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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

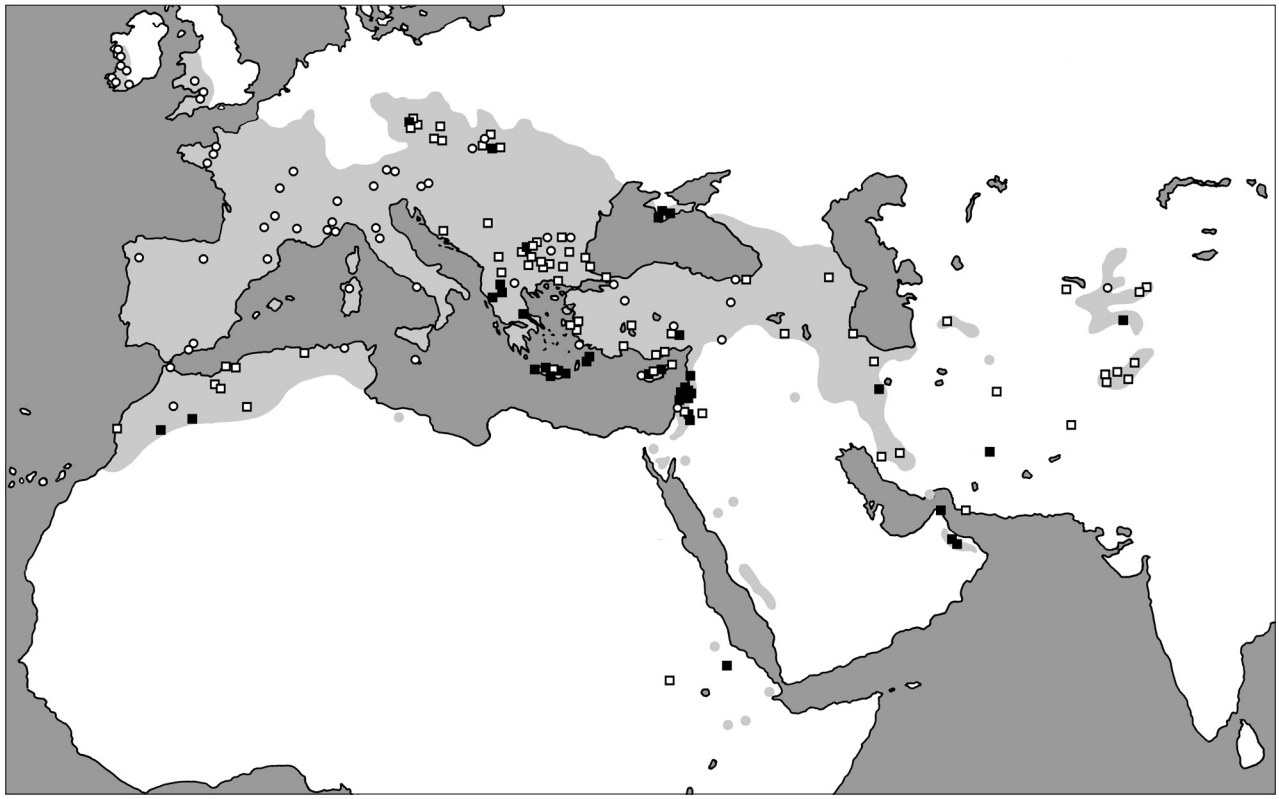

FIG. 1. Map of the distribution range of the *Rhinolophus hipposideros* group (pale grey; after numerous sources) and the localities of origin and grouping of the examined samples (some symbols can denote more sites); full squares indicate the samples examined in both molecular genetic and morphological 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

