontal length of tympanic bulla; CC = rostral width between canines (incl.); M^3M^3 = rostral width between third upper molars (incl.); CM^3 = length of upper tooth-row between canine and third molar (incl.); LMd = condylar length of mandible; ACo = height of coronoid process; CM_3 = length of lower tooth-row between canine and third molar (incl.); (3) Dental dimensions, upper dentition — M^1M^3 = length of tooth-row between first and third molars (incl.); LCs = largest mesio-distal length of canine; LaCs = largest palato-labial width of canine; LP^2 = largest mesio-distal length of first premolar; LaP^2 = largest labio-palatal width of first premolar; LP^41 = largest mesio-distal length of large premolar on the labial cingulum; LP^42 = smallest mesio-distal length of large premolar taken over the talon constriction; LP^43 = mesio-distal length of large premolar on palatal cingulum (largest dimension taken over the palato-mesial to palato-distal points of the talon); LaP^4 = largest palato-labial width of large premolar taken over the mesio-labial and palato-distal cingulum margins; LM^1 = largest mesio-distal length of first molar taken over parastyle and metastyle; LaM^1 = largest palato-labial width of first molar taken over parastyle and palato-distal part of talon; LM^3 = largest mesio-distal length of third molar; LaM^3 = largest palato-labial width of third molar (taken over parastyle and palatal cingulum); (4) Dental dimensions, lower dentition — M_1M_3 = length of tooth-row between first and third molars (incl.); LCi = largest mesio-distal length of canine; LP_2 = largest mesio-distal length of first premolar; LaP_2 = largest labio-lingual width of first premolar; LP_3 = largest mesio-distal length of second (small) premolar; LP_4 = largest mesio-distal length of last premolar; LaP_4 = largest labio-lingual width of last premolar; LMi = largest mesio-distal length of first molar taken over paraconid and hypoconulid. Other abbreviations included: *n* = number of samples; $\bar{x}$ = mean; min, max = range margins; SD = standard deviation.

Echolocation Call Recordings and Analysis

In the *Rhinolophus* bats, the constant frequency component represents a dominant part of the echolocation call in the search phase. This characteristic has maximum energy and thus makes it acceptable to analyse calls from hand-held and flying bats, while avoiding pseudoreplication during the recording of flying bats, respectively. For the echolocation call analysis in the *R. hipposideros* group, we made the acoustic recordings using a portable ultrasound detector D-240x (Pettersson Elektronik AB, Uppsala, Sweden) set on time-expansion mode connected to Edirol R-09HR recorder (Roland Corp., Japan) and an ultrasound detector Batlogger M (Elekon AG, Switzerland). The analysed bat calls were recorded in free flight under natural conditions, usually near the sites where the bats were also mist-netted. Additionally, some echolocation call sequences were recorded when handling the bat in a resting position or hand-releasing the bat.

The recordings were analysed with BatExplorer 2.1.7.0 software (Elekon AG, Switzerland) to evaluate oscillograms, power spectra, and spectrograms. For each echolocation call, the following parameters were measured: pulse duration (PDUR), start frequency (SF), end frequency (EF), frequency of maximum energy (F_{MAXE}) and inter-pulse interval (IPI, the time between two consecutive calls). In most cases, we used only high-quality recordings for analyses, in which all or most of the basic characters were measurable, and only the search phase calls were measured.

For comparison of the geographic variability, mostly published data were used (see Table 5). Original data were obtained from Slovakia, Tajikistan, Saudi Arabia, and Oman; the calls were recorded at the following sites: at the Aksamitka Cave, Slovakia (49°23'N, 20°27'E), 31 August 2015, several individuals, rec. M. Cel'uch; at a small cave near Zingrogh, Tajikistan (38°27'N, 70°49'E), 12 May 2016, one ind., rec. M. Uhrin; at the Umm Jirsan Cave, Saudi Arabia (25°35'N, 39°45'E), 26 October 2022, several inds., rec. M. Uhrin; at a water reservoir near Al Khutaymi, Oman (23°06'N, 57°33'E), 27 March 2011, one ind., rec. M. Uhrin; in Wadi Qatam, Oman (23°05'N, 57°38'E), 31 October 2019, several inds., rec. P. Benda; in a small oasis near Misfah, Oman (23°14'N, 57°08'E), 9 April 2011, one ind., rec. M. Uhrin; and at a pool near Tayma, Oman (22°31'N, 59°20'E), 3 April 2011, one ind., rec. M. Uhrin.

Results

Molecular Genetic Analysis

The resulting *Cyt-b* dataset comprised 81 sequences which were pruned to 54 unique haplotypes. The nuclear dataset comprised 47 *ACOX*, 63 *BGN*, 44 *COPS*, 11 *ROGDI*, and 47 *STAT* sequences that were pruned to 46 haplotypes. For other *Rhinolophus* species, we added 129 sequences from GenBank in total. The *Cyt-b* sequences contained 403 parsimony informative positions (35.73% of total length) and this marker showed a much larger

genetic differentiation within *Rhinolophus* species than the nuclear markers (due to the faster mutation rate). The amount of parsimony informative positions in concatenated nuclear dataset was 386, i.e. 13.33% of its total length (for substitution models of mitochondrial and nuclear trees see Supplementary Table S3).

The ML and BI tree of the nuclear dataset showed slightly different topologies, nonetheless, the different nodes had a low branch support. We showed the ML tree (Fig. 2). The genus *Rhinolophus* was divided into four well supported clades. *Rhinolophus hipposideros* formed a separate clade, however, its exact position remained unclear due to the low branch support of deep nodes. Other groups were: (1) *pusillus* group including the species *R. shameli*, *R. pearsonii*, and *R. pusillus*; (2) *trifoliatus* group including *R. trifoliatus* and *R. luctus*; and (3) Afro-Palaearctic clade that includes the species groups *euryale*, *fumigatus*, *ferrumequinum*, *capensis*, and *landeri*.

The phylogenetic trees obtained by both ML and BI analyses of the *Cyt-b* dataset showed slightly different topologies. The ML tree was fully resolved and had a higher branch support than the BI tree, therefore we showed the ML tree (Fig. 2). The tree

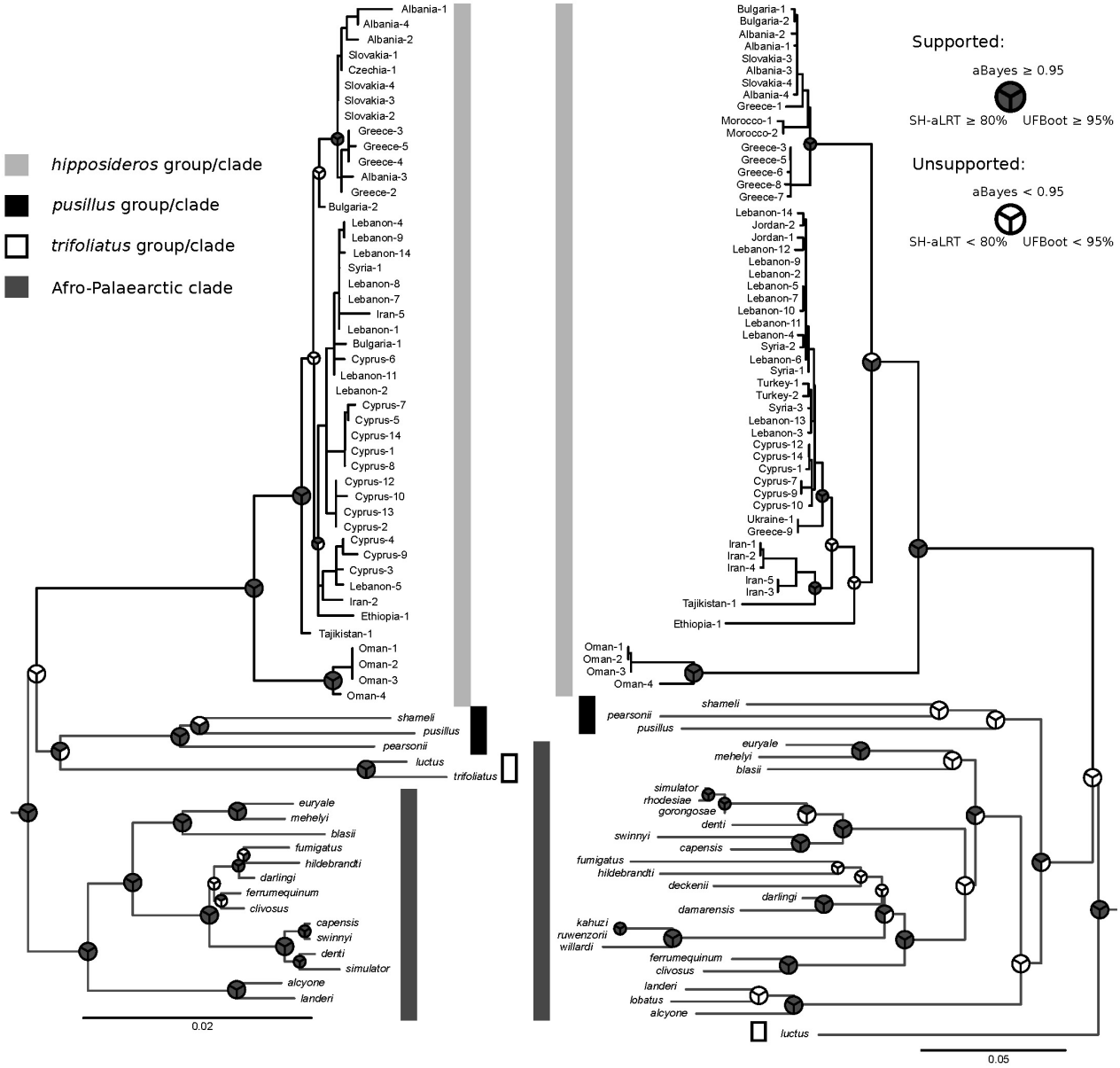

FIG. 2. Maximum likelihood tree of reconstructed phylogenetic relationships of the *Rhinolophus hipposideros* group with selected species of the families Rhinolophidae and Hipposideridae based on the nuclear (left) and mitochondrial (right) datasets, respectively. Branch support values are shown by pie charts on the nodes

topology of the genus *Rhinolophus* was different than the topology of the nuclear tree. Within *Rhinolophus*, *R. hipposideros* formed a separate branch with an uncertain position. Another separate branch led to the *trifoliatus* group including only *R. luctus*. The rest of all *Rhinolophus* species formed a third branch within the genus tree supported by SH-aLRT and aBayes. This clade comprised African, European, and Asian species including the supported species groups *ferrumequinum*, *fumigatus*, *maclaudi*, *capensis*, and *landeri*. Nevertheless, the relationships among these groups and other ungrouped species were not satisfactorily resolved and neither were the relationships between three major clades.

Intraspecifically, *R. hipposideros* split into two lineages in both nuclear and mitochondrial trees. The first lineage ranged from Morocco and Ireland through Central Europe, the Balkans, Levant, and Iran to Tajikistan and Ethiopia; the second lineage comprised samples from north-eastern Oman. In the nuclear tree, the first lineage was not internally branched, and therefore the lineage was genetically uniform through almost the whole *R. hipposideros* range, except Oman which formed the second lineage. In the *Cyt-b* tree, the first lineage was formed by samples from the majority of the *R. hipposideros* distribution range and was further divided into four well-supported sublineages: (1) Afro-European, comprising samples from the Maghreb (Morocco), Central Europe (Slovakia), and the Balkans (Albania, Bulgaria, Greece); (2) Ponto-Levantine, with the samples from Crimea, Rhodes, and the Levant (Cyprus, Syria, Lebanon, Jordan); (3) Central Asian, with the samples from Iran and Tajikistan; and (4) Ethiopian, which includes a single sample from Ethiopia. The relationships among the sublineages were not resolved due to the low branch support. The uncorrected *p*-distances on *Cyt-b* between the two lineages were 8.93–10.75% and between the sublineages 2.30–7.02% (Table 1). The resolution of the nuclear gene trees was in accordance with the genetic variation of each nuclear marker (Supplementary Figs. S2–S6). However, the basic split of *R. hipposideros* into two lineages was evident in all gene trees for which we obtained sequences from both lineages.

For the *R. hipposideros* only mitochondrial tree (Supplementary Fig. S1), we added 155 *Cyt-b* sequences from GenBank to make a dataset of 213 sequences with the total length of 1,103 bp (Supplementary Table S1B). In this tree, five basic lineages in *R. hipposideros* were recovered. It corresponded to the topology with the above division based on the whole *Cyt-b* gene, and it covered almost a complete distribution range of the species; viz. (1) the Afro-European lineage, comprising sequences from the Maghreb (Morocco, Tunisia), Mediterranean Europe (Spain, Italy, France, Slovenia, Albania, Bulgaria, Greece, European Turkey), Mediterranean islands (Malta, Crete), British Isles (Ireland, Great Britain), and Central Europe (Austria, Slovakia, Romania); (2) Ponto-Levantine lineage, composed of the sequences from the Levant (Rhodes, western Anatolia, Cyprus, Syria, Lebanon, Israel, Jordan) and Crimea; (3) Eastern lineage comprising the sequences from the eastern part of the Middle East (eastern Anatolia, Iran) and West Turkestan (Tajikistan); (4) the Ethiopian lineage comprising one sequence from northern Ethiopia; and the last and most distant (5) Omani lineage from the sequences from north-eastern Oman. All five lineages had a high branch support (0.99–1.00 posterior probability [*PP*] and 97–100 bootstrap percentage [BP]). However, the relationships between the lineages did not always show high support, only the sister position of lineages 2 and 3 had marginal to moderate high support (0.82 *PP* and 93 BP), and the ML analysis supported the crown position of lineages 1–4 (98 BP). The uncorrected *p*-distances within lineages were 0–3.42%, between sublineages 2.30–7.02%. The Omani lineage differed from other lineages with the distances of 8.93–11.40%.

TABLE 1. Percentage values of uncorrected genetic *p*-distances of *Cyt-b* among mitochondrial subgroups (lineage/sublineage) of the *Rhinolophus hipposideros* group (below the diagonal). The diagonal corresponds to the within-group genetic divergence estimated for *Cyt-b* in each subgroup

Geographic unit	Europe and Maghreb	Levant and Crimea	Ethiopia	Iran	Tajikistan	Oman
Europe and Maghreb	0.00–1.70					
Levant and Crimea	3.02–3.59	0.00–1.61				
Ethiopia	5.79–6.33	5.83–6.20	x			
Iran	3.55–4.87	2.30–3.23	5.53–6.19	0.00–1.71		
Tajikistan	4.46–5.11	3.56–3.61	7.02	3.39–3.58	x	
Oman	9.25–9.72	8.93–9.94	10.34–10.66	9.09–9.77	10.07–10.75	0.00–3.12

Our results of the Bayesian phylogenetics and phylogeography (BPP) analyses demonstrated the delimitation probabilities of the replicated runs being affected by the prior choice of parameters. It was especially apparent when a large effective population size was chosen in our pruned dataset (Supplementary Table S4). Nevertheless, all the results for *R. hipposideros* and its populations had $PP \geq 0.95$. It means that two clades, one from Oman and another from the rest of the distribution range, were strongly delimited within this lineage.

The topology of the calibrated tree (Fig. 3) showed the same four clades of the genus *Rhinolophus* as displayed by the topology of the nuclear ML/BI tree, however, their positions differed. The basal split occurred 37.8 Ma (95% highest posterior density [HPD]: 37.1–39.0 Ma) and divided the *trifoliatus* group from the rest of *Rhinolophus* species. A second split took place 32.0 Ma (95% HPD: 27.4–36.6 Ma) between the Indomalayan group and the Afro-Palaearctic group including *R. hipposideros*. Finally, the Afro-Palaearctic group diverged from *R. hipposideros* 29.4 Ma (95% HPD: 24.8–34.4 Ma). In the tree, all the nodes were statistically supported except three: between the Indomalayan group and the Afro-Palaearctic group including *R. hipposideros*; between the groups *euryale* and *landeri* (including only *R. alcyone*); and between *R. fumigatus* and *R. hildebrandtii*. In the *R. hipposideros* clade, two lineages used in our study split 7.1 Ma (95% HPD: 4.3–10.0 Ma). For the reconstruction based on a younger root calibration see Supplementary Fig. S7. The topology of both reconstructions remained identical, however, the splits of each group estimated in the alternative reconstruction occurred much later (16.7 Ma [16.1–17.4 Ma], 14.3 Ma [12.3–16.4 Ma], 13.2 Ma [11.0–15.3 Ma], and 3.2 Ma [2.0–4.9 Ma], respectively).

Morphometric Comparison

In accordance with the geographic separation of lineages in the examined mitochondrial markers (see above) and the origin of the comparative material (Appendix I), all of examined material of *R. hipposideros* was sorted into six sample sets (Tables 2–4). The comparison of morphometric characters of the population sets documented a remarkable variation in the body, skull, and tooth sizes as well as in the skull and tooth shapes. In most dimensions, both in their absolute and relative values, the dimension ranges in particular sets overlapped with or exceeded the ranges of other sets. However, metric trends in the population sample sets were easily detectable from the comparison of the basic statistical values (Tables 2 and 3).

Regarding body size, two basic groups could be delimited among the examined samples, the large

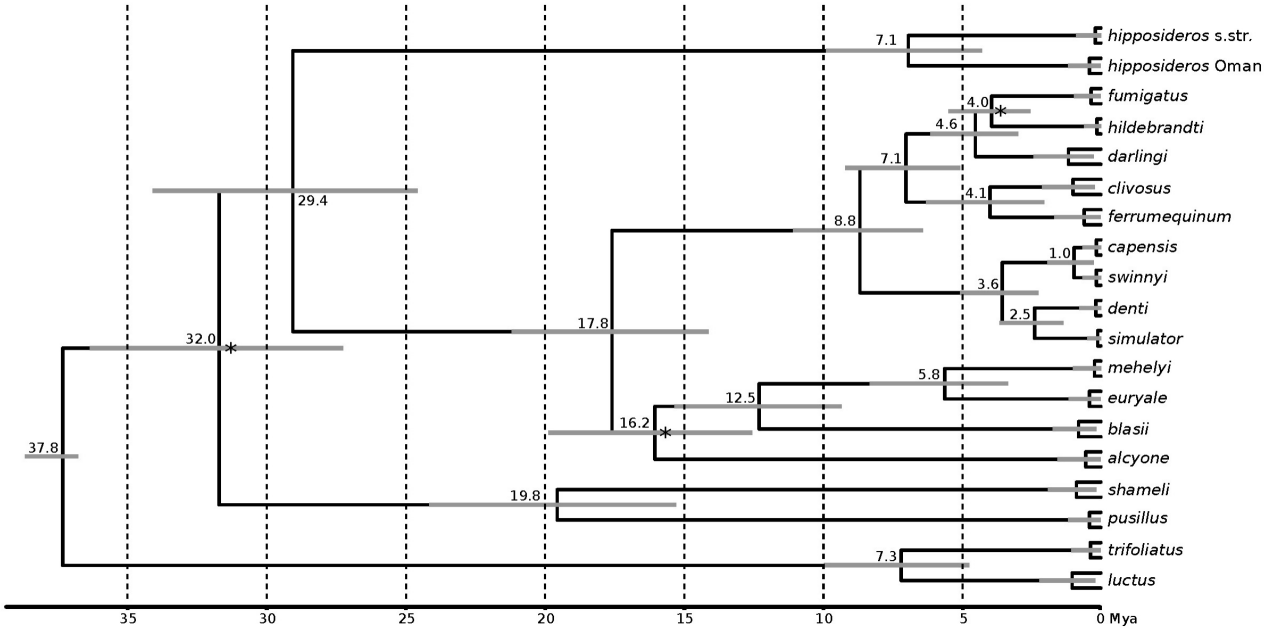

FIG. 3. Chronogram of the family Rhinolophidae based on a Bayesian inference of the nuclear dataset (according to the model by Stoffberg *et al*., 2010). The numbers at nodes show mean divergence time estimates (Ma) and horizontal boxes 95% highest posterior density intervals of these estimates. The asterisk (*) indicates nodes with low branch support, the rest of the nodes were supported ($PP \geq 0.95$)

TABLE 2. External and cranial dimensions of the examined sample sets of the *Rhinolophus hipposideros* group (* — after DeBlase, 1980); *midas*, *escalerae* = dimensions of the respective type specimens; for the sample set delimitations and dimension abbreviations see Materials and Methods. Mean values shown in bold

Character	Central Europe					West Mediterranean					East Mediterranean				
	n	♂	min	max	SD	*n*	♂	min	max	SD	*n*	♂	min	max	SD
LAt	27	**39.42**	37.00	41.30	1.022	91	**37.77**	34.40	40.30	1.333	79	**37.54**	35.20	40.60	1.204
LCr	31	**16.40**	16.08	16.98	0.210	79	**16.01**	15.03	16.76	0.357	48	**15.84**	15.12	16.31	0.270
LOc	51	**15.79**	15.52	16.22	0.184	69	**15.37**	14.75	15.98	0.246	67	**15.10**	14.47	15.84	0.269
LCc	51	**14.14**	13.75	14.58	0.188	93	**13.61**	13.02	14.21	0.264	65	**13.41**	12.82	13.94	0.234
LaZ	51	**7.73**	7.42	8.09	0.153	91	**7.48**	6.65	8.10	0.201	64	**7.33**	6.94	7.81	0.168
LaI	51	**1.71**	1.49	1.97	0.103	100	**1.58**	1.26	2.04	0.131	66	**1.63**	1.24	6.35	0.603
LaInf	51	**3.68**	3.48	3.87	0.085	73	**3.54**	3.28	3.81	0.090	67	**3.50**	3.31	3.68	0.082
LaNc	52	**6.66**	6.38	6.97	0.149	100	**6.56**	6.21	6.89	0.150	66	**6.43**	6.13	7.02	0.147
LaM	51	**7.60**	7.23	7.82	0.125	73	**7.43**	6.98	7.75	0.162	66	**7.27**	6.98	7.49	0.127
ANc	49	**4.79**	4.55	5.10	0.108	93	**4.64**	4.27	4.93	0.117	65	**4.57**	4.23	4.93	0.150
LBT	47	**2.43**	2.13	2.74	0.145	70	**2.38**	2.12	2.69	0.129	51	**2.29**	2.09	2.61	0.118
CC	47	**3.58**	3.42	3.82	0.103	95	**3.40**	2.98	3.88	0.120	63	**3.44**	3.18	3.72	0.119
M^3M^3	52	**5.46**	5.21	5.69	0.105	100	**5.33**	4.93	5.64	0.133	65	**5.25**	4.93	5.49	0.133
CM^3	52	**5.43**	5.23	5.63	0.106	100	**5.30**	4.94	5.53	0.117	66	**5.28**	4.93	5.49	0.121
LMd	52	**10.00**	9.28	10.34	0.204	100	**9.66**	9.05	10.10	0.225	67	**9.50**	9.06	9.87	0.176
ACo	49	**2.04**	1.83	2.21	0.083	100	**1.97**	1.67	2.24	0.116	67	**1.99**	1.75	2.19	0.114
CM_3	52	**5.63**	5.42	5.87	0.108	100	**5.44**	5.11	5.72	0.126	65	**5.44**	5.04	5.74	0.139

	Central Asia					Oman					Ethiopia	*midas*	*escalerae*
LAt	25	**39.37**	36.60	41.00	1.118	4	**37.43**	36.80	38.10	0.556	38.70	37.70*	–
LCr	18	**16.04**	15.64	16.30	0.202	3	**15.91**	15.54	16.35	0.410	16.03	16.31	15.63
LOc	20	**15.35**	14.89	15.94	0.267	3	**15.09**	14.69	15.47	0.391	15.24	–	–
LCc	21	**13.67**	13.25	14.23	0.239	3	**13.43**	13.21	13.64	0.215	13.36	13.96	13.31
LaZ	21	**7.46**	7.17	7.93	0.198	3	**7.26**	7.17	7.33	0.083	7.32	7.36	7.28
LaI	21	**1.59**	1.41	1.77	0.116	3	**1.50**	1.42	1.60	0.092	1.48	1.64	1.58
LaInf	19	**3.61**	3.36	3.92	0.131	3	**3.56**	3.44	3.66	0.111	3.51	3.75	3.57
LaNc	21	**6.46**	5.98	6.82	0.192	3	**6.36**	6.06	6.59	0.273	6.61	6.18	6.66
LaM	20	**7.37**	7.14	7.62	0.116	3	**7.34**	7.13	7.46	0.182	7.42	7.26	7.33
ANc	21	**4.63**	4.38	4.92	0.146	3	**4.42**	4.23	4.59	0.181	4.43	4.34	4.51
LBT	18	**2.38**	2.13	2.69	0.132	3	**3.02**	2.88	3.16	0.140	2.18	2.92	–
CC	18	**3.58**	3.28	3.92	0.166	3	**3.45**	3.38	3.51	0.067	3.25	3.49	3.38
M^3M^3	19	**5.51**	5.21	5.91	0.154	3	**5.30**	5.01	5.46	0.252	5.18	5.58	4.97
CM^3	21	**5.45**	5.22	5.81	0.137	3	**5.44**	5.27	5.57	0.153	5.21	5.58	5.23
LMd	21	**9.81**	9.33	10.22	0.251	3	**9.78**	9.64	9.96	0.164	9.34	10.24	9.58
ACo	21	**1.97**	1.66	2.19	0.124	3	**2.06**	1.89	2.21	0.162	2.03	2.04	2.03
CM_3	21	**5.68**	5.47	6.02	0.146	3	**5.76**	5.58	5.88	0.157	5.39	5.93	5.41

bats (♂ LAt > 39 mm) from Central Europe and Central Asia, and the small bats (♂ LAt < 38 mm) from the Mediterranean and Oman; a single sample from north-eastern Africa is medium-sized in this respect (LAt 38.7 mm).

Large skull size (♂ LCc > 14.0 mm) was found in the bats from Central Europe; a small skull (♂ LCc < 13.5 mm) was seen in the samples from the East Mediterranean, Oman, and north-eastern Africa; and a medium-sized skull (♂ LCc 13.5–13.8 mm) was observed in the bats from the West Mediterranean and Central Asia (Fig. 4). An absolutely and relatively wide skull (♂ LaZ > 7.4 mm; ♂ LaZ/LCc > 0.545) was observed in the bats from Central Europe, West Mediterranean, and Central Asia, whereas an absolutely and relatively narrow skull (♂ LaZ < 7.4 mm; ♂ LaZ/LCc < 0.545) was seen in the bats from Oman and an absolutely narrow but relatively wide skull (♂ LaZ < 7.4 mm; ♂ LaZ/LCc > 0.545) was found in the bats from the East Mediterranean and north-eastern Africa.

An absolutely and relatively wide braincase (♂ LaNc > 6.5 mm; ♂ LaNc/LCc > 0.475) was found in the samples from the West Mediterranean and north-eastern Africa, whereas an absolutely and relatively narrow braincase (♂ LaNc < 6.5 mm; ♂ LaNc/LCc < 0.475) was observed in the bats from Central Asia and Oman; an absolutely wide but relatively narrow braincase (♂ LaNc > 6.5 mm; ♂ LaNc/LCc < 0.475) was observed in the bats from Central Europe; and an absolutely narrow but relatively wide braincase (♂ LaNc < 6.5 mm;

TABLE 3. Dental dimensions of the examined sample sets of the *Rhinolophus hipposideros* group; *midas* = dimensions of the respective type specimen; for the sample set delimitations and dimension abbreviations see Materials and Methods. Mean values shown in bold

Character	Central Europe					West Mediterranean					East Mediterranean				
	n	o	min	max	SD	*n*	o	min	max	SD	*n*	o	min	max	SD
M^1M^3	51	**3.601**	3.40	4.13	0.108	82	**3.505**	3.16	4.08	0.120	41	**3.484**	3.34	3.68	0.083
LCs	51	**1.014**	0.92	1.07	0.028	73	**0.980**	0.90	1.09	0.036	41	**0.936**	0.86	1.00	0.035
LaCs	51	**0.822**	0.75	0.92	0.036	73	**0.791**	0.71	0.88	0.041	41	**0.827**	0.76	0.95	0.042
LP^2	51	**0.535**	0.46	0.59	0.029	73	**0.493**	0.41	0.57	0.041	41	**0.476**	0.38	0.56	0.041
LaP^2	51	**0.511**	0.43	0.59	0.030	73	**0.474**	0.38	0.62	0.049	40	**0.484**	0.36	0.62	0.048
LP^41	51	**0.993**	0.88	1.09	0.041	73	**0.970**	0.86	1.09	0.048	41	**0.922**	0.82	1.01	0.049
LP^42	51	**0.532**	0.46	0.61	0.036	73	**0.496**	0.40	0.61	0.039	41	**0.498**	0.40	0.59	0.037
LP^43	51	**0.735**	0.65	0.86	0.037	73	**0.714**	0.58	0.82	0.048	41	**0.713**	0.59	0.80	0.045
LaP^4	51	**1.548**	1.45	1.76	0.060	73	**1.467**	0.85	1.58	0.091	41	**1.487**	1.35	1.62	0.055
LM^1	51	**1.401**	1.28	1.77	0.062	73	**1.377**	1.30	1.48	0.038	41	**1.361**	1.29	1.46	0.037
LaM^1	51	**1.970**	1.78	2.14	0.066	73	**1.919**	1.80	2.04	0.059	41	**1.894**	1.70	2.11	0.073
LM^3	51	**0.987**	0.89	1.07	0.042	73	**1.076**	0.95	1.22	0.046	41	**1.015**	0.90	1.13	0.068
LaM^3	51	**1.385**	1.30	1.50	0.043	73	**1.359**	1.27	1.49	0.041	41	**1.379**	1.28	1.79	0.075
M_1M_3	51	**3.914**	3.76	4.08	0.071	81	**3.825**	3.39	4.08	0.124	41	**3.814**	3.62	4.00	0.087
LCi	51	**0.716**	0.65	0.78	0.030	72	**0.694**	0.56	0.79	0.045	41	**0.669**	0.62	0.75	0.033
LP_2	51	**0.606**	0.53	0.69	0.032	72	**0.579**	0.46	0.75	0.042	41	**0.567**	0.51	0.63	0.033
LaP_2	51	**0.534**	0.46	0.59	0.025	72	**0.532**	0.45	0.75	0.041	41	**0.529**	0.46	0.60	0.034
LP_3	51	**0.194**	0.02	0.28	0.058	67	**0.173**	0.05	0.28	0.041	36	**0.202**	0.13	0.29	0.040
LP_4	51	**0.778**	0.71	0.85	0.035	72	**0.740**	0.62	0.83	0.037	41	**0.724**	0.63	0.80	0.040
LaP_4	51	**0.645**	0.59	0.71	0.028	72	**0.640**	0.56	0.71	0.032	41	**0.619**	0.56	0.80	0.045
LMi	51	**1.396**	1.33	1.48	0.033	72	**1.380**	1.28	1.64	0.051	40	**1.369**	1.26	1.51	0.048

	Central Asia					Oman					Ethiopia	Sudan	*midas*
M^1M^3	6	**3.688**	3.53	3.79	0.100	3	**3.614**	3.40	3.79	0.201	3.40	3.29	3.81
LCs	6	**0.979**	0.92	1.01	0.031	3	**0.991**	0.97	1.02	0.022	0.94	0.80	0.92
LaCs	6	**0.848**	0.76	0.92	0.067	3	**0.904**	0.88	0.92	0.022	0.84	0.78	0.86
LP^2	6	**0.469**	0.38	0.53	0.064	3	**0.412**	0.38	0.43	0.030	0.51	0.44	0.40
LaP^2	6	**0.480**	0.41	0.57	0.067	3	**0.363**	0.29	0.43	0.068	0.46	0.41	0.37
LP^41	6	**0.962**	0.88	1.03	0.055	3	**0.991**	0.98	1.01	0.012	0.90	0.90	0.98
LP^42	6	**0.506**	0.43	0.56	0.044	3	**0.538**	0.52	0.57	0.024	0.50	0.55	0.56
LP^43	6	**0.754**	0.65	0.82	0.062	3	**0.750**	0.71	0.80	0.042	0.69	0.69	0.79
LaP^4	6	**1.564**	1.50	1.63	0.055	3	**1.553**	1.49	1.59	0.058	1.45	1.41	1.61
LM1	6	**1.421**	1.37	1.50	0.045	3	**1.375**	1.29	1.42	0.076	1.34	1.23	1.47
LaM1	6	**2.007**	1.92	2.07	0.061	3	**1.871**	1.72	1.95	0.133	1.92	1.84	1.91
LM3	6	**1.131**	1.08	1.17	0.037	3	**1.134**	1.05	1.18	0.076	1.04	1.03	1.19
LaM3	6	**1.415**	1.37	1.50	0.050	3	**1.428**	1.32	1.49	0.094	1.40	1.37	1.53
M_1M_3	6	**3.991**	3.84	4.06	0.078	3	**4.028**	3.84	4.16	0.165	3.82	3.58	4.15
LCi	6	**0.710**	0.68	0.75	0.031	3	**0.667**	0.64	0.69	0.026	0.68	0.65	0.68
LP_2	6	**0.581**	0.56	0.61	0.022	3	**0.565**	0.53	0.63	0.054	0.62	0.48	0.45
LaP_2	6	**0.496**	0.38	0.57	0.067	3	**0.454**	0.40	0.50	0.053	0.55	0.48	0.46
LP_3	6	**0.194**	0.16	0.26	0.036	3	**0.251**	0.24	0.26	0.010	0.25	0.19	0.28
LP_4	6	**0.780**	0.74	0.87	0.046	3	**0.789**	0.78	0.81	0.016	0.71	0.69	0.76
LaP_4	6	**0.628**	0.61	0.65	0.018	3	**0.628**	0.56	0.70	0.073	0.64	0.59	0.59
LMi	6	**1.417**	1.38	1.47	0.028	3	**1.428**	1.35	1.49	0.070	1.34	1.30	1.42

o LaNc/LCc > 0.475) was seen in the bats from the East Mediterranean. Two shape types were found concerning the absolute and relative height of braincase; the bats from Central Europe, the Mediterranean, and Central Asia had a high braincase (o ANc > 4.5 mm; o Nc/LCc > 0.335), and the bats from north-eastern Africa and Oman had a low braincase (o ANc < 4.5 mm; o ANc/LCc < 0.335). An absolutely and relatively large tympanic bulla (LBT > 2.9 mm; LBT/LCc > 0.2) was observed in the bats from Oman, whereas a small bulla (LBT < 2.8 mm; LBT/LCc < 0.2) was seen in the bats from all other geographic sample sets (Fig. 5).

The rostral part of the skull was absolutely and relatively long (o CM^3 > 5.4 mm; o CM^3/LCc > 0.395) in the bats from Central Asia and Oman (Fig. 5); an absolutely long but relatively short rostrum (o CM^3 > 5.4 mm; o CM^3/LCc < 0.385) was

TABLE 4. Descriptive features of morphotypes of particular sample of the *Rhinolophus hipposideros* group (average states of absolute metric values are defined)

Character	Central Europe	West Mediterranean	East Mediterranean	Central Asia	NE Africa	Oman
Body size	large	small	small	large	medium	small
Skull size	large	medium	small	medium	small	small
Skull width	large	large	small	large	small	small
Braincase width	large	large	small	small	large	small
Braincase height	large	large	large	large	small	small
Tympanic bulla size	small	small	small	small	small	large
Rostrum length	large	small	small	large	small	large
Upper canine size	medium	small	medium	medium	small	large
Small upper premolar (P^2) size	large	medium	medium	medium	medium	small
Large upper premolar (P^4) size	large	medium	small	medium	small	large
First upper molar (M^1) size	large	medium	medium	large	small	medium
Third upper molar (M^3) size	small	small	small	large	small	large
Lower canine size	large	small	small	large	small	small
First lower premolar (P_2) size	large	large	large	medium	medium	small
Last lower premolar (P_4) size	large	small	small	large	small	large
Small lower premolar (P_3) size	small	small	small	small	small	large
First lower molar (M_1) size	small	small	small	large	small	large

observed in the bats from Central Europe, where-as an absolutely short but relatively long rostrum (o CM^3 < 5.4 mm; o CM^3/LCc > 0.385) was found in the bats from the Mediterranean and north-eastern Africa. A relatively narrow rostrum (o LaInf/LCc < 0.262) was found in the bats from Central Europe and the Mediterranean; a relatively very wide rostrum (o LaInf/LCc > 0.264) was observed in the bats from Oman; and a relatively medium-sized rostrum width (o LaInf/LCc 0.262–0.264) was seen in the bats from Central Asia and north-eastern Africa.

Although the tooth metric characters largely followed the size trends in the skulls, certain shape variability and size trends were detectable in particular teeth. The largest upper canines (Cs; o LaCs

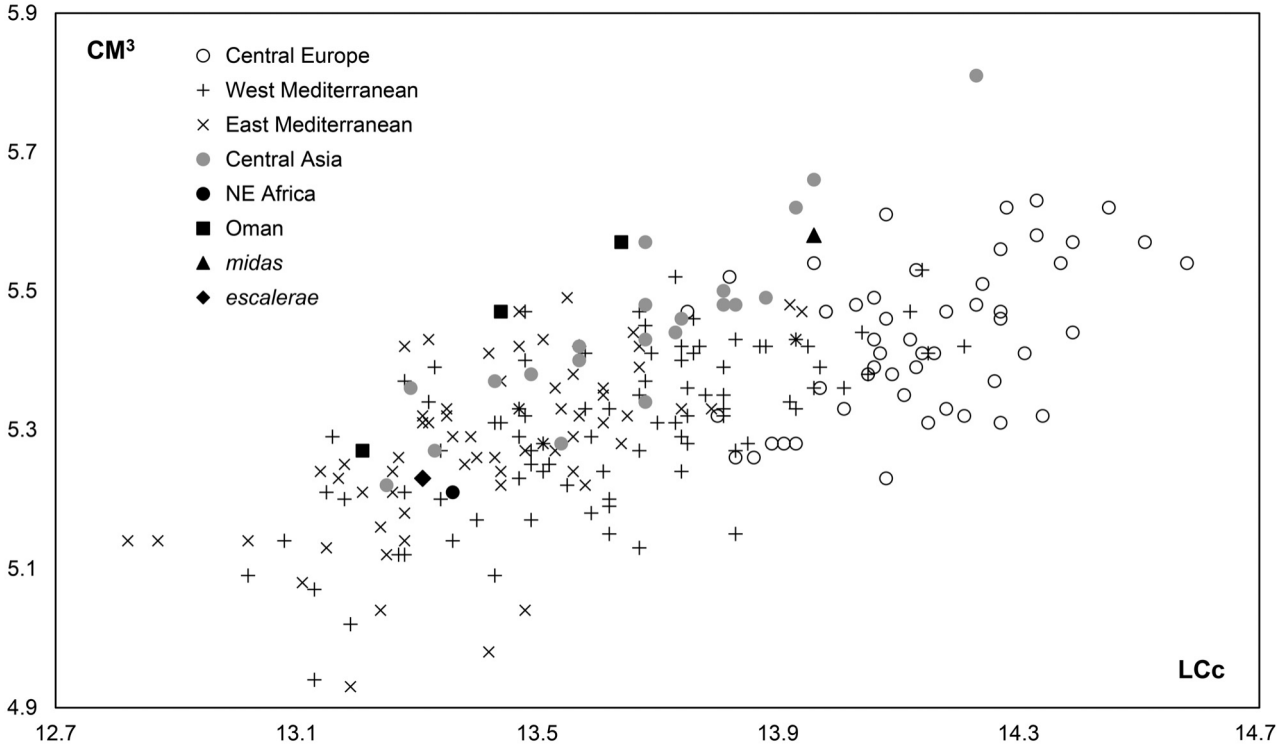

FIG. 4. Bivariate plot of skull dimensions of the examined samples of the *Rhinolophus hipposideros* group: condylocanine length of skull (LCc) against length of the upper tooth-row (CM^3); values in mm

> 0.90 mm) were observed in the bats from Oman, the smallest (o LaCs < 0.81 mm) were seen in the bats from the West Mediterranean and north-eastern Africa, and the upper canines (o LaCs 0.82–0.85 mm) in the bats from Central Europe, the East Mediterranean, and Central Asia were medium size (Fig. 6). The small upper premolar (P^2) was found to be large (LP^2 > 0.52 mm; o $LP^2 \times LaP^2$ > 0.25 mm^2) in the bats from Central Europe, small (o LP^2 < 0.42 mm; o $LP^2 \times LaP^2$ < 0.20 mm^2) in the bats from Oman, and medium-sized (o LP^2 0.46–0.50 mm; o $LP^2 \times LaP^2$ 0.20–0.25 mm^2) in the bats from the Mediterranean, Central Asia, and north-eastern Africa. The large upper premolar (P^4) was found to be large (o LP^41 > 0.98 mm) in the bats from Central Europe and Oman, small (o LP^41 < 0.95 mm) in the bats from the East Mediterranean and north-eastern Africa, and medium-sized (o LP^41 0.95–0.98 mm) in the bats from the West Mediterranean and Central Asia; P^4 was relatively wide (o LaP^4/LP^41 > 1.6) in the bats from the East Mediterranean and Central Asia, and relatively narrow (o LaP^4/LP^41 < 1.6) in the bats from the remaining four sample sets; it was relatively long in its medial portion (i.e., with a smallest posterior concavity in the distal margin of talon; o LP^42/LaP^4 > 0.36) in the bats from north-eastern Africa, short (o LP^42/LaP^4 < 0.34) in the bats from the East Mediterranean and Central Asia, and medium length (o LP^42/LaP^4 0.34–035) in the bats from Central Europe, the West Mediterranean, and Oman.

The first upper molar (M^1) was found to be large (o LM^1 > 1.4 mm; o $LM^1 \times LaM^1$ > 2.7 mm^2) in the bats from Central Europe and Central Asia, small (o LM^1 < 1.3 mm; o $LM^1 \times LaM^1$ < 2.5 mm^2) in the bats from north-eastern Africa, and medium-sized (o LM^1 1.35–1.38 mm; o $LM^1 \times LaM^1$ 2.5–2.7 mm^2) in the bats from the Mediterranean and Oman; M^1 was relatively wide (o LaM^1/LM^1 > 1.4) in the bats from Central Europe, Central Asia, and north-eastern Africa, relatively narrow (o LaM^1/LM^1 1.36) in the samples from Oman, and medium width (o LaM^1/LM^1 1.39–1.40) in the bats from the Mediterranean. The third upper molar (M^3) was large (o LM^3 > 1.1 mm; o $LM^3 \times LaM^3$ > 1.5 mm^2) in the bats from Central Asia and Oman and small (o LM^3 < 1.1 mm; o $LM^3 \times LaM^3$ < 1.5 mm^2) in the bats from Central Europe, the Mediterranean, and north-eastern Africa; M^3 was relatively wide (o LaM^3/LM^3 > 1.4) in the bats from Central Europe, relatively narrow (o LaM^3/LM^3 <1.3) in the samples from the West Mediterranean, Central Asia, and Oman, and medium width (o LaM^3/LM^3 1.3–1.4) in the bats from the East Mediterranean and

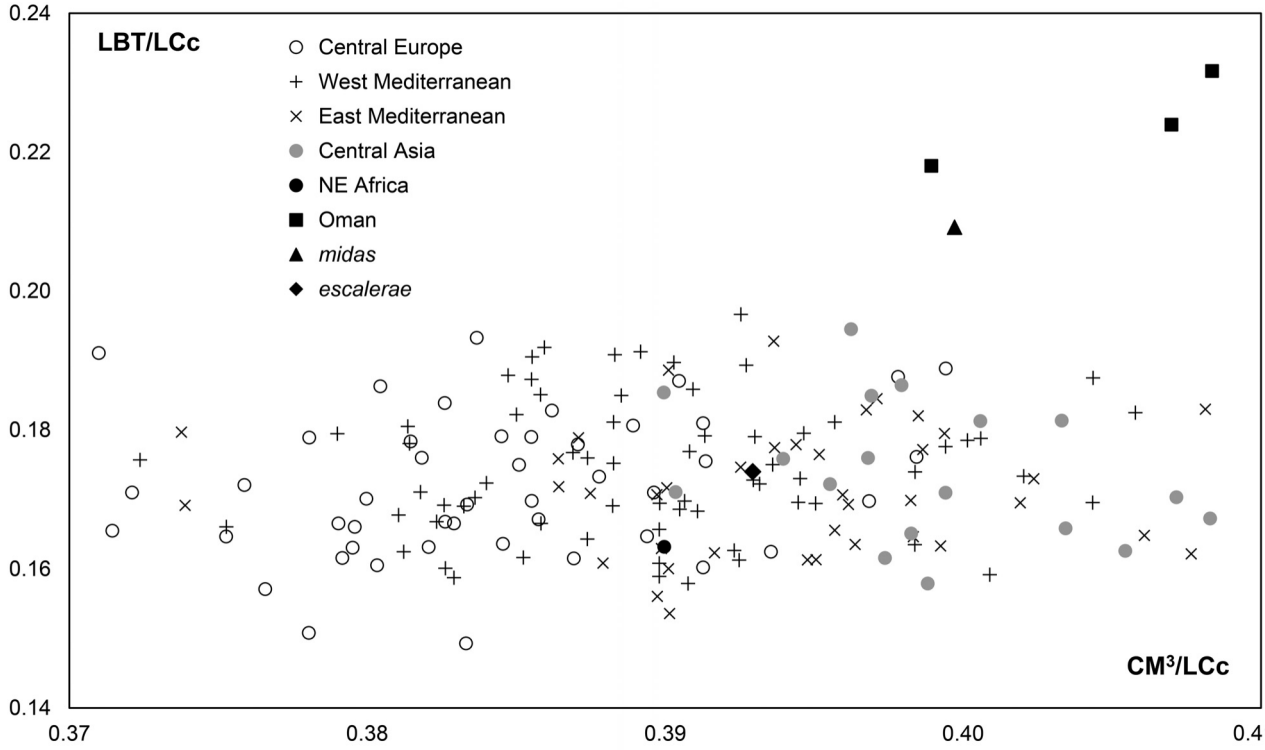

FIG. 5. Bivariate plot of skull dimensions of the examined samples of the *Rhinolophus hipposideros* group: relative length of rostrum (CM^3/LCc) against relative horizontal length of tympanic bulla (LBT/LCc)

north-eastern Africa. In relation to M^1, M^3 was found to be large (o $LM^3 \times LaM^3/LM^1 \times LaM^1 > 0.6$) in the bats from Oman, small (o $LM^3 \times LaM^3/LM^1 \times LaM^1 < 0.5$) in the bats from Central Europe, and medium-sized (o $LM^3 \times LaM^3/LM^1 \times LaM^1$ 0.5–0.6) in the bats from the remaining four sample sets (Fig. 6).

The lower canine (Ci) was observed to be large (o LCi > 0.7 mm) in the bats from Central Europe and Central Asia and small (o LCi < 0.6 mm) in the remaining four sample sets; in relation to the first lower molar (Mi), the Ci was relatively large (o LCi/LMi > 0.5) in the bats from Central Europe, the West Mediterranean, Central Asia, and north-eastern Africa, and relatively small (o LCi/LMi < 0.5 mm) in the bats from the East Mediterranean and Oman. The first lower premolar (P_2) was large (o $LaP_2 > 0.52$ mm; o $LP_2 \times LaP_2 > 0.3$ mm^2) in the bats from Central Europe and the Mediterranean, small (o $LaP_2 < 0.48$ mm; o $LP_2 \times LaP_2 < 0.27$ mm^2) in the bats from Oman, and medium-sized (o LaP_2 0.48–0.52 mm; o $LP_2 \times LaP_2$ 0.28–0.30 mm^2) in the bats from Central Asia and north-eastern Africa. In relation to the last lower premolar (P_4), P_2 was very small (o $LP_2 \times LaP_2/LP_4 \times LaP_4 < 0.53$) in the bats from Oman. In all other sample sets, this tooth was found to be large or very large (o $LP_2 \times LaP_2/LP_4 \times LaP_4 > 0.58$). The last lower premolar (P_4) was large (o $LaP_4 > 0.75$ mm; o $LP_4 \times LaP_4 > 0.48$ mm^2) in the bats from Central Europe, Central Asia, and Oman and small (o $LaP_4 < 0.75$ mm; o $LP_4 \times LaP_4 > 0.48$ mm^2) in the bats from the Mediterranean and north-eastern Africa. The small lower premolar (P_3) was found to be large (o $LP_3 > 0.23$ mm) in the Omani samples, but small (o $LP_3 < 0.23$ mm) in all other sample sets. The first lower molar (Mi) and the lower molar-row were large (o LMi > 1.4 mm; (o $M_1M_3 > 3.95$ mm) in the bats from Central Asia and Oman and small (o LMi <1.4 mm; o $M_1M_3 < 3.95$ mm) in the four remaining sample sets.

