Acta Chiropterologica, 13(2): 245–270, 2011
PL ISSN 1508-1109 © Museum and Institute of Zoology PAS
doi: 10.3161/150811011X624749

Taxonomic revision of the genus *Asellia* (Chiroptera: Hipposideridae) with a description of a new species from southern Arabia

PETR BENDA[1, 2, 5], PETER VALLO[3], and ANTONÍN REITER[4, 2]

[1]*Department of Zoology, National Museum (Natural History), Václavské nám. 68, CZ–115 79 Praha 1, Czech Republic*
[2]*Department of Zoology, Faculty of Science, Charles University, Viničná 7, CZ–128 44 Praha 2, Czech Republic*
[3]*Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, v.v.i., Květná 8, CZ–603 65 Brno, Czech Republic*
[4]*South Moravian Museum in Znojmo, Přemyslovců 8, CZ–669 45 Znojmo, Czech Republic*
[5]*Corresponding author: E-mail: petr_benda@nm.cz*

Two species are currently recognised within the genus *Asellia*, a typical inhabitant of arid areas of northern Africa and south-western Asia. Most of the distribution range of the genus is covered by *Asellia tridens*, while the other species, *A. patrizii*, is restricted to Ethiopia, Eritrea and several Red Sea islands. We analysed the morphological variation in an extensive set of *Asellia* samples covering the range of the genus, including most of the available type material. In a representative subset of samples, we employed molecular genetic analysis to infer the phylogenetic relationships within the broadly distributed *A. tridens*. Morphological comparisons revealed four distinct morphotypes. Except for the endemic *A. patrizii*, almost all African *Asellia* were found to belong to the same morphotype as most of the Middle Eastern specimens. This morphotype was unambiguously identified as *A. tridens*. Two other morphotypes of tentative *A. tridens* were further recognised based on skull shape differences; one in the southern Arabian region of Dhofar, the other in Socotra and Somalia. Phylogenetic analysis of complete sequences of the mitochondrial cytochrome *b* gene yielded three main monophyletic groups, which corresponded to the morphotypes revealed for *A. tridens*. Significant genetic divergences reaching over 5% and 12%, respectively, were discovered between them. Based on the morphological and molecular data obtained, we propose a split of the current *A. tridens* into three separate species: *A. tridens* in northern Africa and most of the Middle East, *A. italosomalica* in Socotra and Somalia, and *Asellia* sp. nov. in southern Arabia. Molecular dating, along with the available paleontological information and geological history of the Arabian Peninsula, supports an Arabian origin of the contemporary *Asellia*. While profound divergence of the Socotran form may be linked to the split of Socotra from the southern Arabian coast in the Middle Miocene, the low sequence variation of *Asellia* in most of Africa and the Middle East suggests a relatively recent colonisation of this vast area during the Pleistocene. The newly described form from southern Arabia most likely represents a relic of aridisation during the Miocene–Pliocene transition.

Key words: *Asellia*, morphology, morphometry, mtDNA, taxonomy, phylogeny

INTRODUCTION

The hipposiderid genus *Asellia* Gray, 1838, or the trident leaf-nosed bats, most typically have three short, pointed processes on the posterior margin of their nose-leafs with two lateral leaflets on each side, rather large ears, and a high sagittal crest on the frontal part of their skulls (Tate, 1941; Koopman, 1994). This genus is a Saharo-Sindian faunal element that inhabits arid and semi-arid areas of northern and north-eastern Africa and south-western Asia (Fig. 1; cf. Koopman, 1994; Simmons, 2005). In Africa, it occurs from Morocco and Senegal to Egypt and Somalia; its Asian range comprises almost the whole Middle East from Syria and Yemen in the west to south-western Afghanistan and western Pakistan in the east (Kock, 1969; Hayman and Hill, 1971; DeBlase, 1980; Owen and Qumsiyeh, 1987; Bates and Harrison, 1997; Horáček *et al*., 2000).

Two species are currently recognised within the genus (Koopman, 1993, 1994; Borisenko and Pavlinov, 1995; Simmons, 2005): large-sized *Asellia tridens* (Geoffroy, 1813) and small-sized *A. patrizii* De Beaux, 1931. While *A. patrizii* is considered to be monotypic and its occurrence is restricted to Ethiopia, Eritrea and several Red Sea islands, *A. tridens* is broadly distributed throughout the range of the genus and well-pronounced morphological variation in this large geographical area has been reported (Koopman, 1994; Simmons, 2005). Up to five subspecies have been regarded as being within the

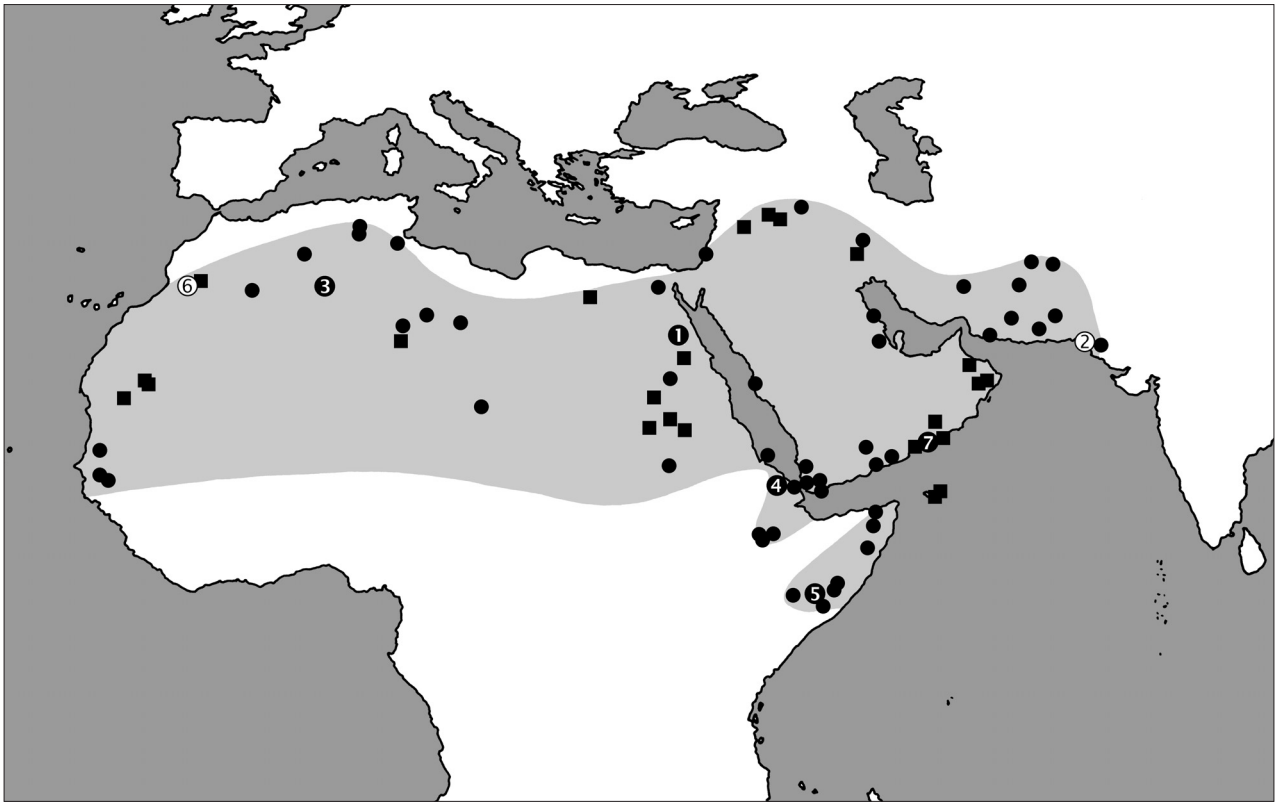

FIG. 1. Map of the approximate distribution of the genus *Asellia* (after Kock, 1969; Largen *et al*., 1974; Koch-Weser, 1984; Harrison and Bates, 1991; Bates and Harrison, 1997) with the sampling sites denoted. The squares show for morphological and genetic samples and the dots show morphological samples only. Circles with numbers show type localities for the described forms of the genus *Asellia* (white numbers in black circles = type material examined). Legend: 1 — *tridens* Geoffroy, 1813; 2 — *murraiana* Anderson, 1881; 3 — *diluta* Andersen, 1918; 4 — *patrizii* De Beaux, 1931; 5 — *italosomalica* De Beaux, 1931; 6 — *pallida* Laurent, 1937; 7 — *Asellia* sp. nov.

latter species (Ellerman and Morrison-Scott, 1951; Harrison, 1957; Kock, 1969; Hayman and Hill, 1971; Koopman, 1975, 1994; Corbet, 1978; Owen and Qumsiyeh, 1987; Kowalski and Rzebik-Kowalska, 1991; Horáček *et al*., 2000; Simmons, 2005): *A. t. tridens* (Geoffroy, 1813) (type locality [TL]: originally Egypt, restricted by Kock (1969) to 'Thebes, Egypt'); *A. t. murraiana* (Anderson, 1881) (TL: Karachi, Sind, Pakistan); *A. t. diluta* Andersen, 1918 (TL: El Golea, Algerian Sahara); *A. t. italosomalica* De Beaux, 1931 (TL: Oddur, southern Somalia); and *A. t. pallida* Laurent, 1937 (TL: Oued Tata, Anti-Atlas Mts., SW Morocco). However, the latter form has often been considered a synonym of *A. t. diluta* (see e.g., Corbet, 1978; Koopman, 1994; Horáček *et al*., 2000), which has been synonymised with either *A. t. murraiana* (Kock, 1969; Anciaux de Faveaux, 1976; Koch-Weser, 1984; Kock *et al*., 2002) or *A. t. tridens* (Laurent, 1942; Owen and Qumsiyeh, 1987). Three subspecies are recognised most frquently in *A. tridens* (see also the review by Benda *et al*., 2006): *A. t. diluta*, living in the Maghreb and West Africa, *A. t. tridens* in Egypt and western and southern Arabia, and *A. t. murraiana* in the eastern portion of the range from Syrian Mesopotamia to the east. In addition, several authors (Allen, 1939; Hayman and Hill, 1971; Koopman, 1975, 1994; Simmons, 2005) reported another subspecies from Somalia, *A. t. italosomalica*.