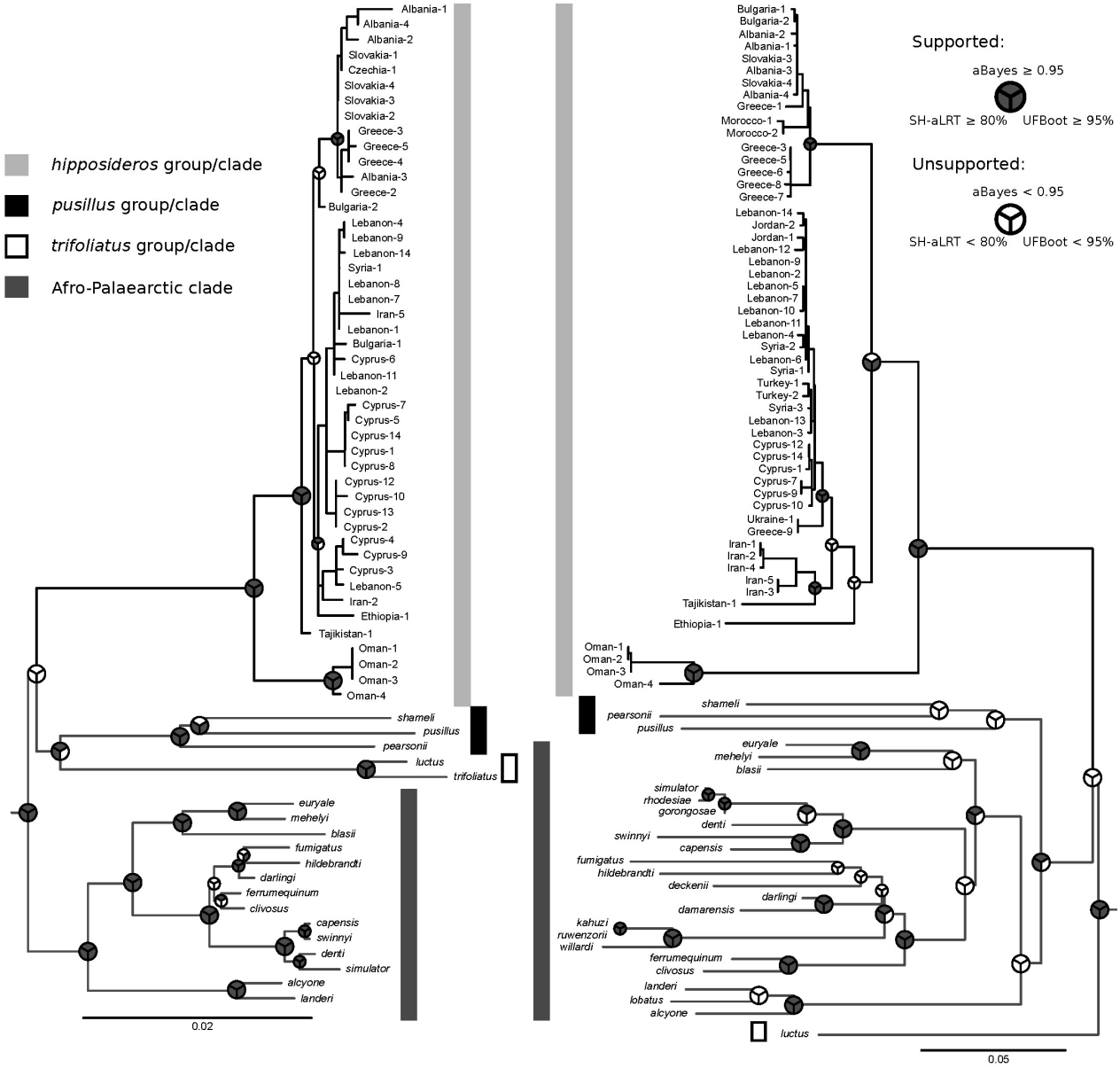

FIG. 2. Maximum likelihood tree of reconstructed phylogenetic relationships of the *Rhinolophus hipposideros* group with selected species of the families Rhinolophidae and Hipposideridae based on the nuclear (left) and mitochondrial (right) datasets, respectively. Branch support values are shown by pie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