In summary, the comparison demonstrated certain characters were unique in four of the six examined sample sets (Tables 2–4 and Supplementary Tables S5–S7; see Appendix II for a review of the state conditions of evaluated metric characters in the particular sample sets). The Omani sample set was shown to be the most distinct among all bats (including the statistical comparison — Supplementary Table S7). Within the matrix of 25 metric characters evaluated above, the Omani bats showed in ten characters a state to be unique in relation to all other sample sets: an absolutely and relatively very narrow skull with a relatively very wide rostrum and an absolutely and relatively very large tympanic

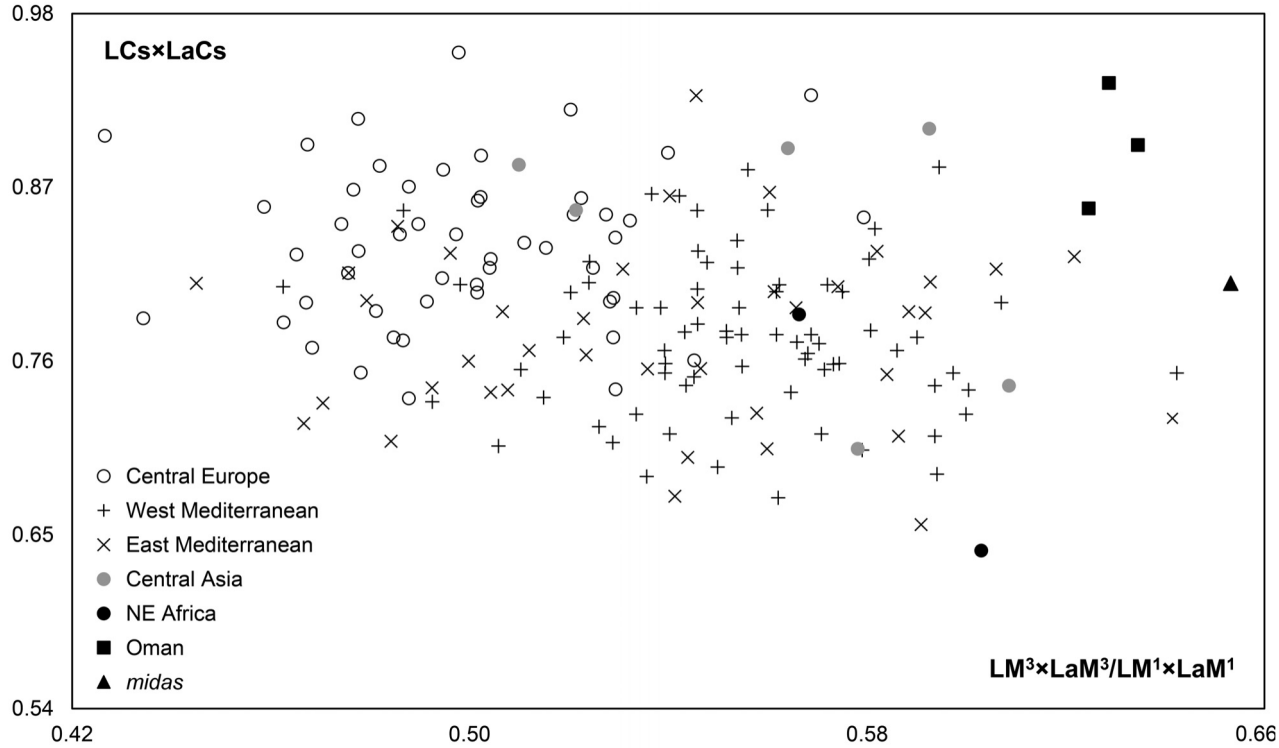

FIG. 6. Bivariate plot of tooth dimensions of the examined samples of the *Rhinolophus hipposideros* group: relative crown size of third upper molar ($LM^3 \times LaM^3/LM^1 \times LaM^1$) against crown size of upper canine (LCs×LaCs)

bulla, very large upper canine, very small first (small) upper premolar (P^2), relatively very narrow (palato-labially short) first upper molar (M^1) and a relatively very large third upper molar (M^3), an absolutely as well as relatively very small first lower premolar (P_2), and a very large small-lower premolar (P_3). Six unique characters were found in the samples from Central Europe: a very large skull size with an absolutely wide but relatively narrow braincase and an absolutely long but relatively short rostrum, a very large first (small) upper premolar (P^2), and a very small and relatively very wide third upper molar (M^3). Only two unique characters were documented in the sample set from north-eastern Africa (large upper premolar (P^4) being relatively narrow as a whole but relatively wide in its medial portion, and a very small first upper molar, M^1), and one was documented in the East Mediterranean set (an absolutely narrow but relatively wide braincase). No unique character among the evaluated metric traits was observed in the bats from the West Mediterranean and Central Asia.

The examined skulls of holotype specimens of two names of the *R. hipposideros* group (*R. midas* and *R. h. escalerae*) were compared with the above-defined morphotypes (Tables 2 and 3). The type of *escalerae* from western Morocco conforms in most characters to the Mediterranean populations, namely in the skull size and shape (i.e., the skull width, absolute and relative length and width of the rostrum, absolute and relative width and height of the braincase, and mandible length). The type of *midas* from southern Iran conforms in most respects to the bats from Oman. Similarities were found in all types of characters, in the skull size and shape and in the sizes and shapes of teeth. The type skull of *R. midas* is large with large tooth rows, although it is absolutely and relatively narrow; the braincase is very low; the rostrum is rather wide; and the tympanic bullae are very large. Although the Omani skulls are slightly smaller than the type skull of *midas* is in absolute dimensions, they well agree in the relative dimensions as well as in the absolute and relative size of tympanic bullae (Figs. 4 and 5). Even more pronounced than in the skull dimensions, the similarity of the *midas* type and Omani bats is apparent in the tooth dimensions. These bats are very similar in the extremely small size of the small upper premolar (P^2), large absolute and mainly relative size of the last upper molar (M^3), small relative and absolute size of the lower canine (Ci), small absolute and relative size of the first lower premolar (P_2), and large absolute size of the smallest lower premolar (P_3).

The separate position of the Omani samples plus the type specimen of *midas* in relation to all other sample sets is also illustrated by the results of the UPGMA cluster analysis (Fig. 7). Both the results calculated from the skull and tooth data showed similar positions of the Omani set together with *midas* positions in separate clusters, whereas the remaining sample sets from Central Europe, the Mediterranean, Central Asia, and north-eastern Africa in other clusters showed variable inner topology of particular sets. The results of a canonical analysis calculated from nine selected plain skull dimensions (LCc, LaZ, LaI, CM^3, CC, M^3M^3, LBT, ACo, CM_3) and seven relative skull dimensions (LaZ/LCc, LaInf/LCc, LaN/LCc, LaM/LCc, ANc/LCc, LBT/LCc, CM^3/LCc) conformed to the results of the empirical comparisons and cluster analysis (Supplementary Fig. S8 — CV1 46.37% of variance, CV2 32.62%). They clearly separated the Omani bats as the most distinct sample set (CV1 > 1.6; CV2 ≤ 6.5) in CV2 without an overlap with the four other

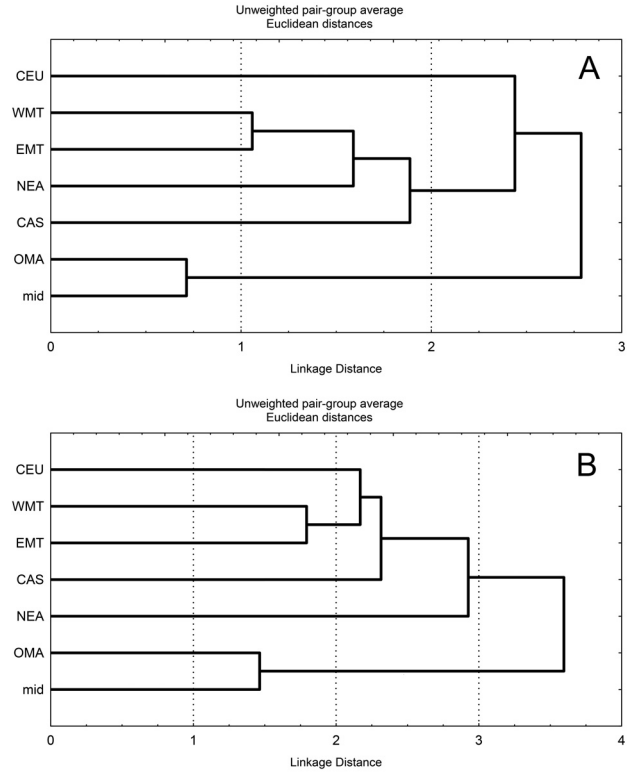

FIG. 7. Results of the cluster analysis (UPGMA): differences between mean values of morphometric traits among the particular sets of samples of the *Rhinolophus hipposideros* group calculated from 27 plain and relative dimensions of skull (A), and from 36 plain and relative dimensions of teeth (B). Samples and sample sets: CEU — Central Europe; WMT — West Mediterranean; EMT — East Mediterranean; CAS — Central Asia; NEA — north-eastern Africa; OMA — Oman; mid — type specimen of *R. midas* Andersen, 1905

sample sets, which, however, overlapped in both canonical variables with each other. Because only one skull was available from north-eastern Africa and the type series of *escalerae* and *midas* are composed only of holotypes, the relative positions of these three samples to other populations were not evaluated by the canonical analysis.

Comparison of Echolocation Call Parameters

The parameters of echolocation calls of *R. hipposideros* show similar values in the majority of characteristics throughout most of its distribution range, including central and southern Europe and south-western Asia, although the samples are rather small in some populations and the descriptive information level of the associated data could be limited (Table 5). Two exceptions among the population samples were found in the data from Malta and Oman (Mifsud and Vella, 2019; own data); whereas in the Maltese bats, the frequency values (start, end, peak) were reported to be much higher than in all other populations (≥ 115 kHz in all these parameters, without a value overlap with other populations), in the Omani bats, the peak frequency was found to be extremely low (< 101 kHz, without an overlap with the respective values from other populations; see Fig. 8).

Discussion

Our analysis uncovered the existence of an unexpected diversity within the *R. hipposideros* group, challenging its existing phylogenetic and taxonomic arrangement as concluded by Koopman (1994), Horáček *et al*. (2000), Csorba *et al*. (2003), Simmons (2005), or Burgin (2019). Genetic and morphological examinations of representative sets of specimens showed two main, geographically exclusive phylogenetic lineages within the group that are well delimited by molecular characteristics and possess two distinct morphotypes and two distinct echotypes. The genetic separation of the lineages is deep and detectable in both nuclear and mitochondrial genomes; in the *Cyt-b* gene, the uncorrected *p*-distance of 8.9–10.8% was found, which is roughly twice that considered sufficient for a taxonomic split (Baker and Bradley, 2006); this distance is even higher than those reported by Demos *et al*. (2019) for various species-pairs in *Rhinolophus* (or than the results for the species-pairs obtained here, e.g., on average 5.80% for *capensis-swinnyi*, 4.99%

Table 5. Echolocation parameters in various populations of *Rhinolophus hipposideros* group; based on published and original data. Abbreviations: SF = start frequency, EF = end frequency, PF = peak frequency (shown in bold), D = pulse duration, IPI = inter-pulse interval

Country	*n*	SF [kHz]	EF [kHz]	PF [kHz]	D [ms]	IPI [ms]	Reference
Great Britain	[call] 33	98.2 ± 0.9	96.3 ± 1.4	**111.0 ± 0.2**	41.7 ± 1.5	–	Parsons and Jones (2000)
Switzerland	[call] 100	–	–	**107.5 ± 3.7**	21.6 ± 4.4	–	Obrist *et al*. (2004)
Italy	[ind]	99.0 ± 3.5	96.6 ± 6.6	**111.1 ± 1.7**	43.6 ± 13.0	70.4 ± 24.5	Russo and Jones (2002)
	34	92.3–107.8	83.4–110.3	**107.3–114.0**	11.9–61.4	14.1–113.7	
Malta	[call]	116.9 ± 1.7	117.2 ± 1.7	**117.5 ± 1.9**	34.5 ± 14.6	80.1 ± 13.5	Mifsud and Vella (2019)
	20	115.3–119.3	115.5–119.3	**115.0–122.0**	6.4–50.6	52.0–100.0	
Greece	[ind]	96.6 ± 10.3	84.8 ± 4.7	**110.6 ± 3.9**	45.2 ± 6.4	98.2 ± 29.1	Papadatou *et al*. (2008)
	5	84.7–107.8	79.0–89.8	**106.4–114.9**	34.3–50.8	68.6–135.5	
Sinai	[call/seq]	89.9 ± 1.1	88.5 ± 1.5	**107.4 ± 0.5**	54.0 ± 6.7	30.7 ± 5.3	Benda *et al*. (2008)
(Egypt)	6/1	88.9–91.5	86.7–90.6	**106.7–108.0**	43.0–61.2	22.9–36.6	
Israel	[call/pass]	92.54 ± 6.8	93.37 ± 8.6	**107.58 ± 0.5**	42.28 ± 12.1	–	Hackett *et al*. (2017)
	57/9	83.9–109.3	80.0–114.2	**103.5–109.3**	–		
Dagestan	[call/seq]	96.0 ± 1.7	91.8 ± 2.4	**113.7 ± 1.6**	47.8 ± 10.6	78.8 ± 10.8	Smirnov *et al*. (2022)
(Russia)	51/7	90.2–98.9	86.0–96.6	**109.6–115.2**	21.8–68.0	39.2–94.0	
Iran	[call/seq]	111.2 ± 0.8	108.5 ± 1.4	**110.3 ± 0.8**	49.9 ± 1.5	41.9 ± 3.8	Benda *et al*. (2012)
	18/2	109.9–112.2	106.2–110.7	**109.0–111.1**	47.8–52.0	36.1–48.7	
	[ind] 4	104.6 ± 0.8	89.9 ± 2.9	**110.7 ± 1.9**	24.9 ± 2.6	62.3 ± 5.3	Shahabi *et al*. (2019)
Slovakia	[call/seq]	103.1 ± 3.6	105.8 ± 2.3	**106.2 ± 2.3**	30.3 ± 13.3	–	This study
	142/6	94.9–110.1	100.3–109.8	**102.2–110.1**	4.6–55.1		
Tajikistan	[call/seq]	105.3 ± 3.8	104.9 ± 6.4	**109.4 ± 0.9**	33.8 ± 12.5	69.1 ± 34.0	This study
	34/3	96.4–110.0	89.9–110.6	**107.6–110.6**	19.5–55.8	26.0–148.0	
Saudi Arabia	[call/seq]	108.4 ± 1.8	108.9 ± 1.6	**109.1 ± 1.5**	31.7 ± 9.7	–	This study
	73/7	103.1–111.0	105.8–111.0	**105.8–111.0**	20.3–54.4		
Oman	[call/seq]	92.3 ± 6.5	92.6 ± 6.8	**98.2 ± 1.6**	42.9 ± 6.6	102.3 ± 25.8	This study
	114/12	72.5–100.6	73.5–99.7	**94.1–100.6**	30.1–59.1	39.0–202.0	

for *euryale-mehelyi*, 4.07% for *ferrumequinum-clivosus*, or 3.65% for *willardi-kahuzi*). Thus, the degree of genetic separation of the two lineages within the *hipposideros* group is sufficient to allow us to consider them as two separate species.

Although one lineage/species was detected in the majority of the distribution range of the *R. hipposideros* group stretching across the whole south-western Palaearctic (Europe, north-western and north-eastern Africa, north of the Middle East, Afghanistan, and West Turkestan), the other lineage/species was discovered in a very limited area in the north-eastern regions of Oman. The divergence of these two lineages is estimated to have occurred in the interval 4.3–10.0 Ma, when the more realistic (concerning the fossil evidence) estimation model of Stoffberg *et al*. (2010) is applied. This age approximately corresponds with the late Miocene period or with the Miocene-Pliocene transition (7.0–5.4 Ma — Herbert *et al*., 2016); that is, with the periods of dramatic environmental changes that could have led to the separation of species lineages. Alternatively, when Dool's *et al*. (2016) model is used, the estimated divergence occurred in the interval of 2.0–4.9 Ma, which is roughly at the Pliocene-Pleistocene transition (2.6 Ma; Gibbard *et al*., 2010) and linked with massive environmental changes as well. However, both time estimations mainly correspond to the main splits of species groups in the Afro-Palaearctic clade of the genus *Rhinolophus* and are associated with much older periods than most of the estimated divergences of crown pairs of species are within this clade (Stoffberg *et al*., 2010; Dool *et al*., 2016).

The first, broadly distributed lineage/species can be easily identified with *R. hipposideros* (André, 1797) s.str. described from Germany, because only this genetic lineage was discovered in Europe. Based on genetic data, this species was confirmed to occur in the prevailing part of the range of the group as described by e.g., Horáček *et al*. (2000), Csorba *et al*. (2003), and Burgin (2019), with the exception of the Caucasus region, southern Iran, and south-western Arabia.

The Omani lineage/species represents a recently discovered population of the lesser horseshoe bat (cf. Harrison and Bates, 1991; Horáček *et al*., 2000; Benda *et al*., 2013). It is known from just six localities (including those where only echolocation call recordings were made) in the Al Hajjar Mountains, situated between Sal Alah (26°02'N, 56°22'E) in the north and Tayman (22°31'N, 59°20'E) in the south-east (some 550–600 km in a line along the Al Hajjar range). Only four specimens were available for examination, which represent a morphotype very distinct from all other examined populations of the lesser horseshoe bat, typified by a very narrow skull with a relatively long rostrum, very large tympanic bullae, a very small first (small) upper premolar (P^2), very large third upper molar (M^3), very small first lower premolar (P_2), and very large second (small) lower premolar (P_3). Because the identical morphotype was also detected in the holotype specimen of *R. midas* Andersen, 1905, this name could also be applied for the Omani species/lineage, and the species has two geographic parts: Omani (known from four bats) and Iranian (known from the type specimen).

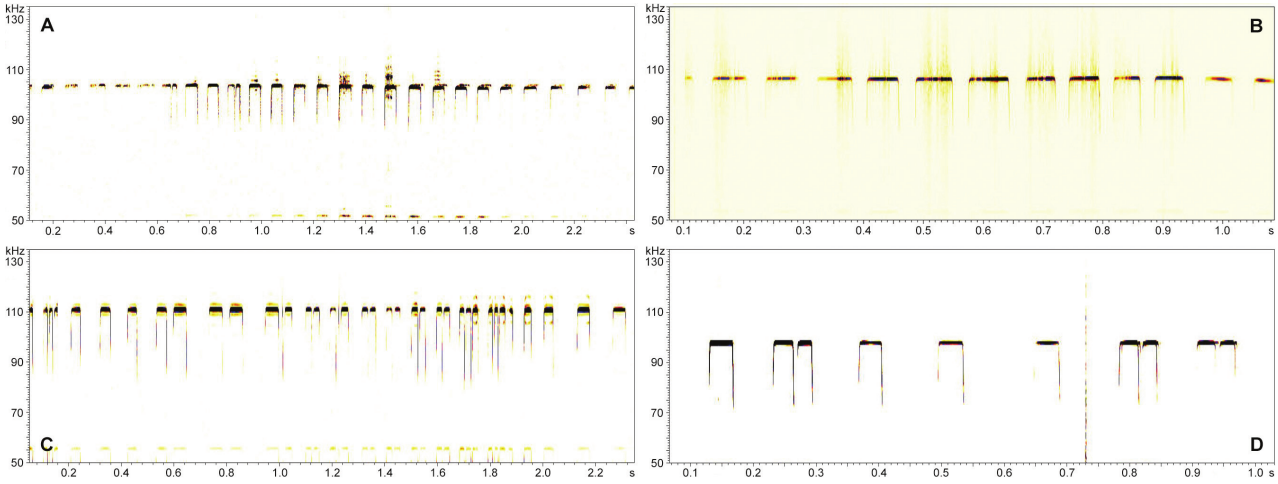

FIG. 8. Spectrograms of echolocation call examples of the *Rhinolophus hipposideros* group (original data); A — an individual recorded inside the Aksamitka Cave, Slovakia; B — an individual foraging at Arjank, Iran (cf. Benda *et al*., 2012); C — a handled individual recorded at Zingrogh, Tajikistan; D — a handled individual recorded at Misfah, Oman. For details see Materials and Methods

The type locality of *R. midas* is Jask, Hormozgan Province, Iran (25°40'N, 57°49'E), on the Iranian side of the Gulf of Oman, just opposite to the Al Hajjar Mountains of Oman. From the biogeographical perspective, the range of *R. midas*, lying in limited areas on both sides of the Gulf of Oman, is understandable. Similar geographical patterns of distribution range have been documented in other bats endemic to the Middle East (Harrison and Bates, 1991; Benda *et al*., 2012); namely, *Rhinopoma muscatellum* Thomas, 1903 (Rhinopomatidae) to a slightly larger geographical extent than in *R. midas* and *Hypsugo arabicus* (Harrison, 1979) (Vespertilionidae), with a distribution pattern very similar to *R. midas*. Besides *R. midas*, also *R. hipposideros* is distributed in Iran. However, according to the available records, it occurs in parapatry with *R. midas*, in uplands of the central and northern parts of the country (Benda *et al*., 2012). Only one record of the lesser horseshoe bat from Iran can be theoretically attributable to *R. midas* besides the type specimen, a bat observed in a cave on Qeshm Island in the Strait of Hormuz (Benda *et al*., 2012), only 106 km north-west of Sal Alah, the northernmost known site of this bat in Oman, and 250 km west–north-west of Jask, the type locality. The closest site of occurrence of *R. hipposideros* s.str. in Iran, confirmed by the genetic analysis (and the closest site as well), is the Tadovan Cave in the Zagros Mts. (1,190 m a.s.l.; Fars Prov., 28°51'N, 53°20'E — Shahabi *et al*., 2019), some 330 km NW of Qeshm Island.

Besides the genetic and morphological differences between *R. midas* and *R. hipposideros* s.str., the two species also differ in the pattern of their echolocation calls. Whereas in *R. hipposideros* s.str. the frequency of maximum energy (peak frequency, PF) of the call was detected around 110 kHz in most populations and only occasionally was it documented within the interval of 100–105 kHz (Benda *et al*., 2010; Győrössy *et al*., 2020), in *R. midas*, the PF was recorded in the interval of 94.1–100.6 kHz (O = 98.2 kHz), i.e., at values much lower than in *R. hipposideros* s.str. Although no important differences in body size were found between *R. hipposideros* s.str. and *R. midas*, the latter species is of a similar size as the Mediterranean populations of the former species, a difference between the species could possibly be present in the auricle size as well as the size of the inner ear (i.e., in characteristics linked with the frequency value of the echolocation call — Huihua *et al*., 2003). The limited available data suggest such type of difference. The ear length in *R. midas* from Oman was 18.0–19.0 mm (O = 18.5 mm), and in *R. hipposideros* s.str. from Iran it was 15.8–18.6 mm (O = 17.1 mm — Benda *et al*., 2012), and in the bats from Lebanon 14.2–18.8 mm (O = 17.4 mm — Benda *et al*., 2016), whereas the forearm length in *R. midas* was 36.8–38.1 mm (O = 37.4 mm), and in *R. hipposideros* s.str., it was 37.7–40.9 mm (O = 39.0 mm) from Iran and 35.3–39.4 mm (O = 37.7 mm) from Lebanon. Therefore, although the body size in *R. midas* is on average smaller than or similar to *R. hiposideros* s.str. from its geographically closest populations, the ear size seems to be on average larger in *R. midas* than it is in *R. hipposideros* s.str. (the species name *midas* also refers to the large ear size; it was selected by Andersen (1905) most probably after King Midas, a character from Greek mythology who had donkey ears). However, the differences in the external characteristics that would allow species identification remain to be found and tested; the currently available number of samples is too small for any conclusion. However, the size and shape of the nose-leaf of *R. midas* from Oman seems to be of identical parameters to those in *R. hipposideros* s.str. (see Fig. 9).

Hence, the *R. hipposideros* group (or the subgenus *Phyllorhina* Leach, 1816) now comprises two species, *R. hipposideros* and *R. midas*, identically as originally suggested by Andersen (1905) when he established the group. This author originally described *R. midas* as a separate species; later, he included it into the species rank of *R. hipposideros* (Andersen, 1918), and this unique morphotype, known from a single specimen until now, was for a long time overlooked. The *midas* morphotype seems to be rather conservative and perhaps more similar to the ancestral one because it exhibits a smaller degree of the reduction of distal molars and tiny premolars than known in the *hipposideros* s.str. morphotype.

The results of our analysis can also contribute to a revision of the intraspecific taxonomy of *R. hipposideros* s.str. Traditionally, the systematic reconstructions were based on body and skull size, nose-leaf shape, and several selected skull and tooth characters, an approach that resulted in delimitations of numerous taxa, namely in the Mediterranean area (see Introduction), a conception introduced by Andersen (1905) and accepted by many authors up to today (see Csorba *et al*., 2003; Simmons, 2005; Burgin, 2019). The molecular genetic analysis and broad evaluation of morphological characters brought a different view of the phylogenetic relationships

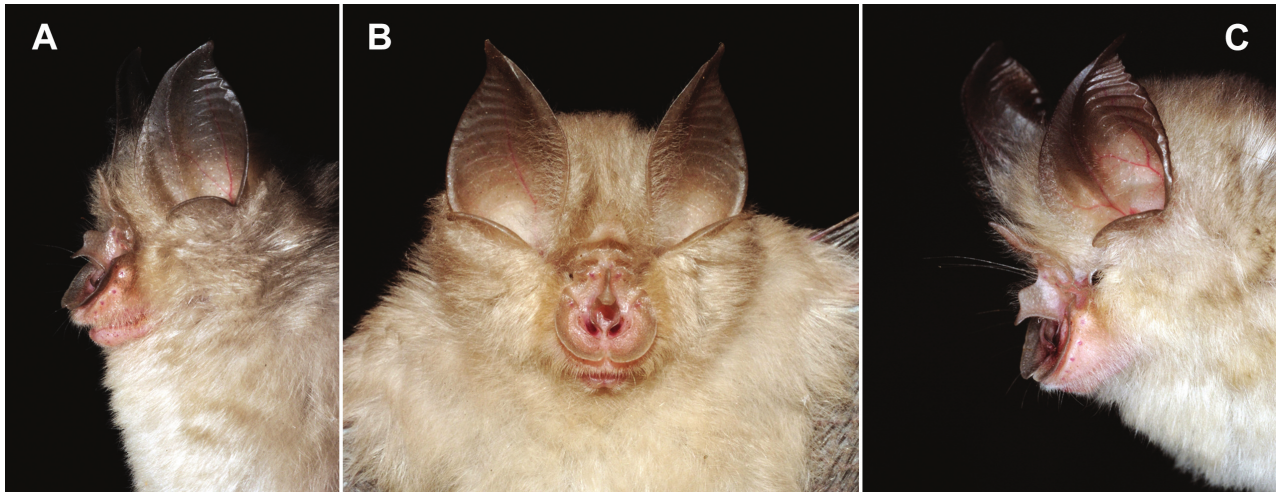

Fig. 9. Portraits of *Rhinolophus midas* Andersen, 1905 from Oman; A, B — ♂ (NMP 93782), Misfah, Ad Dakhiliyah Province, 9 April 2011, lateral and frontal views; C — ♀ (NMP 93994), Sal Alah, Masandam Province, 13 March 2012, lateral view. Photos by A. Reiter

within this species. The genetic analysis revealed the existence of two main genetic sublineages within the species, the western lineage, comprising most populations of Europe, including the British Isles, Sardinia, Malta, and Crete, and the Maghreb, and the eastern lineage, comprising the populations of Asia, including Eastern Mediterranean islands (Rhodes and Cyprus), and of Crimea. Both mitochondrial and nuclear markers showed the single Ethiopian sample to be a part of the eastern lineage, although without support for the mtDNA results (this could be a consequence of relatively large geographical distance between localities of the samples from Ethiopia and the Levant). However, the limited samples from West Turkestan (Tajikistan) were placed differently in the topology of both marker types, either into the eastern lineage (mtDNA) or into a separate lineage (nDNA) in a sister position to the above grouping. However, the West Turkestani samples are very limited and their localities are geographically extremely distant from the remaining analysed samples (the direct distance between the Tajikistani and central Iranian localities is some 1,800 km, across deserts and high mountains). Thus, the phylogenetic position of the easternmost populations of *R. hipposideros* s.str. remains to be investigated more elaborately, employing materials from all parts of Iran and West Turkestan, and from Afghanistan and Kashmir.

Although the geographic division to the western and eastern sublineages was not statistically supported by our results, it conforms to the results of previous analyses (Kůs, 2008; Dool *et al*., 2013), and it is additionally supported by karyological evidence. The geographical boundary between the lineages seems to be localised at the European-Asian transition between the Balkans and Anatolia, and from this location the boundary between the ranges of the 56- and 58-chromosome races is also reported (Zima *et al*., 1992; Zima, 2004; Arslan and Zima, 2014). Hence, the separation of the two lineages could actually be linked to the phylogenetic history of the species.

However, the morphological evidence did not contribute markedly to the reconstruction of the intraspecific relationships within *R. hipposideros* s.str. Two main morphological trends could be demonstrated from the data evaluated: (1) the increase of the body and skull size among the populations along the geographical gradient (latitudinal from the south to the north in the western part of the range, longitudinal from the Mediterranean to the continental climatic zone in the east) and (2) a mosaic-like distribution of characters among populations. The two population sets from the Mediterranean Basin (WMT and EMT) are the most similar to each other in the absolute and relative metric characters, although they belong to two separate sublineages. However, the most distinct population of *R. hipposideros* s.str. in morphometric traits is that of Central Europe. The representatives of the latter population are on average the largest in body size because they originate from the northernmost area of the species occurrence, although they represent a part of the western sublineage. The populations of the western sublineage share identical haplotypes of the mtDNA despite enormous geographical distances between them (e.g., one universal haplotype

was found in Ireland, Great Britain, France, Italy, Austria, Slovenia, Slovakia, Bulgaria, and Greece). This haplotype arrangement suggests relatively recent dispersions of populations across the southern part of Europe and thus a relatively fast evolution of very distinct morphotypes.

The size differences among morphotypes of *R. hipposideros* may correlate with the changes of climatic conditions along a geographical gradient, in accordance with Bergmann's rule (Bergmann, 1847), that are expected to affect also bat populations (Ashton *et al*., 2000). On a smaller geographic scale, Salinas-Ramos *et al*. (2021) recently demonstrated a similar size shift in Italy along approximately 1000 km of the south-north gradient; these authors also explained that it aligned with Bergmann's rule. Other environmental influences that could be responsible for the geography-associated shift in body size, such as the character displacement (cf. Grant, 1972), do not seem to be significant in this bat species. All the evaluated populations come from regions where at least three size categories of horseshoe bats can be observed (i.e., Mediterranean, Central Europe, Central Asia, and north-eastern Africa); therefore, no effects from interspecific competition within the genus and no morphometric or other deflections in particular species were observed (see e.g., Andreas *et al*., 2013). If the character displacement really influenced the morphometry in *R. hipposideros* s.str., it would be primarily observed in the British Isles, where only two horseshoe bat species live in sympatry (the medium-sized category is missing). However, the body size of *R. hipposideros* on these islands is smaller than of the bats in Central Europe (Andersen, 1905; Miller, 1912) where three *Rhinolophus* species occur and where *R. hipposideros* would be much smaller if the character displacement works there. The medium body size of the British bats (in relation to the Mediterranean and Central European ones) is most likely caused by the islands' milder climate compared to Central Europe and harsher climate compared to the Mediterranean.

The Central European morphotype is the most distinct within *R. hipposideros* s.str. because of its extremely large skull size with a relatively narrow braincase and short rostrum, very large first upper premolar (P^2), and very small and relatively wide third upper molar (M^3). However, these differences seem to be a consequence of the allometric size changes of the skull, where the skull is enlarged in length (mainly the braincase), but is not enlarged to the same degree in width and in tooth-row length; the distal molars are enlarged less than the mesial ones and are relatively short (i.e., seem to be more reduced in length) but are not narrow.

The size differences along the geographical latitude from the Mediterranean to Central Europe were first discussed by Andersen (1905, 1907), who distinguished two subspecies at two edges of this gradient: *R. h. hipposideros* in the north and *R. h. minimus* in the south. However, this conception was revised when Saint Girons and Caubère (1966) and Felten *et al*. (1977) demonstrated a cline changes in metric traits, although Miller (1912) had already considered it to be rather dubious. Our results also give no support for such type of taxonomic division. Already Andersen (1918) demonstrated the mosaic-like distribution of morphological characters among populations of *R. hipposideros* s.str. in Europe and the Mediterranean and suggested the existence of six separate taxa within this species in the area between Morocco and Ireland in the west and Turkey and Cyprus in the east. This character distribution was again evaluated by Felten *et al*. (1977), who did not support such division and rather suggested only one, nominotypical subspecies existed in the whole area (except for Crete and Sicily). The echolocation data (another type of evidence) also showed a certain character plasticity within *R. hipposideros* populations in the Mediterranean area; bats living on the islands of Sardinia and Malta exhibited much higher values of call frequencies (up to 117 kHz on average — Russo *et al*., 2007; Mifsud and Vella, 2019) than the bats on the European continent. However, these insular bats represent an inner part of the western lineage of *R. hipposideros* s.str. and do not exhibit any substantial genetic differences from other populations of the lineage (Dool *et al*., 2013).

The documented pattern of morphological and morphometrical variability in *R. hipposideros* s.str. does not help when evaluating phylogenetic relationships among examined populations and the echolocation data show similar relevance when assessing the intraspecific variations in this bat species. Therefore, the results of the molecular genetic analysis remain the only evidence that support the reconstruction of the phylogenetic relationships within this species enough. Splitting the species content into two sublineages for both the nuclear and the mitochondrial genomes represents a well-detected separation event. Therefore, the sublineages could be co-identified with two subspecies. In both sublineages, similar levels of plasticity in morphological characters and similar character diversities in echolocation parameters were ascertained.

The taxonomic affiliation of the western sublineage that occurs throughout most of Europe and in the Maghreb is clear. The species is described from Germany (André, 1797) and therefore, this sublineage must to be identified with the nominotypical subspecies. The majority of the available names for *R. hipposideros* were proposed based on specimens from European type localities, situated in the contemporary countries of Spain, England, Germany, France, Corsica (France), Switzerland, Austria, Czech Republic, and Romania (*minor* Geoffroy, 1803, *minutus* Montagu, 1808, *bihastatus* Geoffroy, 1813, *bifer* de Blainville, 1840, *alpinus* Koch, 1865, *pallidus* Koch, 1865, *typus* Koch, 1865, *kisnyiresiensis* Daday, 1885, *troglophilus* Daday, 1887, *helvetica* Bretscher, 1904, *phasma* Cabrera, 1904, *typicus* Andersen, 1905, *majori* Andersen, 1918, *anomalus* Söderlund, 1921, *intermedius* Söderlund, 1921, *moravicus* Kostroň, 1943). Therefore, all of them should be considered junior synonyms of the nominotypical subspecies, *R. hipposideros hipposideros*. Two additional names were created based on bats from Morocco: *escalerae* Andersen, 1918 and *vespa* Laurent, 1937. Since the Maghrebian populations are a part of the western sublineage, these two names belong among junior synonyms of *R. h. hipposideros*. As summarised in Introduction, in the distribution range of the western sublineage (= *R. h. hipposideros*), up to six different subspecies were reported to occur (*escalerae*, *hipposideros*, *majori*, *minutus*, *minimus*, *vespa* — see Andersen, 1918; Ellerman and Morrison-Scott, 1951; Koopman, 1994; Csorba *et al*., 2003; Simmons, 2005; Burgin, 2019). However, this arrangement is rejected here because we did not find supporting evidence for it in our results, similar to the results by Dool *et al*. (2013).

The eastern sublineage of *R. hipposideros* s.str. is distributed in the Asian range of the species, including the Levant, Asia Minor (including adjacent islands), Crimea, and Iran (except for the Persian Gulf coastal areas). The affiliations of the populations from the eastern parts of the species distribution range (i.e., West Turkestan, Afghanistan, Kashmir) to this sublineage has not been fully resolved. Traditional taxonomic views divided this range into two parts according to the body size: the small-sized Levantine and Turkish populations were assigned to the Mediterranean taxon *R. h. minimus* (Ellerman and Morrison-Scott, 1951; Harrison, 1964; Koopman, 1994; Csorba *et al*., 2003; Burgin, 2019) or *R. h. hipposideros* (Felten *et al*., 1977; Corbet, 1978), whereas the large-sized eastern populations were assigned to *R. h. midas* (Andersen, 1905, 1918; Ellerman and Morrison-Scott, 1951; Harrison, 1964; Corbet, 1978; DeBlase, 1980; Harrison and Bates, 1991; Koopman, 1994; Horáček *et al*., 2000; Csorba *et al*., 2003; Benda *et al*., 2012; Burgin, 2019; see also Benda *et al*. (2006) for a more detailed review). However, our results do not support such west-east separation within the eastern sublineage (see also Dool *et al*., 2013).

As we demonstrated above, the name *midas* Andersen, 1905 is unavailable for designation of the Middle Eastern populations of *R. hipposideros* s.str. because this name is assigned to a different species. Interestingly, in contrast to the western part of the species range of *R. hipposideros* with 19 available names (see above), no synonym of this species name is currently available based on the material from Asia. However, the single Ethiopian sample examined in our analysis was shown to be a part of the eastern sublineage and it originates from the Yohannis Maikudi Church (13°51'N, 39°27'E) at Degum, Tigray State, approximately 240 km south–south-east of Keren, Eritrea, the type locality of *R. minimus* von Heuglin, 1861. Therefore, our Ethiopian sample could serve as a reference for topotype population of the latter name, which could be used as *R. h. minimus* for the eastern sublineage. This name was originally attributed to a separate species by von Heuglin (1861), but was rather early included into the species rank of *R. hipposideros* by Peters (1871). Andersen (1905, 1907, 1918) used this name for the small-sized Mediterranean populations of the species, but this conception was later questioned (Grulich, 1949; Saint Girons and Caubère, 1966; Felten *et al*., 1977; Corbet, 1978; Palmeirim, 1990; Benda *et al*., 2012) and is not supported by our results or the results by Dool *et al*. (2013). Thus, we consider the name *minimus* von Heuglin, 1861 to be unavailable for the European and/or Maghrebian populations of *R. hipposideros* s.str., although numerous recent authors applied this name in a way identical to Andersen's (1905) view (Koopman, 1994; Horáček *et al*., 2000; Roer and Schober, 2001; Csorba *et al*., 2003; Simmons, 2005; Burgin, 2019).

As already indicated, the populations of *R. hipposideros* s.str. that occur in the high mountains of the eastern margin of the species distribution range (Tian Shan, Pamir-Alai, Pamir, Hindu Kush, Karakoram) have an unresolved systematic position because only one specimen was examined for both types of genetic markers. These populations could be a part of the eastern sublineage (*R. h. minimus*), which is supported by the results of

our mitochondrial marker analysis (contra Dool *et al*., 2013). Alternatively, they could pertain to a separate lineage of the species, as the nuclear markers show (again, contra Dool *et al*., 2013), and could represent a taxon of their own. In that case, no name would be available for such taxon/populations and it remains to be created (cf. Bates and Harrison, 1997; Csorba *et al*., 2003).

Samples of two populations of *R. hipposideros*, from the Caucasus region and from the south-western part of Arabia, which are important from a biogeographical point of view, were not included in our analysis. Harrison and Bates (1991) identified *R. h. minimus* in the latter region; whereas from the Caucasus, two forms were reported diversely, *R. h. hipposideros* (Ognev, 1927; Strelkov, 1963; Kuzâkin, 1965; Koopman, 1994; Roer and Schober, 2001) or *R. h. midas* (Horáček *et al*., 2000; Csorba *et al*., 2003; Rahmatulina, 2005). However, based on the available data, we can estimate that both populations are affiliated with the eastern sublineage. The Caucasus region is situated in a space bordered by Crimea in the north-west and Iran in the south-east. In both of these border regions, the eastern sublineage was detected. Similarly, the south-western region of Arabia is bordered by the Levant in the north and the Ethiopian Highlands in the south, where the eastern sublineage was also detected. Therefore, it is most probable that these two populations belong to *R. h. minimus*.

Two additional names appeared in literature among synonyms of *R. hipposideros* (see e.g., Corbet, 1978, 1984; Csorba *et al*., 2003; Simmons, 2005) — *eggenhoeffner* Fitzinger, 1870 and *billanjani* DeBlase, 1972. However, the revisions of original sources showed both names unavailable for zoological nomenclature, being manuscript names (see Fitzinger, 1870; Miller, 1912; DeBlase, 1972, 1980; Benda *et al*., 2012).

To conclude, the revised taxonomic arrangement of the *R. hipposideros* group differs greatly from the most frequently presented views in recent years (see Csorba *et al*., 2003; Simmons, 2005; Burgin, 2019). The group consists of two species: *R. hipposideros*, which is widespread over south-western Eurasia and north-western and north-eastern Africa, and *R. midas*, which is distributed in a small range around the Strait of Hormuz and Gulf of Oman. These two species differ from each other in their morphological, genetic, and echolocation parameters. The extensive range of *R. hipposideros* s.str. is at least inhabited by two subspecies: *R. h. hipposideros* in the Maghreb and in Europe, west of the Dnieper River (cf. Zagorodniuk, 1999), Bosporus, and the Strait of Karpathos, and *R. h. minimus* east of this boundary, including the populations of Crimea, (Caucasus), the Middle East and north-eastern Africa (Sudan, Eritrea, Djibouti, Ethiopia). Besides genetic traits, these two subspecies also differ from each other in karyotype: 2n = 58 was found in *R. h. minimus*, and 2n = 54–56 was found in *R. h. hipposideros*. However, no significant morphological differences were found between the two subspecies of *R. hipposideros*.

Supplementary Information

Contents: Supplementary Figures: Fig. S1. Maximum likelihood tree of reconstructed phylogenetic relationships of the *R. hipposideros* group based on as complete as possible cytochrome-*b* dataset (1,103 bp). Branch support values are shown at the nodes; Fig. S2. Maximum likelihood tree of the reconstructed phylogenetic relationships of the *R. hipposideros* group and selected species of the genus *Rhinolophus* based on *ACOX*. Branch support values are shown above/below the branches in order SH-aLRT/UFBoot; Fig. S3. Maximum likelihood tree of the reconstructed phylogenetic relationships of the *R. hipposideros* group and selected species of the genus *Rhinolophus* based on *BGN*. Branch support values are shown above/below the branches in order SH-aLRT/UFBoot; Fig. S4. Maximum likelihood tree of the reconstructed phylogenetic relationships of the *R. hipposideros* group and selected species of the genus *Rhinolophus* based on *COPS*. Branch support values are shown above/below the branches in order SH-aLRT/UFBoot; Fig. S5. Maximum likelihood tree of the reconstructed phylogenetic relationships of the *R. hipposideros* group and selected species of the genus *Rhinolophus* based on *ROGDI*. Branch support values are shown above/below the branches in order SH-aLRT/UFBoot; Fig. S6. Maximum likelihood tree of the reconstructed phylogenetic relationships of the *R. hipposideros* group and selected species of the genus *Rhinolophus* based on *STAT*. Branch support values are shown above/below the branches in order SH-aLRT/ UFBoot; Fig. S7. Chronogram of the family Rhinolophidae based on a Bayesian inference of the nuclear dataset (according to the model by Dool *et al*., 2016). The numbers at nodes show mean divergence time estimates (Ma) and horizontal boxes 95% highest posterior density intervals of these estimates. The asterisk (*) indicates nodes with low branch support, the rest of the nodes were supported (*PP* ≥ 0.95); Fig. S8. Bivariate plot of skull dimensions of the examined samples of the *R. hipposideros* group: results of the canonical discriminant analysis of selected nine plain and seven relative dimensions (see Results for details). Supplementary Tables: Table S1. A) Original sequences and sequences from GenBank used in the molecular genetic analysis; B) Sequences with the total length of 1103 bp from GenBank used for the *R. hipposideros* tree, see Supplementary Fig. S1; Table S2. Names, sequences, and annealing temperatures of primers used in this study; Table S3. Substitution models as identified by ModelFinder for the different partitions used in MrBayes and IQTREE, respectively; Table S4. Summary of BPP for the nuclear dataset. Values for BPP species are posterior probabilities (*PP*) of delimitation from BPP runs under each of four different schemes under two different algorithms (see Table 1 in Demos

et al., 2019); Table S5. Relative cranial dimensions of the examined sample sets of the *R. hipposideros* group; *midas*, *escalerae* = dimensions of the respective type specimens; for the sample set delimitations and dimension abbreviations see Materials and Methods; Table S6. Relative dental dimensions of the examined sample sets of the *R. hipposideros* group; *midas* = dimensions of the respective type specimen; for the sample set delimitations and dimension abbreviations see Materials and Methods; Table S7. Results of the one-way ANOVA test of skull dimensions between particular sample sets; for the sample set delimitations and abbreviations and for dimension abbreviations see Materials and Methods.

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Appendix I

List of the specimens examined in the morphological analysis; an asterisk (*) denote specimens used also in the molecular genetic analysis. Collection abbrevitations: AUB = American University Beirut, Lebanon; BMNH = Natural History Museum, London, United Kingdom; CUP = Department of Zoology, Charles University, Prague, Czech Republic; ISEA = Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Kraków, Poland; IVB = Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno, Czech Republic; MHNG = Natural History Museum, Geneva, Switzerland; MNHN = National Museum of Natural History, Paris, France; MSNG = Civil Natural History Museum Giacomo Doria, Genoa, Italy; MZLU = Museum of Zoology and Entomology, Lund University, Sweden; NMNHS = National Museum of Natural History, Sofia, Bulgaria; NMP = National Museum (Natural History), Prague, Czech Republic; NMW = Natural History Museum, Vienna, Austria; OHC = Otto von Helversen Collection, Erlangen, Germany; SMF = Senckenberg Museum and Research Institute, Frankfurt am Main, Germany; ZMMU = Zoological Museum, Moscow State University, Moscow, Russia

Afghanistan: 1 ♀ (IVB af547 [S+B]), Abdukil at Shigi, cave above the Kunar river, 1 April 1967, leg. J. Gaisler, D. Povolný, Z. Šebek and F. Tenora; — 1 ♀ (SMF 39214 [S+A]), Barg-i-Matal, Konar, 2010 m, 21 July 1964, leg. D. Meyer-Oehme; — 1S+A]), Dahan Ghar, Wardak, Höhle, 2020 m, 12 March 1965, leg. D. Meyer-Oehme; — 1 ♂ (MZLU L58/3277, L58/3321 [S+A]), Grotte Boulan, 9 April 1958, leg. K. Lindberg; — 2 ♂♂ (IVB af1388 [B], af1389 [S+B]), Jalal Abad, hotel, attic, 19 February 1965, leg. D. Povolný and F. Tenora; — 2 ♂♂ (SMF 39217 [S+A], 39218 [A]), Jalalabad, Nangarhar,

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650 m, 3 August 1965, leg. D. Meyer-Oehme; — 1 ♂ (IVB af1057 [S+B]), Lalanda, Lalanda cave, 20 km S of Kabul, 12 May 1967, leg. J. Gaisler, D. Povolný, Z. Šebek and F. Tenora; — 1 ♀ (IVB af573 [S+B]), Sarobi, cave above the Sarobi–Kabul road, 5 April 1967, leg. J. Gaisler, D. Povolný, Z. Šebek and F. Tenora; — 1 ♂, 1 ♀ (SMF 39213, 39215 [S+A]), Tscharasiaw, Logar, 1850 m, 23 September 1963, 2 October 1963, leg. D. Meyer-Oehme.

Albania: 1 ♂ (NMP 96541 [S+A*]), Gjirokastër, castle, 27 January 2016, leg. F. Bego, P. Benda and M. Uhrin; — 1 ♂ (NMP 96536 [S+A*]), Gollomboç, Hermit Cave, 25 January 2016, leg. F. Bego, P. Benda and M. Uhrin; — 1 ♂ (NMP 96531 [S+A*]), Tren, Treni Cave, 25 January 2016, leg. F. Bego, P. Benda and M. Uhrin; — 1 ♂ (NMP 96551 [A*]), Vithkuq, chapel crypt, 27 June 2016, leg. P. Benda and M. Uhrin.

Algeria: 1 ♂ (ISEA 9586 [S+B]), 20 km NW of Sebdou, 6 November 1981, leg. K. Kowalski and B. Rzebik-Kowalska; — 2 ♂♂ (ISEA 9584, 9585 [S+B]), Brezina, cave, 31 October 1981, leg. K. Kowalski and B. Rzebik-Kowalska; — 1 ♂ (IVB A204 [S+B]), Gorges de Kherrata, tunnel, 15 January 1982, leg. J. Gaisler; — 1 ♂ (ISEA 9587 [S+B]), Misserghin, 14 December 1982, leg. K. Kowalski and B. Rzebik-Kowalska; — 1 ♂, 1 ♀ (ISEA 9588, 9664 [S+B]), Sig, 4 January 1983, 25 January 1983, leg. K. Kowalski and B. Rzebik-Kowalska; — 1 ♂ (IVB A237 [S+B]), Sebdou, 1 May 1982, leg. J. Gaisler.

Azerbaijan: 1 ♀ (NMP 91697 [S+B]), Suçma, Şəki District, 25 April 1976, leg. I. Rakhmatulina.

Bulgaria: 2 ♂♂ (NMP 49788, 49789 [S+A]), Âgodina, Gorna Karanska dupka Cave, 16 August 1978, leg. P. Donát, J. Flegr, J. Janda and V. Vohralík; — 5 ♂♂, 1 ♀ (NMP 49780–49786 [S+A]), Âgodina, Imamova dupka Cave, 15 August 1978, leg. P. Donát, J. Flegr, J. Janda and V. Vohralík; — 1 ♀ (NMP 49807 [S+A]), Bačkovo, cave, 30 July 1979, leg. D. Holečková, P. Donát, I. Horáček, J. Jirouš and V. Vohralík; — 2 ♂♂ (NMP 49434, 49435 [S+A]), Bačkovo, Bačkovski Monastery, 14 July 1976, leg. M. Braniš, V. Hanák, I. Horáček, K. Hůrka, J. Jirouš, V. Švihla and V. Vohralík; — 1 ♀ (NMNHS unnum. [S]), Borovo, 19 March 1968, leg. P. Beron; — 2 ♂♂, 3 ♀♀ (NMP 50091–50095 [S+B]), Brestnica, Saeva dupka Cave, 8 February 1965, leg. J. Figala, J. Gaisler, V. Hanák and K. Hůrka; — 1 ♂ (NMP 49433 [S+A]), Čepelare, 13 July 1976, leg. M. Braniš, V. Hanák, I. Horáček, K. Hůrka, J. Jirouš, V. Švihla and V. Vohralík; — 1 ♂ (NMNHS unnum. [S]), Filipovci, 27 February 1967, leg. P. Beron; — 1 ind. (NMNHS unnum. [S]), Ginci, Tošova dupka Cave, 17 February 1968, leg. P. Beron; — 5 ♀♀ (NMP 50027–50031 [S+A]), Gorna Breznica, 24 July 1981, leg. J. Flousek, R. Fuchs and V. Vohralík; — 2 ♂♂, 1 ♀ (NMP 49354, 49758, 49777 [S+A]), Karlukovo, 5 July 1976, 8 August 1978, 9 August 1978, leg. M. Braniš, P. Donát, J. Flegr, V. Hanák, I. Horáček, K. Hůrka, J. Janda, J. Jirouš, V. Švihla and V. Vohralík; — 1 ♂ (NMP 50080 [S+B]), Karlukovo, Bankova peŝera Cave, 7 February 1965, leg. J. Figala, J. Gaisler, V. Hanák and K. Hůrka; — 1 ♂ (NMP 49753 [S+A]), Karlukovo, Temnata dupka Cave, 7 August 1978, leg. P. Donát, J. Flegr, J. Janda and V. Vohralík; — 5 ♂♂ (NMP 49793–49797 [S+A]), Kotel, 15 July 1979, leg. D. Holečková, P. Donát, I. Horáček, J. Jirouš and V. Vohralík; — 1 ind. (NMNHS N12 [S]), Kričim, date unlisted, leg. I. Bureš; — 2 ♂♂ (NMP 50136, 50137 [S+B]), Lakatnik, Svinskata peŝera Cave, 19 March 1956, collector unlisted; — 1 ind. (NMP 49813 [S+B]), Lakatnik, Temnata dupka Cave, 3 January 1962, leg. J. Sklenář; — 1 ♂, 4 ♀♀ (NMP 49368–49372 [S+A], Lilânovo, 9 July 1976, leg. M. Braniš, V. Hanák, I. Horáček, K. Hůrka, J. Jirouš, V. Švihla and V. Vohralík; — 5 ♂♂, 2 ♀♀ (NMP 49997–50003 [S+A]), Orehovo, 30 August 1980, leg. D. Holečková, J. Jirouš, H. Prágerová and V. Vohralík; — 1 ♀ (NMNHS 739 [S]), Pepelina, Orlova čuka Cave, February 1961, leg. I. Ivanov; — 1 ♀ (IVB 398 [S+B]), Peŝera, Lilova skala Cave, 3 February 1965, leg. J. Figala, J. Gaisler, V. Hanák and K. Hůrka; — 1 ♂ (NMP 50072 [S+B]), Peŝera, Nova peŝera Cave, 4 February 1965, leg. J. Figala, J. Gaisler, V. Hanák and K. Hůrka; — 1 ♂ (NMP 50076 [S+B]), Peŝera, Snežânka Cave, 5 February 1965, leg. J. Figala, J. Gaisler, V. Hanák and K. Hůrka; — 1 ♀ (NMP 49347 [S+B]), Ropotamo, 6 June 1957, leg. V. Hanák; — 2 ♂♂ (NMNHS N63, unnum. [S]), Studen Kladenec, mine, 3 May 1996, leg. T. Ivanova; — 1 ♂ (NMNHS unnum. [S]), Treklâno, date and collector unlisted; — 3 ♂♂, 1 ♀ (NMNHS unnum. [S]), Urvič, 8 April 1971, leg. V. Beškov.