Almost all opinions that have defined geographical variations in *Asellia*, only covered *A. tridens*. An exception was represented by De Beaux (1931), who described two forms of *Asellia* from Eritrea and Somalia: *A. patrizii* and *A. tridens italosomalica*. Unfortunately, his study lacked a broader revision of material coming from the majority of the range of the genus. Harrison (1957) revised populations of *A. tridens* originating in the Middle East and adjacent areas (i.e., most of the species' distribution range) and mentioned a mosaic-like occurrence of two forms of this species, *A. t. tridens* in Egypt and eastern Arabia, and *A. t. murraiana* in Iraq, Pakistan and Yemen, while in Sinai and western

Arabia he found 'intermediate populations'. Subsequently, concerning the Arabian populations, Harrison (1964: 97) reported that "*A. t. murraiana* is found in Iraq and the Aden district; the typical subspecies is found in Oman, Hofuf and Dhufar, while intermediation [between *tridens* and *murraiana*] certainly occurs in Palestine, Sinai and the Hejaz". Kock (1969) reviewed an extensive set of published data on *A. tridens* from most of its distribution range and finally accepted only two subspecies in the whole range: *A. t. tridens* in Upper Egypt, Somalia and eastern Arabia, and *A. t. murraiana* in the Maghreb, Lower Egypt and the Middle East, including Sinai, Israel, and Syria to Afghanistan. He considered other African and Arabian populations to be of an uncertain subspecific position; however, he did not accept any intermediate stages. Corbet (1978), in his review of Palaearctic mammals, introduced a different concept of two subspecies in *A. tridens*. He mentioned the nominotypical form throughout the whole range with exception of the Maghreb, where he suggested the presence of *A. t. diluta*. A broad morphometric analysis of *A. tridens* from throughout almost the whole distribution range was carried out by Owen and Qumsiyeh (1987). They stated the existence of only two subspecies within this range: *A. t. murraiana* in the Middle East (except for Yemen), West Africa and the Maghreb, and *A. t. tridens* in the remaining African areas and in Yemen. However, this revision was not fully accepted with respect to the Arabian part of the distribution range by Harrison and Bates (1991), who referred to *A. t. murraiana* populations from Yemen. A partial morphological revision of *A. tridens* by Benda *et al*. (2006) suggested assigning populations from the Maghreb and the northern part of the Middle East (from Israel to Afghanistan) to *A. t. murraiana*, and those from the Upper Egypt, south-western Libya and Sudan to the nominotypical form.

Most authors (De Beaux, 1931; Harrison, 1956, 1957, 1964; Kock, 1969; Nader and Kock, 1983; Qumsiyeh, 1985; Owen and Qumsiyeh, 1987; Harrison and Bates, 1991; Benda *et al*., 2006) considered body and skull sizes to be the main characteristics distinguishing the species and subspecies in *Asellia*. Representatives of *A. patrizii* are reported to be the smallest within the size range of the genus (forearm length [LAt] 44–46 mm, condylocanine length of skull [LCc] 14.5–15.0 mm), those of *A. t. tridens* medium-sized (LAt 45–53 mm, LCc 15.5–16.7 mm) and individuals of *A. t. murraiana* the largest (LAt 48–56 mm, LCc 15.9–17.8 mm — De Beaux, 1931; Kock, 1969; Benda *et al*., 2006). Harrison (1957) and Harrison and Bates (1991) also suggested the possible importance of pelage colouration and/or presence of rufous phases in colouration for the taxonomic evaluation of respective populations; they concluded that erythristic individuals seemed to be unusual in *A. t. murraiana* but more common in *A. t. tridens*. However, Kock (1969), Owen and Qumsiyeh (1987) and Benda *et al*. (2006) doubted the validity of individual fur colouration for any taxonomic assignment. Benda *et al*. (2006) reported that 40% of the erythristic individuals in an examined population from Syria were referred to *A. t. murraiana*.

In summary, most of the respective authors agree with the taxonomic affiliation of *Asellia* populations from Upper Egypt and northern portions of the Middle East. However, the same authors do not concur with the affiliation of populations from more than half of the genus range comprising southern Arabia, the Horn of Africa, West Africa, and the Maghreb. Thus, we carried out a morphological examination of about 370 museum specimens of *Asellia* with the aim of describing mutual positions of particular populations from the whole distribution area within the genus. To re-define the taxonomic status of these populations, we included most of the available type material in our dataset. We then subjected a geographically representative subset of these specimens to a molecular genetic comparison. In this study, we present a synthesis of the results from these two approaches and propose a new look at the relationships within the genus of trident leaf-nosed bats, *Asellia* Gray, 1838.

Materials and Methods

Analyses

For the morphological analysis, museum material of *Asellia* bats from all parts of the distribution range was used (see Appendix I). We primarily used cranial data for the analysis; for the dimensions taken see Abbreviations and Terminology below. In the comparison, the type material of the taxa *tridens* Geoffroy, 1813, *diluta* Andersen, 1918, *patrizii* De Beaux, 1931, and *italosomalica* De Beaux, 1931, was evaluated; 'topotypic' material was used instead of type material of *pallida* Laurent, 1937. In all morphological comparisons, only adult specimens were used. We did not properly evaluate sexual dimorphism in the taxonomic examination of particular skull characters, however, within all of the compared sample sets both sexes were well represented and balanced (in a ratio from 1:1 to 1:2 — see Appendix I). Thus, we considered that all of the discussed diagnostic characteristics were universally distributed between the sexes within the populations assessed. The examined BMNH material of *A. tridens* from central Ethiopia was not used in the morphometric analysis due to its fragmentation (osteological remains from

cave deposits); it was only evaluated in the biogeographical discussion.

The specimens were measured in the standard way using mechanical or optical callipers, according to Benda *et al*. (2004). Bacula (taken randomly in order to cover all examined populations) were extracted in a 6% solution of KOH and coloured with alizarin red. Only the skull data were used in the statistical comparison. Statistical analyses were performed using Statistica 6.0 software; we used the discriminant function analysis to separate the dimensions most significantly affecting intrageneric and intraspecific variation and a subsequent canonical analysis for the grouping and separation of population samples of similar and different morphotypes, respectively.

A representative subset of sampled *Asellia* specimens was chosen for molecular genetic analysis (Appendix II). The confamilial species *Hipposideros caffer* (Sundevall, 1846) (EU934452) and *H. abae* Allen, 1917 (EU934448) and a species of the sister family Rhinolophidae, *Rhinolophus landeri* Martin, 1838 (FJ457612), were used as outgroups.

Genomic DNA was extracted from alcohol-preserved tissue samples or wing biopsies with a DNeasy Blood and Tissue Kit (Qiagen, Hamburg, Germany) or a JetQuick Spin Tissue Kit (Genomed, Löhne, Germany), following respective manufacturers' protocols. The complete mitochondrial gene for cytochrome *b* (cyt*b*) was PCR amplified using primers F1 (modified; 5'-CCACGACCAATGACAYGAAAA-3') and R1 (5'-CCTT TTCTGGTTTACAAGACCAG-3') by Sakai *et al*. (2003) in a 50 µl reaction volume containing 800 µM dNTP, 200 µM of each primer, 1U of HotMaster *Taq* DNA polymerase with corresponding 10× buffer (Eppendorf, Hamburg, Germany), and 2–5 µl of extracted DNA. Alternatively, Combi PPP Master Mix (Top-Bio, Prague, Czech Republic) was used in preparation for the PCR in a 25 µl reaction volume with proportionally rescaled amounts of primers and DNA. The reaction conditions were 3 min initial denaturation at 94°C, 35 cycles of 40 s denaturation at 94°C, 40 s annealing at 50°C and 90 s extension at 65°C, with a 5 min final extension at 65°C. The products were purified using a QIAquick PCR Purification Kit (Qiagen) or a JetQuick PCR Purification Kit (Genomed) and sequenced commercially (Macrogen, Seoul, Korea) on an ABI 3730XL sequencer using a BigDye Terminator Kit (Applied Biosystems, Foster City, CA, USA). Partially overlapping portions of cyt*b* resulting from sequencing with the F1 and R1 primers were assembled to make complete sequences in Sequencher 4.7 (Gene Codes, Ann Harbor, MA, USA) and Contig Assembly Program (CAP — Huang, 1992) implemented in BioEdit 7.0 (Hall, 1999). All unique, newly obtained sequences were submitted to the GenBank database under the accession numbers JF438999–JF439020.

The sequences were aligned in BioEdit. Genetic divergences between sequences were calculated as Kimura two-parameter (K2P — Kimura, 1980) distances in PAUP* 4.10b (Sinauer Associates, New York, USA). Kimura two-parameter distances were chosen because they have often been used for stating genetic differentiation in studies on bats and thus enable relevant comparisons, particularly in hipposiderids (Bradley and Baker, 2001; Vallo *et al*., 2008; Benda and Vallo, 2009). The sequences were reduced to unique haplotypes, which entered the phylogenetic analysis along with the outgroup sequences of *Rhinolophus landeri*, *Hipposideros caffer*, and *H. abae*. Phylogeny was reconstructed using maximum parsimony (MP) and maximum likelihood (ML) methods in the PAUP* 4.10b program. Maximum parsimony analysis was carried out with unordered, equally weighted characters. Trees were heuristically searched with 100 random additions of sequences and tree bisection-reconnection branch-swapping algorithm. The ML tree was inferred using the same searching procedure under the general time-reversible model of evolution (GTR — Tavaré *et al*., 1986) with a proportion of invariable sites I = 0.046 (Palumbi, 1998), as suggested by the Modeltest 3.7 program (Posada and Crandall, 1998) under Akaike information criterion. Support for topologies inferred under MP and ML criteria was checked by non-parametric bootstrapping (Felsenstein, 1985) using 1,000 and 100 pseudoreplicates, respectively. Phylogenetic relationships were also inferred using Bayesian analysis (BA) in the MrBayes 3.1.2 program (Ronquist and Huelsenbeck, 2003), under the same model of evolution as in the ML analysis. Two simultaneous Metropolis-coupled MCMC processes consisting of one cold and three incrementally heated chains were run for 1000000 generations and sampled each 100 generations. The first 25% of the sampled generations were discarded as burn-in. The relationships among closely related haplotypes were inferred using the phylogenetic network approach, which allows for the contemporary existence of ancestral and descendant haplotypes and offers an alternative explanation for unresolved multifurcations present in phylogenetic trees (Posada and Crandall, 1997; Bandelt *et al*., 1999). A median-joining network (Bandelt *et al*., 1999) was constructed using Network software (Fluxus Technologies, Clare, UK).