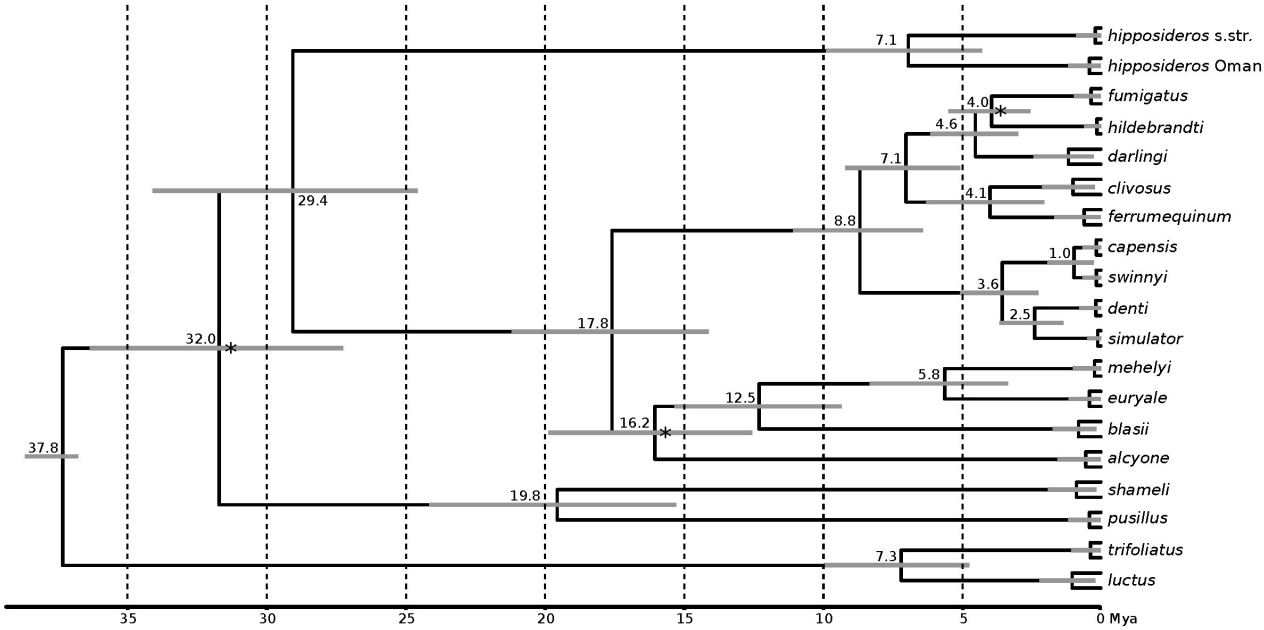

FIG. 3. Chronogram of the family Rhinolophidae based on a Bayesian inference of the nuclear dataset (according to the model by Stoffberg *et al*., 2010). The numbers at nodes show mean divergence time estimates (Ma) and horizontal boxes 95% highest posterior density intervals of these estimates. The 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	x̄	min	max	SD	*n*	x̄	min	max	SD	*n*	x̄	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 (x̄ LAt > 39 mm) from Central Europe and Central Asia, and the small bats (x̄ 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 (x̄ LCc > 14.0 mm) was found in the bats from Central Europe; a small skull (x̄ LCc < 13.5 mm) was seen in the samples from the East Mediterranean, Oman, and north-eastern Africa; and a medium-sized skull (x̄ 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 (x̄ LaZ > 7.4 mm; x̄ LaZ/LCc > 0.545) was observed in the bats from Central Europe, West Mediterranean, and Central Asia, whereas an absolutely and relatively narrow skull (x̄ LaZ < 7.4 mm; x̄ LaZ/LCc < 0.545) was seen in the bats from Oman and an absolutely narrow but relatively wide skull (x̄ LaZ < 7.4 mm; x̄ LaZ/LCc > 0.545) was found in the bats from the East Mediterranean and north-eastern Africa.

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

x̄ 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 (x̄ ANc > 4.5 mm; x̄ Nc/LCc > 0.335), and the bats from north-eastern Africa and Oman had a low braincase (x̄ ANc < 4.5 mm; x̄ 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 (x̄ CM^3 > 5.4 mm; x̄ CM^3/LCc > 0.395) in the bats from Central Asia and Oman (Fig. 5); an absolutely long but relatively short rostrum (x̄ CM^3 > 5.4 mm; x̄ 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, whereas an absolutely short but relatively long rostrum ($\bar{x}$ $CM^3 < 5.4$ mm; $\bar{x}$ $CM^3/LCc > 0.385$) was found in the bats from the Mediterranean and north-eastern Africa. A relatively narrow rostrum ($\bar{x}$ LaInf/LCc < 0.262) was found in the bats from Central Europe and the Mediterranean; a relatively very wide rostrum ($\bar{x}$ LaInf/LCc > 0.264) was observed in the bats from Oman; and a relatively medium-sized rostrum width ($\bar{x}$ 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; $\bar{x}$ LaCs