Croatia: 1 ♂, 1 ♀ (NMP 96815 [S+A], 96816 [A]), Pokrovnik, Škarin Samograd Cave, 5 September 1977, leg. J. Červený and J. Kučera.

Cyprus: 1 ♂ (NMP 97092 [S+A]), Afendrika, Panagia Hrysiotissa, cave, 21 January 2018, leg. P. Benda and M. Uhrin; — 2 ♀♀ (MSNG 44488 [A]), Akantu (Cipro), 12 January 1899, leg. Cecconi; — 1 ♂ (NMP 97121 [S+A]), Alevkaya, Küpö Cave, 2 October 2018, leg. P. Benda and M. Uhrin; — 2 ♂♂ (NMP 90424, 91269 [S+A*]), Cinarli, Inçirli Cave, 6 April 2005, 17 April 2005, leg. P. Benda, V. Hanák, I. Horáček, P. Hulva and R. Lučan; — 4 ♂♂, 2 ♀♀ (NMP 90923–90928 [S+A*]), Troodos Forest, valley south of Kakopetria, mine, 27 July 2006, leg. P. Benda.

Czech Republic: 1 ♀ (NMP 343/64 [S]), Jílové u Prahy, 30 October 1964, leg. V. Hanák; – 1 ♂ (NMP E7 [S]), Karlštejn, mine, 15 February 1957, leg. V. Hanák; — 1 ♀ (NMP 155/62 [B]), Lednice, 9 June 1962, leg. V. Hanák; — 2 ♂♂, 1 ♀ (NMP 341/58, 347/58, 348/58 [S]), Mníšek pod Brdy, 8 March 1958, leg. J. Sklenář; — 1 ♀ (NMP 422/59 [S]), Svitavy, 4 March 1959, leg. Stach; — 1 ♂ (NMP ZN17 [S]), Vranov nad Dyjí, castle attic, 31 July 1957, leg. V. Hanák; — 1 ♂, 1 ind. (NMP ZB11, ZB12 [S]), Zbraslav, 1 December 1956, leg. V. Hanák; — 2 ♀♀ (NMP ZN26, ZN27 [S]), Znojmo, castle attic, 3 August 1957, leg. V. Hanák.

Ethiopia: 1 ♀ (NMP 95890 [S+A*]), Degum, Yohannis Maikudi Church, 31 October 2012, leg. P. Benda.

Greece: 1 ♂, 6 ♀♀ (NMP 48710–48715, 49028 [S+A*]), Kompotades, bunker, 9 September 1996, 10 September 1996, 31 August 2001, leg. M. Andreas, P. Benda and M. Uhrin; — 1 ♂ (NMP 92303 [A]), Krītī, Avdoy, Agios Fōteinīs Cave, 10 October 2007, leg. P. Benda; — 2 ♂♂, 1 ♀ (NMP 91193, 91194 [S+A*], 92292 [A*]), Krītī, Gerani, Geranioy Cave, 6 October 2006, 8 October 2007, leg. P. Benda, V. Hanák and P. Hulva; — 1 ♂ (NMP 92320 [S+A*]), Krītī, Kritsa, Gaidoyrotrypa Cave, 14 October 2007, leg. P. Benda; — 1 ♂, 1 ♀ (NMP 91197, 91198 [S+A*]), Krītī, Milatos, Milatoy Cave, 7 October 2006, leg. P. Benda, V. Hanák and P. Hulva; — 1 ♂ (NMP 92290 [A*]), Krītī, Ploytī, Mikrī Lavyrinthos Cave, 7 October 2007, leg. P. Benda; — 1 ♂ (NMP 92317 [S+A*]), Krītī, Sitanos, Exō Latsidi Cave, 13 October 2007, leg. P. Benda; — 2 ♂♂, 2 ♀♀ (NMP 92297–92300 [S+A*]), Krītī, Theriso, Sarakinas Cave, 8 October 2007, leg. P. Benda; — 1 ♀ (NMP 48643 [S+B]), Marōneia, Kyklōpa Cave, 19 June 1989, leg. R. Chaloupka, V. Hanák and V. Vohralík; — 1 ♂ (NMP 96614 [S+A*]), Rodos, Agios Paylos, 16 August 2012, leg. P. Benda; — 3 ♀♀ (NMP 96615, 96616 [S+A*], 96617 [A*]), Rodos, Gadoyra Dam, hut, 17 August 2012, leg. P. Benda.

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Iran: 1 ♂ (NMP 94427 [A]), Assalem, 3 October 2002, leg. P. Hulva; — 1 ♀ (MHNG 1905.3 [A]), Bouchir, Brazjan, June 1968, leg. A. Arata; — 3 ♂♂ (NMP 48096, 48097, 48439 [S+A*]), Emamzadeh (Esfahan Prov.), 1 May 1997, 6 April 2000, leg. P. Benda and A. Reiter; – 1 ♀ (BMNH 94.11.16.1 [S], holotype of *Rhinolophus midas* Andersen, 1905), Jask, Persian Gulf, date and collector unlisted; — 1 ♂ (NMP 39588 [A]), Karaj River valley, 1934, leg. Kargl; — 1 ind. (NMP 93858 [S+Sk]), Moghan Cave, October 1999, leg. K. Faizolahi; — 1 ♀ (NMP 48117 [S+A*]), Nosrat Abad, 7 May 1997, leg. P. Benda; — 1 ♂ (NMW 21008 [S+A]), Schiras, 1894, leg. B. Wagschal.

Jordan: 1 ind. (NMP 92842 [S+Sk]), Bait Idis, Jesus' Cave, 15 July 2010, leg. P. Benda and A. Reiter; — 2 ♀♀ (NMP 92409, 92410 [S+A*]), Dibbin, Dibbin Forest, underground corridor, 27 October 2008, leg. P. Benda and J. Obuch; — 1 ♂, 2 ♀♀ (NMP 92508–92510 [S+A*]), Zubiya, Zubiya Cave, 24 May 2009, leg. P. Benda and A. Reiter.

Kirghizstan: 1 ♀ (NMP 58323 [S+A]), Kyzyl-Kiâk, cave, 30 June 1988, leg. J. Červený and J. Obuch; — 1 ♀ (NMP 58324/2 [S+A]), Toâ-Moûn, Kolodec Fersmana mine, 12 July 1988, leg. J. Červený and J. Obuch.

Kosovo: 1 ♂ (NMP 96803 [S+A]), Bubël, cave, 27 October 2001, leg. P. Benda.

Lebanon: 3 ♂♂ (NMP 91806, 93709 [S+A*], 91807 [A*]), Aamchit, Saleh Cave, 28 January 2007, 25 March 2009, leg. T. Bartonička, P. Benda, R. Černý, I. Horáček and R. Lučan; — 1 ♂, 1 ♀ (NMP 93552 [S+A*], 93553 [A*]), Aanjar, Aanjar Cave, 5 June 2010, leg. P. Benda and M. Uhrin; — 1 ♀ (NMP 91782 [S+A]), Afqa Cave, 22 January 2007, leg. P. Benda, R. Černý, I. Horáček and R. Lučan; — 1 ♂ (NMP 91798 [S+A*]), Antelias, Kenaan Cave, 25 January 2007, leg. P. Benda, R. Černý, I. Horáček and R. Lučan; — 1 ♂ (AUB M170 [B]), Beit ed Dine, tunnel under building, 7 September 1960, leg. J. E. Stencel; — 1 ♀ (NMP 93711 [A*]), Dahr El Mghara, Aaonamie Cave, 28 March 2009, leg. T. Bartonička, P. Benda, I. Horáček and R. Lučan; — 1 ♂ (NMP 91775 [S+A*]), Er Roueiss Cave, 22 January 2007, leg. P. Benda, R. Černý, I. Horáček and R. Lučan; — 1 ♂ (NMP 91801 [A*]), Faraya, El Qana Cave, 27 January 2007, leg. P. Benda, R. Černý, I. Horáček and R. Lučan; — 2 ♂♂ (NMP 93537, 93538 [S+A*]), Faraya, Raymond Cave, 2 June 2010, leg. P. Benda and M. Uhrin; — 1 ♂, 1 ♀ (NMP 91769 [A*], 91770 [S+A]), Haqel El Azime, Achou Cave, 21 January 2007, leg. P. Benda, R. Černý, I. Horáček and R. Lučan; — 2 ♂♂ (NMP 91802 [A*], 91906 [S+A*]), Hrajel, Seraaya Cave, 27 January 2007, 20 January 2008, leg. P. Benda, R. Černý, I. Horáček, R. Lučan and M. Uhrin; — 1 ♀ (NMP 95792 [S+A*]), Jezzine, Pont El Khalass, 23 June 2006, leg. I. Horáček, P. Hulva, R. Lučan and P. Němec; — 3 ♂♂, 1 ♀ (NMP 91753–91755 [S+A*], 91756 [A*]), Marjaba, mine, 19 January 2007, leg. P. Benda, R. Černý, I. Horáček and R. Lučan; — 1 ♂ (NMP 91809 [S+A*]), Nabaa Es Safa, mine, 29 January 2007, leg. P. Benda, R. Černý, I. Horáček and R. Lučan; — 1 ♂, 1 ♀ (NMP 91789, 91790 [S+A*]), Qadisha Cave, 23 January 2007, leg. P. Benda, R. Černý, I. Horáček and R. Lučan; — 1 ♀ (NMP 93577 [S+A*]), Seraal, 10 June 2010, leg. P. Benda and M. Uhrin; — 1 ♂ (NMP 91786 [S+A]), Tourzaiya, Mebaaj Cave, 23 January 2007, leg. P. Benda, R. Černý, I. Horáček and R. Lučan; — 1 ♀ (NMP 93706 [S+A*]), Wadi Jilo, 22 March 2009, leg. T. Bartonička, P. Benda, I. Horáček and R. Lučan.

Morocco: 1 ♀ (NMP 93602 [S+A*]), Gorges du Dadès, Aït-Ali, 7 October 2010, leg. P. Benda, A. Reiter, M. Ševčík and M. Uhrin; — 1 ind. (BMNH 10.11.24.2. [S], holotype of *Rhinolophus hipposideros escalerae* Andersen, 1918), Mogador, date and collector unlisted; — 2 ♀♀ (NMP 94519, 94520 [S+A*]), Takoumit, small cave, 26 April 2008, leg. P. Benda, J. Červený, A. Konečný and P. Vallo.

North Macedonia: 1 ♂ (NMP 96847 [S+A]), north-eastern bank of the Ohrid Lake, 10 July 1977, leg. V. Tauber.

Oman: 2 ♀♀ (NMP 93717 [S+A*], 93718 [A*]), Bani Habib, house, 28 March 2011, leg. P. Benda, A. Reiter and M. Uhrin; — 1 ♂ (NMP 93782 [S+A*]), Misfah, mosque, 9 April 2011, leg. P. Benda, A. Reiter and M. Uhrin; — 1 ♀ (NMP 93994 [S+A*]), Sal Alah, Birkat Khaldiyah, cistern, 13 March 2012, leg. P. Benda, A. Reiter and M. Uhrin.

Serbia: 1 ♂ (NMP 38955 [S+B]), Petnica, 23 May 1969, leg. J. Hanzák; — 1 ind. (NMP 96856 [S+B]), Serbia (undef.), May 1969, leg. J. Hanzák.

Slovakia: 5 ♂♂ (NMP 118/58, 121–123/58, 125/58, 130/58 [S]), Ardovo, Ardovská Cave, 5 February 1958, leg. V. Hanák; — 2 ♂♂ (NMP 84/63 [S+B], 85/63 [S]), Červený Kláštor, Aksamitka, 2 March 1963, leg. V. Hanák; — 1 ♂, 1 ♀ (NMP 101/58, 102/58 [S]), Domica, Čertova diera Cave, 5 February 1958, leg. V. Hanák; — 1 ♀ (NMP 7712/1957 [S+B]), Domica, Domica Cave, 24 August 1957, leg. J. Hanzák; — 5 ♂♂, 1 ♀ (NMP 109/58–114/58 [S]), Domica, Liščia diera Cave, 5 February 1958, leg. V. Hanák; — 1 ♂ (NMP 154/58 [S]), Drienovec, cave, 6 February 1958, leg. V. Hanák; — 4 ♂♂, 2 ♀♀ (NMP J209–J213, J215 [S]), Gombasek, Ludmila Cave, 20 November 1955, 6 December 1956, 11 December 1956, leg. V. Hanák; — 1 ♂ (NMP 172/58 [S]), Hačava, Hačavská Cave, 7 February 1958, leg. V. Hanák; — 1 ♂ (NMP 7/69 [B]), Jasov, Jasovská Cave, 14 February 1969, leg. J. Gaisler; — 1 ♂, 2 ♀♀ (NMP J185–J187 [S]), Kečovo, mine, 10 December 1956, leg. V. Hanák; — 3 ♂♂, 10 ♀♀ (NMP 160/61, 163/61, 181/61, 193/61, 194/61, 198/61, 200/61, 202/61, 204–206/61, 211/61, 212/61 [S]), Tisovec, Jaskyňa Netopierov Cave, 15 May 1961, 16 February 1961, leg. V. Hanák.

Sudan: 1 ind. (BMNH 47.5.27.48 [S]), Sennar, date and collector unlisted.

Syria: 3 ♀♀ (NMP 48054 [S+A*], 48055, 48056 [A*]), Qala'at Salah Ad Din, ruins, 30 June 1998, leg. M. Andreas and M. Uhrin; — 1 ♀ (NMP 48979 [S+A]), Qanawat, house, 27 April 2001, leg. P. Munclinger and P. Nová.

Tajikistan: 1 ♀ (NMP 95742 [S+A*]), Zingrogh, small cave, 12 May 2016, leg. P. Benda, A. Reiter and M. Uhrin.

Turkey: 1 ♀ (NMW 11731 [S+B]), 5 km W Igneada, Vil. Kirklareli, 15 May 1967, leg. F. Spitzenberger; — 1 ♂ (NMW 24585 [S+B]), Apollohöhle 2 km W Ahmetbeyli, Vil. Izmir, 16 February 1969, leg. F. Spitzenberger; — 2 ♀♀ (NMW 34330, 34331 [S+B]), Efes, Vil. Izmir, 2 August 1984, leg. A. Mayer, F. Spitzenberger and E. Weiss; — 1 ♂, 1 ♀ (NMW 22236, 22237 [S+B]), Ephesus, Westküste, 12 August 1976, leg. P. Wolff; — 1 ♂ (NMW 24587 [S+B]), Höhle Icme Pinari bei Arak, Vil. Isparta, 1 March 1969, leg. F. Spitzenberger; — 1 ind. (SMF 92191 [S]), Höhle Karain (Schwarze Höhle) und Höhle Oküzini (Ochsenhöhle), 450 m, 37.08N, 30.20E, Rand des Taurus-Gebirge an der Ebene von Antalya, 30 km NWN von Antalya, Vil. Antalya, 1990–1994, leg. P. Lacroix; — 2 ♂♂ (NMW 24586, 24588 [S+B]), Höhlen NE Bornova, Vil. Izmir, 6 April 1969, leg. F. Spitzenberger; — 1 ♂ (NMW 13299 [S+A]), Maden köy, Vil. Nigde, 1 August 1970, leg. F. Spitzenberger; — 1 ♀ (CUP T93/63 [S+A]), Narlikuyu, 29 October 1993, leg. P. Benda and I. Horáček; — 3 ♀♀ (NMW 19313–19315 [S+A]), Nestorianische Kirche, Vil. Hakkari,

Appendix I. Continued

16 August 1973, leg. F. Spitzenberger; — 2 ♀♀ (NMP 90488, 90489 [S+A*]), Posyagbasan nr. Adana, 15 June 2003, leg. J. Hájek and J. Hotový; — 1 ♀ (NMW 20510 [B]), Rize, Vil. Rize, 14 July 1961, leg. M. Caglar; — 4 ♂♂ (MHNG 967.48, 967.49 [A], 967.50, 967.51 [S+A]), Satzmal magarasi, ouest de Sile, 29 April 1955, leg. H. Coiffat and P. Strinati; — 1 ♂ (MSNG 44534 [A]), Smirne, 1870, leg. G. Gonzenbach; — 1 ♂, 2 ♀♀ (CUP T93/65, T93/67, T93/68 [S+A]), Yalan Dünya Mağara Cave, 30 October 1993, leg. P. Benda and I. Horáček.

Turkmenistan: 1 ♂ (ZMMU S-169662 [A]), Aj-Derse, Kara-Kalpakskij District, May 1982, collector unlisted.

Ukraine: 1 ♀ (NMP pb4360 [S+A*]), Krym, General'skoe, 18 September 2009, leg. P. Benda, S. Gazarân and M. Uhrin; — 2 ♀♀ (NMP pb4287, pb4289 [S+A*]), Krym, Kujbyševo, 12 September 2009, leg. P. Benda, S. Gazarân and M. Uhrin; — 1 ♀ (NMP pb4342 [S+A*]), Krym, Partizanskoe, 16 September 2009, leg. P. Benda, S. Gazarân and M. Uhrin.

Uzbekistan: 1 ♂ (ZMMU S-13789 [S+B]), Nuratau, Pariš, 26 May 1934, leg. R. Meklenburcev.

Appendix II

Description of morphotypes (review of particular state conditions found in the examined sample sets)

Central Europe: body: large; skull: large in size, absolutely and relatively wide; braincase absolutely wide but relatively narrow, and absolutely and relatively high; tympanic bulla absolutely and relatively small; rostrum absolutely long but relatively short and narrow; teeth: upper canine (Cs) medium-sized; small upper premolar (P^2) large; large upper premolar (P^4) large, relatively narrow, and relatively medium-wide in its medial portion; first upper molar (M^1) large and relatively wide; third upper molar (M^3) small and relatively wide, very small in relation to M^1; lower canine (Ci) is large, large in relation to the first lower molar (Mi); first lower premolar (P_2) large, large in relation to the last lower premolar (P_4); last lower premolar (P_4) large; first lower molar (Mi) and the lower molar-row small.

West Mediterranean: body: small; skull: medium-sized in size, absolutely and relatively wide; braincase absolutely and relatively wide, and absolutely and relatively high; tympanic bulla absolutely and relatively small; rostrum absolutely short but relatively long, and relatively narrow; teeth: upper canine (Cs) small; small upper premolar (P^2) medium-sized; large upper premolar (P^4) medium-sized, relatively narrow, and relatively medium-wide in its medial portion; first upper molar (M^1) medium-sized and relatively medium-wide; third upper molar (M^3) small and relatively narrow, medium-sized in relation to M^1; lower canine (Ci) small, large in relation to the first lower molar (Mi); first lower premolar (P_2) large, large in relation to the last lower premolar (P_4); last lower premolar (P_4) small; first lower molar (Mi) and the lower molar-row small.

East Mediterranean: body: small; skull: small in size, absolutely narrow but relatively wide; braincase absolutely narrow but relatively wide, and absolutely and relatively high; tympanic bulla absolutely and relatively small; rostrum absolutely short but relatively long, and relatively narrow; teeth: upper canine (Cs) medium-sized; small upper premolar (P^2) medium-sized; large upper premolar (P^4) small and relatively wide, and relatively narrow in its medial portion; first upper molar (M^1) medium-sized and relatively medium-wide; third upper molar (M^3) small and relatively medium-wide, medium-sized in relation to M^1; lower canine (Ci) small, small in relation to the first lower molar (Mi); first lower premolar (P_2) large, large in relation to the last lower premolar (P_4); last lower premolar (P_4) small; first lower molar (M_1) and the lower molar-row small.

Central Asia: body: large; skull: medium-sized in size, absolutely and relatively wide; braincase absolutely and relatively narrow, and absolutely and relatively high; tympanic bulla absolutely and relatively small; rostrum absolutely and relatively long, and relatively medium-sized in width; teeth: upper canine (Cs) medium-sized; small upper premolar (P^2) medium-sized; large upper premolar (P^4) medium-sized and relatively wide, relatively narrow in its medial portion; first upper molar (M^1) large and relatively wide; third upper molar (M^3) large and relatively narrow, medium-sized in relation to M^1; lower canine (Ci) large, large in relation to the first lower molar; first lower premolar (P_2) medium-sized, large in relation to the last lower premolar (P_4); last lower premolar (P_4) large; first lower molar (Mi) and the lower molar-row large.

North-eastern Africa: body: medium-sized; skull: small in size, absolutely narrow but relatively wide; braincase absolutely and relatively wide, and absolutely and relatively low; tympanic bulla absolutely and relatively small; rostrum absolutely short but relatively long, and relatively medium-sized in width; teeth: upper canine (Cs) small; small upper premolar (P^2) medium-sized; large upper premolar (P^4) small and relatively narrow, relatively wide in its medial portion; first upper molar (M^1) small and relatively wide; third upper molar (M^3) small and relatively medium-wide, medium-sized in relation to M^1; lower canine (Ci) small, large in relation to the first lower molar (Mi); first lower premolar (P_2) medium-sized, large in relation to the last lower premolar (P_4); last lower premolar (P_4) small; first lower molar (Mi) and the lower molar-row small.

Oman: body: small; skull: small in size, absolutely and relatively narrow; braincase absolutely and relatively narrow, and absolutely and relatively low; tympanic bulla absolutely and relatively large; rostrum absolutely and relatively long, and relatively very wide; teeth: upper canine (Cs) large; small upper premolar (P^2) small; large upper premolar (P^4) large and relatively narrow, relatively medium-wide in its medial portion; first upper molar (M^1) medium-sized and relatively narrow; third upper molar (M^3) large and relatively narrow, very large in relation to M^1; lower canine (Ci) small, small in relation to the first lower molar (Mi); first lower premolar (P_2) small, very small in relation to the last lower premolar (P_4); last lower premolar (P_4) large; first lower molar (Mi) and the lower molar-row are large.

Paper 6: Phylogeny of the *Rhinolophus ferrumequinum* group

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Phylogenetic relationships among horseshoe bats within the *Rhinolophus ferrumequinum* group (Mammalia, Chiroptera)

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Rhinolophus ferrumequinum group phylogeny
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The horseshoe bats of the *Rhinolophus ferrumequinum* group form a well-defined lineage within the Afro-Palaearctic clade of the genus *Rhinolophus*. The group currently comprises four species widely distributed across the Palaearctic and Afrotropic regions: *R. bocharicus* (Central Asia), *R. clivosus* (from northern Africa and the Levant through Arabian Peninsula and eastern Africa to southern Africa), *R. ferrumequinum* (from western Europe and northern Africa through the Balkans and Middle East to Central Asia and India) and *R. nippon* (southern and central China, Korea, and Japan). The broad ranges and geographic variations within these species have led to the proposal of numerous subspecies. The phylogenetic relationships and intraspecific variation of the *R. ferrumequinum* group were investigated using a genetic approach. One mitochondrial marker and five nuclear markers were sequenced and supplemented with available sequences for all four species of the group. Our study revealed five major lineages within the *R. ferrumequinum* group, resulting in the recognition of four currently known species and identification of a new species. The prior name available for this lineage/species is *R. acrotis*. The relationships between the lineages varied depending on the chosen marker, leaving the interspecific relations within the *ferrumequinum* group unresolved. In addition, the results indicated that *R. clivosus* experienced historic introgression from northern Africa and the Levant whose mtDNA was replaced by that of *R. ferrumequinum*. Together, this study introduces a new *Rhinolophus* species, which increases the number of species in the *ferrumequinum* group to five.

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Introduction

The horseshoe bats, genus *Rhinolophus* Lacépède, 1799, is the only genus forming the family Rhinolophidae Gray, 1825. Nevertheless, the family Rhinolophidae is one of five bat families with over 100 described species, and the number of newly recognised species is further increasing (Burgin et al., 2018; Benda et al., 2022; Curran et al., 2022; Mammal Diversity Database, 2023; Simmons & Cirranello, 2023). These bats can only be found in the Old World, including Afrotropical, Palaearctic, Oriental, and Australian regions, with the highest species diversity occurring in the tropics (Csorba et al., 2003). The family Rhinolophidae is further categorised into five to six clades/subgenera within their distribution range, based on both molecular and morphologic data (Guillén-Servent et al., 2003; Zhou et al., 2009).

One of them, the Afro-Palaearctic clade (also known as the subgenus *Rhinolophus*) presently encompasses approximately one-third of the total species number (at least 35 out of 109 species; Demos et al., 2019; Benda et al., 2022; Curran et al., 2022). This clade can be further divided into seven species groups, with one of them being the *Rhinolophus ferrumequinum* group (Horáček et al., 2000; Dool et al., 2016). Previously, this species group included multiple species (Aellen & Brosset, 1968; Bogdanowicz, 1992; Horáček et al., 2000; Csorba et al., 2003), with the largest reported number of species within the group being 17–19 (Andersen, 1905; Tate & Archbold, 1939). However, recent opinions suggest that only 2–4 species belong to the group: *R. bocharicus* Kašenko and Akimov, 1917, *R. clivosus* Cretzschmar, 1828, *R. ferrumequinum* (Schreber, 1774), and *R.* (*f.*) *nippon* (Temminck, 1835) (Bogdanowicz, 1992; Csorba et al., 2003; Stoffberg et al., 2010; Sano et al., 2015; Dool et al., 2016; Burgin, 2019). Some authors put into this group also species with which they do not form monophyletic groupings in molecular genetic analyses, like *R. horaceki* Benda and Vallo, 2012 and *R. xinanzhongguoensis* Zhou Zhaomin, Guillén-Servent, Lim, Eger, Wang Yingxiang and Jiang Xuelong, 2009 (Zhou et al., 2009; Demos et al., 2019) or such species that were not studied by molecular approach – *R. hillorum* Koopman, 1989 and/or *R. sakejiensis* Cotterill, 2002. Here, we follow the grouping classification of Bogdanowicz (1992) and Csorba et al. (2003) that includes four species (according to current taxonomy *sensu* Burgin, 2019), *bocharicus*, *clivosus*, *ferrumequinum*, and *nippon*.

The first of these species, *R. bocharicus*, is the least explored species within the *ferrumequinum* group. To the best of our knowledge, this species was only studied once using molecular methods (Bailey et al., 2016). Morphologically, it closely resembles both *R. clivosus* and *R. ferrumequinum*, and some authors considered it a subspecies of either of these two species (Bobrinskij, 1925; Ognev, 1927; Aellen, 1959; Ognev, 1927; Bauer, 1963; Koopman, 1994). However, most of recent authors recognised *R. bocharicus* as a separate species (Hanák, 1969; Felten et al., 1977; Corbet & Hill, 1992; Horáček et al., 2000; Csorba et al., 2003; Simmons, 2005; Benda et al., 2012, etc.). Although molecular analysis confirmed that *R. bocharicus* is not a subspecies of *R. clivosus*, it did not clarify its phylogenetic position within the *ferrumequinum* group (Bailey et al., 2016). Distributed in a restricted area of Central Asia, from Turkmenistan and Kazakhstan to Kyrgyzstan and Afghanistan (Fig. 1; Csorba et al., 2003; Benda & Gaisler, 2015), *R. bocharicus* is considered a monotypic species (Simmons, 2005; Burgin, 2019).

On the contrary, extensive studies have been conducted on *R. clivosus* (e.g., Andersen, 1904; Koopman, 1966; Dulic & Mutere, 1974; Thomas, 1997; Benda & Vallo, 2012) and molecular genetic tools were employed on multiple occasions (Benda & Vallo, 2012; Stoffberg et al., 2012; Dool et al., 2016; Demos et al., 2019). *Rhinolophus clivosus* has a wide distribution across southern Algeria, the Sahara, the Levant, Arabian Peninsula, eastern Africa including the DR Congo, and southern Africa (Fig. 1; Burgin, 2019). Many subspecies can be distinguished within the species rank of *R. clivosus*, with their number ranging from five to ten (Koopman, 1994; Csorba et al., 2003; Simmons, 2005; Benda & Vallo, 2012; Benda et al., 2017; Birgin, 2019). However, the species rank for some subspecies was warranted (*R. bocharicus*, *R. hillorum*; Hanák, 1969; Cotterill, 2002), and even new species were distinguished in the populations formerly considered to belong to *R. clivosus* (*R.*

sakejiensis, *R. horaceki*, *R. damarensis*; Cotterill, 2002; Benda & Vallo, 2012; Jacobs et al., 2013). At present, about six subspecies are recognised: *clivosus* (including *brachygnathus* and *schwarzi*) from the Sahara, Levant, and Arabia; *socotranus* from Socotra; *acrotis* (incl. *andersoni*) from Sudan, Eritrea, Ethiopia, and Somalia; *keniensis* from Uganda, Kenya, and Tanzania; *augur* from Tanzania, Malawi, Zimbabwe, Botswana, and northern South Africa; and *zuluensis* from coastal South Africa (cf. Csorba et al., 2003, Simmons, 2005; Benda & Vallo, 2012; Benda et al., 2017; Burgin, 2019).

Further, *R. ferrumequinum* is arguably the most extensively studied species of the *ferrumequinum* group (e.g., Andersen, 1905; Strelkov, 1971; Kryštufek, 1993; Thomas, 1997; Benda et al., 2006, 2012; Jiang et al., 2019; Ikeda et al., 2020), including its presence in numerous molecular studies (Rossiter et al., 2007; Flanders et al., 2009, 2011; Stoffberg et al., 2010; Benda & Vallo, 2012; Koh et al., 2014; Dool et al., 2016; Demos et al., 2019). *Rhinolophus ferrumequinum* is the largest horseshoe bat in the western Palaearctic and it morphologically resembles *R. clivosus*. The main difference is in body size, as *R. clivosus* is substantially smaller in the northern parts of its range. However, *R. clivosus* can reach a similar body size to that of *R. ferrumequinum* in southern Africa (Csorba et al., 2003; Benda & Vallo, 2012). Widespread widely through the south of the Palaearctic, *R. ferrumequinum* is distributed from Great Britain and Morocco through central and southern Europe, Maghreb, the Middle East and Central Asia to India and Nepal (Fig. 1; Burgin, 2019). Throughout its range, numerous subspecies were described and synonymised over time (Andersen, 1905; Ellerman & Morrison-Scott, 1951; Sinha, 1973; Corbet, 1978; Ellerman & Morrison-Scott, 1951; Yoshiuki, 1989). Up to seven subspecies can be recognised by taxonomic reviews based on morphology (Thomas, 1997; Csorba et al., 2003; Simmons, 2005). Nevertheless, the distribution range of *R. ferrumequinum* was previously thought to extend further east into China and Japan. It was only through molecular studies that bats from southern and eastern China, Korea, and Japan formed a separate lineage from current *R. ferrumequinum* populations (Thomas, 1997; Rossiter et al., 2007; Flanders et al., 2009; Benda & Vallo, 2012). Based on these results, the rank of full species was assigned to the eastern Asian subspecies *R. f. nippon* (Benda & Vallo, 2012; Burgin, 2019; Ikeda et al., 2020), while *R. ferrumequinum* s.str. was considered a monotypic species (Benda et al., 2012).

As mentioned above, *R. nippon* was for a long time regarded as conspecific with *R. ferrumequinum* (Dobson, 1876; Andersen, 1905; Ellerman & Morrison-Scott, 1951; Corbet, 1978; Koopman, 1994; Simmons, 2005). Currently, *R. nippon* is generally acknowledged as a separate species (Sano, 2015; Burgin, 2019); this is supported by further genetic analyses (Koh et al., 2014; Ikeda & Motokawa, 2021) and morphologic examinations (Ikeda et al., 2020). However, some authors still consider the taxonomic status of *R. nippon* to be unresolved (see e.g., Ransome, 2020). The distribution range of *R. nippon* covers the area formerly assigned to the subspecies *R. f. nippon* s.str. and *R. f. korai* (i.e., *sensu* Csorba et al., 2003; Koh et al., 2014) in southern and eastern China, Korea, and Japan. Molecular studies have recovered two major lineages within this species, one from southern China and the other from eastern China, Korea, and Japan (Fig. 1; Flanders et al., 2009, 2011; Koh et al., 2014). Nevertheless, these lineages are not considered as subspecies. Therefore, *R. nippon* is currently recognised as a monotypic species and the intraspecific taxonomy remains unresolved (see Koh et al., 2014; Ikeda & Motokawa, 2021).

Clearly, the phylogenetic relationships within the *Rhinolophus ferrumequinum* group and the relative positions of individual species and populations have not yet been satisfactorily studied and explained. The reasons for this may vary between species but are generally attributed to the exclusive use of a morphologic approach only, the use of a limited number of samples or markers for genetic analyses or studying the respective species separately. Therefore, in this study, we aim to (1) reconstruct the interspecific relationships within the *ferrumequinum* group, (2) analyse and revise the intraspecific relationships and population structures of the species of the *ferrumequinum* group and (3) express their taxonomic implications. To achieve these objectives, we generated new multi-locus dataset for 179 specimens of the *R. ferrumequinum* species group covering a wide geographical area and combined it with already available data (Dool et al., 2016; Demos et al., 2019).

Material and Methods

Sampling

For the genetic analysis, we used muscle tissue samples from 179 specimens of the *Rhinolophus ferrumequinum* species group (122 specimens of *R. ferrumequinum*, 45 of *R. clivosus*, and twelve of *R. bocharicus*) from the collection of the National Museum, Prague, Czech Republic (NMP) to extract DNA (Supplementary Table S1). We supplemented this dataset with sequences from previous studies (Fig. 1; Benda & Vallo, 2012; Dool et al., 2016; Demos et al., 2019). As an outgroup, we added GenBank sequences of other *Rhinolophus* species (Benda et al., 2022; Dool et al., 2016; Demos et al., 2019) and sequences of three *Hipposideros* species from the sister family Hipposideridae (Teeling et al., 2005; Foley et al., 2015).See Supplementary Table S1 for details of sample origin and GenBank accession numbers.

Amplification and sequencing

The genomic DNA was extracted from the alcohol-preserved tissue samples using the Geneaid Genomic DNA Mini Kit. We targeted one mitochondrial marker (mtDNA), including 1133 bp of cytochrome b (*Cyt-b*), and five nuclear markers (nucDNA) consisting of 536 bp of acyl-coenzyme A oxidase 2 intron (*ACOX*), 614 bp of biglycan intron (*BGN*), 734 bp of COP9 signalsom subunit 7A intron (*COPS*), 493 bp of the rogdi atypical leucine zipper (*ROGDI*) and 525 bp of the signal transducer and activator of transcription 5A intron (*STAT*). The primers used for both PCR amplification and sequencing were specifically designed for the order Chiroptera and provided good amplification in previous studies (see e.g., Puechmaille et al., 2011; Salicini et al., 2011; Thong et al., 2012; Dool et al., 2016). Primer names, sequences and annealing temperatures are listed in Supplementary Table S2. The purified PCR products were Sanger-sequenced from both sides at Macrogen, Inc. (Amsterdam, The Netherlands).

Phylogenetic reconstruction

Sequences were edited and aligned using the MAFFT plugin (Katoh & Standley, 2013) in Geneious 11.0.5 (https://www.geneious.com), and subsequently manually edited and trimmed using Gblocks (Castresana, 2000). Heterozygous positions in the nucDNA markers were coded using IUPAC codes, and ambiguous positions or missing data were coded an 'N'. Indels were treated as gaps. Sequences of protein-coding markers were translated to amino acids to check for the presence of stop codons, which would indicate that pseudogenes were amplified. The two final datasets, mitochondrial and nuclear, were generated. The mitochondrial dataset contained *Cyt-b* sequences with a total length of 1133 bp. The nuclear dataset consisted of *ACOX*, *BGN*, *COPS*, *ROGDI*, and *STAT* sequences with a total length of 2902 bp. Then nuclear dataset was partitioned by gene.

Phylogenetic trees of mitochondrial and nuclear datasets were constructed using Bayesian inference (BI) and maximum likelihood (ML). The appropriate nucleotide substitution model for each partition was selected based on the Bayesian information criterion using ModelFinder (Kalyaanamoorthy et al., 2017) (see Supplementary Table S3). Bayesian analysis was performed using MrBayes v3.2.6 (Ronquist & Huelsenbeck, 2003) on the CIPRES Science Gateway (Miller et al., 2010). Appropriate substitution models were specified for each partition, and all parameters were unlinked across partitions. We ran two independent runs for 20 million generations with trees sampled every 1000 generations. All other parameters were set to default values. The stationarity and convergence of the runs were inspected in Tracer v1.6 (Rambaut et al., 2015) and the value of the average standard deviations of the split frequencies that were lower than 0.01. The burn-in fraction was left at 25% of the sampled trees. Thus, the first 5,000 out of the 20,000 trees generated were discarded. A majority-rule consensus tree was produced from the post-burning trees with posterior probability (PP) values embedded. Then, the ML analysis was done in IQ-TREE (Nguyen et al., 2015; Chernomor et al., 2016). The search for the best-scoring ML was performed by ultrafast bootstrap (UFBoot; Hoang et al., 2018) with 1,000 bootstrap and 1,000 topology replicates. To verify the robustness of the ML tree the branch supports were evaluated by using an SH-like approximate

likelihood ratio test (SH-aLRT; Guindon et al., 2010) and a Bayesian-like transformation of aLRT (aBayes; Anisimova et al., 2011). SH-aLRT was performed with 1000 replications. aBayes branch support was used instead of Bayesian posterior probabilities because aBayes is more conservative, more robust to model violation and exhibits the best power (Anisimova et al., 2011). The ML, SH-aLRT and aBayes analyses were run on the IQtree web server (Trifinopoulos et al., 2016).

We further inferred a phylogenetic network from the *ferrumequinum* group mitochondrial sequences using the neighbor-net algorithm (Bryant & Moulton, 2004) implemented in SplitsTree v.4 (Huson & Bryant, 2006).

Divergence time estimation and species delimitation

For the molecular dating analyses, the nuclear dataset was pruned to one sample per species, except for *R. clivosus*, which was represented by two samples (from Yemen and South Africa) corresponding to two independent lineages in the constituted nuclear tree (see Results). The final alignment was 2895 bp long and included 31 concatenated sequences constituted of at least four out of the five nuclear markers. The divergence time estimation was set up in BEAUti and run in BEAST v1.8.4. We followed the settings from Dool et al. (2016) and Benda et al. (2022) and used strict molecular clocks and the Yule speciation process (Gernhard, 2008; Yule, 1925) for all genes. The substitution model was taken from phylogenetic reconstructions (see above). As a calibration point, we employed the age of the family Rhinolophidae's root, estimated at 37 Mya (million years ago; Stoffberg et al., 2010). For an alternative divergence time reconstruction, we also used a family root age of 16.92 Mya (Foley et al., 2015). We used a lognormal prior distribution for this calibration point. BEAST was run three times for 40 million generations and trees were saved every 4000 generations. Tracer v1.6 was used to confirm adequate mixing of the MCMC chains and acceptable effective sample sizes (ESS >200). LogCombiner was used for burn-in (10%) and merging of tree files, and TreeAnnotator was used to identify the maximum clade credibility tree. All analyses were performed using CIPRES Science Gateway (Miller et al., 2010).

For the first round of species delimitation, the multi-rate Poisson tree process (mPTP) was used (Kapli et al., 2017). The number of substitutions represented by branch lengths was used to model intraspecific and interspecific processes (Zhang et al., 2013). This method accounts for divergent intraspecific variation which improves the estimation of the number of evolutionary lineages in clades with different rates of speciation coalescence across their phylogeny (Štundlová et al., 2019). The mitochondrial ML tree was used to run mPTP using the mPTP web server (http://mptp.h-its.org).

The second round of species delimitation was conducted by Bayesian phylogenetics and phylogeography (BPP v3; Rannala & Yang, 2003; Yang & Rannala, 2010). This analysis was carried out to evaluate the phylogenetic species boundaries. We used a nuclear dataset without outgroup (except for *fumigatus* and *maclaudi* groups) and the sequences were a priori divided into putative species groups based on the results of mPTP analysis combined with the groupings within the nuclear phylogenetic tree. The topology of the nuclear ML tree was used as a fixed guide tree (algorithm A10; Rannala & Yang, 2003; Yang & Rannala, 2010). Analysis runs were replicated twice for each of four different combinations of priors on divergence depth and effective population sizes (τ and θ, respectively; see Table 1 in Demos et al., 2019), as the probability of delimitation by BPP is sensitive to these two parameters (Leaché & Fujita, 2010; Yang & Rannala, 2010). Each replicate was conducted with either the reversible-jump Markov chain Monte Carlo algorithm 0 (with parameter e = 2) or 1 (with parameters a = 2, m = 1; Yang and Rannala, 2010). All eight BPP analyses were then run with the default settings. Lineages were considered statistically supported when the generated delimitation posterior probabilities (PP) exceeded 0.95 under all four prior combinations.

Uncorrected p-distances between haplotypes were calculated for the *Cyt-b* in MEGA11 (Tamura et al., 2021). The bootstrap was performed with 1,000 replications.

Results

In this study, we generated 178 new *Cyt-b* sequences (122 for *Rhinolophus ferrumequinum*, 44 for *R. clivosus*, and 12 for *R. bocharicus*) that represented 62 unique sequences (37 for *R. ferrumequinum*, 22 for *R. clivosus*, and three for *R. bocharicus*). Combined with the GenBank sequences, the final *Cyt-b* dataset comprised 226 unique sequences (52 for *R. ferrumequinum*, 65 for *R. clivosus*, three for *R. bocharicus*, 14 for *R. nippon*, and 92 for outgroups). For the nuclear dataset, we generated 81 *ACOX*, 77 *BGN*, 67 *COPS*, 11 *ROGDI*, and 82 *STAT* sequences which were supplemented with 237 sequences from GenBank. Only individuals with at least three sequenced introns were included in the analyses. This criterion was met by 100 specimens: (56 for *R. ferrumequinum*, 32 for *R. clivosus*, and 12 for *R. bocharicus*). Subsequently, the alignment was reduced to a final nuclear dataset comprising 83 unique haplotypes/sequences (13 for *R. ferrumequinum*, 27 for *R. clivosus*, three for *R. bocharicus*, and 40 for outgroups). Unfortunately, we were unable to obtain a nuclear sequence for *R. nippon*. The final *Cyt-b* dataset was comprised of 475 parsimony informative positions (41.92 % of the total length). The final nuclear dataset was 2902 bp long, the number of parsimony informative positions was 465 (16.06 % of its total length), and missing data accounted for 24.01 % bases of the dataset. For individual nuclear gene trees see Supplementary Figures S2–S7.

Phylogenetic reconstruction

The phylogenetic trees obtained by both ML (Fig. 2) and BI (Supplementary Fig. S1) analyses of the *Cyt-b* dataset showed largely congruent topologies, with notable differences being in relationships between *R. ferrumequinum* and *R. clivosus*, and between species-groups of the Afro-Palaearctic *Rhinolophus* clade. The monophyly of the *ferrumequinum* group was highly supported (bootstrap percentage [BP]=98, posterior probability [PP]=1) and placed as a sister branch to the clade composed of *fumigatus* and *maclaudi* groups. However, the support for this relationship was only moderate (BP=83, PP=0.89). Other lineages within the Afro-Palaearctic clade included *R. horaceki*, *R. xinanzhongguoensis*, and the *euryale*, *capensis*, and *landeri* groups, but the relationships between them were not resolved. Six major lineages were revealed within the *ferrumequinum* group (BP=99–100, PP=1). *Rhinolophus bocharicus* was recovered as sister to the remaining species of *ferrumequinum* group; then *R. nippon* split off; the Ethiopian lineage of *R. clivosus* (ETH lineage); eastern and southern African *R. clivosus* lineage (ESA lineage); and the *clivosus* lineage from southern Arabia and Socotra (ARS lineage), as a sister to a lineage composed of two *R. ferrumequinum* sub-lineages (western and eastern = *ferrumequinum* lineage) and *R. clivosus* from northern Africa and the Levant (NAL *clivosus* lineage; *ferrumequinum* + NAL *clivosus* = 'mixed' lineage). In the 'mixed' lineage, the largest disparity between ML and BI trees arises. Specifically, in the ML tree, the NAL *clivosus* lineage is situated between two sub-lineages of *R. ferrumequinum*, whereas in the BI tree, the NAL *clivosus* lineage is positioned as the sister to the *ferrumequinum* lineage. The relationships between these groups are strongly supported, except for the relationship between the ESA lineage and the ARS + 'mixed' lineages (BP=89, PP=0.87).

ML (Fig. 3) and BI (Supplementary Fig. S2) analyses of the nuclear dataset yielded almost the same topology, except for weakly supported relationships between *R. bocharicus* and *R. clivosus* lineages, and among some lineages within the Afro-Palaearctic clade. The monophyly of the *ferrumequinum* group was unambiguously supported (BP=100, PP=0.98). This group, along with the *fumigatus* group (containing the *maclaudi* group nested within it), formed a strongly supported group within the Afro-Palaearctic clade with high support (BP=98, PP=1). Other lineages within this monophyletic clade (BP=100, PP=1) included the *landeri*, *euryale*, and *capensis* groups. Within the *ferrumequinum* group, four major lineages (BP=97–100, PP=0.96–1.00) were revealed: *R. ferrumequinum* (*ferrumequinum* lineage), *R. bocharicus* (*bocharicus* lineage), *R. clivosus* from Ethiopia to South Africa (ETH + ESA lineages = *acrotis* lineage), and *R. clivosus* from north Africa, Levant, and Arabia and Socotra (NAL + ARS *clivosus* lineages = *clivosus* lineage). The relationships between the *bocharicus* and two *clivosus* lineages remained unresolved due to the low support (BP<62, PP<0.82). In contrast to the mitochondrial analyses, we found strong support for *R.*

ferrumequinum being sister to the remaining species of the *ferrumequinum* group (BP=100, PP=1.00). Unfortunately, we were not able to obtain any nuclear sequences for *R. nippon*.

The phylogenetic network inferred with SplitsTree (Fig. 4) identified similar lineages and a topology that was largely consistent with both the mitochondrial and nuclear trees. The network of the *Cyt-b* dataset showed a close relationship between the Asian species *R. nippon* and *R. bocharicus*, a branching of the ESA *clivosus* lineage, and NAL *clivosus* lineages nested within/in a close contact with the *R. ferrumequinum* lineage. Nevertheless, one line of the network connects the NAL and ARS *clivosus* lineages (see red arrow in Fig. 4). The network of the nuclear dataset showed a clear separation of the *R. bocharicus* and *R. ferrumequinum* lineages from the closely related *R. clivosus* lineages, which could be further subdivided into two major lineages consisting of the ARS + NAL *clivosus* lineages, and the ETH + ESA *clivosus* lineages.

Divergence time estimation and species delimitation

The time-calibrated tree topology of the Afro-Palaearctic *Rhinolophus* clade generally corresponded to the nuclear tree (Fig. 5). From the nodes of our interest, only the divergence among the *acrotis*, *clivosus* and *bocharicus* lineages was not fully supported (0.81 PP). The divergence of the family Rhinolophidae was estimated to have occurred 38.4 million years ago (Ma; 95% highest posterior density [HPD]: 38.0–39.5 Ma). The diversification of the Afro-Palaearctic *Rhinolophus* clade was estimated at 23.0 Ma (95% HPD: 19.1–26.9 Ma). The *ferrumequinum* group diverged from the *fumigatus+maclaudi* group 8.7 Ma (95% HPD: 6.8–10.6 Ma). The split of *ferrumequinum* lineage from the remaining *ferrumequinum* group was assessed to occur at 6.2 Ma (95% HPD: 4.0–8.4 Ma). Subsequently, the *clivosus* lineage from *acrotis* + *bocharicus* lineages at 3.8 Ma (95% HPD: 2.1–5.6 Ma). The split of the *acrotis* lineage and *bocharicus* lineage was estimated at 2.5 Ma (95% HPD: 1.1–3.9 Ma). Noteworthy, the estimated time divergence for the *bocharicus* and *acrotis* lineages exceeds the divergence time of other *Rhinolophus* species (e.g., *R. swinnyi* and *R. capensis* 1.0 Ma, and *R. rhodesiae* and *R. simulator* 1.1 Ma). For the reconstruction based on a more recent root calibration (Foley et al., 2015), see Supplementary Fig. S8. The topology of both reconstructions remained identical, however, the splits of each group estimated in the alternative reconstruction occurred much later: Rhinolophidae divergence – 16.7 Ma [16.1–17.3 Ma], Afro-Palaearctic *Rhinolophus* clade diversification – 10.1 Ma [8.4–11.8 Ma], 3.8 Ma [3.0–4.7 Ma], respectively for the nodes outside of *ferrumequinum* group nodes, and *ferrumequinum* lineage split – 2.7 Mya [1.8–3.7 Ma], *clivosus* lineage split – 1.7 Ma [0.9–2.5 Ma], and *bocharicus* and *acrotis* lineages divergence – 1.1 Ma [0.5–1.7 Ma], respectively, for the inner nodes of the *ferrumequinum* group).