In order to assess the approximate dates of the splitting events, an ML tree was calculated under the molecular clock assumption. The difference in log-likelihood of the tree used in the best model searched in Modeltest 3.7 with and without enforcement of the molecular clock was used for the evaluation of a clock-like evolution within the framework of the likelihood ratio test (Felsenstein, 1981). Due to computational demands, the dataset for molecular dating was reduced to thirteen haplotypes representing the major clades of *Asellia* and three outgroup taxa. Confidence intervals for branch lengths of the clock-like tree were estimated by parametric bootstrapping (Huelsenbeck and Crandall, 1997). Based on the GTR + I evolutionary model and the parameters re-estimated for the reduced dataset, 100 datasets were simulated using Seq-Gen 1.3.2 (Rambaut and Grassly, 1997). A clock-like ML tree was then calculated for each simulated dataset in PAUP*. Resulting branch lengths from the 100 trees were then used to build 95% confidence intervals around internal nodes. Due to a lack of relevant fossil records in Hipposideridae, a split between Rhinolophidae and Hipposideridae set at 37 million years ago (MA) was used to calibrate the molecular clock. The value of 37 MA is referred to as the minimum age from which fossils of the distinct genera *Rhinolophus* and *Hipposideros* are known (Remy *et al*., 1987), and it has already been used to calibrate the bat molecular clock (Hulva *et al*., 2007).

Abbreviations and Terminology

Dimensions (in mm): LAt = forearm length including wrist, LCr = greatest length of skull including premaxillae, LOc = occipitocanine length of skull, LCc = condylocanine length of skull, LaZ = zygomatic width, LaI = width of interorbital constriction, LaInf = rostral width between foramina infraorbitalia, LaN = neurocranium width, LaM = mastoid width of skull, ANc = height of neurocranium, ACr = skull height including tympanic bullae, LBT = largest horizontal length of tympanic bulla, CC = width across upper canines at crowns, PP = rostral width across upper premolars at crowns, M^3M^3 = width across third

upper molars, CM^3 = length of upper tooth-row from front of canine to back of third molar, CP = length of upper tooth-row from front of canine to back of premolar, LMd = condylar length of mandible, ACo = height of coronoid process, I_1M_3 = length of lower tooth-row from from front of first incisor to back of third molar, CM_3 = length of lower tooth-row from front of canine to back of third molar, M_1M_3 = length of lower tooth-row from front of first molar to back of third molar, CP_4 = length of lower tooth-row from front of canine to back of second premolar.

Collections: BCSU = Biological Collection of the Sana'a University, Sana'a, Yemen; BMNH = Natural History Museum, London, United Kingdom; ISEA = Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Cracow, Poland; IVB = Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno, Czech Republic; JOC = Ján Obuch private collection, Blatnica, Slovakia; MNHN = National Museum of Natural History, Paris, France; MSNG = Civil Natural History Museum Giacomo Doria, Genoa, Italy; MZUF = Natural History Museum, Florence, Zoology Section "La Specola", Italy; NMP = National Museum (Natural History), Prague, Czech Republic; NMW = Natural History Museum, Vienna, Austria; SMF = Research Institute and Museum Senckenberg, Frankfurt am Main, Germany; ZFMK = Zoological Institute and Museum Alexander Koenig, Bonn, Germany.

Other abbreviations: A = alcoholic preparation; B = skin (balg); ind. = specimen of sex unidentified; M = mean; max., min. = range margins; S = skull; SD = standard deviation, Sk = skeleton.

Geographical terms (considering origin of the examined material): Dhofar = coastal areas of SE Yemen (Hadramaut and Al Mahra Prov.) and SW Oman (Dhofar Prov.); northern Middle East = Israel, Syria, Iraq, Iran, Afghanistan, and Pakistan; southern Middle East = Saudi Arabia and most of the territories of Oman and Yemen (excluding of some coastal areas, see under Dhofar); Maghreb = Morocco, Algeria, and Tunisia; West Africa = Senegal, Gambia, and Mauretania; North Africa = Egypt, eastern Sahara and the Maghreb; eastern Sahara = southern Libya, northern Tchad, and northern Sudan.

Results

Morphological Analysis

A simple comparison of biometric data showed considerable differences among the geographical groups of the samples examined, both between the two nominate species and within *A. tridens* (Table 1). On the other hand, for the pelage and skin colouration, as well as for the nose-leaf morphology, the samples did not show any considerable differences. Regarding size, the examined bats created four main groups, some of them partly overlapping with others within their dimension ranges. The smallest bats (LAt 36–41 mm, LCc 12.5–13.6 mm) originated from Ethiopia and Eritrea, and the largest ones (LAt 46–56 mm, LCc 15.5–18.2 mm) from a broad area including West Africa and the Maghreb, Lower Egypt and the Middle East (both the northern and southern parts — Figs. 2 and 4). The size range of the latter group, however, very broadly overlapped with the rather larger-sized samples from eastern Sahara and Upper Egypt (LAt 44–53 mm, LCc 15.4–17.3 mm). Bats of the two other geographical groups were medium in size; slightly smaller individuals from Dhofar, a border region of Oman and Yemen (LAt 43–47 mm, LCc 14.2–15.3 mm), and slightly larger individuals from two closely situated areas, south-eastern Somalia and the island of Socotra (LAt 43–48 mm, LCc 14.5–15.8 mm).

Although the bats in the latter two groups overlapped with each other in the margins of their size ranges and were hardly distinguishable solely on the basis of their size, they differed in the shape of their rostra (Fig. 5). The Dhofar bats showed, on average, the narrowest rostra among the examined samples (CC/LCc M = 0.309), whereas bats from Somalia and Socotra showed the widest rostra (CC/LCc M = 0.328) among all of the bats compared (Table 1). The well-separated tiny bats of Ethiopian-Eritrean origin could not be separated from the other samples examined only on the basis of their small size, but they could through their relatively large tympanic bullae (LBT/LCc M = 0.191) and relatively wide braincase (LaN/LCc M = 0.471). Similar but not identically high values of the relative size of the bullae were also present in other groups of rather smaller-sized bats originating from Socotra and Dhofar (Table 1). The Dhofar, Somalian and Socotran samples also concurred in their relatively low braincases (ANc/LCc M = 0.329 [n = 40]) in comparison with the small Ethiopian and Eritrean bats, as well as the large African and Middle Eastern bats (ANc/LCc M = 0.342 [n = 215]). On the other hand, the Dhofar bats showed, on average, the relatively shortest rostrum (CM^3/LCc M = 0.400) and relatively narrowest skull (LaZ/LCc M = 0.605) in all of the *Asellia* populations compared (Table 1).

The populations traditionally assigned to *A. t. tridens* or *A. t. murraiana* s.l., i.e. those from the northern parts of the genus range (North and West Africa and the Middle East, except for Dhofar), represented one common group of samples when their size (LAt 45–56 mm, LCc 15.4–18.2 mm) and skull shape were considered (Table 1). Although these samples created two groups of samples according their average values, viz. Upper Egypt and eastern Sahara (LAt M = 48.50 mm, LCc M = 16.15 mm) vs. West Africa, Maghreb, Lower Egypt and the northern Middle East (LAt M = 51.54 mm [n = 109], LCc M = 16.82 mm [n = 125]), within their dimension ranges these groups broadly overlapped, at least for 60% of the range spans (see Table 1 and Fig. 2).

TABLE 1. Forearm and skull dimensions (in mm) of the examined sample sets of *Asellia*. See Abbreviations and Terminology for explanation of dimension abbreviations