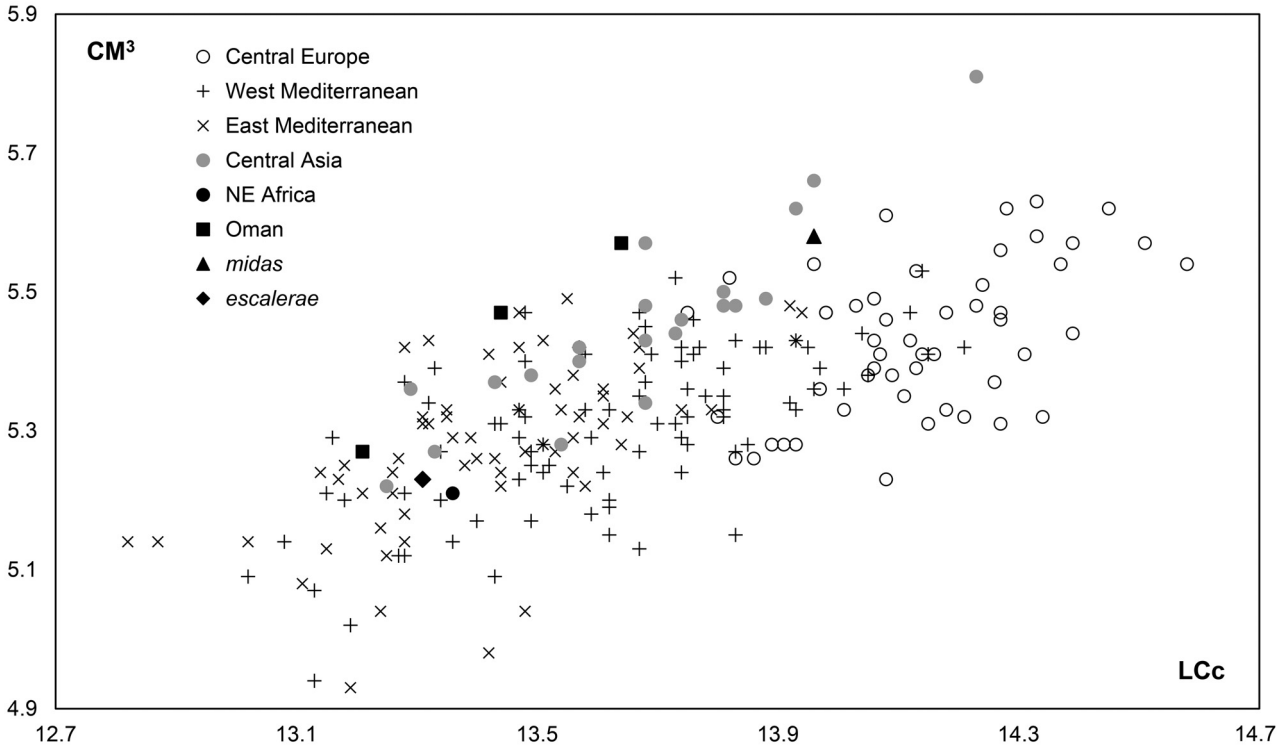

FIG. 4. Bivariate plot of skull dimensions of the examined samples of the *Rhinolophus hipposideros* group: condylocanine length of skull (LCc) against length of the upper tooth-row (CM^3); values in mm