The number of evolutionary lineages identified by mPTP (Fig. 2) in the mitochondrial tree within the *ferrumequinum* group was 14 – one for *R. bocharicus*, *R. clivosus* from Ethiopia (= ETH lineage), and *R. ferrumequinum* (together with the NAL *clivosus* lineages = 'mixed' lineage); three for *R. nippon*; and finally, four for the ARS *clivosus* lineage, and five for southern and east African *R. clivosus* lineage (ESA lineage). The ESA *clivosus* lineages were designated by the abbreviated names of the countries of origin: mmtz (Malawi, Mozambique, Tanzania, and Zambia), keug (Kenya and Uganda), rwa (Rwanda), and bsaz (Botswana, South Africa, and Zimbabwe). With the exception for *R. nippon* (one group), ARS *clivosus* (two groups) and *R. ferrumequinum* (two groups – eastern and western), same groupings as identified by mPTP were used to calculate the uncorrected *p*-distances (Supplementary Table S4). In accordance with mPTP analysis, we found high intraspecific distances in *R. nippon* (0.1 – 4.5%) and ESA *clivosus* (0.0 – 4.3%) lineages. In contrast, the distances within *R. bocharicus* lineage were 0.1–0.4% and the smallest distance to another species, *R. nippon*, was 5.3%. The distances between 'western' and 'eastern' populations of the *R. ferrumequinum* lineage ranged from 0.8 to 2.8%. The NAL *clivosus* lineage differed from the *R. ferrumequinum* lineage by only 1.0–2.6%. In other major lineages, the distance ranges were as follows: ARS *clivosus* 0.0–3.0%, ETH lineage 0.1–2.6%.

For the BPP analyses, the results displayed slightly different delimitation probabilities of the replicated runs when different parameters were chosen (Table 1). Nevertheless, the guided BPP

species delimitation supported (PP≥0.95) all the splits within the *ferrumequinum* group except the split within ARS *clivosus* lineage between samples from Socotra and the Arabian mainland when the effective population size was set up as 'large' ($\theta = \Gamma$ [1, 10]). Therefore, the BPP results supported seven to eight lineages reconstructed in the mPTP analysis, more than reconstructed in the nuclear ML analysis: one *ferrumequinum* lineage, one to two ARS+NAL *clivosus* sub-lineages, one ETH *clivosus* lineage, three ESA *clivosus* sub-lineages (mmtz, keug, and bsaz), and one *bocharicus* lineage. Moreover, the BPP analysis fully supported the branching within the *ferrumequinum* group that were recovered as lowly supported in the nuclear ML tree. Nonetheless, the BPP results implied that all four nuclear lineages of the *ferrumequinum* group were strongly delimited within this group.

Discussion

In this study, we generated multi-locus genetic data for the horseshoe bats of the *Rhinolophus ferrumequinum* species group to investigate phylogenetic relationships among and within species of this group. Our results are partly in agreement with recent molecular genetic studies (Stoffberg et al., 2010; Benda & Vallo, 2012; Dool et al., 2016; Demos et al., 2019). Nevertheless, our extensive collection of new data has allowed us to present more comprehensive and more detailed insights leading to new taxonomic implications.

Phylogeny of the ferrumequinum *group*

The *ferrumequinum* group formed a separate monophyletic group in all our results, in agreement with other molecular phylogenetic studies focusing on the genus *Rhinolophus* (Stoffberg et al., 2010; Benda & Vallo, 2012; Dool et al., 2016; Demos et al., 2019). Moreover, our results also recovered its position within the Afro-Palaearctic *Rhinolophus* clade in sister position to the *fumigatus* group. In agreement is the position of *maclaudi* group within *fumigatus* group (Stoffberg et al., 2010; Benda & Vallo, 2012; Dool et al., 2016; Demos et al., 2019).

The interspecific relationships of horseshoe bats belonging to the *ferrumequinum* group varied between the mitochondrial and nuclear trees (Figs. 2 and 3). This disparity may be partly caused by the presence of *R. nippon* in the mitochondrial tree and its absence in the nuclear tree. The topology of the mitochondrial tree is consistent with previous analyses (Benda & Vallo, 2012; Demos et al., 2019) even though they did not include *R. bocharicus* in their analyses. In our mitochondrial tree, *R. bocharicus* was identified as the sister lineage to all other species within the group. In the previous studies, in this position, sister to the rest, was either *R. clivosus* (Stoffberg et al., 2010) or *R. nippon* (Benda & Vallo, 2012; Demos et al., 2019). *Rhinolophus nippon* was found as the second diverging group. This position, sister to *R. clivosus* and *R. ferrumequinum*, is consistent with the other mitochondrial trees (Benda & Vallo, 2012; Demos et al., 2019), but it differs from the combined tree as it was placed in sister position only to *R. ferrumequinum* (Stoffberg et al., 2010). The remaining supported group comprised *R. ferrumequinum* mixed with north African and Levantine (Saharo-Levantine, NAL) *R. clivosus*; South-Arabian and Socotranese (Arabian, ARS) *R. clivosus*; eastern and southern African *R. clivosus* (ESA lineage); and Ethiopian *R. clivosus* (ETH lineage). The only supported relationship within this group was between the mixed *R. ferrumequinum* and NAL *R. clivosus* lineage and ARS *R. clivosus* from Arabia, which were found to be sister taxa. The mitochondrial trees of earlier studies which examined more than two species revealed a sister relationship between the *ferrumequinum* and *clivosus* lineages (Benda & Vallo, 2012; Demos et al., 2019). Our results also confirm the positioning of NAL *clivosus* within *R. ferrumequinum* as previously demonstrated (Benda et al., 2012; Dool et al., 2016; Demos et al., 2019) even after combining the samples used in those studies.

The study's nuclear tree of the *ferrumequinum* group has expanded to three species, a notable increase from previous studies which only included *R. clivosus* and *R. ferrumequinum* (Dool et al., 2016; Demos et al., 2019). *Rhinolophus ferrumequinum* was found to be the only supported relationship in the nuclear tree with an estimated split of 6.2 Ma from the rest of the group's species.

In this study, *R. bocharicus* joined the sister lineage of *R. clivosus* lineages (NAL+ARS and ETH+ESA). Otherwise, the results agreed with previous studies where northern African *R. clivosus* samples (NAL lineage in this study) formed separate lineages from the sub-Saharan samples (ETH+ESA lineages in this study; Dool et al., 2016; Demos et al., 2019). However, these previous studies lacked nuclear sequences for Arabic *R. clivosus* (ARS lineage).

The split of *R. clivosus* into two separate nuclear lineages supported by the branching in the mitochondrial tree, time-calibrated tree, and species delimitation analysis results, provides compelling evidence to propose the separation of this species into two separate species. The first, the nominotypical *R. clivosus* would consist of the Palaearctic nuclear lineage of this species containing Saharo-Levantine (NAL) and Arabian (ARS) mitochondrial lineages. The type locality of this name (Al Muweileh) is situated in north-western Saudi Arabia (Cretzschmar, 1828; Simmons, 2005). The second species would then be formed by the Afrotropic nuclear lineage of *R. clivosus* (i.e., ETH and ESA mitochondrial lineages). The prior available name for this species is *Rhinolophus acrotis* von Heuglin, 1861 described from Eritrea and which was the name of a subspecies found in Sudan, Eritrea, Ethiopia, and Somalia (Csorba et al., 2003). As such, this name includes our samples collected in Ethiopia as well as those from eastern and southern Africa. Moreover, one of our samples, originating from Axum, Tigray, Ethiopia, is located less than 200 km away from the type locality in Keren, Eritrea.

The estimated split times within the *ferrumequinum* group correspond to those of other species groups within the Afro-Palaearctic clade of the genus, including the *capensis* or *fumigatus* groups based on our time-calibrated tree. The topology of both nuclear trees deviated from the combined data tree presented by Stoffberg et al. (2010), in which *R. nippon* formed a sister clade to *R. ferrumequinum*, and *R. clivosus* held a sister position to these two species. The previous nuclear analyses were limited to two species, either *R. clivosus* or *R. ferrumequinum* s.str. (Dool et al., 2016; Demos et al., 2019) or *R. bocharicus* and *R. clivosus* (Bailey et al., 2016). Despite the limited data, our study was generally consistent with findings from Dool et al. (2016) and Demos et al. (2019).

Relationships between Rhinolophus clivosus *and* R. ferrumequinum

Previous studies have indicated a rather complex relationship between *R. ferrumequinum* and *R. clivosus* (Benda & Vallo, 2012; Dool et al., 2016; Demos et al., 2019). In our study, we analysed mitochondrial and nuclear sequences across the whole distribution range of *R. clivosus* and most of the distribution range of *R. ferrumequinum* (Fig. 1). Notably, we extensively sampled the area where both species are in close proximity. Although we found *R. ferrumequinum* and *R. clivosus* to be separate clades in the nuclear tree, this was not the case for the mitochondrial dataset. The mitochondrial tree showed four major lineages of *R. clivosus* (ETH, ESA, ARS, NAL), with sequences from the Sahara and the Levant (NAL) mixed as a sub-lineage within the *R. ferrumequinum* clade. The mitochondrial tree agrees with the topology recovered by Benda & Vallo (2012) and Dool et al. (2016), where *R. clivosus* individuals from Egypt and Jordan, and Algeria, respectively, were mixed with *R. ferrumequinum*. Additionally, the ARS *clivosus* lineage was found sister to the mixed lineage of *R. ferrumequinum* and NAL *clivosus* in the mitochondrial tree. On the other hand, all Palaearctic *R. clivosus* (NAL+ARS lineage) sequences were ascertained to be monophyletic in the nuclear tree. We suggest that the mitochondrial genome of *R. clivosus* from southern Arabia is the original genome set and it was replaced by the mitochondrial genome of *R. ferrumequinum* in the northern part of the range (Levant), where both species live in parapatry or limited sympatry (Mendelssohn & Yom-Tov, 1999; Benda et al., 2010; own unpubl. data). It is noteworthy that two groups of *clivosus*, NAL and ARS, were found partially connected in the phylogenetic network indicating that a phylogenetic signal is still present in mtDNA.

This discordance between mitochondrial (mtDNA) and nuclear (nucDNA) genomes was recently increasingly reported as the use of both types of markers has become a standard (Toews & Brelsford, 2012). It could be caused by several mechanisms, however, if the differences between mitochondrial and nuclear markers show a geographic pattern, the discordance is mostly caused by an introgression

of a genome part of one species into the genome of another species (Toews & Brelsford, 2012; Mao & Rossiter, 2020). On the other hand, if no evidence for a geographic signal is apparent in the discrepancy between the genomes, the expectable explanation could be incomplete lineage sorting (e.g.,Funk & Omland, 2003). Moreover, the mtDNA introgressions occur more often than the nucDNA introgressions (Bachtrog et al., 2006; Klymus et al., 2010), and are typically asymmetric from a donor species to a receiving species (Mao et al., 2010), and/or from a native species to an invading species (Currat et al., 2008). In bats, the assumed evidence for mtDNA introgression is increasing and is even suggested from the family Rhinolophidae (e.g., Mao et al., 2010, 2013; Sun et al., 2016; Taylor et al., 2018; Mao & Rossiter, 2020), as well as from other bat families such as Vespertilionidae (e.g., Berthier et al., 2006; Vallo et al., 2012; Juste et al., 2013), Mormoopidae (Méndez-Rodríguez et al., 2021), or Pteropodidae (Nesi et al., 2013), and other mammals such as hares, deer or bears (Melo-Ferreira et al., 2009; Senn & Pemberton, 2009; Edwards et al., 2011); for a review see Toews & Brelsford (2012).

Therefore, we suggest that the mtDNA introgression occurred at the present parapatric contact zone of *R. ferrumequinum* and *R. clivosus*, as only one *clivosus* population (NAL *clivosus*) showed a closely related mtDNA with *R. ferrumequinum*. Additionally, according to Currat et al. (2008), the invading species would be *R. clivosus* who was supposedly moving northward until it encountered the local *R. ferrumequinum*. The distribution of *R. ferrumequinum* now follows the Mediterranean and Irano-Turanian bioclimatic zones whereas *R. clivosus* is more commonly found in the Saharo-Arabian climatic zone (Zohary, 1973; Asouti et al., 2015; Miebach et al., 2019). The Irano-Turanian biome expanded especially during colder periods (supposedly during the Pleistocene glacials) and the Saharo-Arabian biome expanded during dry and warmer periods (Miebach et al., 2019). Therefore, it is only possible to hypothesise that *R. clivosus* expanded northward and encountered the resident *R. ferrumequinum* with whom it hybridised during interglacials. Alternatively, the asymmetric introgression could result from the body and genitalia size differences as was demonstrated with Chinese *Rhinolophus* (Mao et al., 2013). In China, the smaller *R. sinicus* and *R. thomasi* introgressed the mtDNA from the larger *R. septentrionalis* which resembles our case where the mtDNA of the larger *R. ferrumequinum* introgressed into the smaller *R. clivosus*. Altogether, in our case, the introgression of mtDNA between *R. ferrumequinum* and *R. clivosus* would be deep historical because (1) their distribution ranges do not overlap currently, and (2) they do not share haplotypes when the smallest genetic distance between *ferrumequinum* and NAR *clivosus* is 1.0% of the *Cyt-b* gene. When the genetic distances were compared with both time-calibrated trees, we could speculate that the introgression might occur around 0.3–0.6 Ma, i.e., in the last second to last fourth interglacial period.

Intraspecific relationships within the ferrumequinum *group*

The mitochondrial tree showed much more branching than the nuclear tree due to the faster coalescence time of mtDNA (Palumbi et al., 2001). Hence, the analysis of a mitochondrial marker can provide better insight into the geography-based division of the particular populations or population groups whereas the analysis of nuclear markers can reveal the phylogeny of all populations together and thus, putative taxa.

To our knowledge, *Rhinolophus bocharicus* was only once studied with the help of genetic methods and it was only with the help of Next-generation sequencing (NGS) methods (Bailey et al., 2016). Therefore, there were no mitochondrial sequences available before our study. We gained sequences from twelve specimens, collected from three localities in south-western Tajikistan, resulting in three haplotypes appearing in both the mitochondrial and nuclear tree. All these haplotypes are composed of monophyletic and compact branch in all the trees, network and with high support in BPP analysis (PP=1). These findings confirm the view presented by the studies that recognised *R. bocharicus* as a separate species based on the evaluation of morphologic characters (Hanák, 1969; Felten et al., 1977; Horáček et al., 2000; Csorba et al., 2003; Benda et al., 2012). Although the used sequences originate from a restricted geographic region of this bat's range, the results of our analysis did not contradict the view that *R. bocharicus* is a monotypic species as was suggested previously

(Horáček et al., 2000; Csorba et al., 2003; Simmons, 2005). However, more data from other parts of its distribution range is needed to complete the knowledge about the phylogenetic relationships within the species, especially concerning the geographically isolated populations, e.g., in north-western Turkestan (cf. Strelkov, 1971).

A quite opposite situation arose in *R. clivosus*. The sampling covered almost the entire distribution range of this bat, including all populations considered to represent separate taxa (see Introduction). This species was divided into multiple lineages in both the mitochondrial and nuclear trees as well as in the phylogenetic network. In the mitochondrial tree, four major lineages (corresponding to NAL, ARS, ESA, and ETH *clivosus* in Figures) occurred within the originally considered *R. clivosus*, comparable to the topology presented by Benda & Vallo (2012) or Demos et al. (2019). The latter authors divided one group into three groups, *R. clivosus* in total to six groups. Notably, the lineage from northern Africa and the Levant (NAL *clivosus*) was found within the branch leading to *R. ferrumequinum* (see above). Sequences from southern Arabia formed a second lineage (ARS *clivosus*). These two lineages correspond to the one of two nuclear *R. clivosus* lineages and thus, they constitute *R. clivosus* s.str. The low differences between samples from northern Africa, the Levant, and the Arabian Peninsula in the nuclear tree support the claim of Benda & Vallo (2012) that a sole subspecies of *R. clivosus* occurs in this area. Additionally, subspecies *brachygnathus* (Egypt) and *schwarzi* (Algeria) were assigned as junior synonyms of *R. c. clivosus* Cretzschmar, 1828. The sequences obtained from Socotra created a separated sub-lineage in the results from both markers and was moderately to highly supported in BPP analysis that could belong to a separate subspecies, namely *R. c. socotranus* Benda, Reiter et Vallo, 2017. Thus, the latter taxon represents the sole supported phylogenetic sub-lineage within the species, other than the nominotypical one, despite the considerable morphometric variation described (Qumsiyeh, 1985; Csorba et al., 2003).

The two remaining major mitochondrial branches, previously assigned to *R. clivosus*, now referred to as *R. acrotis*, correspond to the second nuclear lineage of haplotypes from sub-Saharan Africa (ETH and ESA *clivosus*). One of the major mitochondrial lineages was formed by the sequences from Ethiopia (ETH *clivosus*) and thus, they belong to the nominotypical subspecies, *R. a. acrotis* von Heuglin, 1861 (see above). The other lineage comprised sequences from the rest of the species range south of Ethiopia (ESA *clivosus*) and it could be further divided into three sub-lineages (five sub-lineages based on the mPTP analysis) that correspond to groups 1, 2, and 3 of Demos et al. (2019). These four sub-lineages demonstrate varying levels of support, ranging from low to high branch support, but high support in BPP analysis. The position of the Rwandan subgroup cannot be determined without nuclear sequences. However, some authors argue that the results of BPP analysis diagnose population genetic structure instead of species limits (Sukumaran & Knowles, 2017). As a result, we keep rather a conservative approach in consideration of the putative taxa until more, especially genetic data becomes available. Therefore, we consider the ESA *clivosus* branch as comprising altogether only one well-separated subspecies, *R. acrotis augur* Andersen, 1904. Other subspecies of *R. clivosus* s.l. from sub-Saharan Africa, now potential subspecies of *R. acrotis*, mentioned in Csorba et al. (2003) could be assigned to other sub-lineages – a sub-lineage formed by sequences from Tanzania, Malawi, Mozambique, and Rwanda (currently without an available name), a sub-lineage from Kenya and Uganda as *keniensis* Hollister, 1916, and sub-lineage/s from South Africa, Zimbabwe, and Botswana as *augur* Andersen, 1904. Nevertheless, the division into more subspecies south of Ethiopia was not supported sufficiently by morphological (Benda & Vallo, 2012) or genetic (this study) examinations. Thus, we suggest regarding them just as junior synonyms of *R. a. augur*.

Rhinolophus ferrumequinum s.str. was thoroughly studied using genetic tools several times in the past (e.g., Rossiter et al., 2007; Flanders et al., 2009; Dool et al., 2016; Demos et al., 2019). Our findings largely support the results from these previous studies. They revealed the presence of two mitochondrial branches and one nuclear branch. One mitochondrial branch included sequences from the Middle East from the Levant, Turkey, Cyprus, and Tajikistan (eastern sub-lineage), while the other branch consisted of sequences from Europe, northern Africa, and the eastern Mediterranean (western

sub-lineage). The sequences from Bulgaria, Cyprus, and Syria were found in both sub-lineages, which thus live in an extensive area in sympatry – this pattern was already observed in previous studies (Kůs, 2008; Flanders et al., 2009). However, mPTP analysis, which otherwise rather oversplit, detected only a single mitochondrial lineage. Similarly, the nuclear tree is composed of only one branch from samples originating in a belt extending from Italy to Tajikistan, like in Dool et al. (2016) and Demos et al. (2019). Overall, the results indicate that *R. ferrumequinum* could be best regarded as a monotypic species throughout the whole distribution range, although certain separation between western and eastern sub-lineages could occur between 0.2–0.5 Ma (with the estimation based on the genetic distances) that was later mixed again making their partial overlap in occurrence and genetic mixture.

For *R. nippon* we did not generate any novel sequences and therefore, our results replicated those of previous studies (Flanders et al., 2009, 2011; Benda & Vallo, 2012; Koh et al., 2014). The mitochondrial phylogeny recovered three lineages within this species. The first lineage included sequences from eastern China, and this lineage was a sister to the second lineage that included sequences from eastern China, South Korea, and Japan. The third lineage was a sister to the previous two lineages and consisted of sequences from southern China. Based on mPTP analysis, *R. nippon* was divided into two subgroups, one from eastern China, Korea, and Japan, and the other from central China. However, no nuclear data were available for our analysis, so without a broad nuclear phylogeography it is difficult to determine the position and intraspecific division of individual populations of *R. nippon* without broad nuclear phylogeography. Therefore, based on the currently available data, *R. nippon* remains a monotypic species as it was already discussed by Koh et al. (2014).

Conclusions

In this study, we present a revision of the inter- and intraspecific relationships within a group comprising the *Rhinolophus* species that are closely related to *R. ferrumequinum* and are therefore identified as the *ferrumequinum* group (Csorba et al., 2003; Dool et al., 2016). *Rhinolophus bocharicus* formed a monotypic phylogenetic unit/species, and its position within the *ferrumequinum* group varied according to the marker used, leading to unresolved relations with *R. clivosus* and *R. ferrumequinum*. Similarly, the marker choice caused changes in the position of *R. clivosus* s.str. Nevertheless, the intraspecific relations within *R. clivosus* are complex. The mitochondrial analysis results divided the species into four major groups, with two major groups detectable in the nuclear tree. The main split was between bats from northern Africa, Levant, Arabian Peninsula, and Socotra, forming one population group, and those from eastern and southern Africa from Ethiopia to South Africa creating another group. The differences between these two major groups led us to suggest that these groups represent two separate species, *R. clivosus* and *R. acrotis*. Both species could be considered polytypic, with two subspecies in each species. *Rhinolophus c. clivosus* is found in northern Africa, Levant and Arabia, and *R. c. socotranus* is found in Socotra. Similarly, *R. a. acrotis* is located in Ethiopia, Eritrea and Sudan, and *R. a. augur* inhabits eastern and southern Africa from Kenya to South Africa. This would increase the number of species within the *ferrumequinum* group to five opposing the recent studies (Burgin et al., 2019). Moreover, the results indicated that a historical introgression occurred in *R. clivosus* from northern Africa and the Levant, where its mtDNA was replaced by mtDNA of *R. ferrumequinum*. The introgression was estimated to occur 0.3–0.6 Ma and the distinctiveness of the lineages on the mtDNA tree, together with their genetic distances and non-overlapping ranges, suggests that the gene flow is no longer continuous. The intraspecific relationships detected within the remaining two species, *R. ferrumequinum* and *R. nippon*, were consistent with the previous genetic studies (Flanders et al., 2009, 2011; Benda & Vallo, 2012; Koh et al., 2014; Dool et al., 2016; Demos et al., 2019).

This study presents a newly identified species within the *Rhinolophus* genus and suggests that the number of species in the *ferrumequinum* group may increase with an integrative taxonomic approach and/or the use of phylogenomic markers. On the other hand, the study found that intraspecific

variation in the group was less pronounced than previously thought based on morphologic variation, with only one to two subspecies distinguished within these particular species.

CRediT authorship contribution statement

Marek Uvizl: Methodology, Formal analysis, Data curation, Writing – Original draft. **Zuzana Kotyková Varadínová:** Methodology, Formal analysis, Writing - Review & Editing. **Petr Benda:** Conceptualization, Resources, Writing - Review & Editing, Funding acquisition.

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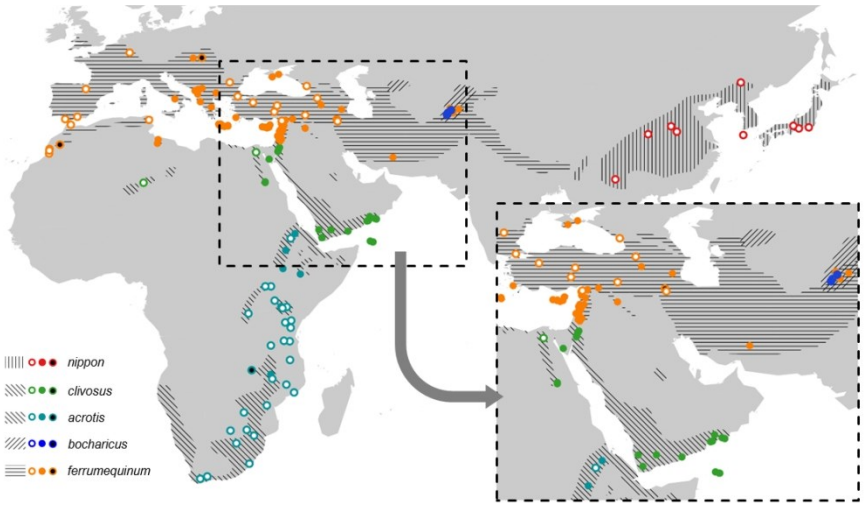
Fig. 1. Map of the distribution range of the Rhinolophus ferrumequinum group and the localities of sequence origin. Open dots indicate the samples from GenBank, full-coloured dots indicate the samples examined in our study, and black dots enclosed in coloured dots indicate the samples from GenBank used in Benda & Vallo, 2012 and Benda et al., 2022b.

Fig. 2. Maximum likelihood tree of reconstructed phylogenetic relationships of the *Rhinolophus ferrumequinum* group with selected species of the family Rhinolophidae based on the Cyt-b dataset (1133 bp). mPTP bar denotes the lineages according to the mPTP species delimitation; Phyl bar denotes the major lineages recognised as higher taxa in this study. The ESA clivosus sub-lineages were denoted by the abbreviation of the country of origin (mmtz for Malawi, Mozambique, Tanzania, and Zambia, keug for Kenya and Uganda, rwa for Rwanda, and bsaz for Botswana, South Africa, and Zimbabwe). Branch support values are shown by pie charts on the nodes.

Fig. 3. Maximum likelihood tree of reconstructed phylogenetic relationships of the *Rhinolophus ferrumequinum* group with selected species of the family Rhinolophidae based on the nuclear dataset (5 introns, 2902 bp). Branch support values are shown by pie charts on the nodes.

Fig. 4. Phylogenetic networks generated by SplitsTree. Bootstrap values are shown for major clades in mtDNA. The ESA *clivosus* sub-lineages were denoted by the abbreviation of the country of origin (mmtz as Malawi, Mozambique, Tanzania, and Zambia, keug as Kenya and Uganda, rwa as Rwanda, and bsaz as Botswana, South Africa, and Zimbabwe). The red arrow shows the line connecting the ARS and NAL *clivosus* lineages in the mtDNA network.

Fig. 5. Chronogram of the family Rhinolophidae based on Bayesian inference of the nuclear dataset (following to the model by Stoffberg et al., 2010). Numbers at nodes indicate mean divergence time estimates (Ma) and horizontal boxes indicate the 95% highest posterior density intervals of these estimates. The asterisk (*) indicates nodes with low branch support, the rest of the nodes were supported (PP ≥ 0.95).



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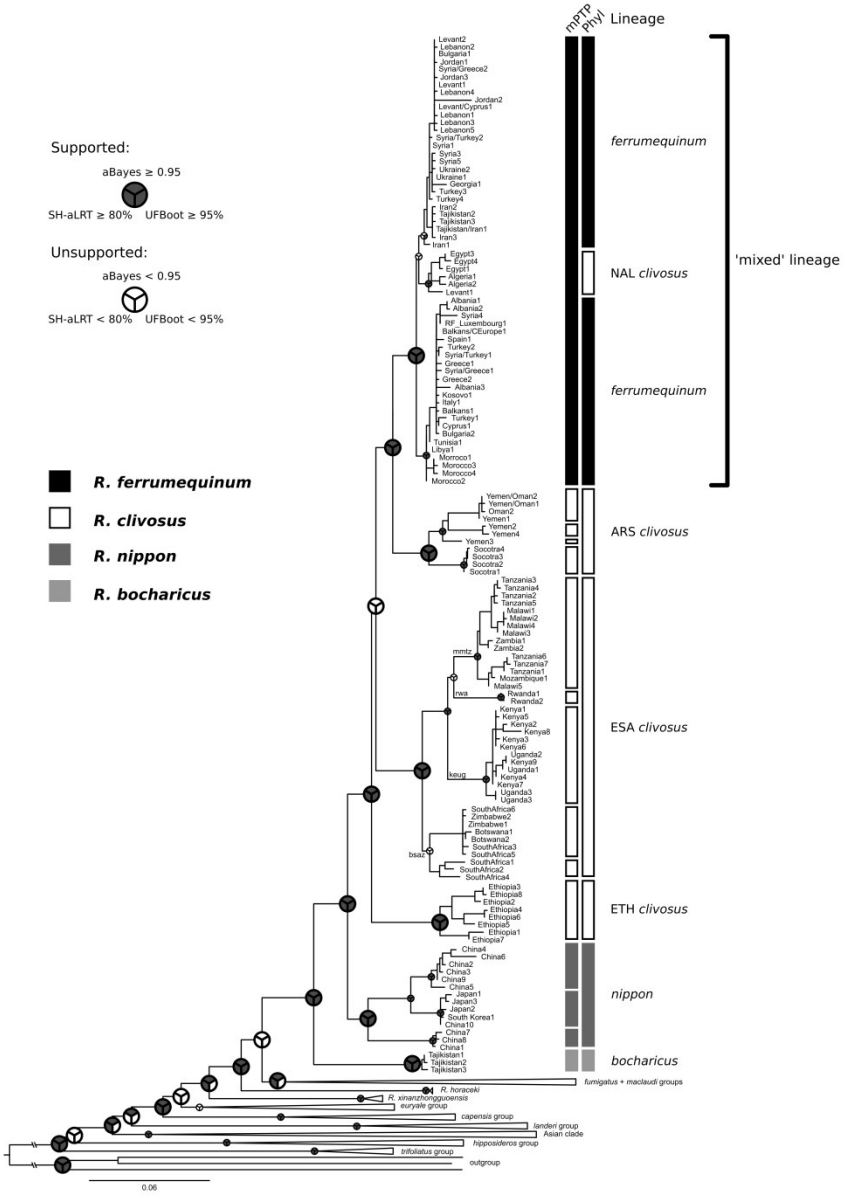
855x481mm (38 x 38 DPI)



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495x699mm (197 x 197 DPI)

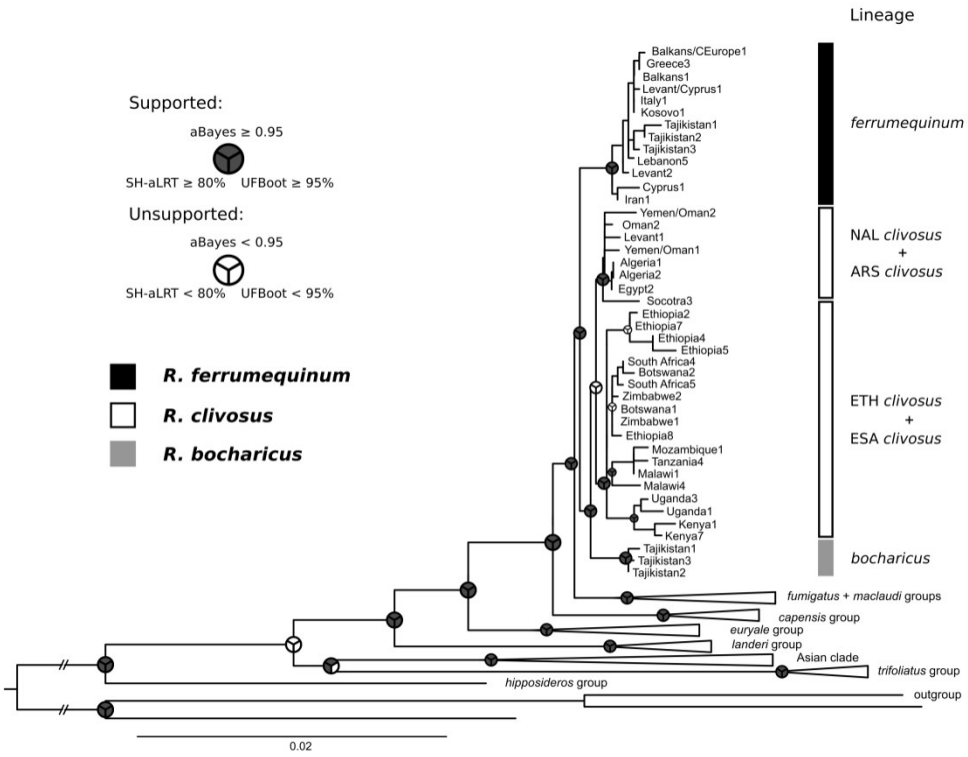

Fig. 3. Maximum likelihood tree of reconstructed phylogenetic relationships of the Rhinolophus ferrumequinum group with selected species of the family Rhinolophidae based on the nuclear dataset (5 introns, 2902 bp). Branch support values are shown by pie charts on the nodes.

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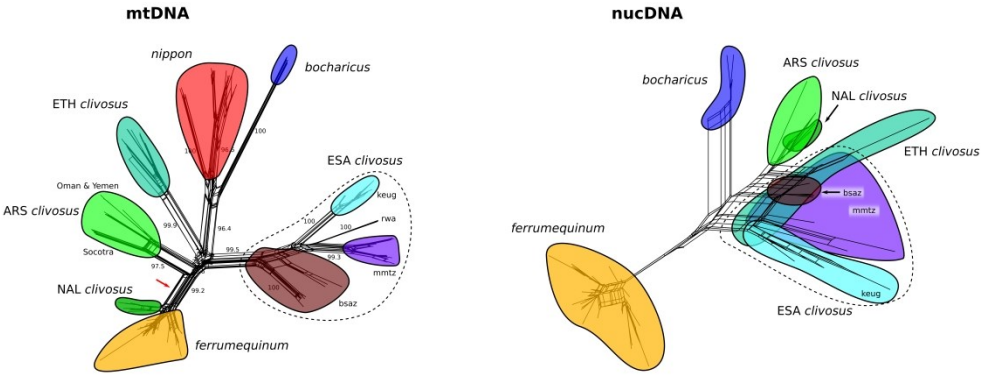

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1116x439mm (197 x 197 DPI)

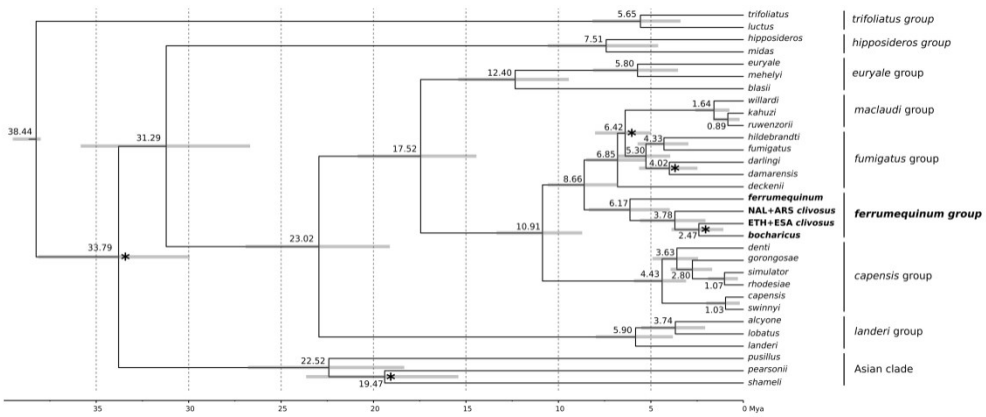

Fig. 5. Chronogram of the family Rhinolophidae based on Bayesian inference of the nuclear dataset (following to the model by Stoffberg et al., 2010). Numbers at nodes indicate mean divergence time estimates (Ma) and horizontal boxes indicate the 95% highest posterior density intervals of these estimates. The asterisk (*) indicates nodes with low branch support, the rest of the nodes were supported (PP ≥ 0.95).

611x261mm (197 x 197 DPI)

Table 1. Summary of BPP for the nuclear dataset. Values for BPP species are posterior probabilities (PP) of delimitation from BPP runs under each of four different schemes under two different algorithms (see Table 1 in Demos et al., 2019). The ESA sublineages were signed by the abbreviate names of countries of origin (mmtz as Malawi, Mozambique, Tanzania and Zambia, keug as Kenya and Uganda, and bsaz as Botswana, South Africa and Zimbabwe), 'soc' in ARS lineage is abbreviation for Socotra.

Split	large deep (e=2)	large deep (a2=2, m=1)	large shallow (e=2)	large shallow (a2=2, m=1)	small shallow (e=2)	small shallow (a2=2, m=1)	small deep (e=2)	small deep (a2=2, m=1)
ESA clivosus mmtz + keug	0.975	0.960	0.981	0.986	0.998	0.998	0.994	0.994
ESA clivosus (mmtz + keug) + bsaz	0.994	0.991	0.996	0.999	1.000	1.000	1.000	1.000
ESA + ETH clivosus	1.000	0.997	1.000	1.000	1.000	1.000	1.000	1.000
ARS clivosus without Soc + Soc	0.920	0.838	0.930	0.921	0.995	0.995	0.994	0.994
(ESA + ETH) + ARS clivosus	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
clivosus + *bocharicus*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
(clivosus + bocharicus) + *ferrumequinum*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Paper 7: Horseshoe bats from Lesotho

Benda, P., Uvizl, M., Eiseb, S., & Avenant, N. On the systematic position of the horseshoe bats (Mammalia: Chiroptera) from Lesotho. Under review in Mammalia.

On the systematic position of the horseshoe bats (Mammalia: Chiroptera) from Lesotho

Petr Benda*, Marek Uvizl, Seth J. Eiseb and Nico L. Avenant

Abstract: The monophyletic Afro-Palaearctic clade of the horseshoe bats (*Rhinolophus*) comprises several species groups whose representatives can be morphologically similar to each other across groups. The only *Rhinolophus* species that occurs in Lesotho was traditionally attributed to the broadly distributed African desert- and savanna-dwelling bat, *R. clivosus*, a member of the *ferrumequinum* group. In this study, we investigated the horseshoe bats from Lesotho with the help of molecular genetic and morphometric analyses to find their position within the group and the clade as well. The genetic analysis resulted in phylogenetic trees with two different topologies, although in both trees the Lesotho bats were a part of the *fumigatus* group instead of the *ferrumequinum* group. In the mitochondrial tree, the Lesotho bats were mixed with *R. damarensis.* On the contrary, the Lesotho bats formed a single distinct lineage on the nuclear tree, closely related to *R. darlingi*, *R. fumigatus*, and *R. damarensis* (in a single lineage each). These results indicate introgressions of mtDNA from the Lesotho bats to *R. damarensis*. Morphologically, the Lesotho bats grouped distinctly from other species of the *fumigatus* and *ferrumequinum* groups. We thus suggest the Lesotho horseshoe bats to be considered a new separate species.

Keywords: Taxonomy; biogeography; *Rhinolophus*; Afro-tropics; southern Africa.

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Introduction

The Kingdom of Lesotho is a small African country (area of 30,355 km^2), encircled by the territory of South Africa. It represents a mountainous island in southern Africa and one of the most mountainous countries of the world; it lies within the altitude range of 1,385–3,482 m a. s. l. and most of the country is situated above 2,000 m. Due to this high elevation, the biota of Lesotho has an alpine character and the diversity of fauna is rather low, in contrast to the diversity-rich subtropical zone of the surrounding parts of southern Africa.

Until now, only eight bat species of five families were documented from Lesotho (Lynch 1994, Bronner et al. 2003, Monadjem et al. 2020). Of the horseshoe bat family, Rhinolophidae, one species is reported to occur in the country, affiliated by all authors to a widespread Afro-Arabian species, *Rhinolophus clivosus* Cretzschmar, 1828 (Lynch and Watson 1990, Lynch 1994, Taylor 2005, Monadjem et al. 2010, 2020, Benda and Vallo 2012; in general sense also e.g., Stoffberg et al. 2012, Bernard and Happold 2013, Odendaal et al. 2014, Jacobs et al. 2017, Burgin 2019). In the traditional sense, this species represents a medium-sized bat of the family, characterised by its high but bluntly rounded connecting process of the rather narrow nose-leaf and a small (or missing) externally positioned small upper premolar, P^2 (Hayman and Hill 1971, Csorba et al. 2003). It is regarded a member of the *R. ferrumequinum* group (Bogdanowicz 1992, Koopman 1994, Csorba et al. 2003, Dool et al. 2016, Demos et al. 2019) belonging to the Afro-Palaearctic clade of the genus *Rhinolophus* Lacépède, 1799 (Guillén Servent et al. 2003, Zhou et al. 2009, Stoffberg et al. 2010, Dool et al. 2016, Demos et al. 2019).

However, the traditional species rank of *R. clivosus* has been recently split into three separate species, two occurring in the Palaearctic, Saharo-Arabian *R. clivosus* s.str. and Cyrenaican endemic *R. horaceki* Benda et Vallo, 2012, and one distributed widely in the Afro-tropics, *R. acrotis* von Heuglin, 1861 (Benda and Vallo 2012, Uvizl et al. in press). Thus, the Lesotho populations should represent a part of the latter species that is composed of two subspecies, well characterised by their phylogenetical, morphological and ecological traits. The small-sized nominotypical form, *R. a. acrotis*, is an endemic of the Ethiopian Highlands in north-eastern Africa, and the large-sized form *R. acrotis augur* Andersen, 1904 occurs in savannahs of eastern and southern Africa, between Kenya and South Africa (Uvizl et al. in press).

However, the Lesotho horseshoe bats occur in the highest parts of the Drakensberg and Maluti Mountains and thus, they cannot be simply considered as a usual savannah-dwelling species. These populations were first discovered in the Sehlabathebe National Park in eastern Lesotho by Lynch nad Watson (1990), in the area situated around 2,500 m a. s. l. Additional records (twelve in total) evidenced this bat from other, somewhat lower parts of Lesotho, showing it to be the second most widespread bat of this country (Lynch 1994; own unpubl. data).

Without any doubts, concerning the ecological characters, the Lesotho horseshoe bat assigned to *R. clivosus* (= *R. acrotis*) differs from other populations of the species, being an inhabitant of montane grassland plateaus, instead of lowland savannahs. However, the phylogenetic position of the Lesotho populations has not been examined till now. A question remains, whether these populations represent just a montane variation of *R. acrotis*, as they are regarded traditionally (see e.g. Lynch 1994, Taylor 1998, 2005, Monadjem et al. 2020), or whether they are phylogenetically more exclusive. Although the specimens of horseshoe bats from Lesotho are scarce in collections, we conducted morphological and genetic examinations of a set of over 30 specimens,

including newly collected bats, and compared them with other African taxa of the family. The results of these two approaches are synthesised here.

Material and methods

Morphometric comparison

For the comparative morphometric analysis and for description of morphologic trends in particular populations of the *Rhinolophus* bats, we used a series of cranial and cranio-dental (tooth-row) measurements and the forearm length (LAt) as a standardised dimension referring to body size; other external dimensions were taken from the freshly collected NMP specimens, in other specimens the data were taken from museum preparations, but were not used in the comparisons. The skulls were measured in a standard way using mechanical and optical callipers with accuracy to 0.02 mm and 0.01 mm, respectively; horizontal dental dimensions (tooth-rows) were taken on cingulum margins of the teeth. We evaluated 15 dimensions in each skull (see Abbreviations below); the skull and tooth shapes we described with the help of relative dimensions (indices) calculated from the plain dimensions (see Table 1). The sexual dimorphism was not considered in the morphometric comparisons. The relevant type materials were examined within the morphologic comparison, viz. *Rhinolophus augur* Andersen, 1904 (BMNH), *Rhinolophus augur zuluensis* Andersen, 1904 (BMNH), *Rhinolophus augur zambesiensis* Andersen, 1904 (BMNH), and *Rhinolophus darlingi damarensis* Roberts, 1946 (TM). For the complete list of specimens examined see Appendix. Statistical analyses (basic statistics, principal component analysis) were performed using the Statistica 6.0 software. The nomenclature of morphological traits follows Csorba et al. (2003: xxv–xxx).

Molecular genetic analyses

In the genetic analysis, we used muscle tissue samples of 12 specimens of *Rhinolophus* from Lesotho and 34 specimens of *R. damarensis* from Namibia from the NMP collection to extract DNA (Table S1). We supplemented this dataset with numerous additional sequences of *Rhinolophus* from the Afro-Palaearctic clade of the genus from previous studies stored in the GenBank (Jacobs et al. 2013, Dool et al. 2016, Demos et al. 2019, Benda et al. 2022, Uvizl et al. 2023). For the taxonomic content of particular species-groups and clades of the genus *Rhinolophus* used in the analysis see Table S1. As an outgroup, we added GenBank sequences of *Rhinolophus* species from other clades of the genus and sequences of three *Hipposideros* species from the sister family Hipposideridae (Teeling et al. 2005, Foley et al. 2015; for the details see Table S1).

The genomic DNA was extracted from the alcohol-preserved tissue samples using Geneaid Genomic DNA Mini Kit. We targeted one mitochondrial marker (mtDNA), including 1057 bp of cytochrome b (*Cyt-b*) and five nuclear markers (nDNA), consisting of 537 bp of acyl-coenzyme A oxidase 2 intron (*ACOX*), 618 bp of biglycan intron (*BGN*), 734 bp of COP9 signalsom subunit 7A intron (*COPS*), 493 bp of the rogdi atypical leucine zipper (*ROGDI*), 525 bp of the signal transducer and activator of transcription 5A intron (*STAT*), and 444 bp of thyrotropin beta chain precursor (*THY*). The primers used have been specifically designed for the order Chiroptera and provided good amplification in previous studies (see e.g., Puechmaille et al. 2011, Salicini et al. 2011, Thong et al. 2012, Jacobs et al. 2013, Dool et al. 2016). For the primer names, their sequences, and annealing temperatures, see Table S2. The PCR products were Sanger-sequenced from both sides using the PCR primers by Macrogen, Inc. (Amsterdam, the Netherlands).

The sequences were edited and aligned using the MAFFT plugin (Katoh and Standley 2013) in Geneious 11.0.5 (https://www.geneious.com), subsequently manually edited and trimmed using Gblocks (Castresana 2000). Heterozygous positions in the nDNA markers were coded with the IUPAC codes and ambiguous positions or missing data were coded with 'N'. Indels were treated as gaps. Sequences of protein-coding markers were translated to aminoacids to check for the presence of stop codons, which would indicate pseudogenes have been amplified. The two final datasets were made according to the mode of inheritance of the markers, mitochondrial and multilocus nuclear dataset. The mitochondrial dataset contained *Cyt-b* sequences of a total length of 1057 bp. The nuclear dataset consisted of *ACOX*, *BGN*, *COPS*, *ROGDI*, and *STAT* sequences of a total length of 2907 bp. The nuclear dataset was partitioned by gene. The supplement dataset was composed of *THY* sequences of a total length of 444 bp. This dataset was prepared to show where our samples belong on the *THY* tree of Jacobs et al. (2013).

Phylogenetic analyses of both datasets were run using Bayesian inference (BI) and maximum likelihood (ML). The appropriate nucleotide substitution model for each partition was selected based on the Bayesian information criterion using ModelFinder (Kalyaanamoorthy et al. 2017; see Table S3). We used MrBayes v3.2.6 (Ronquist and Huelsenbeck 2003) to run the BI analysis. Appropriate substitution models were specified for each partition and all parameters were unlinked across partitions. We ran two independent runs for 20 million generations with trees sampled every 1000 generations. All other parameters were set to default. Stationarity and convergence of the runs were inspected in Tracer v1.6 (Rambaut et al. 2014) and the value of the average standard deviations of the split frequencies that were lower than 0.01. The burn-in fraction was left as the default at 25% of sampled trees. Thus, from the 20,000 produced trees, 5,000 were discarded. A majority-rule consensus tree was produced from the post-burnin trees with posterior probability (PP) values embedded. The BI analyses were run through CIPRES Science Gateway (Miller et al. 2010). Then, we inferred the maximum-likelihood tree using the partition model in IQ-TREE (Nguyen et al. 2015, Chernomor et al. 2016). Searching for the best-scoring ML was performed by ultrafast bootstrap (UFBoot; Hoang et al. 2018) with 1,000 bootstrap and 1,000 topology replicates. To verify robustness of the ML tree, the branch supports were evaluated using SH-like approximate likelihood ratio test (SH-aLRT; Guindon et al. 2010) and a Bayesian-like transformation of aLRT (aBayes; Anisimova et al. 2011). SH-aLRT was performed with 1000 replications; aBayes branch support was used instead of Bayesian posterior probabilities because aBayes is more conservative, more robust to model violation and moreover exhibits the best power (Anisimova et al. 2011). The ML, SH-aLRT, and aBayes analysis were run on IQtree web server (Trifinopoulos et al. 2016).

Uncorrected p-distances between haplotypes were calculated for the *Cyt-b* in MEGA11 (Tamura et al. 2021). The bootstrap was performed with 1,000 replications.

Abbreviations

External dimensions: LC = head and body length; LCd = tail length; LAt = forearm length; LA = ear length; LaFe = horseshoe width.

Cranial dimensions: LCr = greatest length of skull incl. praemaxillae; LCc = condylocanine length; LaZ = zygomatic width; LaI = width of interorbital constriction; LaInf = rostral width between infraorbital foramens; LaNc = neurocranium width; LaM = mastoidal width of skull; ANc = neurocranium height; LBT = largest horizontal length of tympanic bulla; CC = rostral width between canines (incl.); M^3M^3 = rostral width between third upper molars (incl.); CM^3 = length of upper tooth-row between canine and third molar (incl.); LMd = condylar length of mandible; ACo = height of coronoid process; CM_3 = length of lower tooth-row between canine and third molar (incl.).