Parameter	Upper Egipt				Eastern Sahara				Maghreb & West Africa				Northern Middle East				Southern Middle East			
	n	M	min–max	SD	*n*	M	min–max	SD	*n*	M	min–max	SD	*n*	M	min–max	SD	*n*	M	min–max	SD
LAt	33	48.18	44.7–51.6	1.538	34	49.58	45.5–52.7	1.963	60	50.95	47.7–53.5	1.289	67	52.27	48.6–55.8	1.464	40	51.17	48.4–54.3	1.375
LCr	36	18.29	17.52–18.98	0.343	35	18.53	16.93–19.64	0.570	55	19.12	18.36–19.86	0.351	77	18.95	17.95–19.88	0.392	35	18.92	17.66–20.52	0.736
LOc	18	18.08	17.54–18.67	0.347	23	18.22	16.91–19.12	0.566	16	18.69	17.86–19.08	0.320	44	18.85	17.85–19.83	0.421	34	18.94	17.61–20.54	0.792
LCc	38	16.06	15.54–16.64	0.255	34	16.37	15.41–17.27	0.411	57	16.85	15.90–17.54	0.338	85	16.72	15.62–17.75	0.386	38	16.72	15.57–18.14	0.700
LaZ	39	10.19	9.48–11.03	0.312	35	10.37	9.17–10.94	0.398	58	10.59	9.61–11.28	0.315	85	10.58	9.72–11.18	0.285	40	10.48	9.87–11.51	0.458
LaI	39	2.24	2.05–2.51	0.104	35	2.36	2.05–2.72	0.148	58	2.40	2.11–2.67	0.122	90	2.34	2.11–2.65	0.101	41	2.29	2.06–2.62	0.129
LaInf	39	5.75	5.35–6.09	0.159	35	5.84	5.12–6.44	0.268	58	6.09	5.63–6.85	0.237	57	5.96	2.88–6.51	0.476	41	5.94	5.36–6.63	0.342
LaN	39	7.19	6.67–7.53	0.184	35	7.38	6.58–7.78	0.203	58	7.56	6.87–8.11	0.224	84	7.52	6.94–8.07	0.209	41	7.35	6.74–7.88	0.231
LaM	39	8.56	8.16–9.21	0.196	35	8.81	7.76–9.27	0.271	57	8.94	8.53–9.33	0.166	49	8.92	8.59–9.27	0.158	41	8.77	8.37–9.61	0.276
ANc	38	5.48	5.16–5.87	0.207	34	5.54	5.12–6.05	0.232	57	5.69	5.16–6.13	0.186	68	5.54	5.07–6.03	0.216	40	5.47	5.11–6.09	0.227
Acr	37	7.16	6.74–7.61	0.205	34	7.21	6.71–7.75	0.224	56	7.46	6.86–7.83	0.209	47	7.30	6.56–7.71	0.230	37	7.18	5.31–8.03	0.400
LBT	37	2.91	2.68–3.17	0.114	34	2.96	2.76–3.14	0.103	57	2.97	2.74–3.18	0.098	67	2.99	2.80–3.21	0.095	38	2.99	2.76–3.31	0.135
CC	39	5.13	4.78–5.51	0.183	35	5.19	4.61–5.74	0.241	58	5.42	5.05–5.70	0.161	78	5.33	4.75–5.92	0.200	40	5.37	4.64–6.22	0.383
PP	39	6.07	5.73–6.48	0.159	35	6.20	5.67–6.63	0.228	58	6.43	5.92–7.05	0.177	57	6.39	5.93–6.77	0.181	39	6.36	5.70–6.98	0.362
M^3M^3	39	7.23	6.81–7.85	0.203	35	7.38	6.94–7.82	0.244	58	7.57	6.97–8.14	0.218	76	7.52	7.04–7.92	0.183	41	7.51	6.97–8.06	0.308
CM^3	39	6.61	6.38–6.91	0.132	35	6.77	6.32–7.08	0.183	58	6.90	6.49–7.28	0.153	92	6.85	6.44–7.21	0.148	40	6.99	6.54–7.49	0.290
M^1M^3	39	3.94	3.59–4.21	0.131	35	4.03	3.68–4.35	0.155	58	4.10	3.74–4.34	0.123	57	4.11	3.88–4.32	0.112	42	4.14	3.84–4.45	0.131
CP	39	2.91	2.74–3.22	0.107	35	2.97	2.71–4.24	0.244	58	3.02	2.81–3.41	0.123	57	3.04	2.71–3.37	0.136	39	3.03	2.69–3.42	0.199
LMd	38	12.31	11.62–12.71	0.243	35	12.59	11.60–13.42	0.441	58	12.93	12.09–13.58	0.299	89	12.72	11.74–13.57	0.307	41	12.79	11.73–13.88	0.594
ACo	37	3.92	3.52–4.42	0.187	35	4.01	3.43–4.38	0.223	58	4.12	3.67–4.54	0.166	76	4.10	3.67–4.49	0.166	41	4.12	3.74–4.73	0.266
I_1M_3	38	8.07	7.74–8.45	0.158	35	8.25	7.74–8.83	0.254	56	8.45	7.97–8.88	0.178	53	8.32	7.96–8.71	0.168	40	8.46	7.81–9.23	0.374
CM_3	38	7.30	7.03–7.63	0.149	35	7.44	7.03–8.01	0.240	58	7.61	7.14–8.02	0.167	90	7.51	7.02–7.97	0.171	41	7.64	4.67–8.33	0.575
M_1M_3	38	4.69	4.44–5.07	0.135	35	4.78	4.49–5.13	0.157	58	4.91	4.36–7.76	0.406	56	4.81	4.44–5.15	0.131	41	4.93	4.52–5.39	0.207
CP_4	38	2.85	2.56–3.17	0.112	35	2.90	2.57–3.31	0.157	58	2.93	2.66–3.13	0.106	56	2.91	2.64–3.18	0.123	40	2.97	2.64–3.43	0.218
CM^3/LCc	38	0.41	0.40–0.43	0.008	34	0.41	0.40–0.44	0.009	57	0.41	0.40–0.43	0.008	85	0.41	0.39–0.43	0.008	38	0.42	0.40–0.43	0.007
LaZ/LCc	38	0.64	0.58–0.66	0.016	34	0.636	0.60–0.67	0.015	57	0.63	0.60–0.66	0.014	82	0.63	0.61–0.69	0.014	38	0.63	0.59–0.66	0.015
LaN/LCc	38	0.45	0.42–0.47	0.011	34	0.453	0.42–0.47	0.012	57	0.45	0.43–0.47	0.011	83	0.45	0.00–0.49	0.051	38	0.44	0.41–0.46	0.014
LBT/LCc	36	0.18	0.17–0.19	0.007	34	0.181	0.17–0.20	0.006	56	0.18	0.17–0.19	0.007	65	0.18	0.17–0.19	0.005	36	0.18	0.17–0.21	0.008
CC/LCc	38	0.32	0.30–0.34	0.010	34	0.318	0.30–0.34	0.009	57	0.32	0.30–0.34	0.009	71	0.32	0.30–0.34	0.009	38	0.32	0.29–0.35	0.013

TABLE 1. Extended

Parameter	Dhofar				Somalia				Socotra				Ethiopia & Eritrea			
	n	M	min–max	SD	*n*	M	min–max	SD	*n*	M	min–max	SD	*n*	M	min–max	SD
LAt	20	44.65	43.1–46.5	0.950	15	45.17	43.4–47.5	1.606	15	45.62	44.0–48.2	1.189	11	39.40	36.7–40.8	1.064
LCr	14	16.66	16.21–17.14	0.271	13	17.28	16.42–18.21	0.463	13	17.58	16.94–18.04	0.325	15	15.06	14.38–15.62	0.351
LOc	12	16.49	15.91–17.01	0.318	15	17.19	16.21–17.98	0.450	12	17.17	16.63–17.65	0.340	12	14.94	14.58–15.27	0.256
LCc	14	14.76	14.22–15.24	0.264	13	15.26	14.48–15.76	0.397	13	15.17	14.79–15.55	0.205	13	13.18	12.57–13.57	0.325
LaZ	13	8.92	8.68–9.29	0.179	12	9.60	8.98–10.35	0.397	12	9.46	9.08–9.92	0.244	15	7.99	7.43–8.31	0.260
LaI	15	2.27	2.09–2.44	0.104	16	2.27	1.88–2.52	0.186	13	2.04	1.79–2.23	0.125	15	1.84	1.71–2.04	0.091
LaInf	15	5.25	5.06–5.67	0.200	16	5.40	5.13–5.67	0.136	13	5.51	5.21–5.77	0.142	15	4.76	4.51–5.04	0.157
LaN	14	6.64	6.39–6.97	0.166	15	6.97	6.36–7.42	0.305	13	6.84	6.52–7.26	0.187	15	6.18	5.93–6.49	0.164
LaM	14	7.90	7.68–8.07	0.142	15	8.16	7.84–8.49	0.202	12	7.99	7.77–8.17	0.140	14	7.32	7.01–7.69	0.202
ANc	13	4.88	4.73–5.10	0.116	14	5.03	4.67–5.49	0.223	12	4.97	4.71–5.24	0.187	13	4.52	4.23–4.82	0.162
ACr	14	6.48	6.28–6.67	0.123	12	6.68	6.01–7.38	0.349	12	6.73	6.51–7.02	0.173	14	5.96	5.47–6.37	0.253
LBT	12	2.78	2.64–2.86	0.064	13	2.71	2.48–3.09	0.182	13	2.85	2.72–2.97	0.080	14	2.51	2.34–2.87	0.166
CC	15	4.55	4.39–4.77	0.105	15	4.98	4.64–5.45	0.258	13	4.95	4.76–5.24	0.141	15	4.06	3.86–4.36	0.137
PP	12	5.44	5.18–5.67	0.156	16	5.75	5.30–6.13	0.246	13	5.72	5.61–5.89	0.093	15	4.80	4.62–5.11	0.144
M^3M^3	15	6.47	6.24–6.66	0.114	14	6.72	6.39–7.09	0.164	13	6.82	6.62–7.27	0.181	15	5.66	5.21–5.98	0.228
CM^3	15	5.91	5.74–6.12	0.122	16	6.29	6.02–6.50	0.147	13	6.19	5.98–6.42	0.147	15	5.33	5.11–5.53	0.152
M^1M^3	15	3.60	3.45–3.74	0.083	16	3.80	3.68–3.93	0.068	13	3.86	3.74–4.02	0.105	15	3.24	3.03–3.44	0.126
CP	12	2.42	2.34–2.52	0.072	16	2.62	2.41–2.86	0.117	13	2.62	2.47–2.85	0.124	15	2.22	2.04–2.38	0.097
LMd	15	11.12	10.70–11.49	0.194	15	11.88	11.49–12.51	0.342	13	11.70	11.38–12.11	0.235	14	9.96	9.44–10.28	0.240
ACo	15	3.40	3.23–3.58	0.099	16	3.45	3.18–3.67	0.156	13	3.70	3.45–3.84	0.099	14	2.98	2.75–3.24	0.156
I_1M_3	14	7.16	6.95–7.46	0.140	13	7.71	7.34–8.08	0.222	13	7.64	7.36–7.88	0.138	14	6.50	6.23–6.75	0.157
CM_3	15	6.52	6.24–6.82	0.156	15	7.00	6.57–7.27	0.220	13	6.86	6.63–7.18	0.170	15	5.74	3.83–6.37	0.570
M_1M_3	15	4.26	4.01–4.43	0.129	15	4.58	4.19–5.68	0.329	13	4.53	4.28–4.81	0.161	15	3.84	3.53–4.16	0.171
CP_4	12	2.36	2.24–2.52	0.088	16	2.67	2.45–2.92	0.152	13	2.54	2.43–2.74	0.095	15	2.30	2.13–2.74	0.175
CM^3/LCc	14	0.40	0.39–0.42	0.007	13	0.41	0.40–0.42	0.006	13	0.41	0.40–0.42	0.008	13	0.40	0.39–0.42	0.007
LaZ/LCc	12	0.61	0.59–0.62	0.009	12	0.63	0.60–0.66	0.016	12	0.62	0.61–0.65	0.013	13	0.61	0.58–0.63	0.014
LaN/LCc	13	0.45	0.43–0.47	0.010	13	0.46	0.43–0.48	0.015	13	0.45	0.44–0.48	0.010	13	0.47	0.45–0.50	0.013
LBT/LCc	11	0.19	0.19–0.20	0.004	11	0.17	0.16–0.19	0.009	13	0.19	0.18–0.20	0.006	12	0.19	0.18–0.21	0.011
CC/LCc	14	0.31	0.30–0.32	0.008	12	0.33	0.30–0.35	0.016	13	0.33	0.32–0.34	0.007	13	0.31	0.30–0.33	0.007