> 0.90 mm) were observed in the bats from Oman, the smallest ($\bar{x}$ LaCs < 0.81 mm) were seen in the bats from the West Mediterranean and north-eastern Africa, and the upper canines ($\bar{x}$ 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; $\bar{x}$ $LP^2 \times LaP^2$ > 0.25 mm^2) in the bats from Central Europe, small ($\bar{x}$ LP^2 < 0.42 mm; $\bar{x}$ $LP^2 \times LaP^2$ < 0.20 mm^2) in the bats from Oman, and medium-sized ($\bar{x}$ LP^2 0.46–0.50 mm; $\bar{x}$ $LP^2 \times LaP^2$ 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 ($\bar{x}$ LP^41 > 0.98 mm) in the bats from Central Europe and Oman, small ($\bar{x}$ LP^41 < 0.95 mm) in the bats from the East Mediterranean and north-eastern Africa, and medium-sized ($\bar{x}$ LP^41 0.95–0.98 mm) in the bats from the West Mediterranean and Central Asia; P^4 was relatively wide ($\bar{x}$ LaP^4/LP^41 > 1.6) in the bats from the East Mediterranean and Central Asia, and relatively narrow ($\bar{x}$ 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; $\bar{x}$ LP^42/LaP^4 > 0.36) in the bats from north-eastern Africa, short ($\bar{x}$ LP^42/LaP^4 < 0.34) in the bats from the East Mediterranean and Central Asia, and medium length ($\bar{x}$ 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 ($\bar{x}$ LM^1 > 1.4 mm; $\bar{x}$ $LM^1 \times LaM^1$ > 2.7 mm^2) in the bats from Central Europe and Central Asia, small ($\bar{x}$ LM^1 < 1.3 mm; $\bar{x}$ $LM^1 \times LaM^1$ < 2.5 mm^2) in the bats from north-eastern Africa, and medium-sized ($\bar{x}$ LM^1 1.35–1.38 mm; $\bar{x}$ $LM^1 \times LaM^1$ 2.5–2.7 mm^2) in the bats from the Mediterranean and Oman; M^1 was relatively wide ($\bar{x}$ LaM^1/LM^1 > 1.4) in the bats from Central Europe, Central Asia, and north-eastern Africa, relatively narrow ($\bar{x}$ LaM^1/LM^1 1.36) in the samples from Oman, and medium width ($\bar{x}$ LaM^1/LM^1 1.39–1.40) in the bats from the Mediterranean. The third upper molar (M^3) was large ($\bar{x}$ LM^3 > 1.1 mm; $\bar{x}$ $LM^3 \times LaM^3$ > 1.5 mm^2) in the bats from Central Asia and Oman and small ($\bar{x}$ LM^3 < 1.1 mm; $\bar{x}$ $LM^3 \times LaM^3$ < 1.5 mm^2) in the bats from Central Europe, the Mediterranean, and north-eastern Africa; M^3 was relatively wide ($\bar{x}$ LaM^3/LM^3 > 1.4) in the bats from Central Europe, relatively narrow ($\bar{x}$ LaM^3/LM^3 <1.3) in the samples from the West Mediterranean, Central Asia, and Oman, and medium width ($\bar{x}$ LaM^3/LM^3 1.3–1.4) in the bats from the East Mediterranean and