Dental dimensions: LCs = largest mesio-distal length of upper canine; LaCs = largest palato-labial width of upper canine; LP^41 = largest mesio-distal length of large upper premolar on labial cingulum; LP^42 = mesiodistal length of large upper premolar on palatal cingulum (largest dimension taken over the palato-mesial to palato-distal points of the talon); LP^43 = smallest mesio-distal length of large upper premolar taken over the talon constriction; LaP^4 = largest palato-labial width of large upper premolar taken over the mesio-labial and palato-distal cingulum margins; LM^1 = largest mesio-distal length of first upper molar taken over parastyle and metasyle; LaM^1 = largest palato-labial width of first upper molar taken over parastyle and palato-distal part of talon; LM^3 = largest mesio-distal length of third upper molar; LaM^3 = largest palato-labial width of third upper molar taken over parastyle and palatal cingulum; LCi = largest mesio-distal length of lower canine; LP_2 = largest mesio-distal length of first lower premolar; LaP_2 = largest labio-lingual width of first lower premolar; LP_4 = largest mesio-distal length of last lower premolar; LaP_4 = largest labio-lingual width of last lower premolar; LM_1 = largest mesio-distal length of first lower molar taken over paraconid and hypoconulid.

Collections: BMNH – Natural History Museum, London, United Kingdom; DM – Durban Natural Science Museum, Durban, South Africa; KM – Amathole Museum (formerly the Kaffrarian Museum), Qonce, South Africa; MSNG – Civil Natural History Museum Giacomo Doria, Genoa, Italy; MZUF – Natural History Museum, Florence, Zoology Section "La Specola", Italy; NMB – National Museum, Bloemfontein, South Africa; NMP – National Museum (Natural History), Prague, Czech Republic; NMW – Natural History Museum, Vienna, Austria; SMF – Museum and Research Institute Senckenberg, Frankfurt, Germany; SMW – National Museum, Windhoek, Namibia; TM – Ditsong National Museum of Natural History (formerly Transvaal Museum), Pretoria, South Africa; ZFMK – Zoological Institute and Museum Alexander Koenig, Bonn, Germany.

Other abbreviations: A = alcohol preparation; B = skin; f = female; M = mean; m = male; min, max = dimension range margins; S = skull; SD = standard deviation.

Results

Morphometric comparison

The comparison of metric characters showed the Lesotho horseshoe bats to be a morphotype distinct from morphotypes represented by the samples of *R. acrotis* (Fig. 1; Table 1). Concerning body and skull size, the Lesotho bats (LAt 48.6–55.3 mm, LCc 18.4–19.5 mm) are in the middle position to the two population sample sets of *R. acrotis*; they are smaller than the samples of *R. a. augur* from southern and eastern Africa (LAt 51.3–57.4 mm, LCc 18.9–20.6 mm) and larger than the samples of *R. a. acrotis* from the Ethiopian Highlands (45.9–53.2 mm, 17.3–18.8 mm). However, the comparison of plain dimensions selected four specimens from South Africa (Transvaal and Free State), originally labelled as *R. clivosus* (= *R. acrotis*) being small in the skull size and falling into the size range of the Lesotho bats (Fig. 1).

The principal component analysis (PCA) based on all 15 skull and tooth-row dimensions taken (Fig. 2; PC1=71.84% of variance, PC2=8.93%) clearly separated the three size-based groups and showed the Lesoto bats as a separate morpho-group. However, neither the univariate nor multivariate comparisons of plain dimensions solved the positions of all type specimens included in the analyses, since the type specimen of *R. augur zuluensis*, a name considered synonymous with *R. acrotis augur*, conforms in its size traits with the size range of the Lesotho bats (see Figs. 1, 2; Table 3); while the three remaining type specimens fall into the size range of *R. acrotis augur* (*R. augur* and *R. augur zambesiensis*) or of *R. acrotis acrotis* (*R. darlingi damarensis*).

However, the skull shape of the morphotype of the Lesotho bats differs considerably from those of *R. acrotis* (Fig. 3; Table 1). The relative dimensions of their skull and skull parts show completely distinct values than those of *R. acrotis*; in the Lesotho bats the rostrum is relatively very short (CM^3/LCc 0.413–0.430), while in both sample sets of *R. acrotis* the rostrum is relatively long (CM^3/LCc 0.433–0.472). The comparison did not show any overlap between the value ranges of these two groups. Similarly, the relative width of skull separated most of the samples, although with a slight overlap of their value ranges. In the Lesotho bats the skulls are relatively narrow (LaZ/LCc 0.546–0.585), while in the *R. acrotis* morphotype they are relatively wide (LaZ/LCc 0.570–0.614). The three type specimens of taxa traditionally assigned to *R. acrotis* fall into the dimension ranges of this form and not of the morphotype of the Lesotho bats (Fig. 3). The four very small specimens labelled as *R. clivosus* (= *R. acrotis*) from South Africa (Transvaal and Free State) belonged to both different morphotypes of the skull shape, the Transvaal bats ranked among the samples of *R. acrotis*, while the Free State bats fell among the Lesotho bats (Fig. 3).

On the other hand, the comparison of the Lesotho horseshoe bats with the samples of *R. damarensis* from Namibia (demonstrated to be in a close position to the Lesotho bats by the molecular genetic analysis, see below) showed these two sets similar to each other in the skull shape and in this respect, in a similar common position to the sets of *R. acrotis* samples (Fig. 3). Nevertheless, these two sets represented two different morphotypes, since the Namibian bats were much smaller in their body and skull size than the Lesotho bats (Fig. 1), and additionaly, on average they showed a relatively longer rostrum than the Lesotho bats (Fig. 4; Table 1). The type specimen of *R. darlingi damarensis* was placed within the group of specimens from Namibia in all comparisons.

Molecular genetic analysis

In this study, we newly generated 44 of the *Cyt-b* sequences (12 for *Rhinolophus* from Lesotho and 32 of *Rhinolophus* from Namibia) that were pruned to 27 unique sequences (two for *Rhinolophus* from Lesotho and 25 of *Rhinolophus* from Namibia). These unique sequences were supplemented with GenBank sequences and final mitochondrial dataset thus comprised 343 sequences, including two for *Rhinolophus* from Lesotho and 42 for *Rhinolophus* from Namibia. For the nuclear dataset we generated 25 *ACOX*, 23 *BGN*, 25 *COPS*, 25 *ROGDI*, and 25 *STAT* sequences which were supplemented with 632 sequences from GenBank and the sequences of five nuclear introns were concatenated. Only individuals with at least three introns sequenced were employed for the analyses. In total, the final nuclear dataset was composed of sequences for 185 specimens, including eight for *Rhinolophus* from Lesotho and 23 for *Rhinolophus* from Namibia. The *Cyt-b* dataset was 1057 bp long and contained 458 parsimony informative positions (43.33% of total length) and this marker showed much bigger genetic differentiation within the examined samples of the genus *Rhinolophus* than the nuclear markers. The amount of parsimony informative positions in the concatenated nuclear dataset was 492, i.e., 16.92% of its total length. The nuclear dataset was 2907 bp long (537 bp of ACOX, 618 bp of BGN, 734 bp of COPS, 493 bp of ROGDI, and 525 bp of STAT) and missing data accounted for 17.64% of the dataset. For individual nuclear gene trees see Figs. S1–S5.

The phylogenetic trees obtained by both ML and BI analyses of the *Cyt-b* dataset showed almost identical topologies. The deviations between the two methods received generally low support. However, the ML tree showed the topology without any polytomy and thus, we present the tree conducted by this method. In the ML tree (Fig. 5), the *Rhinolophus* sequences from Namibia formed three distinct lineages with moderate to high branch support (bootstrap percentage [BP]=62–97, posterior probability [PP]=0.97–0.99). The first lineage (Namibia1) was composed of samples from northern Namibia and a sample from the Democratic Republic of the Congo (DRC), the second lineage (South Africa) included samples from a large part of southern Africa (South Africa, Lesotho, southern Namibia), and the third lineage (Namibia2) only samples from northern Namibia. The sequences of *Rhinolophus* from Lesotho were thus a part of the lineage otherwise composed of sequences from Namibia and South Africa, identified as of *R. damarensis*. The haplotypes of the third lineage (Namibia2) were obtained from samples originating from the northern part of Namibia, from the same area and, in some cases, also from identical localities as the samples providing the haplotypes of the first lineage (Namibia1). The uncorrected genetic distances between the three lineages lied in the range of 3.97–7.10%, the distances within particular lineages ranged between 0% and 4.88% (Table S4).

Quite different arrangements than resulting from the mitochondrial gene analysis occurred in the results obtained from the nuclear dataset. The ML and BI trees of the nuclear dataset looked almost identical with lowly branch supported deviations. In the ML tree (Fig. 6), a single lineage was constituted from all *Rhinolophus* samples from Namibia, plus one sample from the DRC and two from South Africa. The sequences from Lesotho bats together with one sequence from South Africa (SouthAfrica13), which laid on the basis of two sister *Cyt-b* clades (Namibia1 and South Africa; see Fig. 5), formed a distinct lineage ([BP]=100, [PP]=1.00) in a sister position to the joint clade of *Rhinolophus fumigatus* and *R. darlingi*. All the rest of the *Rhinolophus* samples from Namibia and South Africa formed the second nuclear clade ([BP]=69, [PP]=1.00) that lied on the base of the whole *R. fumigatus* group.

The arrangement of the haplotypes of *Rhinolophus* from Lesotho thus differed between the nuclear and mitochondrial analyses, as well as the haplotypes from *R. damarensis* did. While three mitochondrial lineages of *R. damarensis* haplotypes showed in the analysis of mtDNA, these samples were grouped into two nuclear lineages with a different geographic distribution in the analysis of the nDNA markers.

Discussion

The *Rhinolophus* populations of the Kingdom of Lesotho have been discovered relatively recently, no occurrence of such bats was reported from the territory of Lesotho by Ellerman et al. (1953), Roberts (1954), Meester et al. (1964, 1986), Csorba et al. (2003), or Simmons (2005). Although the horseshoe bats were first collected there at the end of the 19th century (three specimens housed in the MSNG), all other bats were documented in the second half of the 20th century (first in 1978, a specimen in the KM) or even later (see Appendix), and were first published by Lynch and Watson (1990). All recorded *Rhinolophus* bats from Lesotho, both the unpublished MSNG and NMP specimens and published specimens housed in the KM and NMB collections (Lynch and Watson 1990, Lynch 1994, Monadjem et al. 2010, 2020, Benda and Vallo 2012; original data) were originally identified as *R. clivosus*, a species of the *ferrumequinum* group that has a rather unadvanced stage of morphological characters within the genus (Csorba et al. 2003). The universal morphological matrix of the horseshoe bats of this group that is currently composed of five species (*R. ferrumequinum*, *R. nippon*, *R. clivosus*, *R. acrotis*, *R. bocharicus*; see Uvizl et al. in press) led formerly to the inclusion of populations (mostly at the level of subspecies) of other species, which are phylogenetically rather distant and represent not only separate species, but even members of different species groups. This could be illustrated by the cases of *R. horaceki* or *R. damarensis*, both formerly considered a part of the *R. clivosus* species rank, but now representing separate species out of the *ferrumequinum* group – in both cases the current systematic position was demonstrated by a combination of morphological and molecular genetic approaches (see Csorba et al. 2003, Benda and Vallo 2012, Jacobs et al. 2013).

Despite the apparent morphological similarity of the Lesotho horseshoe bats and the representatives of *Rhinolophus clivosus* s.l. from Africa that are currently referred to *R. acrotis* (Uvizl et al. in press), namely in the body size and the structure of nose-leaf, the morphometric comparison demonstrated a unique position of the Lesotho bats in relation to the samples of *R. acrotis*. The skull size of the Lesotho bats does not conform to other African populations, being significantly smaller than the southern African samples and larger than the Ethiopian samples of *R. acrotis*. In the skull shape – the relative width of skull and relative size of rostrum – the Lesotho bats also differ from *R. acrotis*, being smaller in both these parameters than the latter bats. In short, the Lesotho bats represent a unique morphotype that does not conform in its characters with the morphology of *R. acrotis*. Results of the molecular genetic analysis showed the phylogenetic position of the Lesotho bats out of the *ferrumequinum* group and

classified them close to the lineages of *R. damarensis*, *R. fumigatus*, *R. darlingi* and/or *R. hildebrandti* from the *fumigatus* group. Hence, both the morphometric and molecular genetic comparisons demonstrated sufficiently the Lesotho bats as a phylogenetic unit separate from the rank of *R. acrotis* and the *ferrumequinum* group as well.

According to the results of the mitochondrial DNA analysis, the position of the Lesotho bats is among the samples of *R. damarensis* of the southern lineage sensu Jacobs et al. (2013). This group (i.e. the lineage South Africa in Fig. 5) occurs in the southern part of Namibia (south of ca. 24°S, see Maluleke et al. 2017) and north-western part of South Africa, mainly in northern Northern Cape and also in western Transvaal (Jacobs et al. 2013). Besides the southern lineage, Jacobs et al. (2013) documented a northern lineage of *R. damarensis* from the northern part of Namibia, which corresponds with the lineage Namibia1 in Fig. 5, and comprises also a sequence from the DRC (Demos et al. 2019). This lineage contains also the haplotype of the holotype specimen (TM 9474) of *R. darlingi damarensis* Roberts, 1946 (Namibia32), taken from Jacobs et al. (2013). However, the mitochondrial sequences of the samples of *R. damarensis* from northern Namibia created also another lineage (Namibia2) that was mentioned neither by Jacobs et al. (2013), Dool et al. (2016), nor Demos et al. (2019); it was positioned basally in the tree part composed of the *ferrumequinum*, *fumigatus*, and *maclaudi* groups, and of *R. horaceki*, i.e. quite distant from the position of the northern and southern lineages of *R. damarensis* including the Lesotho bats that are embedded within the *fumigatus* group. Nevertheless, the belonging to two mitochondrial lineages did not affect the populations of *R. damarensis* of northern Namibia; both lineages are represented by an identical morphotype (as in the southern lineage), and are present in bats occurring in the same areas and even same roosts (Arnhem Cave, Ghaub Cave, Dragon's Breadth Cave, Karavatu Mine, see Table S1; largest direct distance between these roosts is 385 km).

The analysis of nuclear markers brought a completely different picture of the phylogenetic position of the Lesotho horseshoe bats, which created a lineage of its own, composed of four haplotypes (instead of only two from the mitochondrial marker). This Lesotho lineage additionally contained one haplotype from South Africa (SouthAfrica13), originating from Uintjiesberg Farm, south-eastern Northern Cape, which is the southernmost locality referred to *R. damarensis* by Jacobs et al. (2013) and Maluleke et al. (2017). However, the nuclear sequences of *R. damarensis* from Namibia, DRC, and most of its South African range (Vioolsdrif and Soetfontein, NW and NE of Northern Cape; Dool et al. 2016) created one common lineage, which comprised samples of all three mitochondrial lineages produced by this bat species. The nuclear markers thus demonstrated a considerable geographical separation of the samples of the two lineages, one occurring in Lesotho and south-east Northern Cape, and the other in northern Northern Cape, in Namibia, and in the western DRC (so, most probably also in western Angola); the geographical gap between the ranges of these lineages is at least 250 km. However, besides the strong genetic and geographical (and perhaps also ecological) differences between the lineages, the morphometric comparison also showed morphological differences between them. The bats of the Lesotho lineage are larger and with a relatively shorter rostrum than the bats of the Namibia lineage, they thus represent separate morphotypes. These results suggest that these nuclear lineages represent two separate evolution entities.

The incongruity between the phylogenetic patterns brought by different marker types could be explained by repeated historical introgressions of the mitochondrial DNA between taxa, which however, did not influence the real identity of the taxa as the nuclear DNA was not affected by the introgression. Such past introgressions were suggested to have occurred in many mammal taxa, including bats and even horseshoe bats, and even in southern Africa (see e.g., Vallo et al. 2013, Dool et al. 2016, Taylor et al. 2018). Considering the topologies of both the nuclear and mitochondrial trees obtained in our analysis, the mitochondrial lineage Namibia2 most probably represents the original mitochondrial genetic lineage of *R. damarensis*, its rather basal position to other lineages of the *Cyt-b* tree is similar to the position of the Namibia lineage in the nucDNA tree. The Namibia1 (northern) and South Africa (southern) mitochondrial lineages represent genetic forms close or conforming to the Lesotho genetic material. Since these lineages are two, such introgressions of the Lesotho genetic material into the *damarensis* mitochondrial genes took place twice, the Namibia1 (northern) lineage is a result of an older event and the haplotypes are slightly derived, while the South Africa (southern) lineage is a product of more recent event, since the differences between mitochondrial haplotypes of *R. damarensis* and the Lesotho bats are minute or none at all. This hypothesis suggests that the geographical gap between the distribution ranges of the Lesotho bats and *R. damarensis* is a situation that appeared rather recently and the direct contact between these populations, when the mitochondrial genes flew from one nuclear lineage to the other, discontinued only a short time ago. The Lesotho mitochondrial genes clearly seem to be the maternal basis for the forming of the two lineages, since an unaffected *damarensis* lineage (Namibia2) exists along the two derived. As the mitochondrial DNA is a maternally inherited genome, the large-sized females of the Lesotho bats were perhaps more attractive for the males of *R. damarensis* than the females of their own lineage and mediated the admixture of the mitochondrial genetic material of the two nuclear lineages.

In summary, our study has brought a new view on the phylogenetic position and relationships of the Lesotho horseshoe bats. These populations, considered traditionally to be a part of the species rank of *Rhinolophus clivosus* s.l. of the *ferrumequinum* group, were demonstrated to represent a phylogenetic entity of its own, embedded to the *fumigatus* group. Although this entity shares its mitochondrial genome with *R. damarensis*, it is completely separated from this species as it represents a nuclear genetic lineage of its own. This independence from *R. damarensis* is supported also by a peculiar morphotype of the Lesotho bats that differs from the morphotype of *R. damarensis*.

The results of the genetic analysis suggest that the lineage comprising Lesotho bats is not similar enough to a lineage of any other horseshoe bat species of southern Africa and thus, it represents a separate species of the horseshoe bat. This species occurs in mountainous areas of Lesotho and moreover, it was found at two sites in South Africa, Jagersfontein in the western Free State (confirmed by the morphometry), and Uintjiesberg Farm in south-eastern Northern Cape (confirmed by the genetics). The latter locality was reported as the southernmost locality of *R. damarensis* by Jacobs et al. (2013) and Maluleke et al. (2017) based on the mitochondrial marker analysis, however, the nuclear DNA analysis showed the concerned bats to be a part of the Lesotho lineage and the locality in fact represents the westernmost known point of its occurrence. The distribution range of the Lesotho horseshoe bats is thus much larger than is the territory of the Kingdom of Lesotho. Despite this, there is no name in the synonymy of the genus *Rhinolophus* available for this horseshoe bat species (Allen 1939, Ellerman et al. 1953, Roberts 1954, Csorba et al. 2003, Simmons 2005, Monadjem et al. 2020). Therefore, a new species of *Rhinolophus* from Lesotho and South Africa is described here, again slightly extending the recently enriched southern African diversity of this genus (cf. Taylor et al. 2012, 2018).

Descriptive taxonomy

***Rhinolophus XXX* sp. n.**

Synonymy. *Rhinolophus clivosus* Cretzschmar, 1828: Lynch and Watson 1990: 532; Lynch 1994: 190; Taylor 1998: 35; Taylor 2005: 338; Monadjem et al. 2010: 188, 556; Benda and Vallo 2012: 93; Monadjem et al. 2020: 216, 659.

Type material. *Holotype*: ♀ ad. (NMP 97760, field No. pb5596, alcohol specimen with skull extracted), Sehlabathebe National Park, old park lodge, 17 February 2013, leg. N. Avenant, P. Benda & J. Červený. – *Paratypes*: 11 ♀♀ ad., 1 ♂ sad. (NMB pb5586–5591, NMP 97756–97759, field Nos. pb5586–5595, alcohol specimens with skull extracted; NMB pb5585, NMP 97761, field Nos. pb5585, pb5597, alcohol specimens), locality, date, and collectors as in the holotype.

Type locality. Kingdom of Lesotho, Sehlabathebe National Park, small cave near the old park lodge, 29°52'02"S, 29°07'15"E, 2,425 m a. s. l. (Figs. 7, 8).

Description. *Rhinolophus XXX* sp. n. is a medium-sized horseshoe bat, in most respects similar to the large-sized forms of *R. clivosus* Cretzschmar, 1828 and medium-sized forms of *R. acrotis* von Heuglin, 1861 from the Middle East and Africa, including the structure and relative size of the nose-leaf and the ear size. Forearm length 48–56 mm, ear length 22.8–23.6 mm, horseshoe width 8.3–9.1 mm, condylocanine length of skull 18.4–19.5 mm, length of the upper tooth-row 7.0–7.9 mm.

The horseshoe of *R. XXX* sp. n. is relatively wide (Fig. 9), the connecting process of the nose-leaf is high and rounded in side view, the sella is constricted in the middle, tip of the sella is pointed, lancet is hairy and triangular in shape. One medial groove is present in the lower lip (Fig. 9).

The dorsal pelage of *R. XXX* sp. n. is pale brown, ventral pelage is very pale brown to beige (Figs. 8, 9); in juveniles and subadult animals, the dorsal pelage is grey with very weak brown tinge on cheeks, ventral pelage pale grey (Fig. 8). Nose-leaf and ears are greyish brown, distal parts darker than the proximal, which are pinkish in tinge; in juveniles and subadult animals, the nose-leaf and ears are grey (Figs. 8, 9). Wing membranes are greyish-brown or dark greyish-brown.

Skull is relatively narrow (LaZ 10.1–11.1 mm; LaZ/LCc 0.546–0.585), rostral part of the skull inluding the nasal swellings is medium wide (LaInf 5.2–6.0 mm; CC 5.1–5.9 mm; LaInf/LCc 0.282–0.313), and relatively very short (CM^3/LCc 0.385–0.407; Fig. 10). The braincase is relatively medium-wide, but relatively low (ANc 6.0–6.7 mm; ANc/LCc 0.319–0.362), the sagittal crest is low and rather undeveloped, infraorbital foramen is large and infraorbital bar is long and thin (Fig. 10). Nasal swellings are rather undeveloped, the posterior median swellings are slightly longer and narrower than the anterior median swellings, the lateral swellings (both anterior and posterior) are smaller than the median swellings, the frontal depression is shallow (Fig. 10).

The teeth are relatively weak (Fig. 11); upper molars are relatively narrow (LaM^1/LM^1 1.345–1.454; LaM^3/LM^3 1.502–1.1.663), large upper premolars (P^4) are relatively wide and short in the mesio-distal aspect (LP^4/LaP^4 0.585–0.681), with a well marked concavity in the distal margin of talon (LP^43/LP^41 0.451–0.563). Large lower premolars (P_4) are absolutely small (LP_4 1.06–1.23 mm) and, in relation to the size of smaller lower premolars (P_2), very small (LP_2 0.73–0.87 mm), the area of P_2 is larger more than a half of the area of P_4 ($LP_2 \times LaP_2/LP_4 \times LaP_4$ 0.503–0.691).

The minute first upper premolar (P^2) is present (LP^2 0.29–0.40 mm), it is positioned labially, the upper canine and large premolar (P^4) are not in contact with each other but only with the minute premolar (P^2; Fig. 11). The minute second lower premolar (P_3) is mostly present (in 72.7% of the type series, n=11), it is very small (LP_3 0.17–0.25 mm) and lies out of the premolar tooth-row, the first (P_2) and third (P_4) lower premolars are in direct contact in most cases (63.6% of the type series; Fig. 11).

The baculum of *R. XXX* sp. n. remains unexplored, no adult male specimen was available for examination.

Dimensions of the holotype. See Table 3.

Mitochondrial sequence of the holotype (partial sequence – 1057 bp – of the mitochondrial gene for cytochrome *b*; GenBank Accession Number xxxxx; 5' end). cat gac caa cat tcg caa gtc tca ccc act att caa aat cat caa cga ctc gtt cgt tga cct acc cgc ccc atc aag tat ctc ttc ctg atg aaa ctt cgg atc tct cct agg aat ctg cct agc cat cca aat tct cac cgg act gtt cct agc aat aca cta cac atc aga cac cgc tac agc ctt cca ctc cgt gac cca cat ttg ccg aga tgt caa cta cgg ctg aat cct gcg cta cct cca tgc caa cgg agc ctc cat att ctt tat ctg cct gtt cct aca cgt agg acg agg aat cta tta tgg ctc cta tac att ctc aga aac atg aaa cat cgg aat cat cct cct ctt cgc tgt cat agc cac agc att cat agg cta tgt act ccc atg agg cca aat atc ctt ctg agg ggc aac agt tat cac aaa cct cct ctc agc tat tcc ata cgt cgg aac aac tct agt tga atg agt ctg agg cgg gtt ctc agt tga taa agc tac act cac ccg att ctt cgc cct aca ctt cct ttt acc att cat tat tgc agc tat agt cat agt cca cct act ttt cct cca cga aac agg atc aaa caa ccc aac cgg aat ccc atc aga cgc aga cat aat ccc att cca ccc cta cta cac cat caa aga cat cct agg cct cgt act aat act aat agc act act gtc cct agt act att tgc ccc cga cct act ggg tga ccc aga caa cta cac ccc agc caa ccc act aaa cac ccc acc cca cat taa acc aga gtg gta ctt tct att tgc cta cgc aat cct acg ctc aat ccc aaa taa gct cgg cgg agt tgt agc cct agt cct atc cat cct tat cct agc tgt cat ccc act act cca cac atc aaa aca acg cag cat gac att tcg acc cct aag cca atg cct att ctg act cct agt ggc aga cct cct cac act aac ctg aat cgg agg cca acc tgt cga gca ccc att tat cat cat cgg aca act agc ctc cat tct ata ctt cct aat tat cct cgt cct aat acc act tgc agg cat cgc aga aaa cca tct atg aaa tga aga.

ZooBank No. xxxxx

Derivatio nominis. Eponymous; named in honour of Professor Jaroslav Červený (Prague, Czech Republic) who significantly contributed to the research of the African bat fauna, and mainly, discovered the bat colony that provided the type series of *Rhinolophus XXX* sp. n. Professor Červený took and provided all here presented photographs of the new species and its type locality.

Distribution. *Rhinolophus XXX* sp. n. is known from twelve sites in Lesotho (Lynch and Watson 1990, Lynch 1994; original data) and from two sites in South Africa (western Free State, south-eastern Northern Cape), see Appendix for particular localities. The localities are situated in the altitude range of 1330–3190 m (mean 2,131 m), two thirds of them (64.3%) lie at altitudes above 2,000 m a. s. l.

Echolocation. According to the only available data (Maluleke et al. 2017) from the Uintjiesberg Farm (South Africa), the resting frequency in the echolocation calls of *Rhinolophus XXX* sp. n. was in the range of 83.3–83.2 kHz (mean 84.9 kHz; recordings from 22 females; Maluleke et al. 2017: 7353). However, these data need a confirmation from the Lesotho populations.

Research ethics: Not applicable.

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Appendix

List of the material examined in morphological comparison (arranged in taxonomical and alphabetical order).

***Rhinolophus XXX* sp. n.**

Lesotho (35): 2 ♀♀ (NMB 7350, 7355 [S+B]), Ha Natla, Thaba Tseka, 19–20 October 1989, coll. J. P. Watson; – 2 ♂♂, 1 ♀ (MSNG 42305, 44908a, 44908b [A]), Hermon (Basutoland), October 1891, leg. F. Christol; – 5 ♀♀ (NMB 6983–6986, 6894 [S+B]), Mateanong, Mokhotlong, 1–10 February 1989, coll. J. P. Watson; – 2 ♂♂, 1 ♀ (NMB 6980–6982 [S+B]), 15 km east of Mateanong, near Nkokomele, 1–10 February 1989, coll. J. P. Watson; – 2 ♂♂ (NMB 8481, 8482 [B]), Moqotoane, Thaba-Tseka, 10 March 1992, coll. J. P. Watson; – 1 ♂ (NMB 8222 [S]), Mount Moorosi, Quthing, 13 November 1991, coll. J. P. Watson; – 1 ♀ (NMB 8300 [S+B]), Phallang, Maseru (Semonkong), 12 December 1991, coll. J. P. Watson; – 1 ♀ (KM 21087 [S+B]), cave 3 km S of Roma, September 1978, collector unlisted; – 1 ♂ (NMB 8418 [S+B]), Ski Lodge, Butha-Buthe (Oxbow), 20 February 1992, coll. J. P. Watson; – 2 ♀♀ (NMB 6863, 6864 [S+B]), Sehlabathebe National Park, lodge, 13 November 1988, coll. J. P. Watson; – 1 ♂, 12 ♀♀ (NMB pb5586–5591, NMP 97756–97760 [S+A], NMB pb5585, NMP 97761 [A], type series of *Rhinolophus XXX* sp. n.), Sehlabathebe National Park, Lodge, 17 February 2013, leg. N. Avenant, P. Benda & J. Červený; – 1 ♀ (NMP 97762 [A]), Semonkong, Semonkong Lodge, 25 February 2013, leg. N. Avenant, P. Benda & J. Červený.

South Africa (2): 1 ♂, 1 ♀ (NMB 7626, 7638 [S+B], Jagersfontein, Commonage, Free State, 12–16 February 1990, coll. J. P. Watson.

***Rhinolophus acrotis acrotis* von Heuglin, 1861**

Eritrea (3): 1 ♀ (MZUF 6000 [A]), Asmara, October 1937, leg. Ignesti; – 1 ♂ (MSNG 44312 [S+A]), Assab, July 1893, leg. G. Pestalozzo; – 1 ♂ (MSNG 27583 [S+B]), Assab, Dancalia, February 1929, leg. S. Patrizi.

Ethiopia (11): 1 ♂ (NMP 95891 [S+A]), Aksum, King Basen's Tomb, 1 November 2012, leg. P. Benda; – 1 ♀ (NMP 95913 [S+A], Aman Amba, Simien National Park, 5 November 2012, leg. P. Benda; – 1 ♂ (NMP 95962 [S+A]), Chara, 15 km E of Bonga, 26 November 2012, leg. P. Benda; – 3 ♀♀ (NMP 95937–95939 [S+A]), Dangola Washa Caves, 5 km SW of Kesa, 12 November 2012, leg. P. Benda; – 1 ♂ (MZUF 6029 [S]), Gorgora, Lago Tana, 1828 m, 13 March 1937, leg. G. Dainelli; – 1 ♀ (MSNG 18243 [S+B]), Harrar, date unlisted, leg. P. Felter; – 2 ♀♀ (MSNG 45630a, 45630b [S+A]), Harrar, 1893, leg. Salimbeni; – 1 ♂ (MZUF 5649 [S]), Lago Tana, 1937, leg. G. Dainelli.

Sudan (1): 1 ♀ (MSNG 46965 [S+A]), Port Sudan, Mar Rosso, January 1908, leg. G. Nicolosi.

***Rhinolophus acrotis augur* Andersen, 1904**

Kenya (6): 1 ♂, 1 ♀ (NMW 32251, 32253 [S+B]), Chepnyalil Cave, Mt. Elgon NP, 24 December 1980, leg. F. Spitzenberger & Kenya Expedition 1980/1981; – 2 ♂♂ (NMW 32256, 32258 [S+B]), Kitum Cave, Mt. Elgon National Park, 24 December 1980, leg. F. Spitzenberger & Kenya Expedition 1980/1981; – 1 ♂ (NMW 32260 [S+B]), Makingeny Cave, Mt. Elgon National Park, 24 December 1980, leg. F. Spitzenberger & Kenya Expedition 1980/1981; – 1 ♀ (SMF 39427 [S]), Naibei's Great Cave, Kapasakwany, Süd Seite des Mt. Elgon, 13 December 1970, leg. Dr. Mutuku.

Malawi (4): 1 ♂ (BMNH 97.10.1.18. [S+B], holotype of *Rhinolophus augur zambesiensis* Andersen, 1904), Fort Hill, N. Nyasa, July 1896, leg. A. Whyba; – 1 ♂, 2 ♀♀ (NMP mw199, mw231, mw232 [S+A]), Ntchisi Forest Reserve, 8–9 July 2008, leg. J. Šklíba.

Mozambique (1): 1 ♀ (MSNG 18316 [S+B]), Quelimane, 5 November 1908, collector unlisted.

Rwanda (1): 1 ind. (SMF 92961 [S+Sk]), Lava-Höhle Ubuvumo, 11 December 2004, leg. Laumanns.

South Africa (30): 1 ♀ (SMF 55037 [S+B]), Doornhoek, Pietermaritzburg, Natal, 27 March 1976, leg. I. W. Espie; – 1 ♂, 2 ♀♀ (DM 8373–8375 [S+A]), Fort Yolland Farm, Eshowe-Melmoth, Entumeni Dist., KwaZulu-Natal, 14 May 2005, leg. P. J. Taylor; – 4 ♂♂, 3 ♀♀ (NMW 26126–26132 [S+B]), Guano Cave, Tsitsikama, Coastal National Park, Cape Prov., 4 December 1975, leg.

F. Spitzenberger & B. Herzig; – 1 ♂ (TM 46882 [S+A]), Haffenden Heights, Limpopo, 3 November 2002, leg. L. Cohen; – 1 ♀ (BMNH 4.5.1.8. [S+B], holotype of *Rhinolophus augur zuluensis* Andersen, 1904), Jususic Valley, 20 mi NW of Eshowe, Zululand, 17 November 1903, leg. C. H. B. Grant; – 1 ♂ (TM 47619 [S+A]), Kaalrug, Mpumalanga, 25 October 2004, leg. L. Cohen; – 1 ♂ (MSNG 44467 [S+A]), Kenilworth, soborgo della Citta del Capo, 15 March 1906, leg. W. L. Sclater; – 2 ♂♂ (NMB 11072, 11075 [S+B]), Koegelbeen Caves, Hay (Griekwastad), Northern Cape, 22 February 1997, coll. N. Avenant; – 1 ♂ (BMNH 4.10.1.1. [S+B], holotype of *Rhinolophus augur* Andersen, 1904), Kuruman, Bechuana, 19 April 1904, leg. R. B. Woosnam; – 3 ♂♂, 1 ♀ (DM 8376–8379 [S+A]), Melmoth, Woodlands Estate, KwaZulu-Natal, 15 May 2005, leg. P. J. Taylor; – 2 ♀♀ (NMB 10573, 10638 [S+B]), Merrimetzie, Winburg Dist., Free State, 6 February 1996, coll. J. P. Watson; – 1 ♂ (MSNG 73 [A]), Ookiep, Namaqualand, 1906, collector unlisted; – 1 ♂ (SMF 44809 [S+A]), Rhin. Z., Transvaal, 27 December 1952, leg. Zumpt; – 1 ♂ (MSNG 42112 [A]), Sud Africa, Rhodesia Mus., date and collector unlisted; – 1 ♀ (TM 46643 [S+A]), Sudwala Caves, Mpumalanga, 18 December 2008, leg. H. C. Schoeman & S. Stoffberg; – 1 ♀ (SMF 19557 [S]), Uitkoms, Transvaal, 19 January 1958, leg. J. Meester; – 1 ♂ (MSNG 44381 [A]), Wunderfontin Caves, Petchepton District, Transvaal, April 1907, collector unlisted.

Tanzania (4): 2 ♂♂ (SMF 91227, 91228 [S+A]), Amani-Sigi Forest Reserve, E Usambara Mts., Tanga Reg., 05°07'S, 38°39'E, 14 March 1999, leg. Frontier; – 1 ♂ (SMF 92505 [S+A]), Nilo Forest Reserve, 3 August 2000, leg. Frontier; – 1 ♀ (NMW 19822 [S]), Ugano, Ruvumq Prov., 1935–1936, leg. H. Zerny.

Uganda (1): 1 ♀ (SMF 44092 [S+A]), Kisoro, Kigezi Dist., 01°17'S, 29°42'E, 30 October 1975, leg. A. B. C. Killango.

***Rhinolophus damarensis* Roberts, 1946**

Namibia (94): 1 ♂, 8 ♀♀ (SMW 1348–1353 [S+B], 6809–6811 [S]), Aar 16, 22 km E Aus, Lüderitz Dist., 18 October 1970, leg. P. J. Buys; – 2 ♂♂ (ZFMK 77.423 [B], 77.428 [S]), Abram Botha Cave, 1 April 1972, leg. H. Roer; – 1 ♂, 3 ♀♀ (NMP pb7050, pb7051, pb7053 [S+A], pb7052 [A]), Ameib Farm, 7 October 2020, leg. P. Benda; – 1 ♀ (SMW 6568 [S]), Arnhem Cave, Arnhem 222, Windhoek Dist., 16 February 1971, leg. C. G. Coetzee; – 1 ♂, 1 ind. (SMW 12214, 12215 [S]), Arnhem Cave, Arnhem 222, Windhoek Dist., 15 October 1988, leg. J. R. Pallett; – 2 ♀♀ (NMP pb5760, pb5761 [S+A]), Arnhem Cave, 21 December 2013, leg. P. Benda & M. Uhrin; – 1 ♂, 2 ♀♀ (NMP pb6556–6558 [S+A]), Arnhem Cave, 27 May 2017, leg. P. Benda & J. Červený; – 1 ♂ (SMW 13397 [A]), Awa Aab, 16 km E of Homeb, Namib Desert Park, 2 July 1977, leg. M. Griffin; – 1 ♂ (NMP pb7064 [S+A]), Bramberg West Mine, 9 October 2020, leg. P. Benda; – 1 ♂, 1 ♀ (SMW 6812, 6813 [S+A]), Daan Viljoen, Windhoek, 13 February 1970, leg. C. Klingel; – 1 ♂ (ZFMK 83.318 [S+B]), Farm Awagobibtal, Raum Grootfontein, 14 January 1982, leg. H. Roer; – 1 ♂ (ZFMK 77.584 [S+B]), Farm Scheidthof, Windhoek Bez., 15 January 1975, leg. H. Roer; – 1 ♂ (KM 31805 [A]), Farm Tjirundo 91, Omaruru Dist., 34.5 km N / 7.8 km W Omaruru, 1300 m, 5 May 1990, collector unlisted; – 1 ♂ (ZFMK 77.583 [S+B]), Farmhaus Dr. Schulz, Naos, 1 February 1969, leg. H. Roer; – 1 ♂ (NMP pb5818 [S+A]), Fransfontein, 10 January 2014, leg. P. Benda, S. Eiseb & M. Uhrin; – 1 ♂ (SMW 7851 [S]), Geduld III, Outjo, 3 July 1978, leg. A. P. Simoes; – 1 ♀ (SMW 10345 [A]), Geduld III, Outjo, 21 October 1982, leg. C. G. Coetzee; – 3 ♂♂, 4 ♀♀ (NMP pb5886–5890 [S+A], pb5891, pb5892 [A]), Ghaub, Dragon's Breadth Cave, 16 July 2014, leg. P. Benda & Uhrin; – 4 ♀♀ (NMP pb5867–5869 [S+A], pb5870 [A]), Ghaub, Ghaub Cave, 14 July 2014, leg. P. Benda & M. Uhrin; – 2 ♂♂ (SMW 7071, 7072 [S]), Gobabeb, Namib Desert Park, April 1977, leg. R. Tilson; – 1 ♂, 7 ♀♀ (KM 1744–1751 [S+B]), Karibib, S.W. Africa, 5 November 1923, leg. G. C. Shortridge; – 1 ♂ (SMW 14404 [A]), Hardap Dam (dam gallery), Mariental Dist., 20 June 1991, leg. R. E. Griffin; – 2 ♀♀ (NMP pb6316 [S+A]), Karavatu Mine, !Uris, 20 August 2016, leg. P. Benda & M. Uhrin; – 3 ♂♂, 4 ♀♀ (NMP pb6434, pb6435, pb6438 [S+A], pb6436, pb6437, pb6439, pb6440 [A]), Karavatu Mine, !Uris, 13 May 2017, leg. P. Benda & J. Červený; – 1 ♀ (NMP pb5790 [S+A]), Khowarib Lodge, 7 January 2014, leg. P. Benda, S. Eiseb & M. Uhrin; – 2 ♂♂ (SMW 6817, 6818 [S+A]), Klein Windhoek, Waterpoint at R.C.M., August 1970, leg. C. G. Coetzee & M. Raath; – 7 ♂♂, 3 ♀♀ (TM 8288, 8290, 8291, 8293, 8294 [S+B], SMF 19555 [S], TM 8289, 8292, 8295, 8296 [B]), Kochina, Gt. Karas Mts., 10 August 1937, leg. Barlow Transvaal Museum Expedition; – 1 ♀ (SMW 14436 [A]), Mara 114, Bethanien Dist., 24 November 1992, leg. E. Marais; – 1 ♂ (KM 32606 [B]), Matchless Leopper Mine, betw. Windhoek and Rehoboth, Rehoboth Dist., 1800 m, 9 June 1937, leg. F. M. Lanham; – 1 ♀ (ZFMK 89.339 [A]), Naukluft Park, Höhle, 26 February 1958, leg. G. Niethammer; – 1 ♀ (TM 8297 [B]), Neudam, Windhoek, 15 July 1937, leg. Barlow Transvaal Museum Expedition; – 1 ♂ (SMW 10148 [S+A]), Oberdorf 43, Bethanie Dist., 17 October 1975, leg. M. Griffin & C. G. Coetzee; – 1 ♂ (SMW 6780 [S]), Okongava Ost 72, Karibib Dist., 12 August 1972, leg. C. G. Coetzee; – 1 ♀ (TM 9474 [S+B], holotype of *Rhinolophus darlingi damarensis* Roberts, 1946), Oserikari, Okahandja, 10 October 1941, leg. A. Roberts; – 1 ♀ (ZFMK 97.574 [A]), Otjiwarongo, ca. 1982, leg. Schoershoever; – 1 ♀ (ZFMK 34.142 [S+B]), Otjosongombe, 20 May 1934, leg. W. Hoesch; – 1 ♀ (SMW 8092 [S+A]), Otjovasandu, Etosha Petroleum, Etosha National Park, 5 May 1978, leg. J. E. W. Dixon; – 1 ♂ (NMP pb6062 [S+A]), Puros, Hoarusib River, 9 September 2015, leg. P. Benda & S. Eiseb; – 1 ind. (SMW 10143 [S]), Reinfels 125, Keetmanshoop, 14 October 1975, leg. M. Griffin; – 1 ♂ (SMW 2699 [S+A]), Sinclair Mine, Helmeringhausen, Lüderitz, 14 March 1969, leg. A. J. Tree; – 1 ♂ (NMP pb7058 [S+A]), Spitzkoppe Camp, 8 October 2020, leg. P. Benda; – 1 ♂ (TM 12925 [S+B]), Uatab, Kuiseb River, 19 mi. upstream from Gobabeb, 10 May 1959, leg. B. Carp Expedition; – 1 ♀ (NMP pb5837 [S+A]), Uis, Ugab River, 12 January 2014, leg. P. Benda, S. Eiseb & M. Uhrin; – 1 ♂, 2 ♀♀ (SMW 4311 [S+B], 4325, 4335 [B]), Waterberg 416, Otjiwarongo Dist., 27 March 1969, leg. P. J. Buys; – 1 ♀ (SMW 11270 [S+B]), Zwartmodder 101, 70 km W of Maltahöhe, 3 November 1984, leg. C. G. Coetzee; – 1 ♀ (TM 37615 [S+B]), Zwartmodder 101, 70 km W of Maltahöhe, 5 November 1984, leg. I. L. Rautenbach; – 2 ♂♂ (ZFMK 59.407, 59.408 [B]), SW Afrika [not specified], date unlisted, leg. G. Niethammer; – 1 ind. (ZFMK 77.526 [S]), Namibia [not specified], date unlisted, leg. H. Roer.

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Captions

Figure 1 Bivariate plot of skull dimensions of the examined samples of the Lesotho bats and comparative taxa: condylocanine length of skull (LCc) against length of the upper tooth-row (CM^3); values in millimetres. Explanations: Abyssinia = samples of *R. acrotis acrotis* from Etiopia, Eritrea and the Sudan; E Africa = samples of *R. acrotis augur* from East Africa (Uganda, Kenya, Rwanda, Tanzania, Malawi); S Africa = samples of *R. acrotis augur* from southern Africa (Mozambique, South Africa); types = holotype specimens of *Rhinolophus augur* (G), *Rhinolophus augur zambesiensis* (B), *Rhinolophus augur zuluensis* (U), and

Rhinolophus darlingi damarensis (D); S Africa II = extremely small-sized specimens from South Africa originally identified as *R. clivosus* (see text for details); polygon = range extremes of the values for *R. damarensis* from Namibia.

Figure 2 Bivariate plot of skull dimensions of the examined samples of the Lesotho bats and comparative taxa: first two roots of the principal component analysis of 15 plain skull dimensions; for explanations see Fig. 1.

Figure 3 Bivariate plot of skull dimensions of the examined samples of the Lesotho bats and comparative taxa: relative length of rostrum (CM^3/LCc) against relative width of skull (LaZ/LCc); for explanations see Fig. 1.

Figure 4 Bivariate plot of skull dimensions of the examined samples of the Lesotho bats and comparative taxa: condylocanine length of skull (LCc) against length of the upper tooth-row (CM^3); values in millimetres. Explanations: Lesotho I = samples from Lesotho examined by molecular genetic analysis; Lesotho II = samples from Lesotho examined only by the morphological analysis; Namibia 0 = samples of *R. damarensis* examined only by the morphological analysis; Namibia 1 = samples of *R. damarensis* of the Namibia 1 lineage; Namibia 2 = samples of *R. damarensis* of the Namibia 2 lineage; *damarensis* T = holotype specimen of *Rhinolophus darlingi damarensis*; S Africa II = extremely small-sized specimens from South Africa (Free State) originally identified as *R. clivosus* (see text for details).

Figure 5 Maximum likelihood tree of reconstructed phylogenetic relationships of the Lesotho horseshoe bats with species of the *fumigatus* group and other *Rhinolophus* groups based on the *Cyt-b* dataset. Branch support values are shown by pie charts on the nodes.

Figure 6 Maximum likelihood tree of reconstructed phylogenetic relationships of the Lesotho horseshoe bats with species of the *fumigatus* group and other *Rhinolophus* groups based on the nuclear dataset. Branch support values are shown by pie charts on the nodes.

Figure 7 General view of the type locality of *Rhinolophus XXX* sp. n.: upper parts of the Sehlabathebe National Park, Lesotho (photo by J. Červený).

Figure 8 Type locality of *Rhinolophus XXX* sp. n.: small cave near the old park lodge in the Sehlabathebe National Park, Lesotho, and the bat colony containing the type series (photo by J. Červený).

Figure 9 Portrait and a frontal (middle) and lateral views (right) of the horseshoe of *Rhinolophus XXX* sp. n. (photo by J. Červený).

Figure 10 Skull in lateral view (top) and in dorsal view (below) of *Rhinolophus XXX* sp. n. (top – NMP 97760, holotype; below – NMP 97758, paratype). Scale bar – 5 mm.

Figure 11 Occlusal views on the mesial part of the left upper tooth-row ($C–M^1$) and the right lower toothrow ($I_3–M_1$) of *Rhinolophus XXX* sp. n. (NMP 97760, holotype). Scale bar – 2 mm.

Table 1 External and cranial biometric data on the examined samples of the Lesotho horseshoe bats and comparative taxa; for dimension explanations see Abbreviations.

Table 2 Dental biometric data on the examined samples of the Lesotho horseshoe bats and *Rhinolophus augur* from southern and eastern Africa; for dimension explanations see Abbreviations.

Table 3 Biometric data on the holotype specimens examined, Explanations: cer = *Rhinolophus XXX* sp. n.; aug = *Rhinolophus augur*; zam = *Rhinolophus augur zambesiensis*; zul = *Rhinolophus augur zuluensis*; dam = *Rhinolophus darlingi damarensis* (dental data unavailable).

Captions of the Supplementary files

Figure S1 Maximum likelihood tree of reconstructed phylogenetic relationships of the Lesotho and Namibian horseshoe bats with species of the *fumigatus* group and other *Rhinolophus* groups based on the ACOX alignment. Branch support values are shown by pie charts on the nodes.

Figure S2 Maximum likelihood tree of reconstructed phylogenetic relationships of the Lesotho and Namibian horseshoe bats with species of the *fumigatus* group and other *Rhinolophus* groups based on the *BGN* alignment. Branch support values are shown by pie charts on the nodes.

Figure S3 Maximum likelihood tree of reconstructed phylogenetic relationships of the Lesotho and Namibian horseshoe bats with species of the *fumigatus* group and other *Rhinolophus* groups based on the *COPS* alignment. Branch support values are shown by pie charts on the nodes.

Figure S4 Maximum likelihood tree of reconstructed phylogenetic relationships of the Lesotho and Namibian horseshoe bats with species of the *fumigatus* group and other *Rhinolophus* groups based on the *ROGDI* alignment. Branch support values are shown by pie charts on the nodes.

Figure S5 Maximum likelihood tree of reconstructed phylogenetic relationships of the Lesotho and Namibian horseshoe bats with species of the *fumigatus* group and other *Rhinolophus* groups based on the *STAT* alignment. Branch support values are shown by pie charts on the nodes.

Table S1 Original sequences and sequences from GenBank used in the molecular genetic analysis. X denotes samples from our study and GenBank number will be added.

Table S2 Names, sequences, and annealing temperatures of primers used in this study.

Table S3 Substitution models as identified by ModelFinder for the different partitions used in MrBayes and IQTREE, respectively.

Table S4 Percentage values of uncorrected genetic *p*-distances of *Cyt-b* among mitochondrial lineages and groups of the examined sample sets of *Rhinolophus* bats (below the diagonal). The diagonal corresponds to the within-lineage/group genetic divergence estimated for *Cyt-b* in each lineage/group.