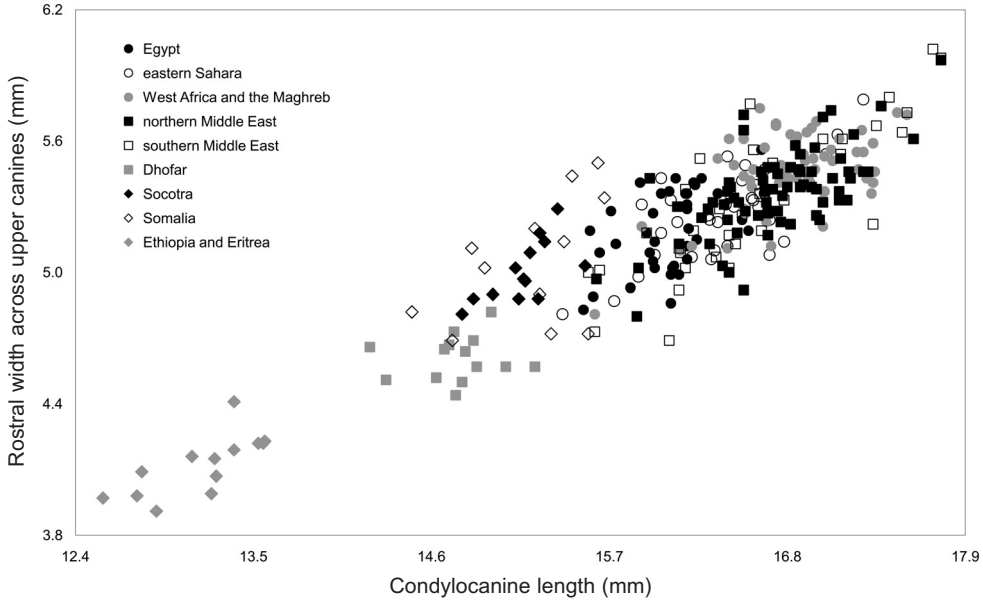

FIG. 2. Bivariate plot of the *Asellia* samples compared: condylocanine length against the width of the rostrum across the upper canines

The *A. tridens* samples from the southern Middle East represented a rather dimensional transition between these two groups (LAt M = 51.19 mm, LCc M = 16.48 mm); however, the range of dimensions of this group covered whole ranges of the other groups (see also Fig. 2). The SDs of most of the dimensions of the southern Middle Eastern group were twice as high as in the other groups (Table 1).

Fom the whole set of 23 cranial dimensions (see Abbreviations and Terminology) the discriminant function analysis separated a selection of 13 skull dimensions (LCr, LCc, LaZ, LaI, LaM, CC, M^3M^3, M^1M^3, CP, LMd, ACo, CM_3, CP_4) that most affected the geographical variation within the genus *Asellia*. The resulting canonical analysis [CA] of these selected dimensions (CV1 = 80.5% of variance; CV2 = 6.3% — Fig. 3) clearly separated four groups of samples corresponding to the defined geographical groups. While the group of bats from Ethiopia and Eritrea (CV1 < -7) and a group of samples from the northern part of the genus range (North and West Africa and the Middle East, except of Dhofar; CV1 > -2) were separated from the other groups along the CV1, i.e., according to their size characters, the remaining two groups (-7 < CV1 < -2) also differed from each other along the CV2, i.e., according to their distinctive skull shapes (see above), viz. the Dhofar group (CV2 < 0) and the group of Somalian and Socotran samples (CV2 > 0). The results of this analysis thus confirmed the previous morphological comparison that showed the mutual proximity between Socotran and Somalian skull morphotypes and also the mutual proximity and inseparability of morphotypes that originated from the northern parts of the genus range (North and West Africa, and the Middle East, except for Dhofar). The separate CA of the samples of the latter group did not separate the particular geographical sets and again confirmed their inseparability, as shown by the metric comparison as well as the CA of the complete *Asellia* material (not shown, cf. Fig. 3).

The examined bacula, extracted from *Asellia* samples originating from all of the main parts of the genus range, were similar in shape (Fig. 6). The bones were simple sticks with enlarged epiphyses; the proximal epiphyses were usually larger than the distal ones. In some samples the distal epiphyses were structured, and in others they were simply widened. The total length of a baculum varied from 0.98 mm in the Ethiopian sample to 1.72 mm in the Syrian sample. The most distinctive ones in terms of shape were the Socotran samples (Fig. 6j–k); the two examined bones were robust, 1.44 and 1.56 mm long, with wide diaphyses and simple distal epiphyses. The proximal epiphyses were very wide, 0.66 and 0.62 ≥ 40% of the baculum length), and the distal ones 0.36 and 0.33 mm, respectively. Another distinct baculum morphotype was shown in the samples from Dhofar (Fig. 6l–n). These bones were tiny, 1.07–1.24 mm wide (< 18% of baculum length) and with rather narrow proximal epiphyses, 0.32–0.37 mm wide (< 30% of baculum length). The only Ethiopian specimen examined had small bone

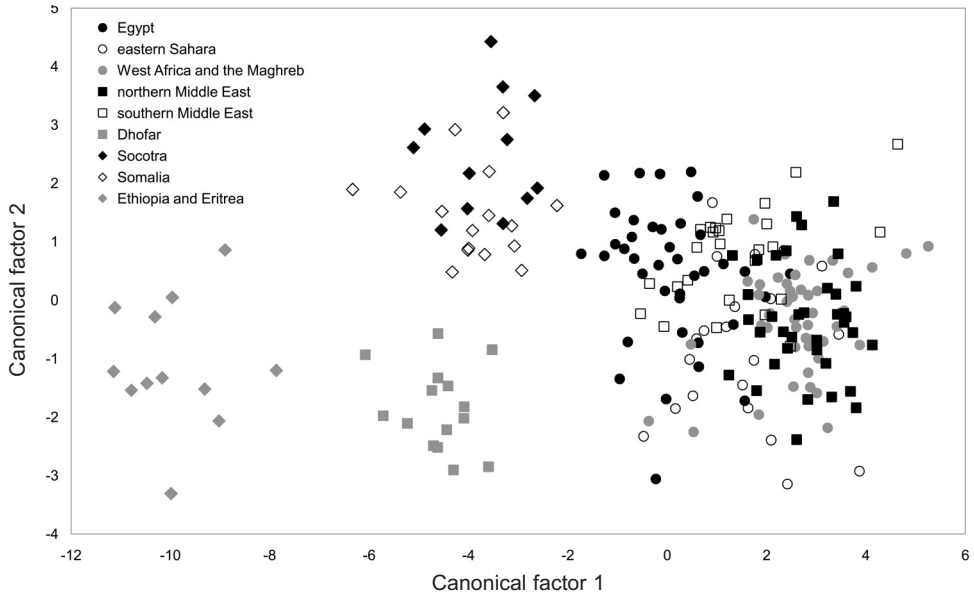

FIG. 3. Bivariate plot of the *Asellia* samples compared: results of the canonical analysis of 13 selected skull dimensions (see Results for details)

(Fig. 6o), 0.98 mm long, with a laterally flattened diaphysis and a small distal epiphysis and broad proximal epiphysis (0.44 mm wide, 45% of baculum length). The samples examined from Egypt and the Middle East (Syria, Iran, Oman) were very similar in shape (Fig. 6a–i), 1.28–1.72 mm long (M 1.45, n = 9); the most variable part of these bones were their proximal epiphyses, being 0.40–0.65 mm wide (26–38% of baculum length); the distal epiphyses were found to have various structures and/or were extensively widened.

To summarise, the morphological comparison and analysis of *Asellia* specimens from all parts of the genus range showed the existence of four basic morphotypes which correspond to their geographical origin; (1) the small bats from Ethiopia and Eritrea with a relatively high and wide braincase and relatively large tympanic bullae, and with a laterally flattened baculum; (2) the medium-sized bats from Dhofar with a relatively narrow skull (across the zygomatic arches), relatively narrow and short rostrum, low braincase, rather large tympanic bullae, and a very delicate baculum with a narrow distal epiphysis; (3) the medium-sized bats from Somalia and Socotra with a relatively wide rostrum, low braincase, rather large tympanic bullae, and a massive and relatively large baculum; and (4) the large bats from the wide range stretching from West Africa to Pakistan, including most of the Middle East, with relatively small tympanic bullae, a rather broad skull, a high braincase, and a long rostrum, and with a rather gracile baculum with large distal and proximal epiphyses.

Molecular Genetic Analysis

From the subset of *Asellia* specimens we obtained 49 complete sequences of cyt*b*, which corresponded to 23 unique haplotypes (see Appendix III). Genetic divergences among them ranged from 0.1–13.4%, while divergences among *Asellia* and the outgroup ranged from 16.0–19.6% (Table 2). In the alignment, 351 positions were variable and 237 parsimony informative. Approximately 14.5% of the substitutions occurred at first, 4.0% at the second, and 81.5% at the third codon positions. Base composition did not differ among the taxa (χ^2 = 10.83, *d.f.* = 75, P = 1.00) and mean values for the base frequencies were A = 0.27969, C = 0.32699, G = 0.14015, and T = 0.25317, which was in concordance with the typical skew of mammalian mtDNA towards a lack of guanin residues.