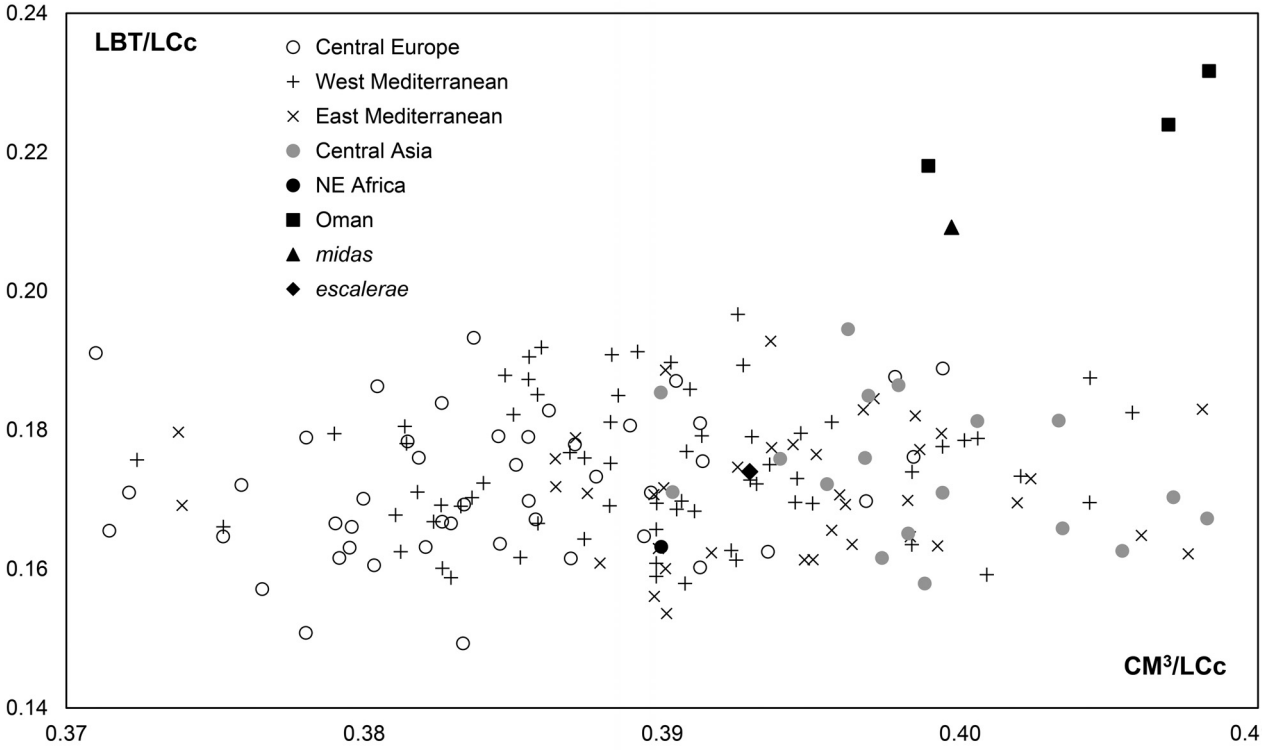

FIG. 5. Bivariate plot of skull dimensions of the examined samples of the *Rhinolophus hipposideros* group: relative length of rostrum (CM^3/LCc) against relative horizontal length of tympanic bulla (LBT/LCc)