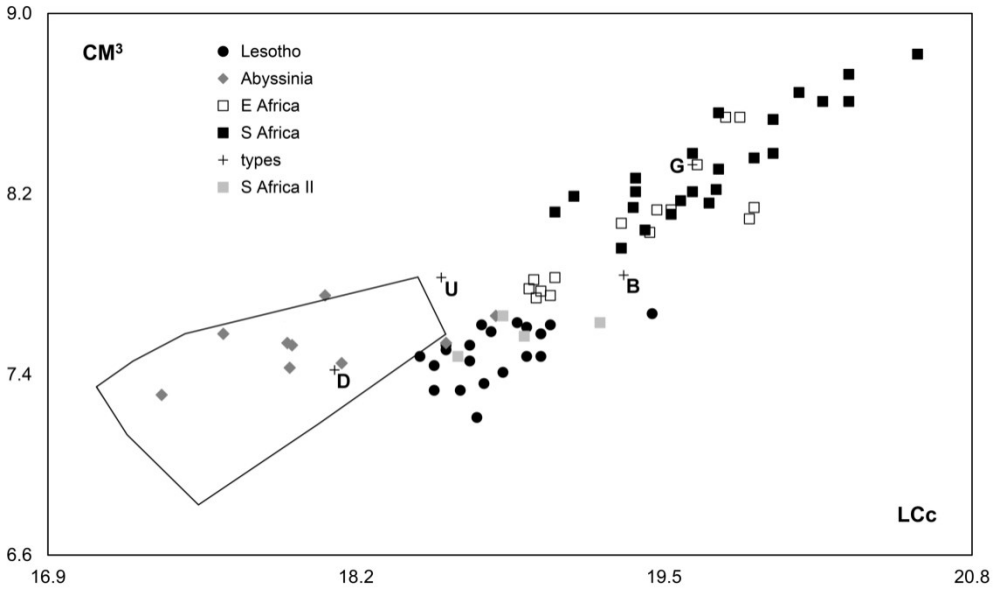


Figure 1 Bivariate plot of skull dimensions of the examined samples of the Lesotho bats and comparative taxa: condylocanine length of skull (LCc) against length of the upper tooth-row (CM3); values in millimetres. Explanations: Abyssinia = samples of R. acrotis acrotis from Etiopia, Eritrea and the Sudan; E Africa = samples of R. acrotis augur from East Africa (Uganda, Kenya, Rwanda, Tanzania, Malawi); S Africa = samples of R. acrotis augur from southern Africa (Mozambique, South Africa); types = holotype specimens of Rhinolophus augur (G), Rhinolophus augur zambesiensis (B), Rhinolophus augur zuluensis (U), and Rhinolophus darlingi damarensis (D); S Africa II = extremely small-sized specimens from South Africa originally identified as R. clivosus (see text for details); polygon = range extremes of the values for R. damarensis from Namibia.

249x155mm (300 x 300 DPI)

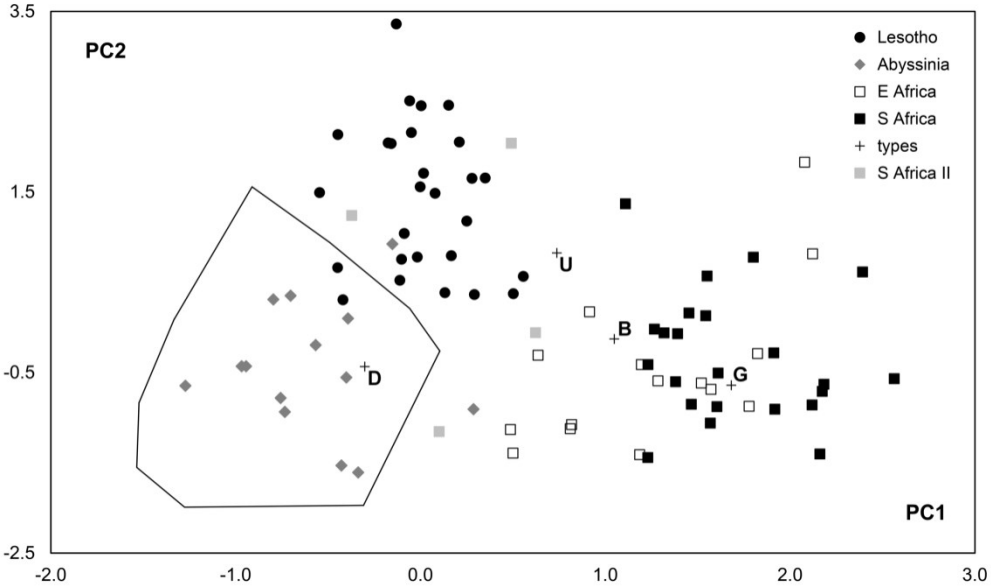


Figure 2 Bivariate plot of skull dimensions of the examined samples of the Lesotho bats and comparative taxa: first two roots of the principal component analysis of 15 plain skull dimensions; for explanations see Fig. 1.

249x155mm (300 x 300 DPI)

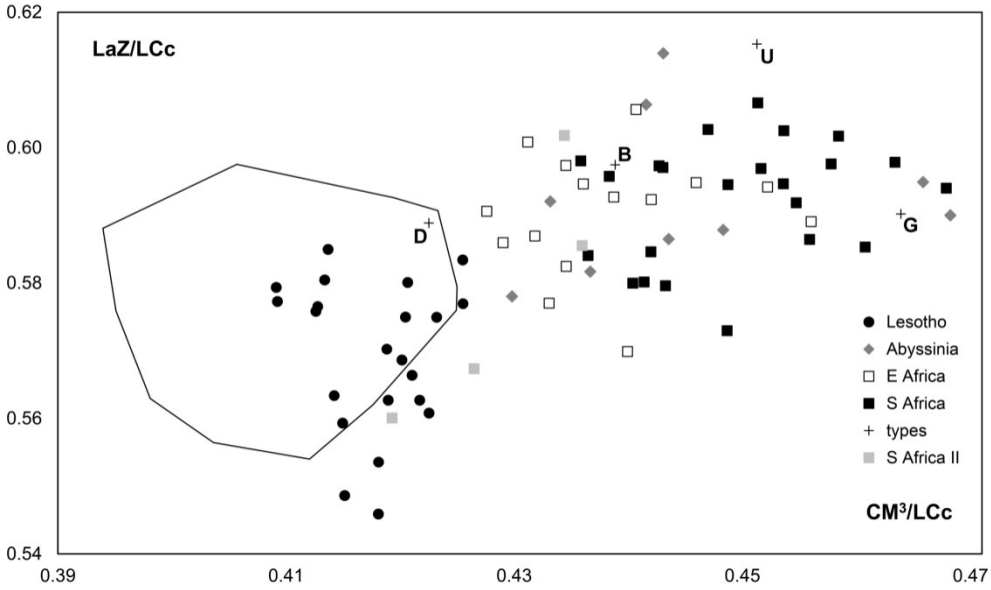


Figure 3 Bivariate plot of skull dimensions of the examined samples of the Lesotho bats and comparative taxa: relative length of rostrum (CM3/LCc) against relative width of skull (LaZ/LCc); for explanations see Fig. 1.

249x155mm (300 x 300 DPI)

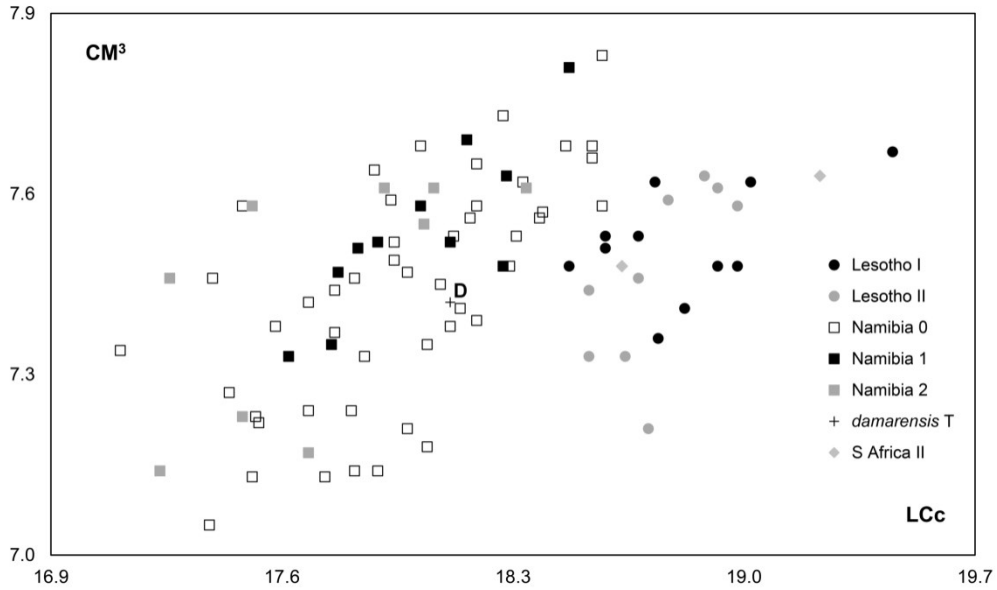


Figure 4 Bivariate plot of skull dimensions of the examined samples of the Lesotho bats and comparative taxa: condylocanine length of skull (LCc) against length of the upper tooth-row (CM3); values in millimetres. Explanations: Lesotho I = samples from Lesotho examined by molecular genetic analysis; Lesotho II = samples from Lesotho examined only by the morphological analysis; Namibia 0 = samples of R. damarensis examined only by the morphological analysis; Namibia 1 = samples of R. damarensis of the Namibia 1 lineage; Namibia 2 = samples of R. damarensis of the Namibia 2 lineage; damarensis T = holotype specimen of Rhinolophus darlingi damarensis; S Africa II = extremely small-sized specimens from South Africa (Free State) originally identified as R. clivosus (see text for details).

249x155mm (300 x 300 DPI)

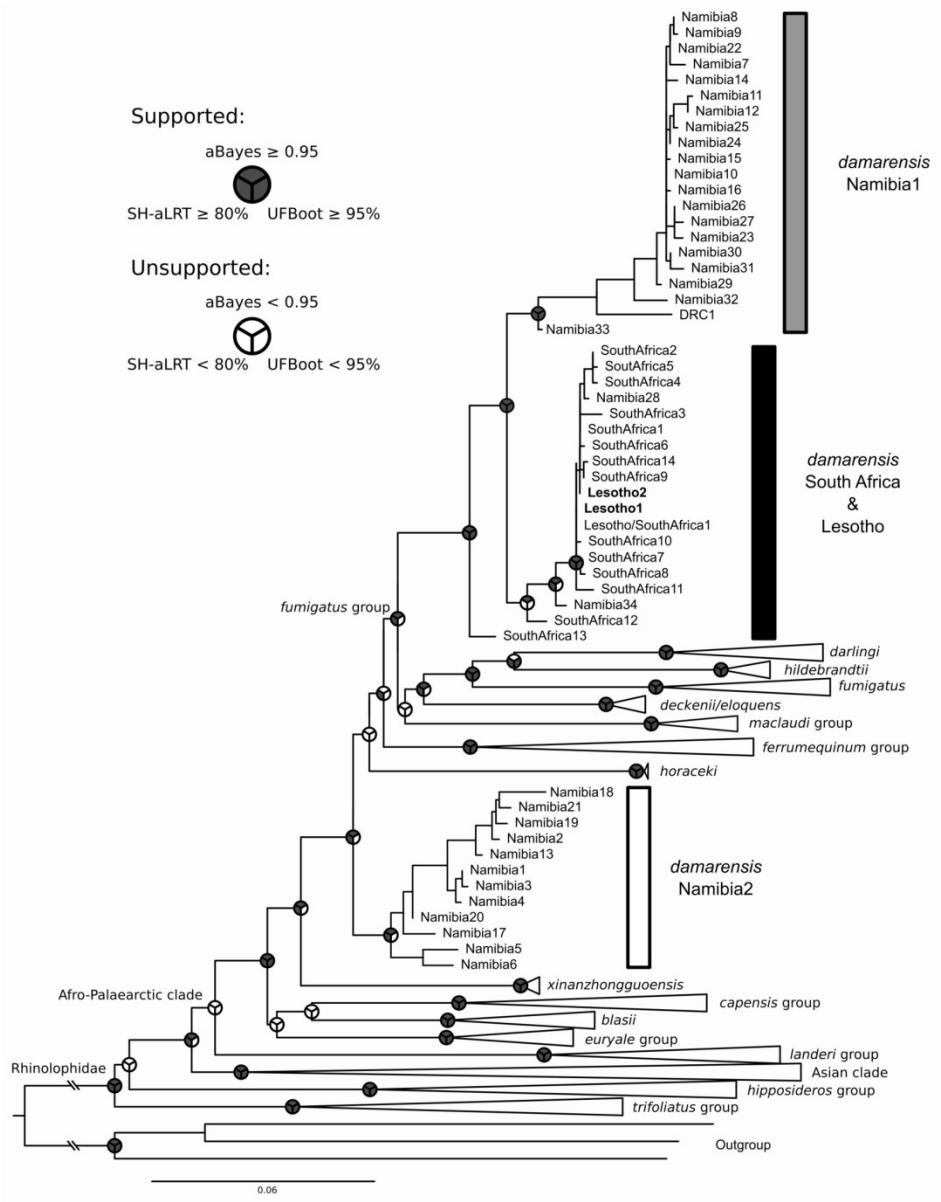


Figure 5 Maximum likelihood tree of reconstructed phylogenetic relationships of the Lesotho horseshoe bats with species of the fumigatus group and other Rhinolophus groups based on the Cyt-b dataset. Branch support values are shown by pie charts on the nodes.

149x190mm (300 x 300 DPI)

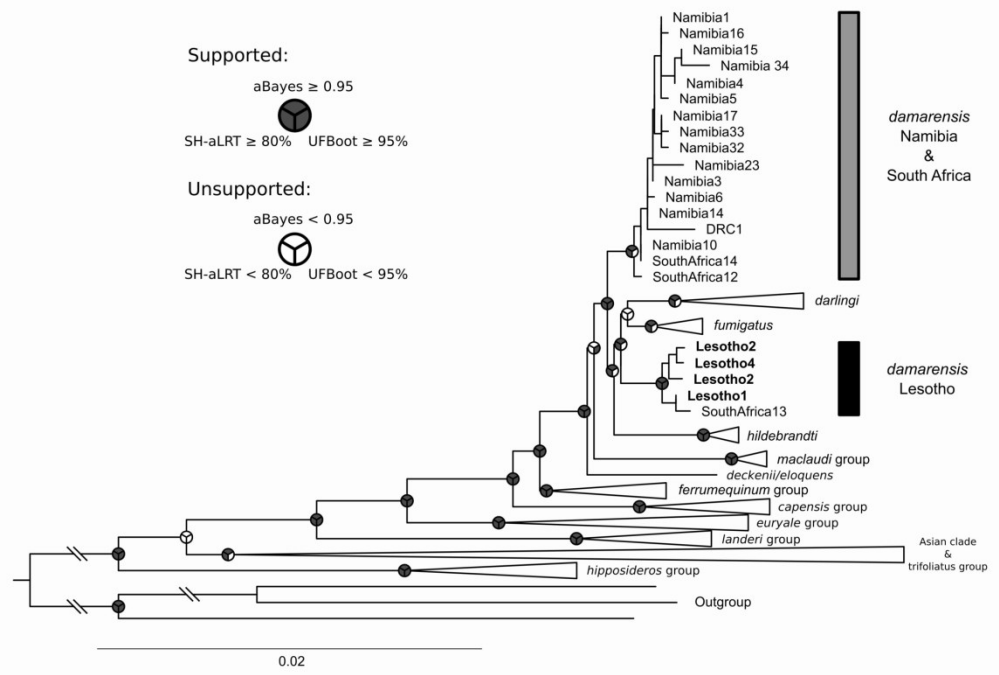


Figure 6 Maximum likelihood tree of reconstructed phylogenetic relationships of the Lesotho horseshoe bats with species of the fumigatus group and other Rhinolophus groups based on the nuclear dataset. Branch support values are shown by pie charts on the nodes.

179x121mm (300 x 300 DPI)

Figure 7 General view of the type locality of Rhinolophus XXX sp. n.: upper parts of the Sehlabathebe National Park, Lesotho (photo by J. Červený).

600x240mm (300 x 300 DPI)

Figure 8 Type locality of Rhinolophus XXX sp. n.: small cave near the old park lodge in the Sehlabathebe National Park, Lesotho, and the bat colony containing the type series (photo by J. Červený).

600x240mm (300 x 300 DPI)

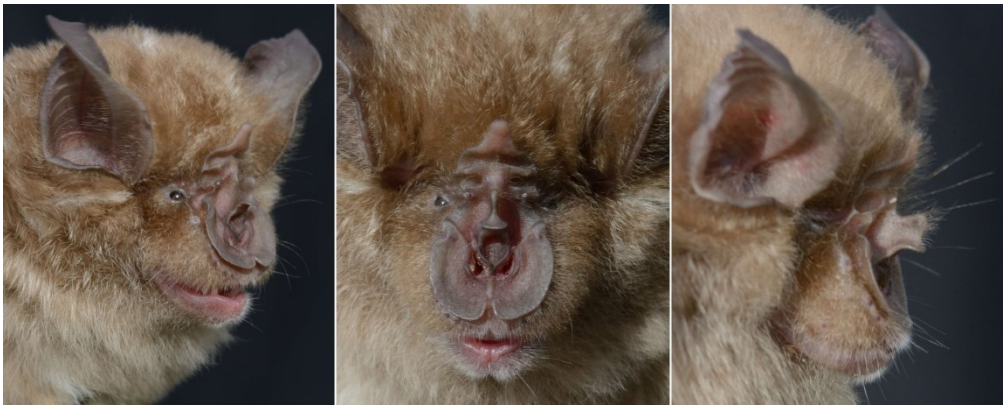


Figure 9 Portrait and a frontal (middle) and lateral views (right) of the horseshoe of Rhinolophus XXX sp. n. (photo by J. Červený).

600x240mm (300 x 300 DPI)

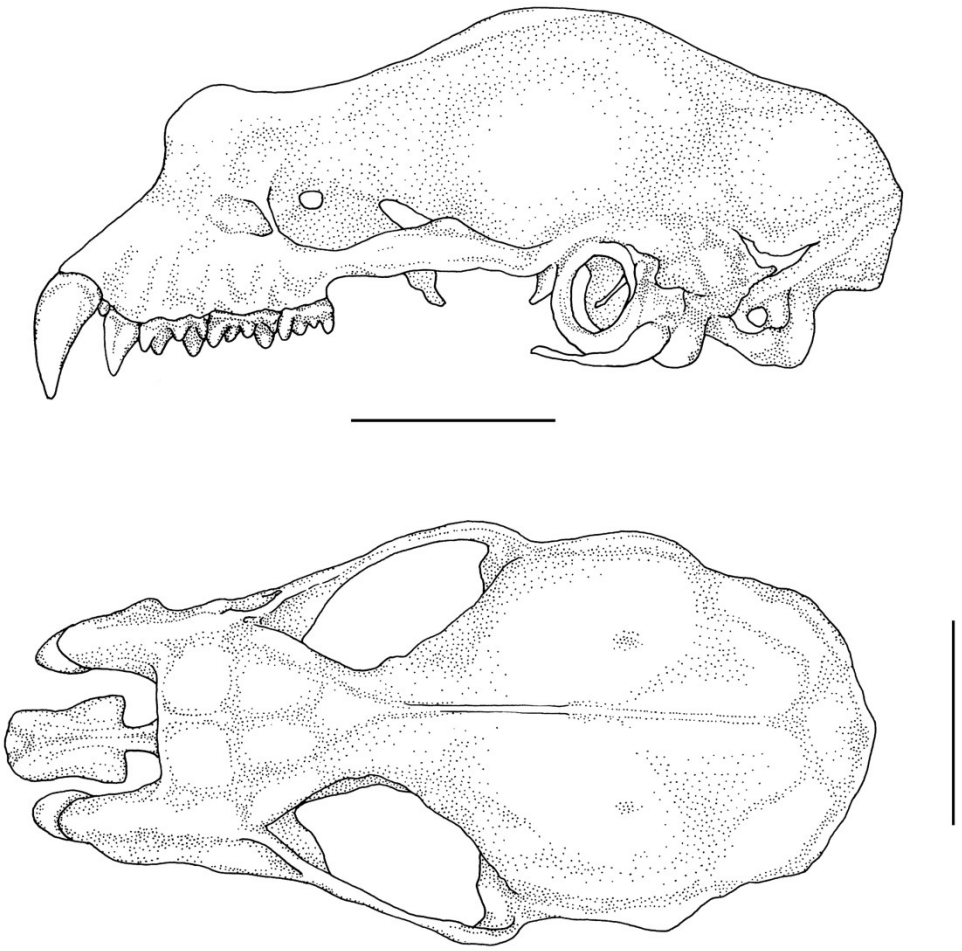


Figure 10 Skull in lateral view (top) and in dorsal view (below) of Rhinolophus XXX sp. n. (top – NMP 97760, holotype; below – NMP 97758, paratype). Scale bar – 5 mm.

219x224mm (300 x 300 DPI)

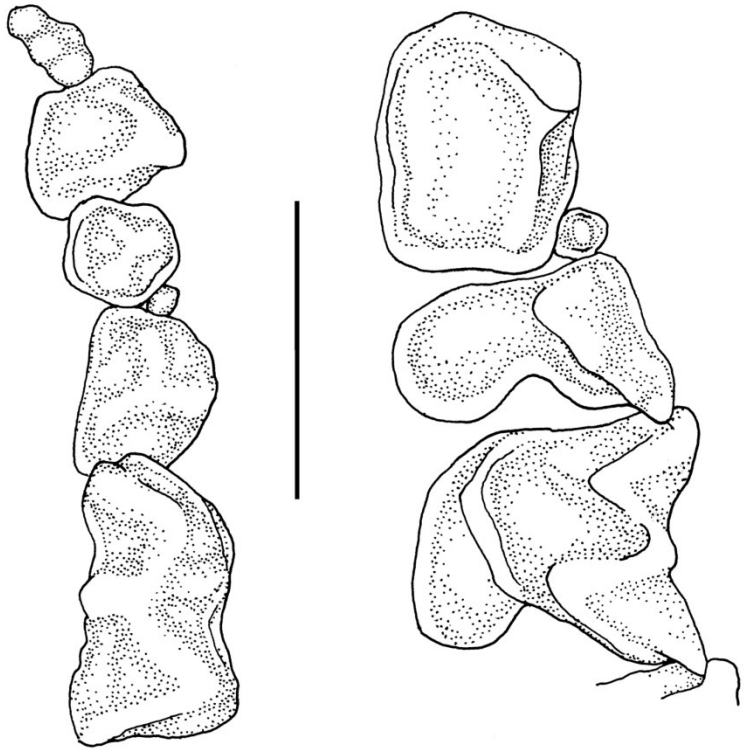


Figure 11 Occlusal views on the mesial part of the left upper tooth-row (C–M1) and the right lower toothrow (I3–M1) of Rhinolophus XXX sp. n. (NMP 97760, holotype). Scale bar – 2 mm.

163x179mm (300 x 300 DPI)

Table 1 External and cranial biometric data on the examined samples of the Lesotho horseshoe bats and comparative taxa; for dimension explanations see Abbreviations.

	Rhinolophus XXX sp. n.					*Rhinolophus acrotis acrotis*					*Rhinolophus acrotis augur*					*Rhinolophus damarensis*				
	n	M	min	max	SD	n	M	min	max	SD	n	M	min	max	SD	n	M	min	max	SD
LC	14	72.7	69	78	2.555	6	66.3	64	69	2.251	14	60.3	55	64	2.627	34	62.4	59	67	1.952
LCd	14	33.2	28	41	3.766	6	31.2	29	33	1.602	15	32.5	27	36	2.875	38	32.3	27	38	2.774
LAt	34	52.72	49.2	55.3	1.689	13	50.38	45.9	53.2	2.849	45	54.12	50.4	57.4	1.627	85	50.22	46.5	53.4	1.564
LA	14	23.20	22.8	23.6	0.269	6	22.12	20.5	23.1	0.966	16	20.32	17.0	23.0	1.786	38	22.18	18.0	24.3	1.295
LaFe	14	8.64	8.3	9.1	0.206	8	7.76	6.8	8.8	0.655	18	8.12	7.20	8.90	0.457	34	8.54	7.3	8.9	0.339
LCr	19	21.92	21.43	22.62	0.325	13	20.98	19.93	22.11	0.624	32	22.65	21.63	23.57	0.497	57	20.93	20.23	21.68	0.397
LCc	22	18.79	18.47	19.45	0.240	10	18.04	17.38	18.79	0.434	43	19.52	18.56	20.57	0.443	69	17.94	17.11	18.57	0.353
LaZ	27	10.67	10.12	11.06	0.245	11	10.60	9.94	10.93	0.271	43	11.55	11.02	12.06	0.269	71	10.33	9.83	10.82	0.229
LaI	29	2.72	2.38	2.98	0.172	14	2.46	2.16	2.75	0.182	44	2.62	2.29	3.11	0.181	75	2.38	1.84	2.78	0.193
LaInf	29	5.51	5.28	5.92	0.145	14	5.26	4.93	5.48	0.164	43	5.72	5.38	6.14	0.167	73	5.36	5.04	5.61	0.112
LaN	26	8.83	8.47	9.13	0.170	13	8.54	8.22	8.93	0.241	43	9.26	8.88	9.81	0.234	71	8.55	8.07	9.02	0.189
LaM	22	9.90	9.58	10.11	0.128	13	9.47	9.01	9.83	0.222	42	10.16	9.73	10.60	0.160	70	9.48	8.96	9.97	0.188
ANc	23	6.39	6.03	6.73	0.178	12	6.20	5.64	6.61	0.305	42	6.68	6.16	7.18	0.221	68	6.18	5.74	6.62	0.200
LBT	25	3.62	3.28	4.08	0.225	12	3.32	2.81	3.79	0.306	42	3.32	2.86	3.75	0.226	67	3.36	2.93	3.91	0.206
CC	27	5.47	5.12	5.84	0.162	14	5.50	5.19	5.76	0.158	43	6.10	5.38	6.60	0.238	72	5.40	4.75	5.78	0.177
M^3M^3	28	7.68	7.23	8.11	0.183	14	7.66	7.29	7.98	0.190	43	8.37	7.76	8.96	0.319	74	7.55	7.03	7.98	0.203
CM^3	29	7.49	7.09	7.82	0.153	14	7.52	7.31	7.75	0.112	44	8.16	7.56	8.82	0.318	78	7.43	6.83	7.83	0.200
LMd	29	13.88	13.37	14.48	0.252	14	13.55	12.92	14.54	0.420	44	14.78	13.93	15.68	0.459	71	13.52	12.87	14.28	0.338
ACo	29	3.30	2.93	3.61	0.157	14	3.24	2.91	3.48	0.150	44	3.63	3.16	3.93	0.164	73	3.18	2.61	3.62	0.171
CM_3	29	7.93	7.64	8.33	0.163	14	8.04	7.77	8.49	0.209	44	8.75	8.17	9.56	0.349	73	7.93	7.45	8.31	0.197
LaZ/LCc	22	0.569	0.546	0.585	0.011	10	0.592	0.578	0.614	0.011	43	0.592	0.570	0.615	0.009	69	0.576	0.554	0.597	0.010
ANc/LCc	22	0.340	0.319	0.362	0.011	10	0.346	0.325	0.357	0.011	42	0.342	0.323	0.368	0.012	69	0.344	0.309	0.365	0.010
CM3/LCc	22	0.422	0.413	0.430	0.005	10	0.450	0.434	0.472	0.013	43	0.449	0.432	0.472	0.011	70	0.415	0.390	0.443	0.009
LBT/LCc	22	0.194	0.175	0.220	0.012	10	0.183	0.151	0.213	0.019	42	0.170	0.150	0.198	0.011	65	0.187	0.164	0.220	0.012

Table 2 Dental biometric data on the examined samples of the Lesotho horseshoe bats and *Rhinolophus augur* from southern and eastern Africa; for dimension explanations see Abbreviations.

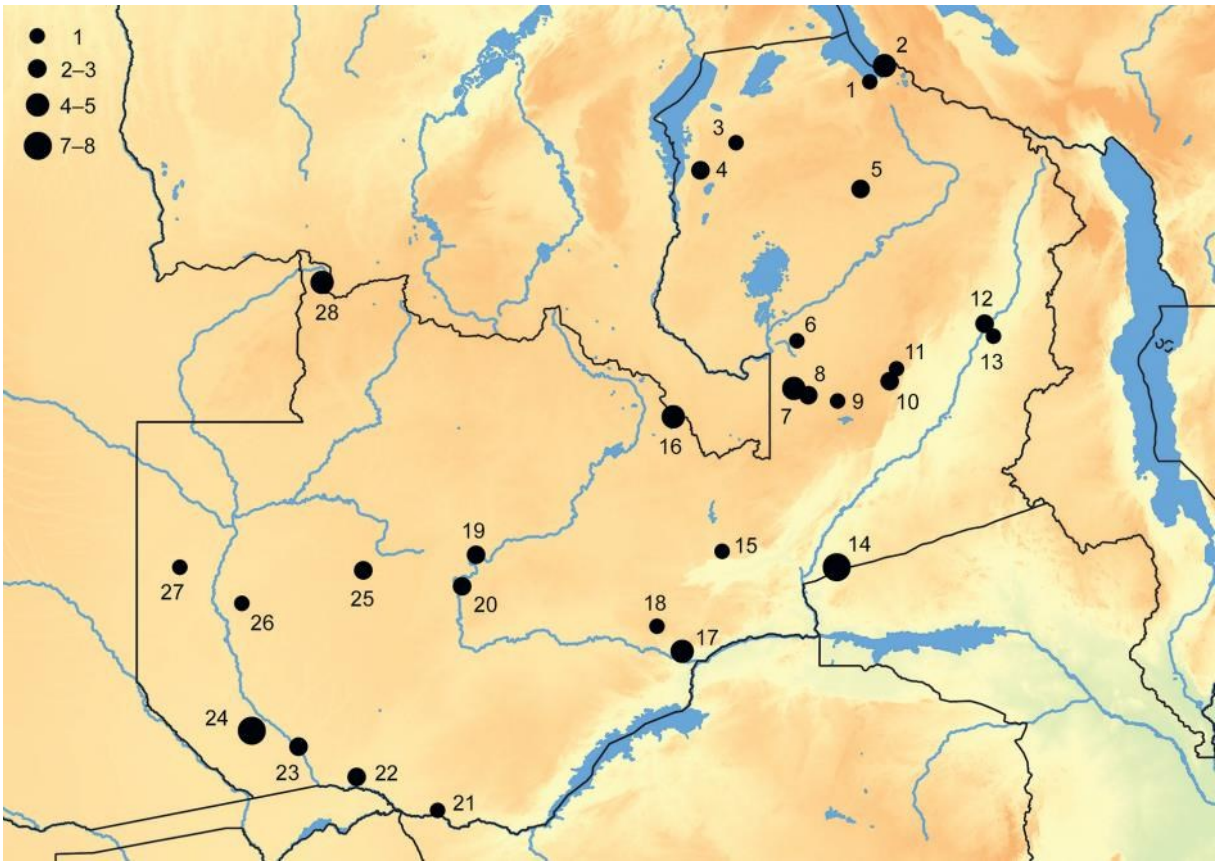
	Rhinolophus XXX sp. n.					*Rhinolophus acrotis augur*				
	n	M	min	max	SD	n	M	min	max	SD
LCs	11	1.723	1.45	1.86	0.107	26	2.054	1.90	2.27	0.108
LaCs	11	1.383	1.28	1.45	0.051	26	1.684	1.50	2.03	0.128
LP^2	11	0.346	0.29	0.40	0.036	24	0.335	0.25	0.45	0.051
LP^41	11	1.427	1.36	1.54	0.052	26	1.557	1.32	1.74	0.120
LP^42	11	0.959	0.88	1.07	0.057	26	1.087	0.90	1.26	0.092
LP^43	11	0.735	0.67	0.82	0.053	26	0.872	0.72	1.02	0.079
LaP^4	11	2.263	2.18	2.40	0.063	26	2.527	2.03	2.83	0.192
LM^1	11	1.940	1.90	2.00	0.035	26	2.114	1.93	2.32	0.107
LaM^1	11	2.696	2.60	2.83	0.068	26	3.086	2.69	3.31	0.159
LM^3	11	1.283	1.21	1.35	0.039	26	1.312	1.17	1.42	0.067
LaM^3	11	1.993	1.93	2.09	0.046	26	2.199	1.95	2.40	0.108
LCi	11	0.952	0.83	1.07	0.074	26	1.181	1.01	1.36	0.094
LP_2	11	0.792	0.73	0.87	0.047	26	0.868	0.73	1.03	0.085
LaP_2	11	0.806	0.72	0.88	0.050	26	0.979	0.80	1.13	0.087
LP_3	8	0.209	0.17	0.25	0.032	2	0.225	0.21	0.24	0.022
LP_4	11	1.131	1.06	1.23	0.058	26	1.269	1.12	1.37	0.070
LaP_4	11	0.973	0.86	1.09	0.061	26	1.204	1.06	1.34	0.080
LM_1	11	2.046	1.97	2.15	0.055	26	2.209	2.04	2.39	0.098

Table 3 Biometric data on the holotype specimens examined, Explanations: cer = *Rhinolophus* XXX sp. n.; aug = *Rhinolophus augur*; zam = *Rhinolophus augur zambesiensis*; zul = *Rhinolophus augur zuluensis*; dam = *Rhinolophus darlingi damarensis* (dental data unavailable).

	cer	aug	zam	zul	dam		cer	aug	zam	zul
LAt	54.5	55.7	53.9	51.8	50.5	LCs	1.80	2.08	1.98	1.92
LCr	21.97	22.64	22.43	22.01	21.12	LaCs	1.39	1.59	1.50	1.57
LCc	18.82	19.62	19.33	18.56	18.11	LP^41	1.38	1.66	1.65	1.59
LaZ	10.59	11.58	11.37	11.42	10.82	LP^42	0.95	1.01	1.12	0.97
LaI	2.64	2.67	2.58	2.66	2.62	LP^43	0.71	0.89	0.76	0.79
LaInf	5.39	5.61	5.77	5.59	5.38	LaP^4	2.20	2.59	2.52	2.41
LaN	9.02	9.42	9.04	9.23	8.56	LM^1	1.95	2.14	2.02	2.06
LaM	9.88	10.18	10.07	10.07	9.75	LaM^1	2.66	3.11	3.17	3.07
ANc	6.38	6.64	6.58	6.49	6.02	LM^3	1.28	1.42	1.30	1.37
LBT	3.44	3.35	3.34	3.67	3.14	LaM^3	1.95	2.23	2.11	1.95
CC	5.51	6.29	6.02	5.86	5.51	LCi	0.98	1.15	1.09	1.04
M^3M^3	7.66	8.67	8.29	8.23	7.83	LP_2	0.84	0.74	0.87	0.73
CM^3	7.41	8.33	7.84	7.83	7.42	LaP_2	0.77	1.03	0.91	0.87
LMd	13.76	14.73	14.61	13.98	13.76	LP_4	1.23	1.30	1.24	1.29
ACo	3.38	3.71	3.59	3.48	3.49	LaP_4	0.86	1.20	1.09	1.20
CM_3	7.96	9.18	8.38	8.45	8.02	LM_1	1.97	2.35	2.21	2.15

Paper 8: Zambian bat fauna

Benda P., Uvizl M., Šklíba J., Mazoch V., & Červený J. (2022). African bats in the collection of the National Museum, Prague (Chiroptera). I. Bats from Zambia. Lynx, n. s. (Praha). 53, 291–332.



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African bats in the collection of the National Museum, Prague (Chiroptera). I. Bats from Zambia

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Abstract. A list of 139 specimens of bats belonging to 32 species of eight families originating from Zambia, housed in the collection of the National Museum, Prague, Czech Republic, is presented in a systematical review. The species lists are complemented by comments on distribution and morphometry data. The specimens represent 73 new records (species vs. locality) of bats from Zambia. The collection contains two species new for the Zambian fauna, *Afropipistrellus grandidieri* and *Neoromicia somalica*, and the bat fauna of Zambia now comprises 76 species in total. Two species, *Rhinolophus sakejiensis* and *Chaerephon bivittatus* are documented from Zambia for the second time, the former bat for the first time since the species description. The record localities of *Epomophorus labiatus*, *Rhinolophus mossambicus*, and *Neoromicia somalica* shift margins of the hitherto known distribution ranges of these bats. In *Epomophorus dobsonii*, *Nyctinomus aegyptiacus*, *Glauconycteris variegata*, *Pipistrellus rusticus*, *Scotophilus leucogaster*, and *S. viridis*, the collection specimens represent new peripheral records making their distribution range margins more precise. Molecular genetic analysis revealed new extents of distribution for particular mitochondrial lineages of otherwise common species in Zambia, *Hipposideros caffer*, *Nycteris thebaica*, and *Miniopterus natalensis* s.str.

Key words. National Museum, collection, catalogue, bats, distribution, Afrotropics, southern Africa, Northern Rhodesia.

INTRODUCTION

Zambia is a large country of south-central Africa, occupying 752,617 square kilometres of high plateaus, covered mainly by woodland savannas with large areas of wetlands and floodplains and small patches of dry broadleaf forests (Burgess et al. 2004). In comparison with the faunas of many other African countries, the bat fauna of Zambia is relatively well known. Already Ansell (1978) mentioned 62 species of bats from this country, and recently, at the time of immense taxonomic changes in the African mammal fauna, Monadjem et al. (2020a) reported the confirmed occurrence of 73 bat species from Zambia (Appendix 1).

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Bat specimens from Zambia are scattered throughout many collections; Monadjem et al. (2020a) reported almost 500 bat records from the country based on museum specimens. More than three quarters of these specimens (77.4%) are housed in four collections, viz. the Natural History Museum of Zimbabwe, Bulawayo, Zimbabwe; the Amathole Museum, King William's Town, South Africa; the Natural History Museum, London, UK; and the Livingstone Museum, Livingstone, Zambia; while the remaining specimens are reported to be spread across 13 collection institutions.

A small collection of bats from Zambia is also housed in the National Museum, Prague (NMP). The collection comprises mostly specimens gathered by the staff and students of the Department of Zoology, University of South Bohemia, České Budějovice, Czech Republic, during various research projects focused on the diversity of small mammal fauna of central Africa (see e.g., Bryja et al. 2012, 2014, 2018, McDonough et al. 2015, Mizerovská et al. 2019, etc.). The bats were collected relatively recently during several research trips to Zambia in the period 2009–2018 and have been transferred to the NMP collection in the last few years. The NMP series of Zambian bats contains 139 specimens belonging to 32 species of eight families. These bats originate from 29 localities covering the entire country (Fig. 1) and represent 73 records (species vs. locality), i.e. about 13% of the available country's amount of bat records. Thus, concerning the information potential, such a collection has a certain value. In this catalogue, we describe the NMP collection of Zambian bats in the context of the last and most comprehensive compendium of bats of Zambia and surrounding countries by Monadjem et al. (2020a).

We intend this contribution to be an initial part of a catalogue series of African bats housed in the NMP collection and an informal continuation of several geography-based catalogues of the NMP bat specimens, until now focused on the Palearctic fauna only (Gaisler 1956, Benda et al. 2008, 2011, 2018).

METHODS

The lists of specimens from the collection of the National Museum, Prague (NMP), are arranged in alphabetical order (according to the collection locality name) and then, in chronological order (according to collection date). The lists include, for each item, the following information: (1) indication of sex, (2) NMP collection ID, (3) preparation type (see Abbreviations below), (4) name of the locality (primarily listed by the name of the closest settlement or notable physical feature), (5) date of collection, and (6) collector name/s. For the names of the first level administrative divisions and geographic coordinates of the localities see gazetteer (Appendix 2; in alphabetical order). The lists of specimens of particular species are complemented by a list of references reporting the particular specimen/s or the finding/s, i.e., additional data concerning the specimens.

Basic biometric data taken from the NMP specimens are presented in Tables 1–10. The specimens were measured in a standard way with the use of mechanical calliper. Horizontal dental dimensions were taken on cingulum margins.

Molecular genetic examinations

The genomic DNA was extracted from alcohol-preserved tissue of the museum specimens using Geneaid Genomic DNA Mini Kit. We targeted the complete mitochondrial gene for cytochrome *b* (Cyt-*b*). When we were not able to obtain any sequence for this marker we targeted shorter parts of Cyt-*b* and completed the mitochondrial gene for NADH dehydrogenase subunit 1 (ND1). These markers were used frequently in previous studies dealing with African bats. The genes were amplified with the primers mtDNA-R3-F (TGGCATGAAAAATCACCGTTGT; Puechmaille et al. 2011) and CytB-H (CTTTTCTGGTTTACAAGACCAG; Weyeneth et al. 2008) for complete Cyt-*b*, F3.1-R (CGGTTGGGTTATTGGACCCA) and

R3.2-F (AGAATGAGTCTGAGGTGGCTTTT; Puechmaille et al. 2011), and Cytb1 (CCATCCAACAT-CTCAGCATGATGAAA) and Cytb2 (CCCTCAGAATGATATTTGTCCTCA; Kocher et al. 1989) for short Cyt-*b*, and ER65 (5'-CCTCGATGTTGGATCAGG-3') and ER66 (5'-GTATGGGCCCGATAGCTT-3; Dietz et al. 2016) for ND1. The PCR amplifications of complete Cyt-*b* were treated as in Uvizl et al. (2019), of shorter Cyt-*b* as in Puechmaille et al. (2011) and Šmíd et al. (2013), respectively, and of *ND1* as in Dietz et al. (2016). The PCR products were Sanger-sequenced from both sides using the PCR primers by Macrogen, Inc. (Amsterdam, the Netherlands).

Sequences were edited and aligned using the MAFFT plugin (Katoh & Standley 2013) in Geneious 11.0.5 (https://www.geneious.com), subsequently manually edited and trimmed using Gblocks (Castresana

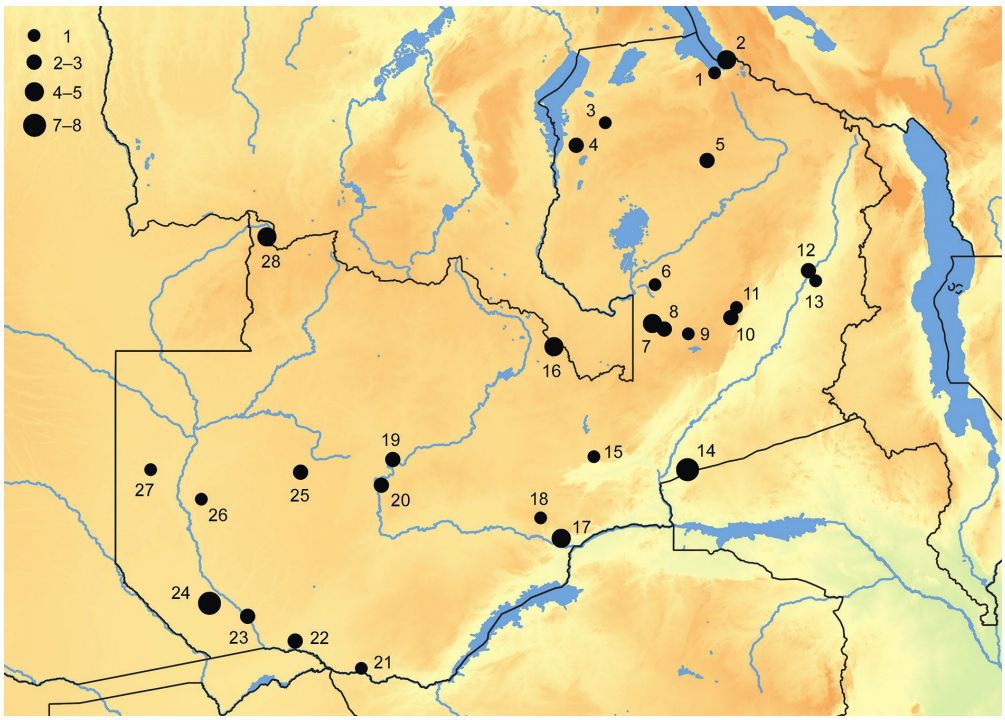

Fig. 1. Map of Zambia with the localities of recorded bat specimens housed in the collection of the National Museum, Prague, Czech Republic; the size of circle corresponds with the number of species collected (see legend in top left corner of the map). Localities: 1 – Kasakalawe, Lake Tanganyika Lodge, 2 – Kalambo Falls, 3 – Lumangwe Falls, 4 – Ntumbachushi Falls, 5 – Chishimba Falls, 6 – Bangweulu Game Reserve, Chikuni, 7 – Kasanka National Park, Luwombwa Camp, 8 – Kasanka National Park, Pontoon Camp & Fibwe Camp, 9 – Nsalu Cave, 10 – Mutinondo, Mayense Camp, 11 – Mutinondo, Kankonde Camp, 12 – North Luangwa National Park, Chifunda Camp, 13 – Chifunda, Old Luelo Ranger Post, 14 – Kacholola, 15 – Mkushi River Camp, 16 – Ndola Hill, 17 – Chisakila, Bwarenunka Cave, 18 – Lusaka, Lusaka East Forest Reserve, 19 – Kafue National Park, Lufupa River Camp, 20 – Kafue National Park, Chunga Camp, 21 – Livingstone, No Name Camp, 22 – Simungoma, Nulubeti village, 23 – Kabula Lodge, 24 – Sioma Bush Camp, 25 – Kaoma, Farmers Rendezvous Lodge, 26 – Newa, 27 – Liuwa Plain National Park, Lyangu Camp, 28 – Sakeji, Nchila Wildlife Reserve.

2000). Sequences of protein-coding markers were translated to amino acids to check for the presence of stop codons, which would indicate pseudogenes have been amplified. Basic Local Alignment Search Tool (BLAST; Altschul et al. 1990) was used to search for the most related sequences and therefore to identify the species of some samples that were difficult to identify by morphological examination. The GenBank Accession Numbers of the newly defined haplotypes are listed in Appendix 3, comparative haplotypes were extracted from the studies by Juste et al. (2013), Koubínová et al. (2013), Monadjem et al. (2013b, 2021), Goodman et al. (2015), Benda et al. (2016), Hutterer et al. (2019), and Moir et al. (2020).

Phylogenetic analyses of the obtained datasets were run maximum likelihood (ML). The appropriate nucleotide substitution model for each partition was selected based on the Bayesian information criterion (BIC) ModelFinder (Kalyaanamoorthy et al. 2017). We inferred the maximum-likelihood tree using the partition model in IQ-TREE (Nguyen et al. 2015). Searching for the best-scoring ML was performed by ultrafast bootstrap (UFBoot; Hoang et al. 2018) with 1,000 bootstrap and 1,000 topology replicates. To verify robustness of the ML tree, the branch supports were evaluated using SH-like approximate likelihood ratio test (SH-aLRT; Guindon et al. 2010) and a Bayesian-like transformation of aLRT (aBayes; Anisimova et al. 2011). SH-aLRT was performed with 1000 replications. aBayes branch support was used instead of Bayesian posterior probabilities because aBayes is more conservative, more robust to model violation and moreover exhibits the more confident resolution (Anisimova et al. 2011). The ML, SH-aLRT and aBayes analysis were run on IQtree web server (Trifinopoulos et al. 2016).

Abbreviations

Preparation type. A = alcohol specimen; – B = skin (balg); – S = skull; – Sk = skeleton.
Dimensions. **External**: G = weight; – LAt = forearm length. – **Cranial**: LCr = greatest length of skull (including praemaxilla); – LOc = occipitocanine length; – LCb = condylobasal length; – LCc = condylocanine length; – LaZ = zygomatic width; – LaI = width of interorbital constriction; – LaP = width of postorbital constriction; – LaInf = infraorbital width; – LaN = neurocranium width; – LaM = mastoidal width; – ANc = neurocranium height; – LBT = largest horizontal length of tympanic bulla; – CC = rostral width between labial margins of canines; – M^2M^2 = rostral width between labial margins of second upper molars; – M^3M^3 = rostral width between labial margins of third upper molars; – CM^2 = length of upper tooth-row between mesial margin of canine and distal margin of second molar; – CM^3 = length of upper tooth-row between mesial margin of canine and distal margin of third molar; – LMd = condylar length of mandible; – ACo = height of coronoid process; – CM_3 = length of lower tooth-row between mesial margin of canine and distal margin of third molar.
Collections. AMNH – American Museum of Natural History, New York, United States of America; – MHNG = Natural History Museum, Geneva, Switzerland; – NMP = National Museum (Natural History), Prague, Czech Republic; – ZMB = Natural History Museum, Berlin, Germany.
Others. leg. = legit [presented, bequeathed, sended]; – M = mean; – max., min. = dimension range margins; – SD = standard deviation.

ANNOTATED LIST OF SPECIMENS

Pteropodidae

Epomophorus crypturus Peters, 1852

Material (31 specimens). 1 ♂ (NMP 97595 [S+A]), Kabula Lodge, shrubland, 20 May 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera;
1 ♂, 1 ♀ (NMP 97692, 97693 [S+A]), Kacholola, riverine forest, 15 June 2010, leg. J. Šklíba, V. Mazoch & E. Knotková;
1 ♀ (NMP 97617 [S+A]), Kafue National Park, Chunga Camp, 28 May 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera;

2 ♂♂ (NMP 97615 [S+A], 97616 [A]), Kafue National Park, Lufupa River Camp, 26 May 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera;
1 ♂, 1 ♀ (NMP 97667, 97668 [S+A]), Kaoma, Farmers Rendezvous Lodge, small pool, 5 June 2010, leg. J. Šklíba, V. Mazoch & E. Knotková;
1 ♂, 4 ♀♀ (NMP 97559–97561, 97563 [S+A], 97562 [A]), Kasanka National Park, Luwombwa Camp, 27 November 2018, leg. P. Benda & J. Červený;
1 ♂, 1 ♀ (NMP 97629, 97630 [S+A]), Kasanka National Park, Pontoon Camp, riverine forest, 15 June 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera;
1 ♂, 1 ♀ (NMP 97671, 97672 [S+A]), Liuwa Plain National Park, Lyangu Camp, woodland, 8 June 2010, leg. J. Šklíba, V. Mazoch & E. Knotková;
1 ♀ (NMP 97662 [S+A]), Lumangwe Falls, chalet, 4 August 2009, leg. V. Mazoch & J. Zima;
1 ♀ (NMP 97666 [S+A]), Lusaka, Lusaka East Forest Reserve, miombo forest, 29 May 2010, leg. J. Šklíba & H. Patzenhauerová;
1 ♂ (NMP 97637 [S+A]), Mkushi River Camp, river bank, 4 July 2009, leg. V. Mazoch & J. Zima;
1 ♀ (NMP 97642 [S+A]), Mutinondo, Kankonde Camp, river bank, 9 July 2009, leg. V. Mazoch & J. Zima;
1 ♂, 1 ♀ (NMP 97622, 97623 [S+A]), Ndola Hill, 7 June 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera;

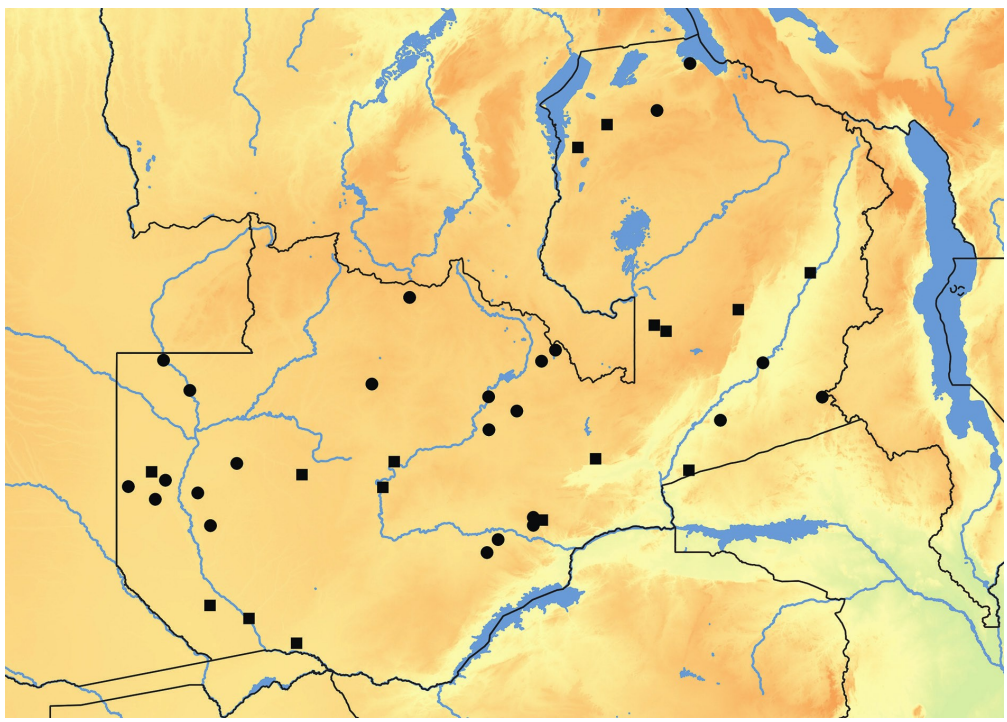

Fig. 2. Distribution of *Epomophorus crypturus* in Zambia based on museum specimens; circles – published data (Bergmans 1988, Monadjem et al. 2020a), squares – new data.