All of the phylogenetic methods basically produced identical tree topologies that only differed in minor rearrangements of the terminal taxa, showing three well-supported clades corresponding to the geographical divisions of the sampled area of *A. tridens* (Fig. 7). A deep split separated the basal clade, exclusively comprising haplotypes from the island of Socotra. Genetic divergence of the Socotran clade from the rest of *Asellia* ranged from 12.3–13.4%.

Among the remaining haplotypes, those originating from Dhofar diverged from the other mainland haplotypes in the Middle East and North/West Africa, differing by 5.3–5.8% from them. The haplotypes in the large Middle Eastern and African clade showed a rather low genetic variation, reaching up to 3.4%. This clade could be subdivided into three distinct subclades grouping the respective haplotypes from North/West Africa, the Middle East except for Dhofar, and a solitary haplotype from the Shisr oasis of south-western Oman. The North African subclade contained haplotypes from Morocco, Libya, Egypt, and Sudan, and exhibited very little difference between the haplotypes, up to two substitutions. The haplotype NA1 was identified in Morocco and SW Libya, a distance of almost 2000 km, and the haplotype NA2 was even identified in Morocco, Egypt, and Sudan, i.e. over a distance of more than 4000 km. The West African subclade included haplotypes from three sites in NW Mauretania, again differing by up to two substitutions from each other. The subclade of the Middle East contained haplotypes from Syria and Iran, and from north-eastern Oman, i.e. the northern and southern Middle East, respectively. Differences within the Middle East subclade ranged up to 0.7%. The solitary haplotype OM3 from the Shisr oasis differed by 2.1–3.4% from the Middle Eastern and both African subclades. Its relationships to these subclades could not be definitely resolved. In 9 out of the 12 recovered MP trees (557 steps), the haplotype OM3 was placed in a sister position to the other Middle Eastern haplotypes, while in the other three trees, the haplotype OM3 was in a paraphyletic position to other Middle Eastern and North/ West African haplotypes. The latter position of OM3 was also shown in the two ML trees obtained (-lnL = 4059.61646). In the Bayesian consensus tree, the haplotype OM3 was placed in a trifurcation with the Middle Eastern and both African subclades (Fig. 7). The MP analysis did not resolve the position of the West African subclade but according to high support from the ML and Bayesian analyses this subclade is a sister to the North African subclade. Another insight into the relationships within the Middle Eastern and North/West African *Asellia* was provided through the phylogenetic network. Four groups of haplotypes were

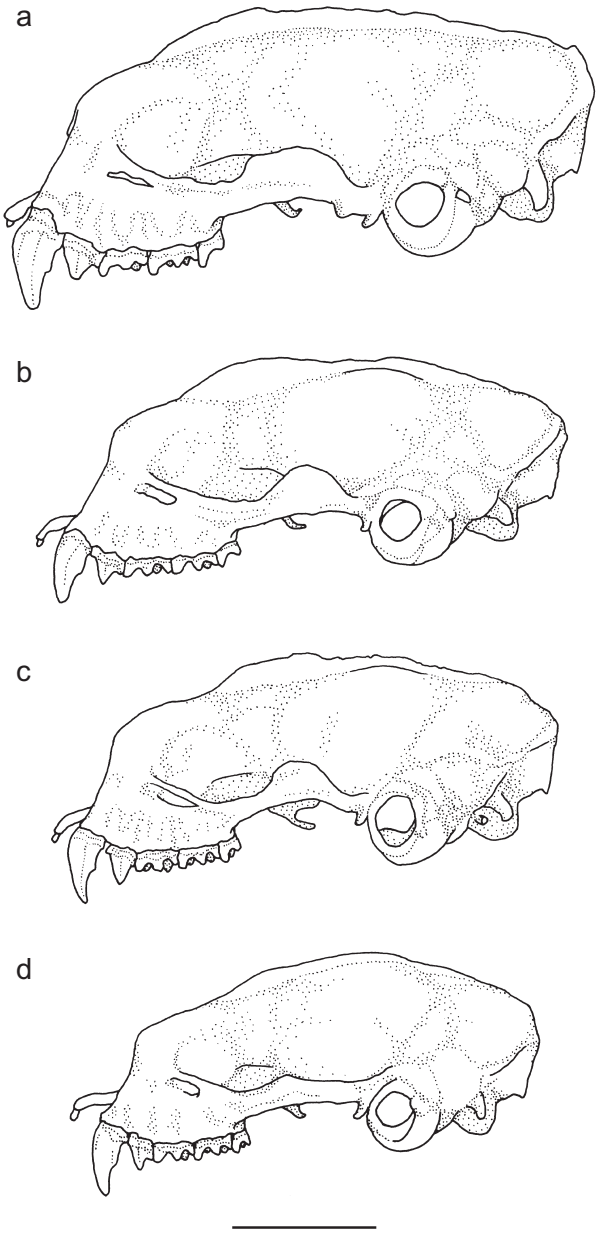

FIG. 4. Skulls of the *Asellia* bats representing the distinct morphotypes revealed (see Results): a — *A. tridens*, ♀, NMP 48026, Halabiyyeh, Syria; b — *A. italosomalica*, ♀, NMP 90574, Kam, Socotra; c — *Asellia* sp. nov., ♀, NMP 92796, Damqaft, Yemen; d — *A. patrizii*, ♀, SMF 52377, Metahara, Ethiopia. Left, ventral/dorsal view, right, lateral/semilateral view. Scale bar = 5 mm

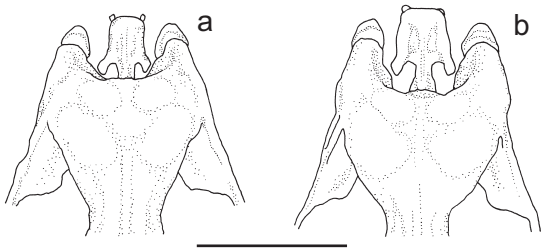

FIG. 5. Rostral parts of the skulls of two *Asellia* morphotypes, similar in size: a — *Asellia* sp. nov., ♀, NMP 92796, Damqaft, Yemen; b — *A. italosomalica*, ♀, NMP 90574, Kam, Socotra. Scale bar = 5 mm. Note the size and shape of the nasal swellings and the large size of the maxillae

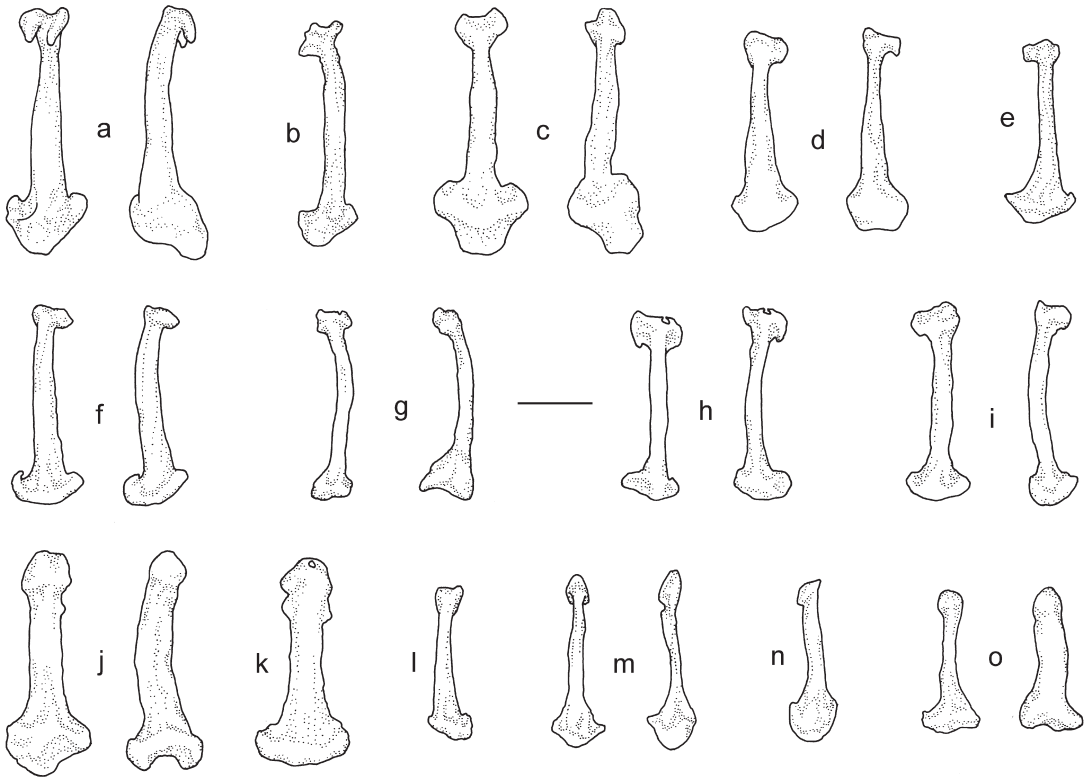

FIG. 6. Baculum preparations of the *Asellia* bats from various parts of their distribution range: a — *A. tridens*, NMP 48039, Tadmor, Syria; b — *A. tridens*, NMP 48175, Choqa Zanbil, Iran; c — *A. tridens*, NMP 92591, Aswan, Egypt; d — *A. tridens*, NMP 92587, Aswan, Egypt; e — *A. tridens*, NMP 90353, Siwa, Egypt; f — *A. tridens*, NMP 92770, Muqal, Oman; g — *A. tridens*, NMP 92629, Al Ghubrah, Oman; h — *A. tridens*, NMP 92687, Shisr, Oman; i — *A. tridens*, NMP 92686, Shisr, Oman; j — *A. italosomalica*, NMP 90579, Suq, Socotra, Yemen; k — *A. italosomalica*, NMP 90592, Mazaaba, Socotra, Yemen; l — *Asellia* sp. nov., NMP 92721, Ain Jarziz, Oman; m — *Asellia* sp. nov., NMP 92798, Damaqawt, Yemen; n — *Asellia* sp. nov., holotype, NMP 92790, Hawf, Yemen; o — *A. patrizii*, MZUF 6545, Metahara, Ethiopia. Scale bar = 0.5 mm

identified in the network, corresponding with clustering in the phylogenetic trees (Fig. 8). The OM3 haplotype from the Shisr oasis was positioned as a separate group without any close affinity to the North/West African and Middle Eastern groups. The West African group clearly shared the last common ancestor with the North African group. Haplotypes within the respective haplogroups mostly differed in one or two substitutions and showed a star-like pattern, confirming the presence of ancestral polymorphism in the respective evolutionary units of *A. tridens*. Only one haplotype in the Middle Eastern haplogroup, OM2, differed by six substitutions from its closest relative and was depicted as a terminal node in the network. Such an arrangement contradicted the topology of the phylogenetic trees revealed, which highly supported a paraphyletic position of this haplotype compared to the other haplotypes within the Middle Eastern subclade.