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

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

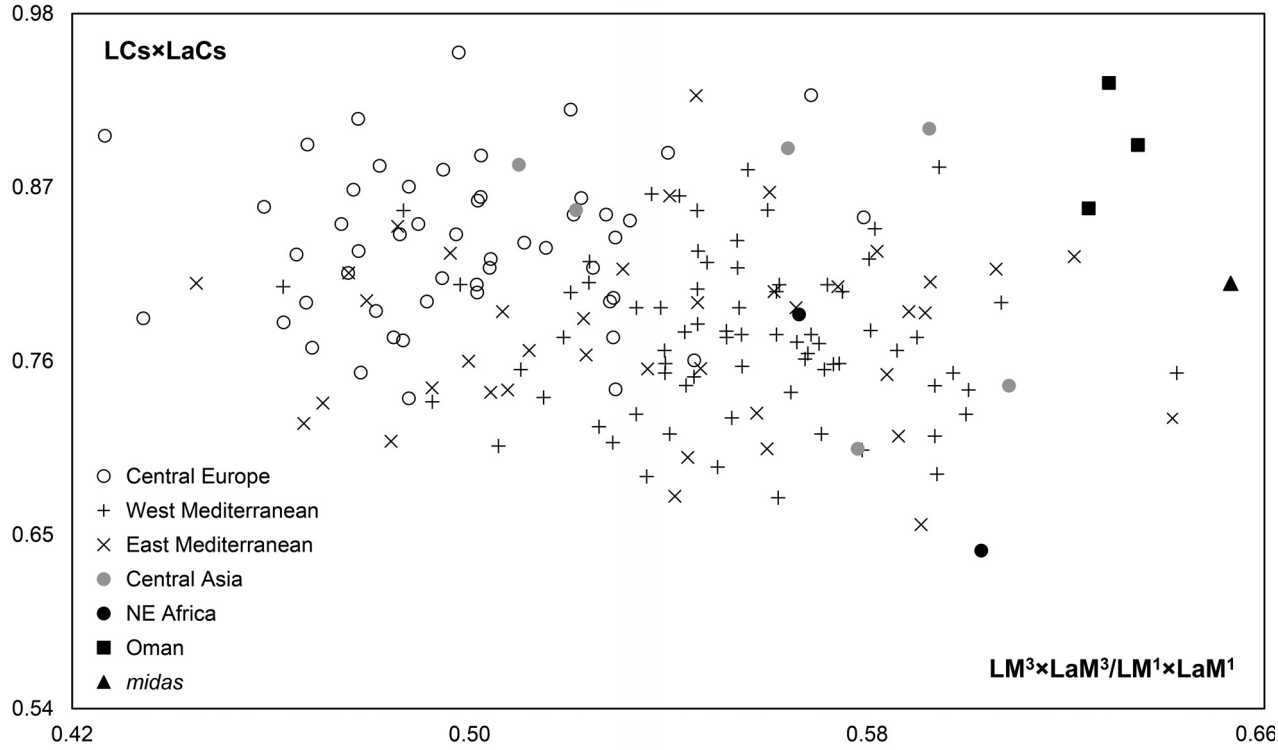

FIG. 6. Bivariate plot of tooth dimensions of the examined samples of the *Rhinolophus hipposideros* group: relative crown size of third upper molar ($LM^3 \times LaM^3/LM^1 \times LaM^1$) against crown size of upper canine (LCs×LaCs)

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

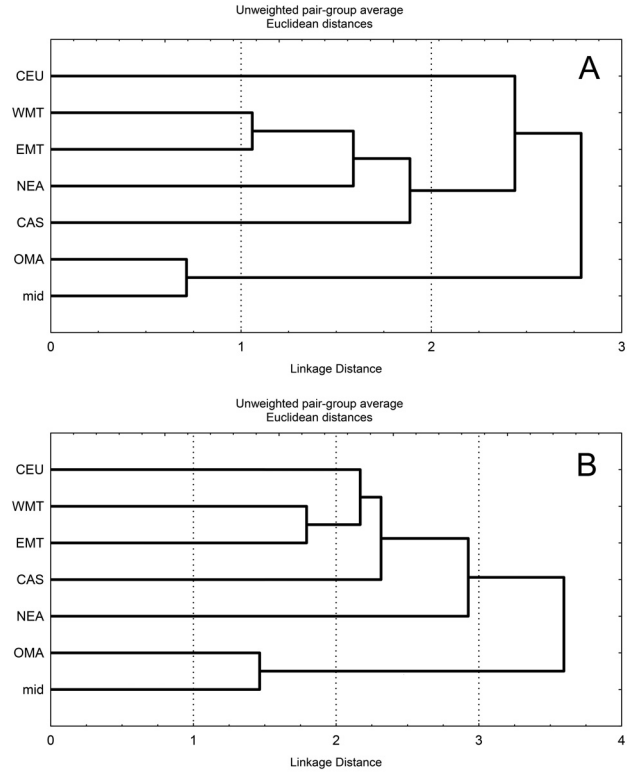

FIG. 7. Results of the cluster analysis (UPGMA): differences between mean values of morphometric traits among the particular sets of samples of the *Rhinolophus hipposideros* group calculated from 27 plain and relative dimensions of skull (A), and from 36 plain and relative dimensions of teeth (B). Samples 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 the *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 = interpulse 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).