Table 1. Basic biometric data on the NMP specimens of *Epomophorus crypturus* from Zambia (only adult specimens are included). For abbreviations see Methods

dimension	males					females				
	n	**M**	min	max	SD	*n*	**M**	min	max	SD
G	*8*	**99.35**	90.0	105.0	4.593	*18*	**76.91**	52.0	88.0	8.278
LAt	*9*	**85.27**	83.2	87.6	1.575	*18*	**79.79**	72.8	86.3	2.976
LCr	*9*	**53.12**	45.75	55.56	2.964	*17*	**46.63**	40.76	53.06	2.503
LCb	*9*	**53.22**	45.28	55.75	3.167	*17*	**46.33**	40.35	53.13	2.673
LaZ	*9*	**26.43**	24.81	28.11	0.945	*17*	**24.55**	21.84	26.76	1.064
LaI	*9*	**8.65**	7.36	9.98	0.741	*17*	**7.64**	7.18	8.16	0.290
LaP	*9*	**10.17**	9.44	11.02	0.546	*17*	**9.71**	8.41	10.84	0.708
LaInf	*9*	**11.43**	10.27	12.36	0.662	*17*	**10.55**	9.45	11.92	0.566
LaN	*9*	**17.03**	16.56	18.13	0.607	*17*	**16.52**	15.61	17.61	0.555
LaM	*9*	**18.14**	17.34	19.08	0.570	*17*	**17.31**	16.23	18.63	0.620
ANc	*9*	**10.82**	10.41	11.62	0.349	*17*	**10.78**	10.22	11.71	0.440
LBT	*9*	**4.73**	4.46	4.98	0.192	*17*	**4.65**	4.18	5.12	0.238
CC	*9*	**9.85**	8.64	10.51	0.543	*17*	**9.25**	8.14	9.98	0.443
M^2M^2	*9*	**13.70**	12.54	14.42	0.558	*16*	**12.94**	11.32	14.17	0.661
CM^2	*9*	**17.86**	14.92	19.07	1.242	*16*	**15.80**	13.38	17.98	0.947
LMd	*9*	**42.42**	36.04	44.33	2.525	*17*	**36.73**	31.69	42.05	2.179
ACo	*9*	**16.28**	14.38	17.64	0.906	*17*	**14.17**	12.24	17.24	1.152
CM_3	*9*	**19.27**	16.35	20.21	1.193	*17*	**17.31**	14.85	19.76	0.995

1 ♀ (NMP 97643 [S+A]), North Luangwa National Park, Chifunda Camp, 12 July 2009, leg. V. Mazoch & J. Zima;
1 ♂ (NMP 97664 [S+A]), Ntumbachushi Falls, above road, 6 August 2009, leg. V. Mazoch & J. Zima;
3 ♂♂, 1 ♀ (NMP 97589 [S+A], 97590–97592 [A]), Simungoma, Nulubeti village, 19 May 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera;
1 ♂, 1 ♀ (NMP 97597, 97598 [S+A]), Sioma Bush Camp, 22 May 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera.

Epomophorus crypturus is an endemic of south-eastern Africa and the territory of Zambia represents a large segment of its distribution range (Bergmans 1988). Monadjem et al. (2020a) reported 25 confirmed record sites of this bat from Zambia and we added 15 new sites that well complement the distribution evidence across the country (Fig. 2); from the Lufupa Camp, this bat was reported also by Kearney et al. (2010). In the NMP collection, *E. crypturus* is the most numerous species of Zambian bats, it was recorded at 17 of 29 sampled localities, its specimens represent almost a quarter of the collection (22.3%). This bat is widespread in Zambia, it was collected from the whole territory of the country with the exceptions of the Chambeshi/Luapula river basin and the "Ikelenge Pedicle" of north-western Zambia (Fig. 2). The dimensions of the adult NMP specimens of *E. crypturus* from Zambia are shown in Table 1.

Epomophorus labiatus (Temminck, 1837)

Material (7). 2 ♀♀ (NMP 97659, 97660 [S+A]), Chishimba Falls, river bank, 25 July 2009, leg. V. Mazoch & J. Zima;

2 ♂♂, 1 ♀ (NMP 97655–97657 [S+A]), Kalambo Falls, camp above river, 18 July 2009, leg. V. Mazoch & J. Zima;
1 ♀ (NMP 97558 [S+A]; Fig. 3), Kasanka National Park, Luwombwa Camp, 27 November 2018, leg. P. Benda & J. Červený;
1 ♀ (NMP 97663 [S+A]), Ntumbachushi Falls, 6 August 2009, leg. V. Mazoch & J. Zima.

Epomophorus labiatus is distributed in the central and eastern parts of Africa and in southern Arabia (Happold 2013a). In Zambia, this species occurs only in the north-eastern section of the country and reaches there a segment of the south-western margin of its distribution range (Monadjem et al. 2020a). The NMP specimens of *E. labiatus* originate from four sites in Zambia, they fell roughly into the known range of distribution; from the Kalambo Falls this bat was reported already by Bergmans (1988), though as *E. minor* Dobson, 1880, a junior synonym of *E. labiatus* (Claessen & De Vree 1991, Bergmans & van Strien 2004). Two newly documented sites of occurrence of this bat, Luwombwa Camp in the Kasanka NP and Ntumbachushi Falls, demarcate newly the south-western margin of the species range (cf. Monadjem et al. 2020a; Fig. 4). A record of *E. labiatus* from the Kasanka NP was already marked by Happold (2013a), however, without reference to specimens and in a tentative version of map. Now, the occurrence of *E. labiatus* in this marginal part of the species range is confirmed and definitely represents a distribution extreme (Fig. 4).

The dimensions of the NMP specimens of *E. labiatus* from Zambia are shown in Table 2. The metric data conform to the characteristics of this species as defined by Bergmans & van Strien (2004).

Fig. 3. A female of *Epomophorus labiatus* (NMP 97558) netted at the Luwombwa Camp, Kasanka National Park, on 27 November 2018. All photos by J. Červený.

Table 2. Basic biometric data on the NMP specimens of *Epomophorus labiatus* and *E. wahlbergi* from Zambia. For abbreviations see Methods

dimension	*Epomophorus labiatus* males		*Epomophorus labiatus* females					*Epomophorus wahlbergi*
	NMP 97655	NMP 97656	*n*	**M**	min	max	SD	NMP 97583
G	45.0	50.0	*5*	**41.44**	37.2	45.0	3.297	91.0
LAt	66.3	65.6	*5*	**63.40**	62.1	65.0	1.405	80.3
LCr	39.66	39.38	*5*	**36.17**	33.86	37.98	1.674	44.97
LCb	38.86	38.84	*5*	**35.47**	33.03	36.94	1.628	44.76
LaZ	21.61	20.86	*5*	**19.93**	19.14	20.48	0.702	24.92
LaI	6.58	6.28	*5*	**6.20**	5.81	6.44	0.249	7.75
LaP	8.61	8.05	*5*	**8.78**	8.47	8.95	0.183	9.34
LaInf	9.57	9.03	*5*	**8.52**	8.21	8.74	0.232	11.32
LaN	13.90	13.63	*5*	**13.95**	13.16	14.42	0.542	16.13
LaM	14.43	14.74	*5*	**14.09**	13.54	14.53	0.490	17.12
ANc	10.38	9.77	*5*	**10.20**	9.83	10.51	0.279	10.38
LBT	3.98	3.69	*5*	**3.70**	3.55	3.89	0.127	4.64
CC	7.58	7.68	*5*	**6.83**	6.51	7.23	0.266	9.38
M^2M^2	11.44	10.74	*5*	**10.16**	9.81	10.38	0.223	–
CM^2	13.64	13.34	*5*	**12.12**	11.63	12.48	0.346	15.47
LMd	31.06	31.02	*5*	**28.08**	26.61	28.88	0.924	35.58
ACo	11.25	11.94	*5*	**10.86**	9.38	12.09	1.042	13.75
CM_3	14.84	14.37	*5*	**13.43**	12.55	14.21	0.637	17.38

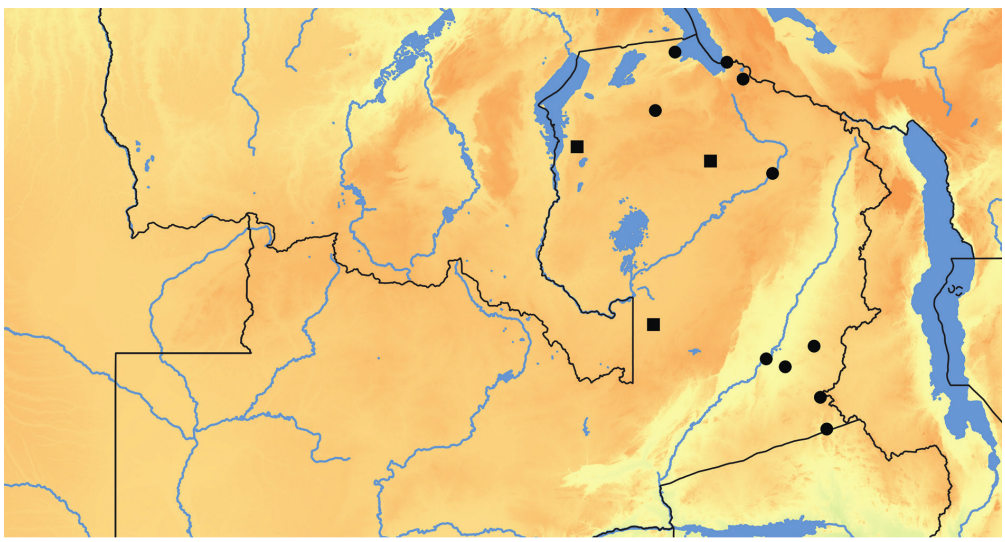

Fig. 4. Distribution of *Epomophorus labiatus* in Zambia based on museum specimens; circles – data published by Monadjem et al. (2020a), squares – new data.

Epomophorus wahlbergi (Sundewall, 1846)

Material (1). 1 ♀ (NMP 97583 [S+A]), Sakeji, Nchila Wildlife Reserve, 27 April 2009, leg. R. Šumbera, M. Lövy, H. Patzenhauerová & J. Šklíba.

Epomophorus wahlbergi is a widespread and common bat in the eastern and central parts of southern Africa (Bergmans 1988, Monadjem et al. 2020a), its rarity in the NMP collection from Zambia is thus rather surprising. A single specimen of *E. wahlbergi* was documented among almost fifty fruit bat specimens collected in Zambia. It was obtained from the area where it was documented already by Ansell (1978). The dimensions of the NMP specimen of *E. wahlbergi* are shown in Table 2.

Epomophorus dobsonii de Bocage, 1889

Material (8). 1 ♀ (NMP 97661 [S+A]), Chishimba Falls, river bank, 25 July 2009, leg. V. Mazoch & J. Zima;
1 ♂ (NMP 97691 [S+A]), Kacholola, riverine forest, 15 June 2010, leg. J. Šklíba, V. Mazoch & E. Knotková;
1 ♀ (NMP 97621 [S+A]), Ndola Hill, 7 June 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera;

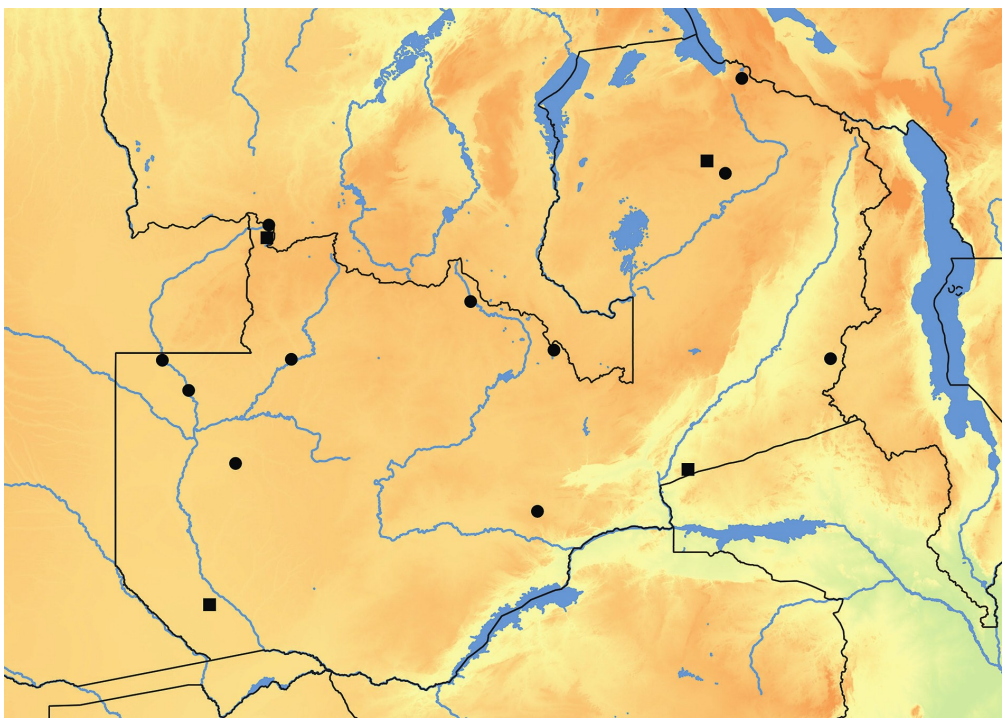

Fig. 5. Distribution of *Epomophorus dobsonii* in Zambia based on museum specimens; circles – published data (Bergmans 1989, Monadjem et al. 2020a), squares – new data.

Table 3. Basic biometric data on the NMP specimens of *Epomophorus dobsonii* from Zambia (only adult specimens are included). For abbreviations see Methods

dimension	males					females				
	n	**M**	min	max	SD	*n*	**M**	min	max	SD
G	*3*	**102.67**	88.0	112.0	12.858	*3*	**95.67**	90.0	104.0	7.371
LAt	*3*	**86.47**	82.0	90.7	4.355	*3*	**86.23**	83.4	90.3	3.612
LCr	*3*	**53.14**	50.13	55.03	2.637	*3*	**49.82**	47.38	53.29	3.088
LCb	*3*	**52.55**	48.88	54.61	3.186	*3*	**48.86**	46.98	51.93	2.679
LaZ	*3*	**26.28**	24.68	27.11	1.383	*3*	**24.78**	23.94	25.87	0.990
LaI	*3*	**7.29**	6.88	7.83	0.488	*3*	**6.90**	6.54	7.61	0.612
LaP	*3*	**10.51**	9.98	11.02	0.520	*3*	**10.47**	9.54	11.44	0.951
LaInf	*3*	**11.87**	11.16	12.34	0.626	*3*	**11.21**	10.71	11.58	0.449
LaN	*3*	**18.34**	18.04	18.64	0.300	*3*	**17.84**	17.29	18.48	0.599
LaM	*3*	**18.37**	18.05	18.78	0.374	*3*	**17.47**	16.84	18.38	0.809
ANc	*3*	**11.41**	11.21	11.64	0.217	*3*	**11.52**	10.86	12.33	0.746
LBT	*3*	**4.97**	4.83	5.08	0.129	*3*	**4.97**	4.51	5.24	0.400
CC	*3*	**10.34**	10.08	10.73	0.344	*3*	**10.18**	10.10	10.28	0.092
M^2M^2	*3*	**14.80**	13.75	15.56	0.938	*3*	**13.97**	13.83	14.14	0.158
CM^2	*3*	**15.92**	14.74	16.53	1.019	*3*	**14.48**	14.25	14.68	0.217
LMd	*3*	**41.62**	38.11	43.81	3.069	*3*	**39.00**	37.63	41.45	2.127
ACo	*3*	**13.67**	13.23	14.04	0.410	*3*	**13.31**	12.82	14.08	0.675
CM_3	*3*	**17.54**	16.21	18.48	1.184	*3*	**15.76**	13.73	17.18	1.806

2 ♀♀ (NMP 97578, 97584 [S+A]), Sakeji, Nchila Wildlife Reserve, 27 April 2009, leg. R. Šumbera, M. Lövy, H. Patzenhauerová & J. Šklíba;
2 ♂♂ (NMP 97585, 97586 [A]), Sakeji, Nchila Wildlife Reserve, 28 April 2009, leg. R. Šumbera, M. Lövy, H. Patzenhauerová & J. Šklíba;
1 ♂ (NMP 97606 [S+A]), Sioma Bush Camp, 23 May 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera.

The territory of Zambia represents a large part of the distribution range of *Epomophorus dobsonii* (Bergmans 1989). As a faunal element of central Africa, it reaches a part of the southern margin of its distribution in this country. Published records of *E. dobsonii* are available from all parts of Zambia, except the areas south of Lusaka. The NMP collection contributes to the distribution picture at the southern margin of the range of this bat (Fig. 5); the finding of a male specimen at the Sioma Bush Camp represents the second southernmost record and that from Kacholola the fifth southernmost record of this species (Bergmans 1989, Monadjem et al. 2020a). The dimensions of the NMP specimens of *E. dobsonii* from Zambia are shown in Table 3.

Rhinolophidae

***Rhinolophus clivosus* Cretzschmar, 1828**

Material (2). 1 ♀ (NMP 97631 [S+A]), Kasanka National Park, Fibwe Camp, chalet, 16 June 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera;

1 ♂ (NMP 97665 [S+A]), Kasanka National Park, Fibwe Camp, chalet, 10 August 2009, leg. V. Mazoch & J. Zima.

Rhinolophus clivosus is a locally abundant species throughout southern Africa; however, in Zambia it is a rather rare species, only seven confirmed record sites were reported from the country (Monadjem et al. 2020a). The available records are spread over the whole country, the new locality of the NMP specimens from the Kasanka NP does not represent an important contribution to the distribution picture of this bat.

The dimensions of the NMP specimens of *R. clivosus* from Zambia are shown in Table 4. This bat is considered a polyphyletic species, although this matter has not yet been resolved definitely (see Csorba et al. 2003, Benda & Vallo 2012, Stoffberg et al. 2012, Bernard & Happold 2013, Benda et al. 2017, Demos et al. 2019a, Monadjem et al. 2020a, etc.). Three groups of populations being worthy of possible species status were suggested to occur within the distribution range of this bat, from the Middle East in the north and southern Africa in the south (Benda et al. 2017). These groups differ from each other in morphometric and molecular genetic traits (mtDNA) (Aellen 1939, Benda & Vallo 2012). The Zambian samples represent the large-sized morphotype that comprises the populations occurring between Uganda and South Africa, tentatively referred to *R.* (*clivosus*) *augur* Andersen, 1904 (LAt 49.2–57.4 mm, LCc 18.47–20.57 mm, CM^3 7.09–8.82 mm; Benda et al. 2017) that possesses a genetic lineage of its own (at least on the mitochondrial genome; Benda & Vallo 2012: 81, Fig. 6).

Table 4. Basic biometric data on the NMP specimens of Rhinolophidae and Hipposideridae from Zambia. For abbreviations see Methods

dimension	*Rhinolophus clivosus*		*Rhinolophus sakejiensis*	*Rhinolophus fumigatus*		*Rhinolophus mossambicus*		*Hipposideros caffer*			*Macronycteris vittata*
	97631	97665	97587	97641	97650	97612	97640	97570	97571	97572	97569
G	18.5	18.0	21.0	16.0	11.5	22.0	28.5	7.8	7.2	7.3	74.2
LAt	52.5	52.5	56.6	53.8	48.7	66.3	66.4	46.9	47.0	46.7	99.0
LCr	22.82	23.08	25.47	23.72	21.96	29.17	29.96	–	17.05	17.44	33.25
LCO	22.19	22.31	24.76	23.06	21.28	28.44	28.51	–	16.93	17.31	33.44
LCc	19.61	19.87	21.86	20.14	18.65	25.09	25.34	–	14.88	15.06	29.83
LaZ	11.62	11.82	13.39	11.77	11.09	14.23	14.27	–	9.18	9.28	17.18
LaI	2.62	2.52	3.18	2.62	2.13	2.89	2.66	–	2.54	2.32	3.11
LaInf	5.91	5.98	6.91	6.19	5.49	7.79	7.43	–	4.81	4.93	9.26
LaN	9.29	9.29	10.27	9.49	8.74	10.81	11.03	–	7.49	7.46	11.54
LaM	10.27	10.47	11.56	10.86	10.08	12.76	12.68	–	9.28	9.32	14.87
ANc	6.76	7.04	7.84	6.51	6.34	8.13	8.21	–	5.74	5.74	10.54
LBT	3.28	3.48	3.45	4.41	3.88	4.88	5.27	–	3.15	3.38	4.33
CC	6.29	6.37	7.60	6.44	5.86	7.79	7.96	–	3.93	4.16	8.85
M^3M^3	8.31	8.65	9.89	8.54	8.01	10.07	10.49	–	6.14	6.25	11.91
CM^3	8.17	8.48	9.61	8.43	7.75	10.32	10.12	–	5.95	6.15	11.96
LMd	14.69	14.93	17.13	15.41	14.14	19.41	19.33	–	10.48	10.73	22.48
ACo	3.62	3.74	4.68	4.09	3.28	4.98	4.94	–	2.47	2.54	7.43
CM_3	8.87	9.02	10.66	9.08	8.38	11.22	10.89	–	6.32	6.67	13.57

Rhinolophus sakejiensis Cotterill, 2002

Material (1). 1 ♂ (NMP 97587 [A]), Sakeji, Nchila Wildlife Reserve, 28 April 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera.

Rhinolophus sakejiensis is a rather enigmatic taxon, until recently known only from the type series. The type series composed of three male specimens was collected on 11 October 1990 and twelve years later formally described as a new species (Cotterill 2002). The NMP specimen reported here represents a fourth known specimen of *R. sakejiensis*, and is the first collected after the type series, i.e. after almost 20 years. The locality of collection of the NMP specimen of *R. sakejiensis* is the Nchila Wildlife Reserve, a site situated some 4 km WNW of the type locality marked in a map by Cotterill (2002: 167, Fig. 1). All known specimens of *R. sakejiensis* come from a limited area of the eastern part of "Ikelenge Pedicle" of north-western Zambia; so, the species remains known from only a small upland region north of the source of the Zambezi.

The dimensions of the NMP specimen of *R. sakejiensis* from Zambia are shown in Table 4. The correct identification of the species is indisputable, the dimensions of the bat conform to those mentioned by Cotterill (2002) for the type series, the structure of noseleaf of the NMP bat is identical to that figured by Cotterill (2002: 170, Fig. 2). In most of the dimensions that allow comparison (LCr 24.6–25.6 mm; LCc 21.7–22.3 mm; LaZ 12.9–13.5 mm; CM^3 9.4–9.7 mm), the NMP specimen falls within the respective ranges of the type series (Cotterill 2002: 169, Table 3); in three dimensions (LAt 52.5–55.2 mm; LaM 11.0–11.5 mm; M^3M^3 9.2–9.5 mm) the ranges of the type specimens lie slightly below the values of the NMP specimen. However, these differences are tiny and insignificant, regarding the small sample size of the type series.

Rhinolophus fumigatus Rüppell, 1842

Material (2). 1 ♂ (NMP 97650 [S+A]), Kalambo Falls, camp above river, 18 July 2009, leg. V. Mazoch & J. Zima;
1 ♂ (NMP 97641 [S+A]), Mutinondo, Mayense Camp, camp office, 8 July 2009, leg. V. Mazoch & J. Zima.

In southern Africa, the distribution of *Rhinolophus fumigatus* has two separate areas, the western patch in north-western Namibia and western Angola, and the eastern patch in Zimbabwe and Malawi, slightly exceeding to surrounding countries (Monadjem et al. 2020a). In Zambia, this bat is a rather rare faunal element; only six confirmed record sites are known from the eastern part of the country. The new localities of the NMP specimens of *R. fumigatus* originate from north-eastern Zambia and do not represent an important contribution to the distribution picture of this bat. The dimensions of the NMP specimens of *R. fumigatus* from Zambia are shown in Table 4.

Rhinolophus mossambicus Taylor, Stoffberg, Monadjem, Schoeman, Bayliss et Cotterill, 2012

Material (2). 1 ♀ (NMP 97612 [S+A]), Kafue National Park, Lufupa River Camp, 26 May 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera;
1 ♀ (NMP 97640 [S+A]), Mutinondo, Mayense Camp, camp office, 8 July 2009, leg. V. Mazoch & J. Zima.

Recently described *Rhinolophus mossambicus* is an endemic of the eastern parts of southern Africa (Taylor et al. 2012, Monadjem et al. 2020a). Its confirmed distribution range stretches from northern South Africa, through Mozambique and Zimbabwe to Malawi and Zambia. In the latter country, two distribution areas were documented, one in the lower Kafue river basin

and the other in the broader Luangwa river basin. Concerning the two new localities here presented, each falls into one of these range segments (Fig. 6). The record from the Lufupa River Camp marks a new geographical extreme at the north-western margin of the species distribution range in central Africa.

The dimensions of the NMP specimens of *R. mossambicus* from Zambia are shown in Table 4. In their description of the new species *R. mossambicus*, Taylor et al. (2012) gave very little information on the dimensions of this and other newly defined taxa. Only three of them could be compared, based on five specimens: forearm length (LAt 60–65 mm; mean [M] 62.8 mm), greatest length of skull (LCr 27–29 mm; M 28.1 mm), and condylocanine length (LCc 24–25 mm; M 24.4 mm). Monadjem et al. (2020a) added new data for LAt (59.5–66.0 mm; M 63.9 mm), based on an enlarged set of specimens (n=11). In all cases, the published ranges of dimensions are below the values of the data collected from the NMP specimens (see Table 4); the two bats from NMP are larger in body size than other examined specimens of *R. mossambicus*. These differences could be a consequence of cline shift in body size in this bat along the geographic (south-north) or climatic (south-east to north-west) gradient (observable also in other mammal species in Africa), or just of a difference in the method of measuring. In any case, this observation seems to be in a need of further examination.

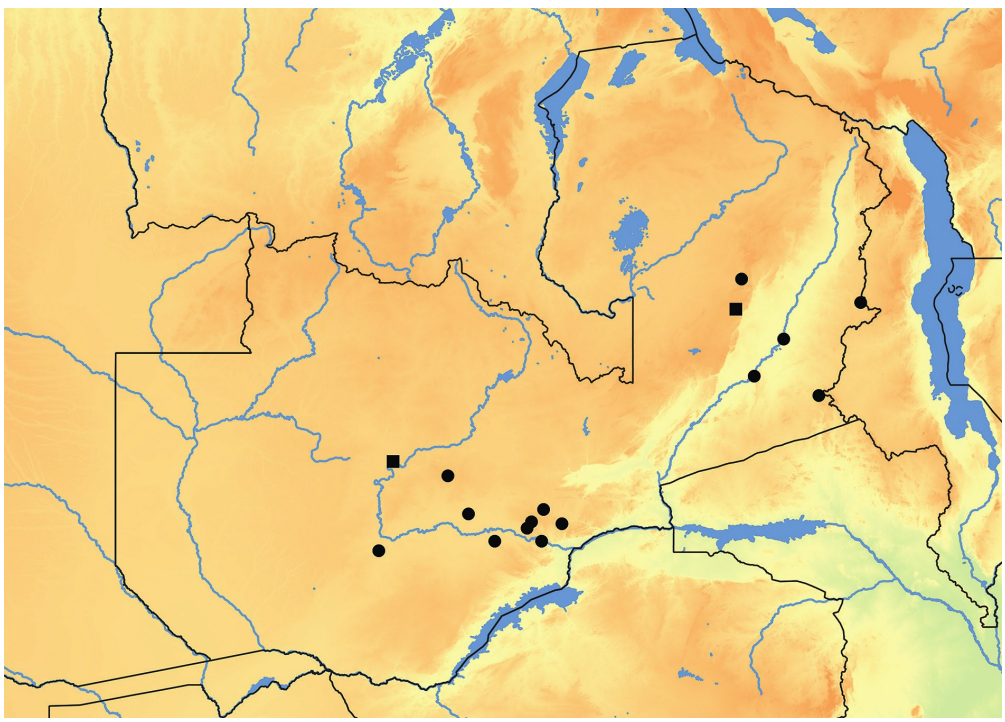

Fig. 6. Distribution of *Rhinolophus mossambicus* in Zambia based on museum specimens; circles – data published by Monadjem et al. (2020a), squares – new data.

Hipposideridae

***Hipposideros caffer* (Sundevall, 1846)**

Material (3). 3 ♀♀ (NMP 97571, 97572 [S+A], 97570 [A]), Chisakila, Bwarenunka Cave, 1 December 2018, leg. P. Benda & J. Červený.

Hipposideros caffer s.l. is a common bat in most of the savanna habitats of sub-Saharan Africa and it occurs also in south-western Arabia and western Maghreb (Spanjer Wright 2009). In southern Africa, it is distributed more abundantly mainly in the northern and eastern parts. It is also a common bat in Zambia, where 30 confirmed record localities are available according to Monadjem et al. (2020a). The here presented single record from the south-central part of Zambia does not represent an important contribution to the distribution picture of this bat; in the karst area at the Zambezi Escarpment south-east of Lusaka this bat has been documented repeatedly (Ansell 1978, Monadjem et al. 2020a).

The NMP specimens from Zambia belong to the A1 mitochondrial genetic lineage sensu Vallo et al. (2008), i.e. *H. caffer* s.str. However, their haplotypes create a sub-lineage of their own within this lineage (A1c; Fig. 7), which is rather deeply separated from the A1[a] sub-lineage from South Africa, Swaziland/Eswatini, and southern Mozambique, as originally defined by Vallo et al. (2008), and the A1b sub-lineage from northern Mozambique defined by Monadjem et al. (2013b). The bats of the new sub-lineage represent the first record of the population of A1 lineage from Zambia and the westernmost and the most inland record of this lineage, until now known to occur only in the rather low situated areas of south-eastern Africa along the Mozambique Channel; other extremes of its occurrence are known from northernmost Mozambique – the northernmost record from 12°11'S, 37°33'E and the easternmost from 12°52'S, 37°41'E (Monadjem et al. 2013b, 2020a). The A1 lineage of the *H. caffer* complex is now known from a triangular area stretching between southern Zambia, northern Mozambique and south-eastern South Africa (Mkuzi Reserve; Vallo et al. 2008). This geographical pattern suggests that the bats of the *caffer*-morphotype (sensu Koopman 1975) from this part of distribution range belong to the A1 lineage, since all genetically examined specimens of A1 lineage belong to this morphotype (Vallo et al. 2008, Monadjem et al. 2013b, 2020a; Table 4). This could also refer to the bats from north-western Mozambique, including the type specimen of *Phylorhina gracilis* Peters, 1852. This bat (ZMB 364) was collected at Tete (NW Mozambique; Peters 1852) and represents the *caffer*-morphotype (LAt 43.6 mm, LCc 15.39 mm, LaZ 9.14 mm, CM^3 6.02 mm; own data). This suggests that this name really represents a synonym of *Hipposideros caffer* as it is traditionally considered, based on biogeographic grounds (Allen 1939, Ellerman et al. 1953, Meester et al. 1986, Koopman 1993, Simmons 2005, Spanjer Wright 2009).

A colony of this species composed of ca. 1000 bats (adults and non-flying juveniles) was discovered in the Bwarenunka Cave near Chisakila on 1 December; all examined bats were lactating females. The dimensions of the NMP specimens of *H. caffer* from Zambia are shown in Table 4.

***Macronycteris vittata* (Peters, 1852)**

Material (1). 1 ♀ (NMP 67569 [S+A]), Chisakila, Bwarenunka Cave, 1 December 2018, leg. P. Benda & J. Červený.

The limits of the distribution range of *Macronycteris vittata* are still a subject of research; however, this bat is not rare in the central and north-eastern parts of southern Africa (Monadjem et

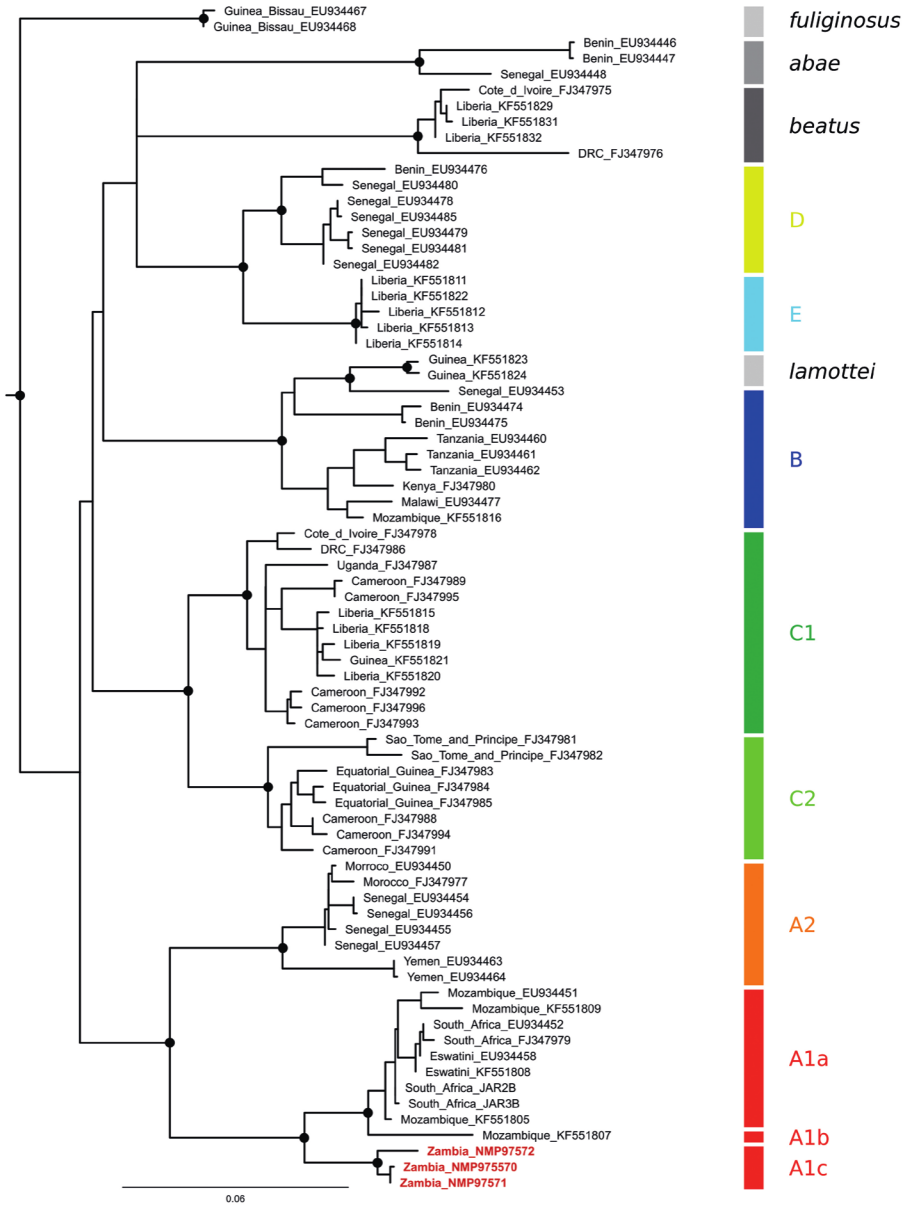

Fig. 7. Maximum likelihood tree of reconstructed phylogenetic relations of the *Hipposideros caffer* complex (cf. Vallo et al. 2008, Monadjem et al. 2013b) and *H. fuliginosus* (Temminck, 1853) as an outgroup, based on the cytochrome *b* sequences (the Zambian bats in red; the labeling of the lineages sensu Vallo et al. 2008). Black dots on the nodes denote that these nodes have high branch support (e.g., SH-aLRT ≥80%, aBayes ≥0.95, UFBoot ≥90%).

Fig. 8. A group of local boys hunting for *Macronycteris vittata* in the Bwarenunka Cave near Chisakila.

al. 2020a). In Zambia, nine confirmed record sites of *M. vittata* are available from central and southern parts of the country, including the karst area at the Zambezi Escarpment south-east of Lusaka (Ansell 1978, Monadjem et al. 2020a), where the single NMP specimen of this bat comes from.

A colony of this bat was discovered in the Bwarenunka Cave near Chisakila; the examined adult female did not show any signs of current reproduction. The colony is used as a food source by the local community, we found a group of boys from a village nearby that hunted these bats inside the cave (Fig. 8). The dimensions of the NMP specimen of *M. vittata* from Zambia are shown in Table 4.

Rhinonycteridae

***Cloeotis percivali* Thomas, 1901**

Material (5). 4 ♂♂, 1 ♀ (NMP 97565–97568 [S+A], 97564 [A]), Chisakila, Bwarenunka Cave, 1 December 2018, leg. P. Benda & J. Červený.

Cloeotis percivali is an uncommon bat in southern Africa; in Zambia, its distribution is limited to the south-eastern part of the country, four record sites are available from a belt stretching between Lusaka and Chipata (Monadjem et al. 2020a). The new locality of the NMP specimens of *C. percivali* falls into this range and conforms with the current picture of the occurrence of this bat.

A colony of this species composed of ca. 100 bats (adults and non-flying juveniles) was discovered in the Bwarenunka Cave near Chisakila on 1 December (Fig. 9); the examined female was lactating. The dimensions of the NMP specimens of *C. percivali* from Zambia are shown in Table 5.

N y c t e r i d a e

***Nycteris thebaica* Geoffroy, 1813**

Material (3). 2 ♂♂ (NMP 97613, 97614 [S+A]), Kafue National Park, Lufupa River Camp, 26 May 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera.
1 ♂ (NMP 97628 [S+A]), Kasanka National Park, Pontoon Camp, riverine forest, 15 June 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera;

Nycteris thebaica is a common bat throughout Africa except the rain forest zone and a large part of the Sahara; furthemore, it occurs also in the western and southern parts of the Middle East (Gray et al. 1999). In Zambia, this species ranks among the most common bats; it was collected from at least 34 localities (Monadjem et al. 2020a). The NMP specimens of *N. thebaica* originate from only two sites. This is rather surprising considering the common occurrence of this bat in Zambia. However, at least one of these localities lies in a region where this bat

Fig. 9. Colony of *Cloeotis percivali* (partly mixed with a colony of *Hipposideros caffer*) in the Bwarenunka Cave near Chisakila observed on 1 December 2018.

Table 5. Basic biometric data on the NMP specimens of *Cloeotis percivali*, *Nycteris thebaica*, and *Chaerephon pumilus* from Zambia. For abbreviations see Methods

dimension	*Cloeotis percivali*					*Nycteris thebaica*					*Chaerephon pumilus*				
	n	**M**	min	max	SD	*n*	**M**	min	max	SD	*n*	**M**	min	max	SD
G	*5*	**3.44**	3.1	3.9	0.344	*3*	**8.67**	8.0	9.0	0.577	*4*	**9.50**	6.0	11.0	2.380
LAt	*5*	**34.32**	33.8	35.0	0.536	*3*	**42.50**	42.5	42.5	0.000	*4*	**37.60**	37.3	38.0	0.294
LCr	*3*	**13.38**	13.14	13.82	0.379	*1*	**19.43**	–	–	–	*3*	**16.58**	15.83	17.13	0.673
LCO	*4*	**13.11**	12.88	13.32	0.209	*3*	**19.08**	18.83	19.31	0.240	–	–	–	–	–
LCb	–	–	–	–	–	–	–	–	–	–	*2*	**15.20**	14.66	15.73	0.757
LCc	*4*	**11.00**	10.93	11.04	0.051	*3*	**16.74**	16.58	16.92	0.170	–	–	–	–	–
LaZ	*4*	**7.17**	7.04	7.26	0.092	*3*	**10.87**	10.83	10.93	0.051	*3*	**10.30**	9.98	10.63	0.325
LaI	*4*	**1.44**	1.32	1.58	0.116	*3*	**4.75**	4.66	4.83	0.085	*3*	**3.72**	3.68	3.81	0.075
LaP	–	–	–	–	–	*3*	**4.59**	4.57	4.61	0.021	–	–	–	–	–
LaInf	*4*	**3.48**	3.38	3.61	0.099	*3*	**4.64**	4.62	4.68	0.032	*3*	**4.29**	4.21	4.41	0.108
LaN	*4*	**5.57**	5.25	5.88	0.321	*3*	**8.71**	8.63	8.87	0.139	*3*	**8.46**	8.36	8.59	0.117 *3*
LaM	*4*	**6.81**	6.71	6.93	0.109	**8.41**	8.28	8.53	0.125	*3*	**9.85**	9.81	9.92	0.064	*2*
ANc	*4*	**4.56**	4.35	4.71	0.165	**6.70**	6.58	6.86	0.143	*3*	**5.75**	5.61	5.89	0.198	*3*
LBT	*4*	**2.21**	1.84	2.47	0.280	**3.39**	3.35	3.42	0.035	*3*	**3.58**	3.44	3.73	0.145	*3*
CC	*4*	**2.89**	2.81	2.97	0.066	**4.63**	4.43	4.88	0.230	*3*	**4.36**	4.18	4.63	0.236	*3*
M^3M^3	*4*	**4.63**	4.48	4.74	0.111	**6.89**	6.72	6.98	0.150	*3*	**7.31**	6.84	7.61	0.412	*3*
CM^3	*4*	**4.03**	3.84	4.12	0.129	**6.40**	6.28	6.59	0.164		**6.24**	5.86	6.48	0.333	
LMd	*4*	**7.49**	7.32	7.63	0.130	*3*	**11.99**	11.97	12.01	0.020	*3*	**10.90**	10.34	11.33	0.506
ACo	*4*	**1.46**	1.36	1.53	0.071	*3*	**3.57**	3.42	3.65	0.130	*3*	**2.91**	2.73	3.02	0.157
CM_3	*4*	**4.06**	3.91	4.16	0.114	*3*	**6.75**	6.68	6.84	0.081	*3*	**6.47**	6.15	6.76	0.307

has not been documented before, the Kasanka National Park (see Ansell 1978, Monadjem et al. 2020a).

According to the results of examination of the mitochondrial genome from the samples from both the above mentioned localities, the populations of *N. thebaica* from Zambia belong to the clade *thebaica* 4 sensu Demos et al. (2019b). Until now, this clade was known only from two regions in south-eastern Kenya (Kilifi and Kwale), while from the regions between Kenya and Zambia, other clades were detected (*thebaica* 2, *thebaica* 6; Demos et al. 2019b). The dimensions of the NMP specimens of *N. thebaica* from Zambia are shown in Table 5.

Molossidae

***Chaerephon pumilus* (Cretzschmar, 1830)**

Material (4). 1 ♂, 1 ♀ (NMP 97619, 97620 [S+A]), Kafue National Park, Chunga Camp, 28 May 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera;
1 ♂ (NMP 97673 [S+A]), Newa, 10 km E of Mongu, grassland at a river, 12 June 2010, leg. J. Šklíba, V. Mazoch & E. Knotková;
1 ♂ (NMP 97605 [A]), Sioma Bush Camp, 23 May 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera.

Bats traditionally assigned to *Chaerephon pumilus* are currently considered a complex composed of several species (Naidoo et al. 2016, Monadjem et al. 2020a). However, the phylogenetic relations within the complex as well as its taxonomic arrangement still remain to be resolved. If the complex really comprises more species, there is a high probability that the name *C. pumilus* is not applicable for the populations of southern and central Africa (Naidoo et al. 2016). The morphotype of *C. pumilus* s.l. represents one of the most common bat forms in the Afro-tropics except the southern Sahara and the arid regions of south-western Africa (Bouchard 1998). In Zambia, this bat is the most commonly recorded molossid (Ansell 1978, Monadjem et al. 2020a). The localities of four specimens of the NMP collection are situated in the western part of Zambia, where this bat has been previously collected most frequently (Monadjem et al. 2020a).

The dimensions of the NMP specimens of *C. pumilus* s.l. from Zambia are shown in Table 5. A genetic analysis of the mitochondrial ND1 gene showed these specimens to be in agreement by 98.3–99.7% with the haplotypes referred to *Chaerephon leucogaster* (Grandidier, 1869) from Madagascar (sensu Ammerman et al. 2012), i.e. to one of the possible separate species within the *Chaerephon pumilus* species complex (Naidoo et al. 2016).

Chaerephon bivittatus (von Heuglin, 1861)

Material (4). 3 ♂♂, 1 ♀ (NMP 97651–97654 [S+A]), Kalambo Falls, camp above river, 18 July 2009, leg. V. Mazoch & J. Zima.

Chaerephon bivittatus occurs in a long belt of savannas across the eastern part of Africa, stretching from Eritrea to Zimbabwe (Eger & Peterson 1979). Only one published record of this bat is available from Zambia, from Abercorn (= Mbala) in the north-eastern part of the country (Hayman & Harrison 1966, Monadjem et al. 2020a). The here presented second record of *C. bivittatus* from Zambia originates from an almost identical region as the first record; the Kalambo Falls are situated only some 30 km north-west of Mbala. The dimensions of the NMP specimens of this bat from Zambia are shown in Table 6.

Chaerephon nigeriae Thomas, 1913

Material (1). 1 ♀ (NMP 97670 [S+A]), Kaoma, Farmers Rendezvous Lodge, small pool, 5 June 2010, leg. J. Šklíba, V. Mazoch & E. Knotková.

Chaerephon nigeriae is a widely distributed savanna bat of sub-Saharan Africa (Willis et al. 2002). The region of south-central Africa, including Zambia, represents one of the distribution centres of this bat (Willis et al. 2002, Monadjem et al. 2020a). At least eight record localities are available from Zambia (Ansell 1978, Monadjem et al. 2020a); the highest concentration of records comes from the western part of the country, from where the NMP specimen also originates. The dimensions of the NMP specimen of *C. nigeriae* from Zambia are shown in Table 6.

Mops condylurus (Smith, 1833)

Material (5). 3 ♂♂, 2 ♀♀ (NMP 97632–97636 [S+A]), Bangweulu Game Reserve, Chikuni, swamp, 17 June 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera.

Mops condylurus ranks among the most common and widespread savanna bats of sub-Saharan Africa (Happold 2013b). However, with just seven confirmed records, this species is only the fourth most common molossid bat of Zambia (Monadjem et al. 2020a). The available record

Table 6. Basic biometric data on the NMP specimens of *Chaerephon bivittatus*, *C. nigeriae*, *Mops condylurus*, *Nyctinomus aegyptiacus*, and *Myotis welwitschii* (*Mw*) from Zambia. For abbreviations see Methods

dimension	*Chaerephon bivittatus*					*Chaerephon nigeriae*	*Mops condylurus*					*Nyctinomus aegyptiacus*		*Mw*
	n	**M**	min	max	SD	97670	*n*	**M**	min	max	SD	97638	97639	97579
G	*4*	**16.25**	14.0	17.0	1.500	16.0	*5*	**27.40**	23.0	36.0	5.177	17.0	17.0	13.0
LAt	*4*	**45.65**	44.4	46.3	0.850	46.0	*5*	**48.70**	46.8	50.0	1.447	46.6	45.7	57.0
LCr	*4*	**19.06**	18.88	19.38	0.238	18.98	*5*	**21.40**	20.34	22.68	0.991	18.78	19.06	19.62
LCb	*4*	**17.71**	17.45	18.14	0.302	17.98	*5*	**19.01**	18.48	19.58	0.484	18.08	18.68	18.72
LaZ	*4*	**11.54**	11.36	11.72	0.191	12.27	*5*	**13.49**	13.02	13.98	0.377	11.48	11.86	12.71
LaI	*4*	**3.85**	3.71	3.91	0.093	3.82	*5*	**4.53**	4.48	4.61	0.062	4.12	4.55	4.58
LaInf	*4*	**4.93**	4.58	5.21	0.263	4.74	*5*	**6.06**	5.52	6.53	0.360	4.47	4.43	5.38
LaN	*4*	**9.70**	9.36	9.84	0.228	10.41	*5*	**10.81**	10.42	11.18	0.296	9.48	9.84	9.31
LaM	*4*	**10.75**	10.54	10.88	0.147	11.12	*5*	**12.39**	12.13	12.87	0.280	10.88	11.13	9.81
ANc	*4*	**6.69**	6.57	6.85	0.130	6.58	*5*	**7.96**	7.39	9.17	0.741	5.66	5.74	6.57
LBT	*4*	**4.13**	3.87	4.42	0.234	4.11 CC	*5*	**3.86**	3.67	4.04	0.171	4.41	4.34	3.44
	4	**5.25**	4.93	5.44	0.220	5.18	*5*	**6.47**	6.03	6.98	0.440	4.68	4.88	5.14
M^3M^3	*4*	**8.47**	8.38	8.61	0.101	8.68	*5*	**9.58**	9.21	9.83	0.269	7.88	8.08	8.09
CM^3	*4*	**7.31**	7.13	7.46	0.155	7.56	*5*	**8.00**	7.69	8.31	0.258	7.09	7.39	7.87
LMd	*4*	**12.89**	12.63	13.11	0.236	13.07	*5*	**14.51**	13.94	15.19	0.586	12.95	13.14	15.06
ACo	*4*	**3.28**	3.14	3.39	0.105	3.53	*5*	**4.01**	3.73	4.24	0.231	3.92	3.71	4.76
CM_3	*4*	**7.87**	7.61	8.03	0.195	8.05	*5*	**8.72**	8.38	9.04	0.270	7.71	7.93	8.47

sites of *M. condylurus* are spread over the whole territory of Zambia, and the NMP series thus does not contribute significantly to more precise understanding of its distribution. The dimensions of the NMP specimens of *M. condylurus* from Zambia are shown in Table 6.