A dating procedure was carried out using the reduced 16-taxon dataset under topological constraints forcing the respective haplotypes from the subclades revealed as being monophyletic and the position of the subclades in agreement with the recovered phylogeny in order to reduce computation time. The solitary Shisr haplotype OM3 was constrained to monophyly with the Middle Eastern and North/West African subclades. The likelihood ratio test of the evolutionary model with and without the molecular clock did not reject the clock-like evolution within the dataset (Δ lnL = 31.16542, *d.f.* = 24, $P = 0.1490$). Three clock-like ML trees (-lnL = 4010.39115) resulted from this analysis (Fig. 9). These differed in the mutual arrangement of the three North African haplotypes, but the differences were negligible. Based on the split between *Rhinolophus* and *Hipposideros* set at 37 MA and the length of the branch leading from the root to *Rhinolophus*, relevant branch lengths for hipposiderids were calculated including 95% confidence intervals (CI) inferred from tabulated and ordered branch lengths of 100 simulated clock-like ML trees. These trees were

generated under topological constraints resulting from the original clock-like ML tree. The split between the genera *Hipposideros* and *Asellia* was dated as 24.3 MA (95% CI: 20.9–30.1 MA). The basal split in *Asellia*, resulting in separation of the Socotran clade, was estimated to have occurred ca. 12.3 MA (95% CI: 9.9–14.8 MA). Splitting of the mainland *Asellia* then followed ca. 4.4 MA (95% CI: 3.2–5.9 MA), resulting in the Dhofar clade and the remaining *Asellia* of the Middle East and North/West Africa. Within the last clade, radiation of the Middle Eastern and North/West African subclades probably occurred ca. 1.8 MA (95% CI: 1.4–2.3 MA).

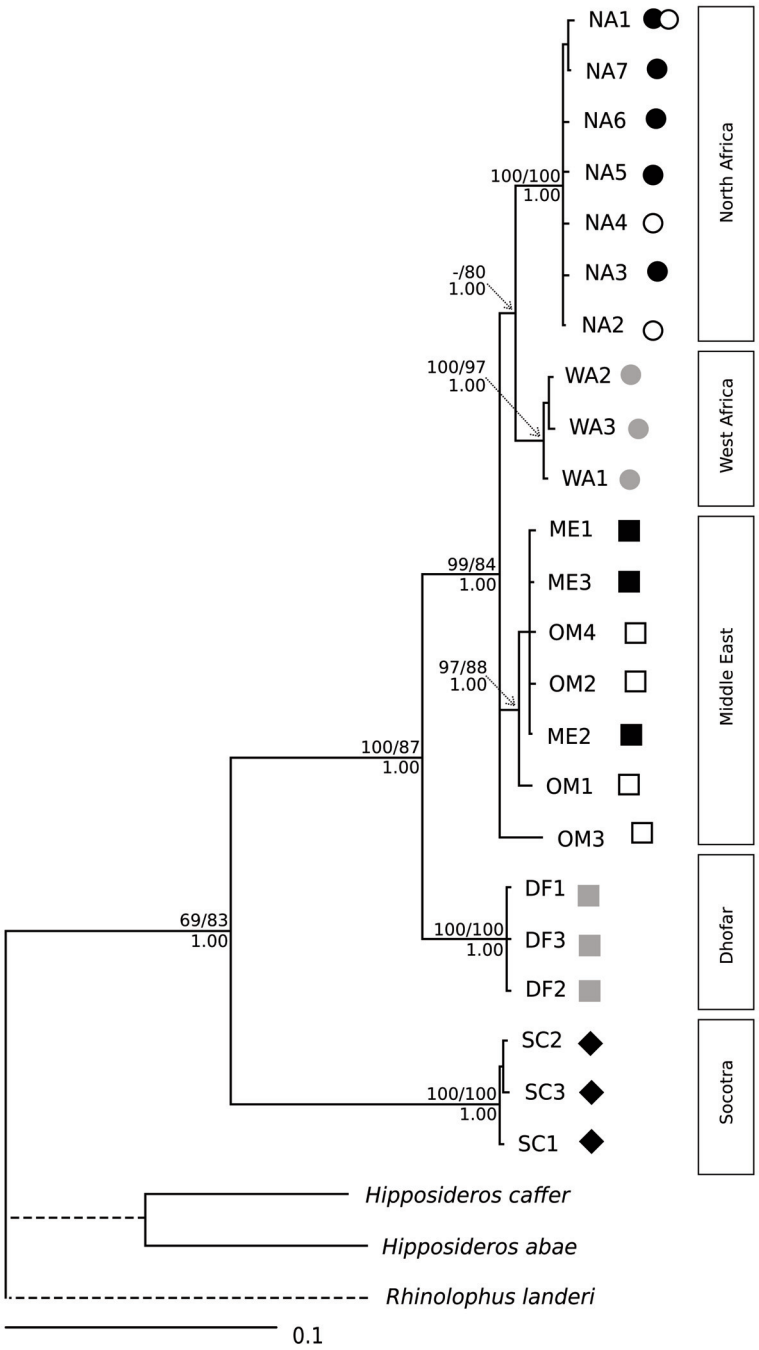

FIG. 7. Bayesian 50% majority rule consensus tree depicting the phylogenetic relationships in *Asellia* based on cyt*b* sequences. Bootstrap supports for MP and ML are indicated above the branches, posterior probability for BA below the branches. See Appendix II for an explanation of the haplotype names. Graphical coding according to geographical origin of the haplotypes corresponds to the morphological analysis (Figs. 2 and 3)

Discussion

Phylogeny

Molecular genetic analysis of a representative subset of *Asellia* samples showed that three distinct phylogroups exist within the currently acknowledged species *A. tridens*, confined to the island of Socotra, the Dhofar region in SW Oman and E Yemen, and the vast area of most of the Middle East and Africa. These phylogroups largely corresponded to groups that were revealed by morphological examination. The fourth distinct morphogroup of *Asellia* was found to be confined to Eritrea and Ethiopia and pertains to the other currently acknowledged species *A. patrizii*, which unfortunately was not included in our phylogenetic analysis due to repeatedly unsuccessful attempts to extract DNA from old museum specimens.

Although the specimens included in our genetic analysis were originally considered to belong to just one currently recognised trident leaf-nosed bat species, *Asellia tridens*, we discovered profound genetic divergences between them. The basal phylogroup from Socotra differed from the rest of *Asellia* by more than 12%, which is much higher than the commonly reported values of intraspecific variability of cyt*b* sequences in mammals (Bradley and Baker, 2001; Baker and Bradley, 2006). Within the family Hipposideridae in particular, similar divergences were found between the well-defined outgroup species *H. caffer* and *H. abae*, as well as among other *Hipposideros* species (Vallo *et al*.,

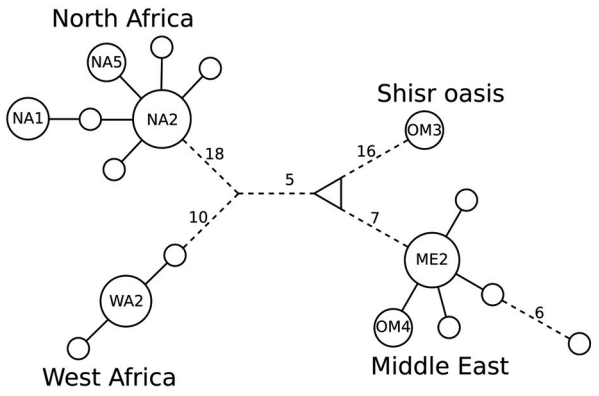

Fig. 8. Median-joining network of Middle Eastern and African *Asellia* haplotypes. Solid lines indicate one substitution. Dashed lines are not proportional to the evolutionary change, but the numbers of substitutions are indicated numerically. The size of the nodes corresponds to the frequency of the particular haplotype. Solitary haplotypes are left blank, more frequent haplotypes are labelled according to Appendix II

Table 2. Kimura two-parameter distances (in %) among and within geographical groups of haplotypes of the cytochrome *b* gene found in the genus *Asellia*

K2P [%]	*R. landeri*	*H. caffer*	*H. abae*	N Middle East	NE Oman	Shisr Oasis	North Africa	West Africa	Dhofar	Socotra
Rhinolophus landeri	n/a									
Hipposideros caffer	21.3	n/a								
H. abae	20.4	11.2	n/a							
northern Middle East	16.5–16.7	16.7–17.0	17.7–17.9	0.1–0.2						
NE Oman	16.8	16.6	18.0	0.1–0.7	0.2–0.7					
Shisr Oasis	17.5	17.1	17.9	2.1	2.1	n/a				
North Africa	16.7–16.9	17.7–17.9	18.4–18.6	2.6–3.0	2.6–3.0	3.3–3.4	0.1–0.3			
West Africa	16.8	17.3	18.4	2.1–2.2	2.2	2.9	2.5–2.7	n/a		
Dhofar	17.4–17.5	16.4–16.5	17.7–17.8	5.3–5.6	5.5–5.6	5.7–5.8	5.6–5.8	5.5–5.6	0.1–0.2	
Socotra	19.5–19.6	16.0–16.3	17.9–18.2	12.3–12.7	12.5–12.7	13.2–13.4	12.6–13.0	12.4–12.6	12.4–12.8	0.1–0.2