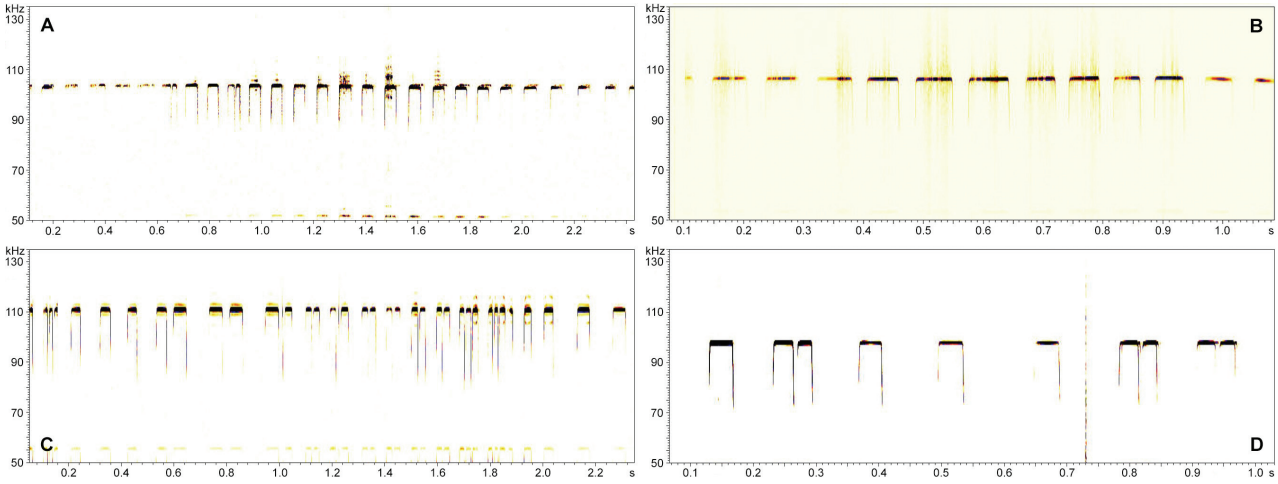

FIG. 8. Spectrograms of echolocation call examples of the *Rhinolophus hipposideros* group (original data); A — an individual recorded inside the Aksamitka Cave, Slovakia; B — an individual foraging at Arjank, Iran (cf. Benda *et al*., 2012); C — a handled individual recorded at Zingrogh, Tajikistan; 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 ($\bar{x}$ = 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 ($\bar{x}$ = 18.5 mm), and in *R. hipposideros* s.str. from Iran it was 15.8–18.6 mm ($\bar{x}$ = 17.1 mm — Benda *et al*., 2012), and in the bats from Lebanon 14.2–18.8 mm ($\bar{x}$ = 17.4 mm — Benda *et al*., 2016), whereas the forearm length in *R. midas* was 36.8–38.1 mm ($\bar{x}$ = 37.4 mm), and in *R. hipposideros* s.str., it was 37.7–40.9 mm ($\bar{x}$ = 39.0 mm) from Iran and 35.3–39.4 mm ($\bar{x}$ = 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

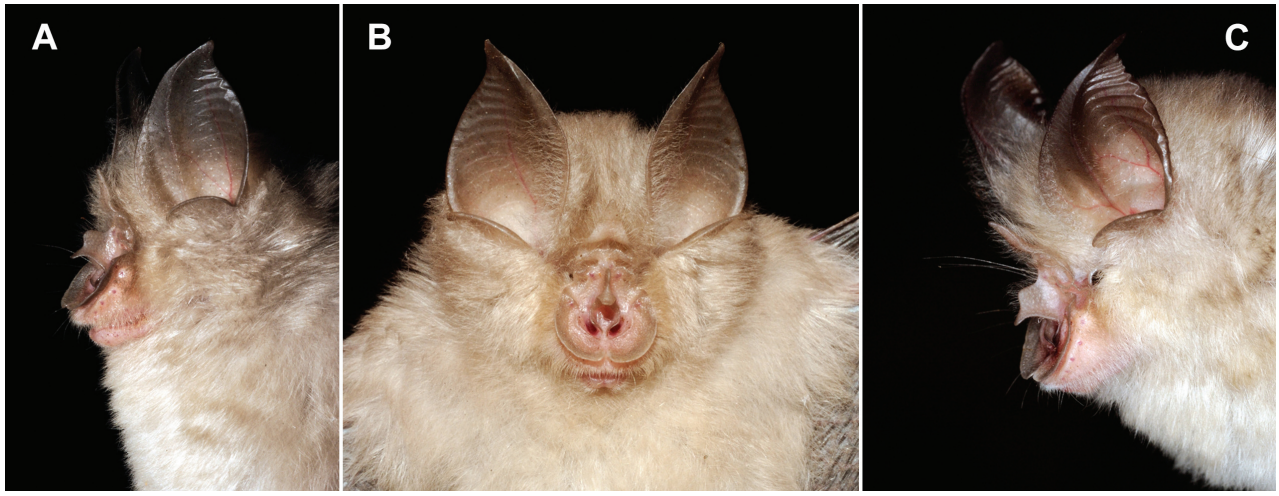

FIG. 9. Portraits of *Rhinolophus midas* Andersen, 1905 from Oman; A, B — ♂ (NMP 93782), Misfah, Ad Dakhiliyah Province, 9 April 2011, lateral and frontal views; C — ♀ (NMP 93994), Sal Alah, Masandam Province, 13 March 2012, lateral view. Photos by A. Reiter

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 southwestern 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 northwest 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 ($PP \geq 0.95$); 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 1,103 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. Supplementary Information is available exclusively on BioOne.

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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,

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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.