Nyctinomus aegyptiacus Geoffroy, 1818

Material (2). 2 ♂♂ (NMP 97638, 97639 [S+A]), Nsalu Cave, 7 July 2009, leg. V. Mazoch & J. Zima.

Nyctinomus aegyptiacus was traditionally assigned to the genus *Tadarida* Rafinesque, 1814 (Freeman 1981, Koopman 1993, Simmons 2005, Monadjem et al. 2020a, etc.); however, the results of molecular genetic analyses by Lamb et al. (2011) and Ammerman et al. (2012) revealed this genus to be paraphyletic in respect to the relationships of *N. aegyptiacus* and the type species of this genus, *T. teniotis* (Rafinesque, 1814). Therefore, we classify the former species into a genus of its own, *Nyctinomus* Geoffroy, 1818, of which *N. aegyptiacus* is the type species (Geoffroy Saint-Hilaire 1818).

This bat is the most widely distributed molossid of the Old World; it occurs in the whole of non-forested parts of Africa, from South Africa to Egypt and Morocco, in southern Arabia, Iran, and broadly in the Indian subcontinent, from Afganistan to Bangladesh and Ceylon (Simmons 2005). In southern Africa, *N. aegyptiacus* is one of the most common and widespread bats in arid habitats; however, only three confirmed records are available from Zambia, namely from its southern part (Ansell 1978, Monadjem et al. 2020a). Thus, the NMP specimens from the

Nsalu Cave represent the northernmost record not only from Zambia, but from the eastern part of the region as well (see Monadjem et al. 2010, Curran et al. 2012).

Nyctinomus aegyptiacus is considered to be a complex composed of more than one species (Benda et al. 2012, Monadjem et al. 2020a). The dimensions of the NMP specimens of this bat from Zambia are shown in Table 6, they suggest these specimens to belong to the medium-sized morphotype of the complex, corresponding with the morphotype of *N. thomasi* (Wroughton, 1919), so far known from India and Arabia only (Benda et al. 2012).

Vespertilionidae

Myotis welwitschii (Gray, 1866)

Material (1). 1 ♂ (NMP 97579 [S+A]), Sakeji, Nchila Wildlife Reserve, 27 April 2009, leg. J. Šklíba, M. Lövy, H. Patzenhauerová & R. Šumbera.

Myotis welwitschii is a bat with patchy distribution in sub-Saharan Africa, it occurs almost exclusively in upland areas and was documented from all main mountain ranges and highland

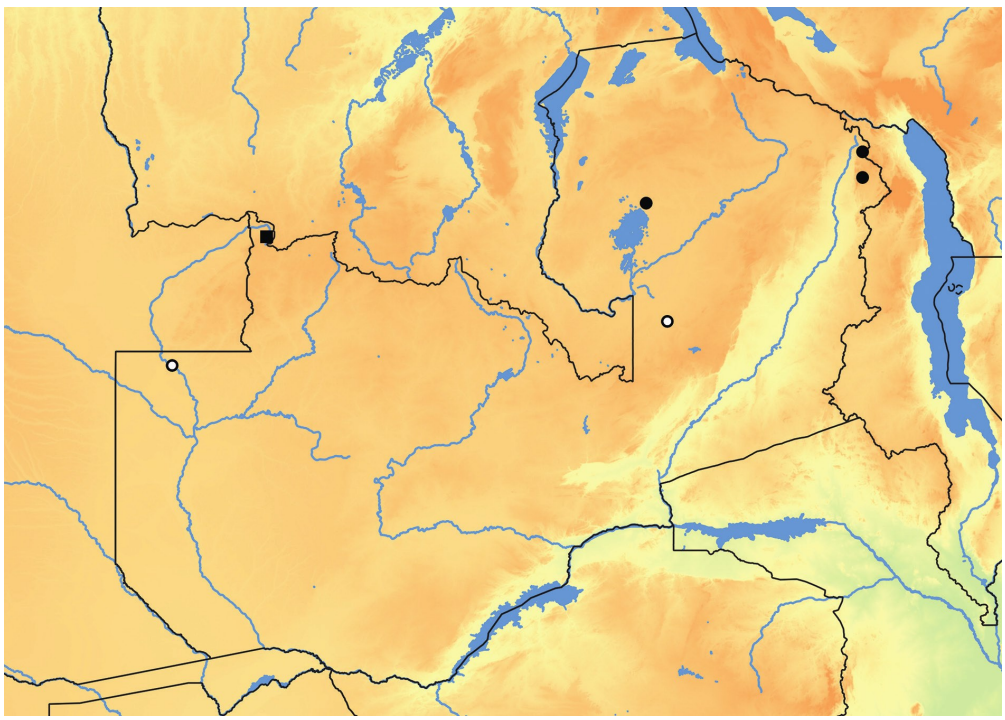

Fig. 10. Distribution of *Myotis welwitschii* in Zambia based on the data published by Fahr & Ebigbo (2003) and new data; closed circles – published localities of museum specimens, open circles – other published localities, square – locality of the NMP specimen.

plateaus of this region except for dry zones (Fahr & Ebigbo 2003, Sedláček et al. 2006). The new record from Zambia is in accordance with this pattern, the Nchila Wildlife Reserve near Sakeji is situated in an upland above 1400 m a. s. l. Fahr & Ebigbo (2003) mentioned five localities of *Myotis welwitschii* from Zambia. However, Monadjem et al. (2020a) considered only three of them as indisputable, being based on museum specimens, all situated in the north-eastern part of Zambia. The new finding here reported thus represents the first record from the western part of the country which is confirmed by a collected specimen (Fig. 10). The dimensions of the NMP specimen of *M. welwitschii* from Zambia are shown in Table 6.

Glauconycteris variegata (Tomes, 1861)

Material (3). 1 ♂ (NMP 97669 [S+A]), Kaoma, Farmers Rendezvous Lodge, small pool, 5 June 2010, leg. J. Šklíba, V. Mazoch & E. Knotková;
2 ♂♂ (NMP 97602, 97603 [S+A]), Sioma Bush Camp, 23 May 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera.

Glauconycteris variegata is a bat widely distributed in the savanna zone of sub-Saharan Africa (Rambaldini 2010). In southern Africa, it occurs mainly in the central and eastern parts of the region and in all countries it ranks as an uncommon species (Monadjem et al. 2020a). Eight record localities are available from Zambia (Ansell 1978, Monadjem et al. 2020a), which is the largest number from any country of southern Africa (Monadjem et al. 2020a). However, with the exception of an eastern record from Chipata at the boder with Malawi, all localities are situated in a narrow belt of meridian arrangement, aproximately between 26°20'E and 27°55'E, across the central part of the country. This north-south stretching chain of sites also represents the western border of the distribution range of *G. variegata* in the central/northern part of southern Africa (Monadjem et al. 2020a). The NMP specimens of this bat come from western Zambia, the record from the Sioma Bush Camp shifts the margin of known distribution by ca. 320 km westwards. The dimensions of the NMP specimens of *G. variegata* from Zambia are shown in Table 7.

Pipistrellus rusticus (Tomes, 1861)

Material (7). 1 ♀ (NMP 97626 [A]), Ndola Hill, 7 June 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera;
3 ♂♂ (NMP 97580, 97581 [S+A], 97582 [A]), Sakeji, Nchila Wildlife Reserve, 27 April 2009, leg. J. Šklíba, M. Lövy, H. Patzenhauerová & R. Šumbera;
1 ♀ (NMP 97588 [S+A]), Sakeji, Nchila Wildlife Reserve, 28 April 2009, leg. J. Šklíba, M. Lövy, H. Patzenhauerová & R. Šumbera;
2 ♂♂ (NMP 97593, 97594 [S+A]), Simungoma, Nulubeti village, 19 May 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera.

The distribution range of *Pipistrellus rusticus* comprises two separate patches in sub-Saharan Africa, one in the savanna belt stretching from Senegal to Ethiopia and Kenya, the other in southern Africa in a triangle of savannas, delimited by central Angola, central Malawi, northern Namibia, and Swaziland/Eswatini (Kearney 2013, Monadjem et al. 2020a). In Zambia, this bat is known from eight localities, all of which are situated only in the western part of the country (Ansell 1978, Monadjem et al. 2020a); the NMP specimens of *P. rusticus* also originate from three sites in this part of the country. However, the record from the Nchila Wildlife Reserve at Sakeji seems to define newly the northern margin of the southern African distribution range segment, to which the nominotypical subspecies of *P. rusticus* is

Table 7. Basic biometric data on the NMP specimens of *Glauconycteris variegata*, *Pipistrellus rusticus*, and *Afropipistrellus grandidieri (Ag)* from Zambia. For abbreviations see Methods

dimension	*Glauconycteris variegata*					*Pipistrellus rusticus*					*Ag*
	n	**M**	min	max	SD	*n*	**M**	min	max	SD	97557
G	*3*	**12.67**	12.0	14.0	1.155	*7*	**5.36**	5.0	6.0	0.476	10.7
LAt	*3*	**44.73**	43.8	45.7	0.950	*7*	**29.81**	27.4	31.1	1.678	38.2
LCr	*3*	**13.26**	13.21	13.29	0.044	*5*	**11.79**	11.32	12.13	0.345	14.58
LCb	*3*	**13.35**	13.24	13.48	0.121	*5*	**11.33**	10.64	11.71	0.431	14.42
LaZ	*3*	**10.71**	10.64	10.81	0.089	*3*	**7.98**	7.66	8.21	0.287	10.33
LaI	*3*	**4.54**	4.47	4.62	0.075	*5*	**3.44**	3.31	3.56	0.119	4.02
LaInf	*3*	**4.82**	4.75	4.89	0.070	*5*	**3.82**	3.75	4.02	0.114	4.95
LaN	*3*	**8.48**	8.42	8.54	0.060	*5*	**6.35**	6.12	6.61	0.180	6.98
LaM	*3*	**9.42**	9.39	9.45	0.031	*5*	**7.33**	7.04	7.57	0.230	8.58
ANc	*3*	**6.14**	6.07	6.23	0.081	*5*	**4.45**	4.24	4.59	0.132	5.79
LBT	*3*	**3.68**	3.53	3.78	0.131	*5*	**2.60**	2.35	2.77	0.176	3.22
CC	*3*	**4.96**	4.88	5.08	0.104	*5*	**4.07**	3.94	4.11	0.073	4.92
M^3M^3	*3*	**7.02**	6.88	7.21	0.171	*5*	**5.40**	5.21	5.67	0.186	6.58
CM^3	*3*	**4.94**	4.91	4.97	0.031	*5*	**4.24**	4.08	4.38	0.123	5.28
LMd	*3*	**10.05**	9.89	10.17	0.144	*5*	**8.73**	8.33	9.02	0.268	11.04
ACo	*3*	**3.31**	3.29	3.32	0.015	*5*	**2.65**	2.53	2.78	0.117	3.81
CM_3	*3*	**5.37**	5.33	5.41	0.040	*5*	**4.53**	4.41	4.64	0.101	5.68

referred (Ansell 1978, Kearney 2013). The dimensions of the NMP specimens of this bat from Zambia are shown in Table 7.

Afropipistrellus grandidieri (Dobson, 1876)

Material (1). 1 ♀ (NMP 97557 [S+A]; Fig. 11), Kasanka National Park, Luwombwa Camp, 27 November 2018, leg. P. Benda & J. Červený.

Throughout its range covering the central parts of Africa between Cameroon, southern Kenya, Angola and central Mozambique, *Afropipistrellus grandidieri* is a rare bat; Thorn et al. (2007) mentioned only 27 known specimens from 18 localities in this extensive area (they did not include six bats from four sites in the DR Congo reported by Hayman et al. 1966). From southern and central Africa, Monadjem et al. (2020a) list nine localities in four countries, but none from Zambia. Our finding of a female in the Kasanka NP thus represents the first record of *A. grandidieri* from Zambia.

The dimensions of the NMP specimen of *A. grandidieri* from Zambia are shown in Table 7. By its skull size (LCr 14.58 mm, CM^3 5.28 mm), the Zambian specimen corresponds well with the dimensions of the large-sized southern African subspecies *A. g. angolensis* (Hill, 1937), see Thorn et al. (2007: Table 1: LCr 14.1–14.7 mm, CM^3 4.9–5.4 mm). However, by the forearm length, this female specimen shows the largest value ever recorded (LAt 38.2 mm). As the largest specimen of this species, Thorn et al. (2007) reported a male from Angola (AMNH 85535, holotype of *Eptesicus capensis angolensis*) that showed LAt 37.0 mm. Nevertheless, an identical forearm length as in the Zambian female was found in a male from southern Malawi

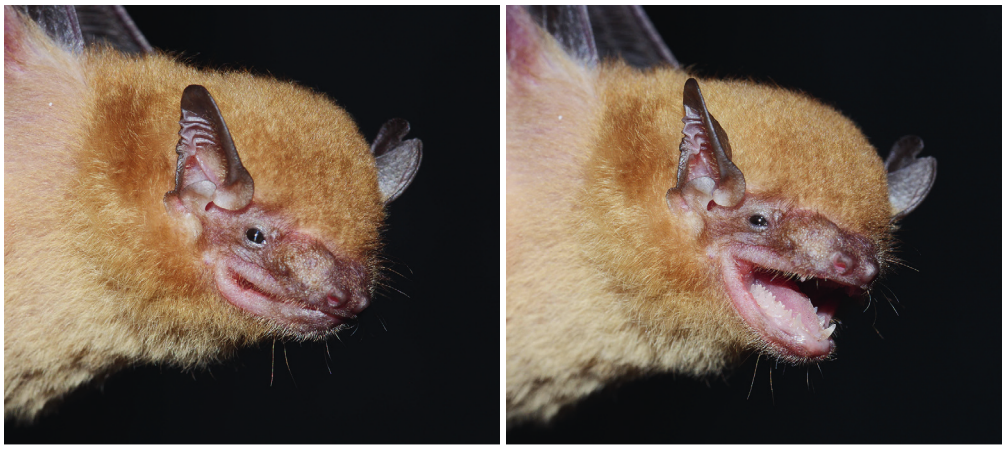

Fig. 11. Portraits of *Afropipistrellus grandidieri* female (NMP 97557) netted at the Luwombwa Camp, Kasanka National Park, on 27 November 2018. It is the first individual of this bat recorded from Zambia.

(LAt 38.2 mm, LCr 14.58 mm, CM^3 5.33 mm; MHNG 1971.044 [S+A], Mt. Mulanje foothills, Tea Research Foundation Forest, 1 December 2007, leg. M. Curran & M. Kopp; own data, cf. Curran et al. 2012).

Based on an analysis of mitochondrial marker, Monadjem et al. (2021) suggested to include *A. grandidieri* into the genus *Nycticeinops* Hill et Harrison, 1987, until then considered a monotypic genus. However, since only the mtDNA was employed in the analysis and the genus *Nycticeinops* s.str. possesses a markedly distinct condition of several morphologic traits (usual for generic separation, namely the dentition or, in lesser extent, the baculum morphology) than other species suggested to be included to this taxon, we prefer to retain the latter genus in its traditional taxonomic structure in the sense by Hill & Harrison (1987). For *A. grandidieri*, formerly frequently referred to the genera *Eptesicus* Rafinseque, 1820 or *Pipistrellus* Kaup, 1829, we prefer to use the genus name *Afropipistrellus* Thorn, Kock et Cuisin, 2007 (original-ly a subgenus for *Pipistrellus grandidieri*), at least tentatively, until the positions of various mitochondrial lineages found within the group of pipistrelloid bats of Africa are elucidated by a profound analysis of various genetic markers, including the nuclear ones.

Afronycteris nana (Peters, 1852)

Material (6). 1 ♂, 1 ♀ (NMP 97686, 97687 [S+A]), Kacholola, riverine forest, 15 June 2010, leg. J. Šklíba, V. Mazoch & E. Knotková;
1 ♂ (NMP 97658 [A]), Kasakalawe, Lake Tanganyika Lodge, lake bank, 20 July 2009, leg. V. Mazoch & J. Zima;
1 ♂ (NMP 97611 [S+A]), Livingstone, No Name Camp, 24 May 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera;
1 ♂, 1 ♀ (NMP 97644, 97645 [S+A]), North Luangwa National Park, Chifunda Camp, 12 July 2009, leg. V. Mazoch & J. Zima.

References. Benda et al. (2016), Monadjem et al. (2021), Taylor et al. (2022).

Afronycteris nana is a bat inhabiting most of the savanna habitats of sub-Saharan Africa. In southern Africa it is distributed mostly in its eastern part; the territory of Zambia lies on the southern margin of the continuous African range (Happold 2013d, Monadjem et al. 2020a). Despite this, *A. nana* ranks among the most common and widespread bats of the country, Monadjem et al. (2020a) reported 25 confirmed record localities from Zambia. The new specimens come from four sites spread over the whole country, situated in its northern, southern, western and eastern parts. The dimensions of the NMP specimens of *A. nana* from Zambia are shown in Table 8.

Neoromicia capensis (Smith, 1829)

Material (9). 2 ♂♂ (NMP 97689, 97690 [S+A]), Kacholola, riverine forest, 15 June 2010, leg. J. Šklíba, V. Mazoch & E. Knotková;
1 ♂ (NMP 97618 [S+A]), Kafue National Park, Chunga Camp, 28 May 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera;
2 ♀♀ (NMP 97600, 97601 [S+A]), Sioma Bush Camp, 22 May 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera;
2 ♂♂, 2 ♀♀ (NMP 97607, 97609, 97610 [S+A], 97608 [A]), Sioma Bush Camp, 23 May 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera.

References. Benda et al. (2016), Monadjem et al. (2021), Taylor et al. (2022).

Neoromicia capensis is a common and perhaps the most widespread bat of southern Africa, although in Zambia it is less abundant compared to the more southward situated regions. According to the data by Monadjem et al. (2020a) and regarding the territories of particular countries,

Table 8. Basic biometric data on the NMP specimens of *Afronycteris nana*, *Neoromicia capensis*, *N. somalica* (*Ns*), and *Laephotis angolensis* (*La*) from Zambia. For abbreviations see Methods

dimension	*Afronycteris nana*					*Neoromicia capensis*					*Ns*	*La*
	n	**M**	min	max	SD	*n*	**M**	min	max	SD	97648	97599
G	*6*	**3.58**	3.0	4.0	0.492	*9*	**5.83**	5.0	7.0	0.750	4.0	5.0
LAt	*5*	**30.42**	28.9	33.5	1.862	*9*	**32.28**	31.0	33.1	0.646	30.6	36.0
LCr	*5*	**11.27**	10.98	11.63	0.252	*8*	**13.22**	12.65	13.62	0.344	11.88	14.03
LCb	*5*	**10.76**	10.42	11.22	0.330	*8*	**12.77**	12.21	13.08	0.296	11.47	13.43
LaZ	*5*	**7.21**	7.11	7.53	0.180	*7*	**8.77**	8.61	8.91	0.116	7.57	8.07
LaI	*5*	**3.15**	2.99	3.24	0.100	*8*	**3.39**	3.22	3.54	0.097	2.87	3.28
LaInf	*5*	**3.21**	2.98	3.44	0.200	*8*	**4.29**	4.11	4.64	0.162	3.48	3.93
LaN	*5*	**5.98**	5.79	6.13	0.130	*8*	**6.77**	6.56	6.93	0.122	6.76	6.94
LaM	*5*	**6.62**	6.33	6.83	0.190	*8*	**7.76**	7.58	8.13	0.181	6.76	7.73
ANc	*4*	**4.06**	3.91	4.25	0.148	*8*	**4.37**	4.15	4.51	0.123	4.34	4.43
LBT	*5*	**2.58**	2.51	2.68	0.076	*8*	**3.04**	2.53	3.34	0.257	2.88	3.35
CC	*5*	**3.34**	3.23	3.46	0.093	*8*	**4.25**	4.04	4.49	0.161	3.38	3.93
M^3M^3	*5*	**4.56**	4.38	4.66	0.109	*8*	**5.61**	5.43	5.73	0.108	4.94	5.42
CM^3	*5*	**3.81**	3.66	3.98	0.136	*8*	**4.71**	4.41	4.83	0.132	4.29	4.59
LMd	*5*	**7.82**	7.48	8.13	0.305	*8*	**9.50**	9.19	9.88	0.229	8.48	9.38
ACo	*5*	**2.31**	2.14	2.44	0.130	*8*	**3.21**	3.12	3.41	0.092	2.61	2.88
CM_3	*5*	**4.06**	3.87	4.25	0.166	*8*	**5.09**	4.74	5.26	0.170	4.41	4.96

N. capensis is three times more abundant in Botswana (with 41 record sites) and ten times more frequently recorded in Zimbabwe (92 sites), compared to the evidence from Zambia (18 sites). Nevertheless, in the NMP collection of Zambian bats, *N. capensis* is one of the most numerous bats, nine specimens were collected from three localities in southern Zambia. The dimensions of the NMP specimens of *N. capensis* from Zambia are shown in Table 8.

Neoromicia somalica (Thomas, 1901)

Material (1). 1 ♀ (NMP 97648 [S+A]), Chifunda, Old Luelo Ranger Post, 13 July 2009, leg. V. Mazoch & J. Zima.

The small bat of the genus *Neoromicia* collected at Old Luelo Ranger Post of Chifunda, in the upper Luangwa river basin, eastern Zambia, fits by its body size to the category of medium-sized brown-winged *Neoromicia* bats, traditionally affiliated to *N. somalica* s.l., a bat widely distributed in savannas of sub-Saharan Africa (cf. Peterson et al. 1995, Kearney et al. 2002, Lavrenchenko et al. 2004, Simmons 2005, Benda et al. 2011, 2016). However, several cryptic species were recognised within this morphotype, originally defined only by body and skull size within the genus limits, see the review by Benda et al. (2011). Based on molecular genetic analysis, Monadjem et al. (2021) restricted the distribution of *N. somalica* s.str. to East Africa, with confirmed records spread in a belt of savannas stretching from Somaliland via Kenya to central Tanzania.

The molecular genetic comparison clustered the Zambian specimen (NMP 97648) among the haplotypes of *N. somalica* s.str. sensu Monadjem et al. (2021); the position of this bat was close to the specimens from southern Kenya and central Tanzania, while the samples from western and central Kenya were slightly more distant (Fig. 12). The bat collected in Chifunda thus represents the first record of *N. somalica* from Zambia and from southern and central Africa as well (in the sense of Monadjem et al. 2020a). The closest confirmed locality of *N. somalica* is Maji Moto, Ruaha National Park, Tanzania (08°02'S, 34°30'E), ca. 475 km NNE of Chifunda. The new record from Zambia has thus extended the known distribution range of this species significantly southwards. The dimensions of the NMP specimen of *N. somalica* from Zambia are shown in Table 8.

Laephotis angolensis Monard, 1935

Material (1). 1 ♂ (NMP 97599 [S+A]), Sioma Bush Camp, 22 May 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera.

References. Benda et al. (2016), Monadjem et al. (2021), Taylor et al. (2022).

Although the NMP specimen from Zambia was originally identified as and referred to *Laephotis botswanae* Setzer, 1971 (Benda et al. 2016, Monadjem et al. 2021), here its determination is corrected to *L. angolensis*, in accordance with the conclusions by Taylor et al. (2022). Based on the results of a molecular genetic analysis, the latter authors suggested to consider these names as synonyms. This conclusion is supported by the morphological similarity of bats identified as these two species and difficulties to dinstinguish between them based on external or cranial characters (Kearney & Seamark 2005, Monadjem et al. 2020a, own data).

Of the genus *Laephotis* s.s., only *L. angolensis* was reported from Zambia till present (under *L. botswanae*, see Ansell 1978, Monadjem et al. 2020a) and this species is the most wide-

spread member of the genus in southern and central Africa. The four available Zambian record localities of *L. angolensis* come only from the western part of the country. The locality of the here presented record lies also in this section of the country, although it is the first finding of

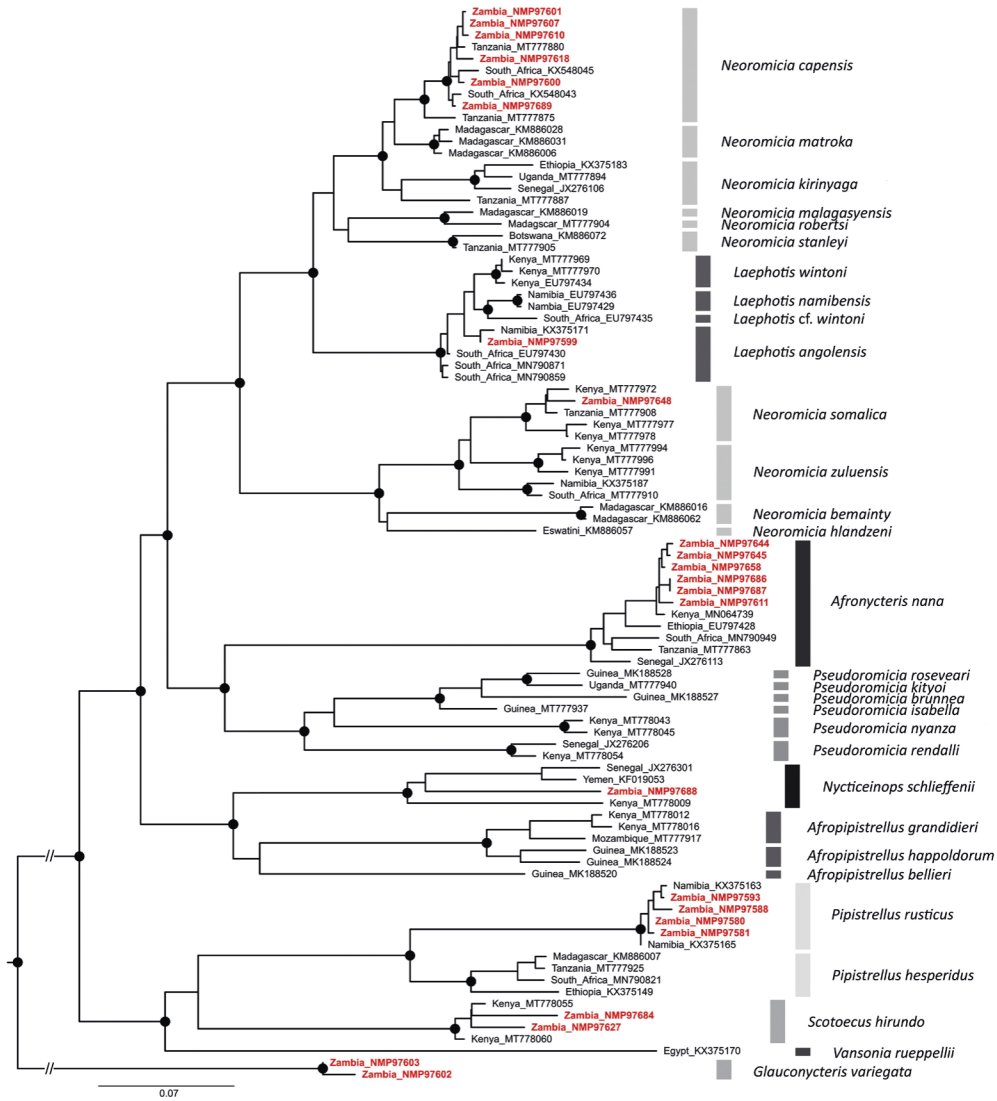

Fig. 12. Maximum likelihood tree of reconstructed phylogenetic relations of African pipistrelloid bats (Vespertilioninae) based on the cytochrome *b* sequences (the Zambian bats in red). Black dots on the nodes denote that these nodes have high branch support (e.g., SH-aLRT ≥80%, aBayes ≥0.95, UFBoot ≥90%).

this bat from southern Zambia. The dimensions of the NMP specimen of *L. angolensis* from Zambia are shown in Table 8.

Nycticeinops schlieffenii (Peters, 1859)

Material (4). 1 ♂ (NMP 97596 [S+A]), Kabula Lodge, shrubland, 20 May 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera;
1 ♀ (NMP 97688 [S+A]), Kacholola, riverine forest, 15 June 2010, leg. J. Šklíba, V. Mazoch & E. Knotková;
2 ♀♀ (NMP 97555, 97556 [S+A]), Kasanka National Park, Luwombwa Camp, 27 November 2018, leg. P. Benda & J. Červený.

Nycticeinops schlieffenii is a bat broadly distributed in dry savannas and arid steppes of sub-Saharan Africa, from the southern Sahara to South Africa (Happold 2013c). In southern and central Africa, it is widespread over central and eastern parts of the region, although in Zambia it is less abundant compared to the more southern regions. According to the data by Monadjem et al. (2020a) and regarding the territories of particular countries, *N. schlieffenii* is almost twice more abundant in Botswana (with 11 record sites) and 13 times more frequently recorded in Zimbabwe (56 sites), compared to the evidence from Zambia (8 sites). The Zambian localities cover mainly areas of the central and northern parts of the country. In the NMP collection of Zambian bats, specimens of this species are available from three localities, including two in southern Zambia. The dimensions of the NMP specimens of *N. schlieffenii* from Zambia are shown in Table 9.

Scotoecus hirundo (de Winton, 1899)

Material (3). 1 ♂, 1 ♀ (NMP 97684, 97685 [S+A]), Kacholola, riverine forest, 15 June 2010, leg. J. Šklíba, V. Mazoch & E. Knotková;
1 ♀ (NMP 97627 [S+A]), Ndola Hill, 7 June 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera.

The taxonomy of the dark-winged species of the genus *Scotoecus* Thomas, 1901 remains unresolved, with existence of one to three species being suggested within this group (Koopman 1993, Simmons 2005). The analyses of geographic variation showed small-sized bats to occur in the savanna belt from Senegal to Ehiopia and Uganda, medium-sized bats in eastern Africa, and large-sized bats in southern Africa, plus marked sexual dimorphism where males are larger than females (Hill 1974, Robbins 1980, Taylor & van der Merwe 1998, Cotterill 2001, own data). These findings can indicate either an existence of more taxa within this group or a cline of increasing body size from north to south within one taxon. Hence, without an employment of molecular genetic methods in a broad geographical scale, this problem will perhaps remain unresolved for good. Therefore, we here temporarily assign the Zambian specimens under a broadly defined taxon *Scotoecus hirundo* s.l., sensu Hayman & Hill (1971), Koopman (1975, 1993), and Happold (2013e).

This form is a widespread but uncommon savanna bat of sub-Saharan Africa (Happold 2013e). Only a limited number of records of *S. hirundo* are available from southern Africa, with most numerous records from Mozambique and Zambia (Cotterill 2001, Monadjem et al. 2020a). In Zambia, the confirmed record localities are situated in the eastern, central, and western parts of the country (Monadjem et al. 2020a, under *S. hindei* / *S. albigula*), which includes the areas where the NMP specimens of this bat also come from (Ndola and Petauke regions). So, the

Table 9. Basic biometric data on the NMP specimens of *Nycticeinops schlieffenii*, *Scotoecus hirundo*, and *Scotophilus leucogaster* from Zambia. For abbreviations see Methods

dimension	*Nycticeinops schlieffenii*					*Scotoecus hirundo*					*Scotophilus leucogaster*	
	n	**M**	min	max	SD	*n*	**M**	min	max	SD	97646	97647
G	*4*	**6.20**	4.5	7.4	1.273	*3*	11.67	10.5	13.0	1.258	15.0	15.0
LAt	*4*	**31.55**	28.7	33.8	2.381	*3*	34.93	33.6	36.9	1.739	45.2	46.3
LCr	*3*	**12.53**	11.87	13.28	0.709	*3*	14.24	13.83	14.85	0.540	16.46	16.47
LCb	*3*	**12.19**	11.54	13.04	0.771	*3*	14.01	13.53	14.66	0.585	15.53	15.75
LaZ	*3*	**8.77**	8.29	9.19	0.452	*3*	10.95	10.62	11.41	0.411	12.37	12.16
LaI	*4*	**3.63**	3.54	3.69	0.070	*3*	4.53	4.36	4.62	0.150	4.44	4.56
LaInf	*4*	**4.14**	3.86	4.34	0.212	*3*	5.61	5.38	5.98	0.326	6.18	5.94
LaN	*4*	**6.52**	6.21	6.85	0.334	*3*	7.84	7.58	8.07	0.246	8.63	8.65
LaM	*4*	**7.20**	7.08	7.44	0.168	*3*	9.55	9.18	10.04	0.444	10.32	10.36
ANc	*3*	**4.52**	4.38	4.65	0.135	*3*	5.39	5.06	5.58	0.289	7.08	6.74
LBT	*4*	**2.82**	2.56	3.05	0.225	*3*	3.43	3.39	3.45	0.032	3.68	3.73
CC	*4*	**3.94**	3.54	4.17	0.276	*3*	5.39	5.21	5.74	0.303	5.96	5.75
M^3M^3	*4*	**5.59**	5.09	6.02	0.397	*3*	7.41	7.33	7.52	0.098	7.88	7.74
CM^3	*4*	**4.62**	4.37	4.83	0.219	*3*	5.81	5.54	6.13	0.299	5.96	5.75
LMd	*4*	**9.22**	8.52	9.71	0.540	*3*	11.27	10.88	11.81	0.484	11.87	12.38
ACo	*4*	**3.11**	2.79	3.45	0.270	*3*	3.52	3.38	3.75	0.203	4.89	5.04
CM_3	*4*	**4.94**	4.63	5.19	0.288	*3*	6.09	5.87	6.47	0.328	6.64	6.66

NMP bats do not represent an important contribution to the distribution picture of *S. hirundo* in Zambia. The dimensions of these specimens are shown in Table 9.

Scotophilus leucogaster (Cretzschmar, 1830)

Material (2). 2 ♂♂ (NMP 97646, 97647 [S+A]), North Luangwa National Park, Chifunda Camp, 12 July 2009, leg. V. Mazoch & J. Zima.

The distribution range of *Scotophilus leucogaster* comprises two separate patches in the Afrotropics, one in the savanna belt stretching from Mauritania to Yemen and Kenya, the other in the central part of southern Africa, between central Angola, central Zambia and southern Mozambique (Van Cakenberghe & Happold 2013). Monadjem et al. (2020a) reported only one confirmed record of this bat from Zambia, from Mfuwe near Kakumbi, in the South Luangwa National Park. The here presented specimens of *S. leucogaster* originate from a locality in the same region of the Luangwa river valley, some 150 km upstream along the river. Currently, this record represents the northernmost confirmed occurrence site in the southern distribution patch of the species in Africa; it is inhabited by the subspecies *S. l. damarensis* Thomas, 1906 (Vallo & Van Cakenberghe 2017). The dimensions of the NMP specimens of *S. leucogaster* from Zambia are shown in Table 9.

Scotophilus viridis (Peters, 1852)

Material (5). 2 ♂♂, 2 ♀♀ (NMP 97679–97682 [S+A]), Kacholola, riverine forest, 15 June 2010, leg. J. Šklíba, V. Mazoch & E. Knotková;

Table 10. Basic biometric data on the NMP specimens of *Scotophilus viridis*, *S. dinganii*, *Miniopterus natalensis* (*Mn*), and *M. mossambicus* from Zambia. For abbreviations see Methods

dimension	*Scotophilus viridis*					*Scotophilus dinganii*		*Mn*	*Miniopterus mossambicus*				
	n	**M**	min	max	SD	97624	97625	97649	*n*	**M**	min	max	SD
G	*5*	**18.30**	16.5	21.0	1.891	36.0	36.0	10.0	*11*	**9.23**	8.0	10.9	0.771
LAt	*5*	**48.38**	46.7	50.1	1.268	58.9	58.8	45.2	*11*	**44.48**	43.5	45.5	0.704
LCr	*5*	**17.51**	17.02	17.98	0.345	21.75	21.64	14.88	*10*	**14.99**	14.68	15.16	0.142
LCb	*5*	**16.54**	15.98	16.83	0.338	20.10	20.14	14.42	*10*	**14.45**	14.31	14.68	0.114
LaZ	*5*	**12.71**	12.49	12.98	0.190	15.01	15.06	8.44	*10*	**8.26**	8.11	8.48	0.142
LaI	*5*	**4.50**	4.32	4.68	0.162	5.13	5.18	3.68	*10*	**3.62**	3.51	3.78	0.109
LaInf	*5*	**6.19**	6.01	6.35	0.132	7.75	7.97	3.93	*10*	**3.73**	3.58	3.93	0.096
LaN	*5*	**8.89**	8.74	9.07	0.138	10.61	9.90	7.88	*10*	**7.71**	7.48	7.92	0.147
LaM	*5*	**10.83**	10.62	10.94	0.138	13.19	12.63	8.54	*10*	**8.37**	8.26	8.52	0.095
ANc	*5*	**7.03**	6.68	7.33	0.234	8.88	8.62	6.27	*10*	**6.08**	5.93	6.27	0.107
LBT	*5*	**3.73**	3.64	3.92	0.114	4.69	4.63	2.93	*10*	**2.97**	2.82	3.18	0.101
CC	*5*	**5.98**	5.87	6.11	0.086	7.74	7.48	4.38	*10*	**4.28**	3.98	4.44	0.126
M^3M^3	*5*	**8.26**	8.13	8.43	0.122	9.88	9.38	6.14	*10*	**6.14**	5.67	6.33	0.186
CM^3	*5*	**6.21**	6.11	6.28	0.073	7.29	7.54	5.66	*10*	**5.75**	5.67	5.93	0.078
LMd	*5*	**12.90**	12.71	13.06	0.159	15.59	16.06	10.34	*10*	**10.44**	10.32	10.66	0.128
ACo	*5*	**4.94**	4.68	5.16	0.189	6.53	6.14	2.63	*10*	**2.46**	2.28	2.61	0.096
CM_3	*5*	**6.96**	6.92	7.02	0.043	8.24	8.58	6.01	*10*	**6.09**	6.02	6.28	0.078

1 ♀ (NMP 97604 [S+A]), Sioma Bush Camp, 23 May 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera.

Scotophilus viridis s.str. is nearly endemic to southern Africa (Vallo & Van Cakenberghe 2017), it occurs mainly in the eastern part of the region and the north-western margin of its distribution range stretches through Zambia (Monadjem et al. 2020a). The NMP specimens of this bat come from southern Zambia, from the area of known distribution of *S. viridis*; however, the record from the Sioma Bush Camp creates a new marginal point of the species' western distribution border in south-western Zambia. The dimensions of the NMP specimens of *S. viridis* from Zambia are shown in Table 10.

Scotophilus dinganii (Smith, 1833)

Material (2). 1 ♂, 1 ♀ (NMP 97624, 97625 [S+A]), Ndola Hill, 7 June 2009, leg. J. Šklíba, M. Lövy, V. Mazoch & R. Šumbera.

Scotophilus dinganii is a bat distributed abundantly in southern and eastern Africa (Vallo & Van Cakenberghe 2017), in southern Africa it is the most widespread species of the genus (Monadjem et al. 2020a). This is also true for Zambia, where 13 confirmed sites of occurrence are known from all parts of the country (Ansell 1978, Monadjem et al. 2020a). The NMP specimens thus do not improve its distribution picture. The dimensions of the NMP specimens of *S. dinganii* from Zambia are shown in Table 10.

Miniopteridae

***Miniopterus natalensis* (Smith, 1833)**

Material (1). 1 ♂ (NMP 97649 [S+A]), Kalambo Falls, camp above river, 18 July 2009, leg. V. Mazoch & J. Zima.

The distribution range of *Miniopterus natalensis* s.str. is only imperfectly defined; according to Monadjem et al. (2020a), this bat occurs mainly in savannas of the temperate zone of southern Africa. Confirmed records of *M. natalensis* s.str. come from the countries southwards and westwards of Zambia, while in Zambia, Mozambique and the DR Congo, this species still awaits confirmation of its occurrence. Thus, the here presented bat from the Kalambo Falls in northern Zambia represents the first specimen of *M. natalensis* s.str. confirmed by the molecular genetic methods from the country and one of the northernmost known records. The dimensions of the NMP specimen of this species from Zambia are shown in Table 10.

***Miniopterus mossambicus* Monadjem, Goodman, Stanley et Appleton, 2013**

Material (11). 2 ♂♂, 3 ♀♀ (NMP 97574–97577 [S+A], 97573 [A]; Fig. 13), Chisakila, Bwarenunka Cave, 1 December 2018, leg. P. Benda & J. Červený;
3 ♂♂, 3 ♀♀ (NMP 97674–97678, 97683 [S+A]), Kacholola, riverine forest, 15 June 2010, leg. J. Šklíba, V. Mazoch & E. Knotková.

Fig. 13. A male of *Miniopterus mossambicus* collected from the Bwarenunka Cave near Chisakila on 1 December 2018.

The known distribution range of recently described *Miniopterus mossambicus* stretches discontinuously across the savanna belt of eastern and south-eastern Africa; it covers a triangular area in the eastern part of southern Africa (Monadjem et al. 2020a), and recently this bat has been discovered in central Tanzania and southern Kenya (Demos et al. 2020). Besides Mozambique that gave the name to the bat, the confirmed records of *M. mossambicus* from southern Africa are known only from southern Zambia. Two record sites are available from the latter country, the Leopards Hill Cave near Lusaka (15°36'S, 28°43'E; Miller-Butterworth et al. 2005, Monadjem et al. 2013a) and Old Mine at Missale (14°07'S, 32°52'E; Monadjem et al. 2013a, 2020b). The NMP specimens of *M. mossambicus* originate from southern Zambia, from the area geographically bordered by the previous two sites. This pattern suggests the distribution that follows the Zambezi watercourse from lowlands of Mozambique to the inland uplands of Zambia and Zimbabwe. A colony of this species composed of ca. 2000 bats of both sexes was discovered in the Bwarenunka Cave near Chisakila on 1 December; all examined females were in the lactation stage.

So, although *M. mossambicus* is a rare bat in collections (cf. Monadjem et al. 2020a), the new NMP samples do not contribute significantly to its distribution picture. However, the eleven NMP specimens identified with help of genetic analysis can be useful for the description of metric traits, until now defined based on a small number of samples of which only several were diagnosed genetically (Monadjem et al. 2013a, 2020b). The dimensions of the NMP specimens of *M. mossambicus* from Zambia are shown in Table 10. The values of the dimensions are slightly larger than those given by Monadjem et al. (2013a, 2020b), both external and cranial, of the latters, both lengths and widths (LAt 41.0–44.9 mm, mean [M] 43.9 mm; LCr 14.38–15.20 mm, M 14.71 mm; LaZ 7.85–8.40 mm, M 8.06 mm; CM^3 5.27–5.87 mm, M 5.52 mm; Monadjem et al. 2020a, b). These differences could indicate a cline shift in body and skull size in this bat along the geographic and/or climatic gradient from east to west, from warm lowlands to continental uplands.

CONCLUSIONS

The NMP collection contains 139 specimens of bats from Zambia belonging to 32 species of eight families (Table 11). These bats originate from 29 localities covering the whole territory of the country (Fig. 1), with a frequency 1–8 species per locality, on average 2.5 species and 4.8 specimens per locality. Particular species originate from 1–17 localities, together representing 73 records (species vs. locality), on average 2.3 records per species, and the species are represented by 1–31 specimens, on average 4.3 specimens per species. Most of the specimens belong to common species, which could be frequently found in other collections containing material from Zambia and the broader region of south-central Africa (Monadjem et al. 2020a). However, some of the species series have an undoubted value for zoological research. Generally, the collection as a whole contributes significantly to the description of both distribution and physical traits of the bat fauna of Zambia.

According to the review by Monadjem et al. (2020a), the bat fauna of Zambia is composed of 73 species (Appendix 1, Table 11; or 74 species, when *Miniopterus* cf. *natalensis* is included) belonging to ten families; of these, 29 species are housed in the NMP collection, making up 39.7% of the known fauna. The evaluation of the NMP collection brought confirmation of two more species for the Zambian fauna, *Afropipistrellus grandidieri* and *Neoromicia somalica*, plus confirmation of the occurrence of *Miniopterus natalensis* s.str. in the country based on

molecular genetic evidence. The bat fauna of Zambia now comprises 76 species in total, 42.1% of them are housed in the NMP collection. One species, *Neoromicia somalica*, is now confirmed as a new bat also for the whole region of southern and central Africa as defined geographically by Monadjem et al. (2020a).

Until now, *Rhinolophus sakejiensis* has been known only from the type series, composed of three bats collected in north-western Zambia in 1990; the NMP collection contains a new specimen of this bat, first documented after the species description. The NMP collection includes also four specimens of *Chaerephon bivittatus*, representing the second record of this bat from Zambia.

The record localities of the NMP specimens of *Epomophorus labiatus*, *Rhinolophus mossambicus*, and *Neoromicia somalica* changed the known distribution ranges of these bats as a whole, not only in Zambia. The genetic analysis revealed a new distribution extension of several mitochondrial lineages of the otherwise common species, like *Hipposideros caffer* (A1 lineage) and *Nycteris thebaica* (clade *thebaica* 4), to the territory of Zambia; the genetic analysis also confirmed the occurrence of *Miniopterus natalensis* s.str. in Zambia. In several species, the NMP specimens represent new marginal records, making the distribution ranges more precise, viz. *Epomophorus dobsonii*, *Nyctinomus aegyptiacus*, *Glauconycteris variegata*, *Pipistrellus rusticus*, *Scotophilus leucogaster*, and *S. viridis*.

In two species, *Rhinolophus mossambicus* and *Miniopterus mossambicus*, the basic morphometric comparison suggests an increase of body size along a gradient from south-eastern African lowlands towards central African uplands resembling a cline shift in metric traits according to Bergmann's rule (although not in the north-south direction). However, this brief observation needs further studies based on examinations of more extensive materials.

In summary, the small collection of bats from Zambia, created in a relatively short time between 2009 and 2018, represents a valuable series of specimens, providing an important addition to the knowledge of composition, distribution and morphometry of the bat fauna of the country.

Table 11. Composition of the bat fauna of Zambia according to Monadjem et al. (2020a) [M20] and the composition of the NMP bat collection from Zambia (record = species vs. locality); the new species for the Zambian fauna are typed in **bold**

family	species fauna M20	species NMP	specimens NMP	records NMP
Pteropodidae	11	4	47	27
Rhinolophidae	9	4	7	6
Hipposideridae	3	2	4	2
Rhinonycteridae	2	1	5	1
Megadermatidae	1	0	0	0
Emballonuridae	2	0	0	0
Nycteridae	6	1	3	2
Molossidae	13	5	16	7
Vespertilionidae	24	11+**2**	45	25
Miniopteridae	2	1+**1**	12	3
total	73	29+**3**	139	73

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APPENDIX 1

Composition of the bat fauna of Zambia, as summarised by Monadjem et al. (2020a); in brackets are the numbers of records based on museum specimens

Pteropodidae: *Eidolon helvum* [11], *Rousettus aegyptiacus* [5], *Myonycteris angolensis* [2], *M. torquata* [1], *Epomophorus crypturus* [26], *E. labiatus* [11], *E. wahlbergi* [14], *E. dobsonii* [12], *Epomops franqueti* [1], *Micropteropus pusillus* [1], *Plerotes anchietae* [2];
Rhinolophidae: *Rhinolophus clivosus* [7], *R. sakejiensis* [1], *R. darlingi* [2], *R. fumigatus* [6], *R. mossambicus* [15], *R. rhodesiae* [4], *R. lobatus* [4], *R. simulator* [7], *R. blasii* [3];
Hipposideridae: *Hipposideros caffer* [30], *H. ruber* [4], *Macronycteris vittata* [9];
Rhinonycteridae: *Triaenops afer* [1], *Cloeotis percivali* [4];
Megadermatidae: *Lavia frons* [4];
Emballonuridae: *Taphozous perforatus* [1], *T. mauritianus* [11];
Nycteridae: *Nycteris thebaica* [34], *N. major* [1], *N. macrotis* [10], *N. grandis* [4], *N. woodi* [7], *N. hispida* [13];
Molossidae: *Chaerephon pumilus* [16], *C. major* [1], *C. chapini* [4], *C. ansorgei* [1], *C. bivittatus* [1], *C. nigeriae* [8], *Mops midas* [4], *M. condylurus* [7], *Mops niveiventer* [12], *Otomops martiensseni* [1], *Nyctinomus aegyptiacus* [3], *Tadarida fulminans* [2], *T. ventralis* [1];
Vespertilionidae: *Myotis tricolor* [1], *M. welwitschii* [3], *M. bocagii* [2], *Kerivoula lanosa* [3], *K. argentata* [7], *Eptesicus hottentotus* [2], *Glauconycteris variegata* [8], *Pipistrellus hesperidus* [7], *P. rusticus* [8], *Vansonia rueppellii* [5], *Afronycteris nana* [25], *Neoromicia zuluensis* [9], *N. capensis* [17], *N. stanleyi* [6], *N. anchietae* [11], *Laephotis angolensis* [4], *Pseudoromicia rendallii* [2], *Nycticeinops schlieffenii* [8], *Scotoecus hirundo* s.l. [7], *S. albofuscus* [1], *Mimetillus thomasi* [4], *Scotophilus leucogaster* [1], *S. viridis* [4], *S. dinganii* [13];
Miniopteridae: *Miniopterus inflatus* [1], *M. mossambicus* [1].

Annex

These studies were published or submitted during the PhD study and are not part of the PhD thesis:

Šmíd, J., Göçmen, B., Crochet, P. A., Trape, J. F., Mazuch, T., **Uvizl, M.**, & Nagy, Z. T. (2019). Ancient diversification, biogeography, and the role of climatic niche evolution in the Old World cat snakes (Colubridae, *Telescopus*). *Molecular Phylogenetics and Evolution*, 134, 35-49. https://doi.org/10.1016/j.ympev.2019.01.015

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