2008). A split within the mainland *Asellia* haplotypes delimited a geographically restricted Dhofar population that differed by more than 5% from the other sampled populations. Although substantially lower than the divergence of the Socotran population, such a value lies within the interval of known divergences between sister species of bats (Bradley and Baker, 2001; Baker and Bradley, 2006). It is generally accepted that percentage sequence divergence alone cannot be considered as the only evidence for the distinction of morphologically similar forms. On the other hand, a 5% divergence was suggested by Baker and Bradley (2006) as a substantiated threshold for a potential specific distinction of sister phylogroups. According to these authors, given such divergence, a search for other distinctive traits (e.g., morphological) is highly recommended, which could contribute information towards the acceptance or refusal of the suspected species. In this case, the information resulting from detailed morphological analyses clearly supported a specific distinction of the phylogroup from Dhofar. On the contrary, divergences within the remaining *Asellia* phylogroup from the Middle East

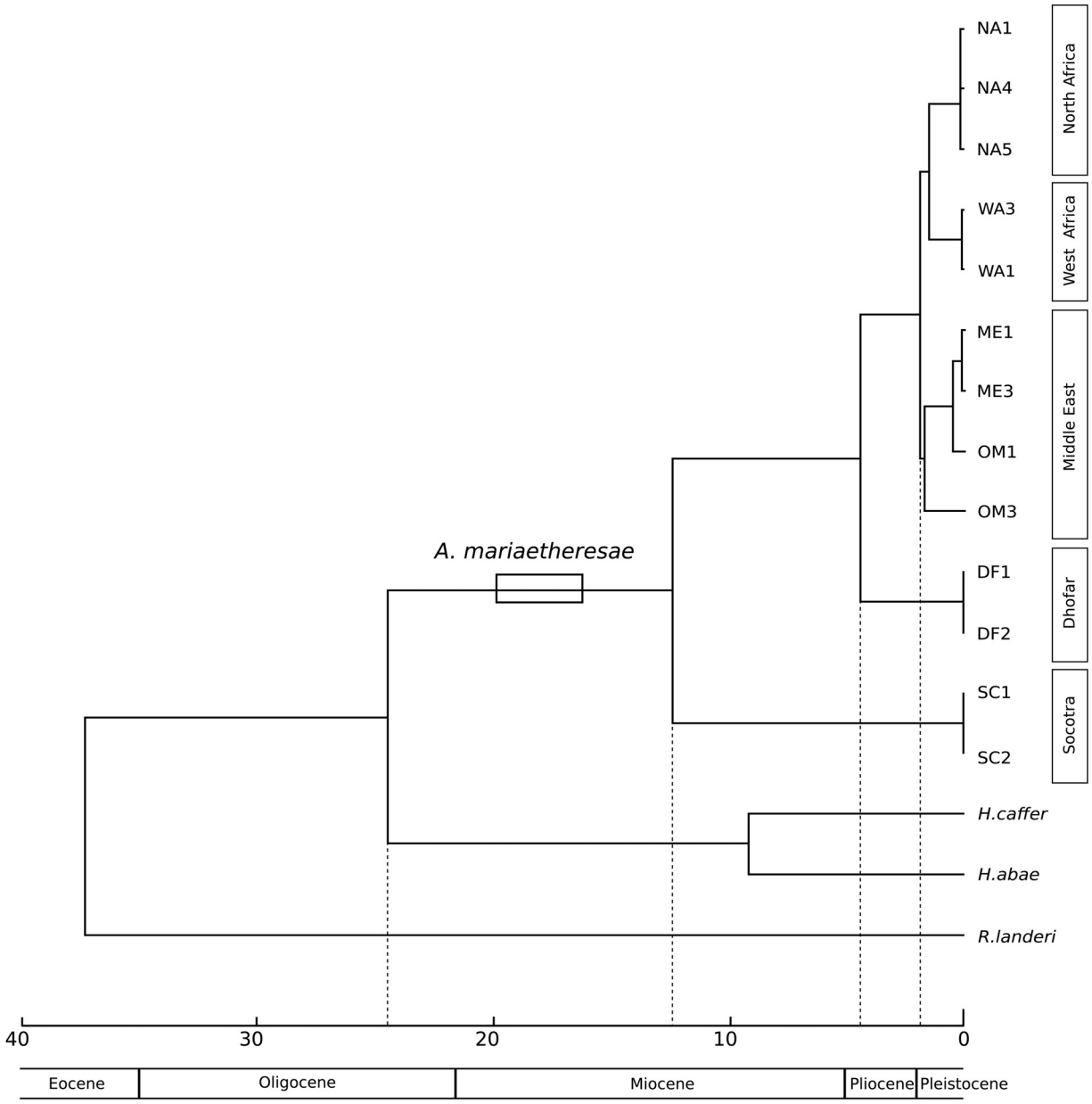

FIG. 9. Clock-like ML tree from the reduced, 16-taxon dataset, containing representative samples of each *Asellia* phylogroup. Scale bar depicts an approximate division of the Cenozoic era. Time on the X-axis is indicated in millions of years (MA). Estimated position of the fossil *A. mariaetheresae* (see Discussion) is marked on the internal branch leading to extant *Asellia* species

and North and West Africa reached up to 2.7% and the populations included were found to be similar in morphology, which unambiguously indicates intraspecific variability, especially with regard to the broad geographical distribution of haplotypes.

The phylogroup comprising the Middle Eastern and North and West African haplotypes was rather well structured into two African subclades and one Middle Eastern subclade, and a solitary haplotype from the Shisr oasis in south-western Oman (OM3). The North and West African haplotypes were found to be sister groups. Although the MP analysis did not support this relationship, both likelihood-based methods agreed on it. The probable reason for this is that the latter methods use a model of evolution that considers variations in substitution rates and multiple substitutions, unlike the MP method. When the weighting of characters (e.g., downweighting transitions or the third codon position) was applied in the MP analysis, a sister position of both African subclades appeared, even in the MP trees (data not shown). On the other hand, the position of the Shisr haplotype could not be fully resolved by any of the three methods used, as all of the trees showed a trifurcation in the basal node of the Middle Eastern/African clade. Such a pattern may indicate radiation of the three lineages within a short time period, but a poor resolution of the relationships between closely related haplotypes can also be attributed to a lack of informative characteristics for traditional phylogenetic analysis (Posada and Crandall, 2001). The median-joining network constructed as an alternative to the phylogenetic trees corroborated this trichotomic pattern, showing the Shisr haplotype as a separate group, and it also confirmed the sister relationship between the two African subclades. However, a strong discordance was found in the position of the haplotype OM1 within the Middle Eastern subclade. While all of the phylogenetic trees showed the haplotype OM1 as a basal subclade, in the network it was placed as a descendant of the compact Middle Eastern group, differing by six substitution steps from its ancestor. The star-like resolution of the Middle Eastern haplogroup explained the unresolved multifurcation at the shallowest level of phylogeny as a contemporary occurrence of ancestral and descendant haplotypes differing by one to two mutations (Bandelt *et al*., 1999; Posada and Crandall, 2001). Nevertheless, it seems unlikely that the haplotype OM1, which differed by six mutations from its most closely related haplotype ME3, is the youngest one among the Middle Eastern haplotypes as suggested from the network pattern because the basal position of OM1 was highly supported in the bifurcate trees. Therefore, we considered this haplotype as a separate lineage and suggest the existence of ancestral polymorphism only within the remaining crown haplotypes, as shown in the depicted phylogenetic tree (Fig. 7).

The pronounced phylogenetic structure and extant geographical distribution of *Asellia* could be plausibly explained with regard to the geological history of the Arabian Peninsula. Despite the obvious limitations of our molecular dating caused by both stochastic errors of phylogenetic inference and arbitrary setting of only one calibration point, the timing of splitting events in phylogeny coincided with the acknowledged palaeohistorical events. Very few fossil records of the family Hipposideridae are suitable for relevant molecular dating. The oldest hipposiderids are known from karst sediments of the Middle Eocene in Europe. The genus *Asellia* is documented by only one fossil species, *A. mariaetheresae* Mein, 1958, from the Early and Middle Miocene of the north-western Mediterranean (Legendre, 1982). According to the inferred molecular dating, the genus *Asellia* may have appeared in the Late Oligocene around 24 MA. This indeed corresponds with the first fossil evidence of *A. mariaetheresae* dated to the Burdigalian period of the Early Miocene (ca. 20.5–16 MA). Since that time, the morphology of this genus remained virtually unchanged, and it was hypothesised that the recent *A. tridens* is closely related to the Miocene *A. mariaetheresae* (Legendre, 1982). Because *Asellia* remains have not been found at any African locality harbouring relevant fossils of hipposiderid bats (Legendre, 1982), this could suggest that *Asellia* was not present in Africa in that period. In the Middle Miocene, a harsh climate might have caused the disappearance of *Asellia* from Europe (Legendre, 1982). Roughly in the same period, the connection of Arabia with Eurasia may have opened an escape route to the warmer areas of the Arabian Peninsula, which originated after the Arabian Plate drifted away from the African Plate and collided with the Eurasian Plate (Samuel *et al*., 1997). The Red Sea and the Gulf of Aden could then have functioned as barriers against the dispersal of *Asellia* to Africa. The Arabian Peninsula could thus plausibly be the region where the evolutionary history of the modern *Asellia* began.

The island of Socotra separated from the Arabian mainland during the formation of the Gulf of Aden (Samuel *et al*., 1997; Fleitmann *et al*., 2004). The split in the Socotran *Asellia* lineage, dated at 12.3 MA, corresponds to the origin of Socotra, for which

the latest estimate lies at 15 MA (Fleitman *et al*., 2004). Although the island of Socotra is now situated closer to the Horn of Africa than to the Arabian Peninsula, evidence of floristic affinities between Socotra and the southern Arabian coast (Yuan *et al*., 2005; Meister *et al*., 2007) support the hypothesis on the Arabian origin of Socotran *Asellia*. Despite the lack of molecular data on Somalian *Asellia* populations, their morphological identity with the Socotran population allowed speculation on the colonisation of the Horn of Africa from Socotra in recent times, for example, during Pleistocene glaciations, which caused the sea level to decrease by up to 120 m (Beydoun and Bichan, 1969), providing a possible connection between Socotra and the Somalian coast for volant mammals. In the Arabian Peninsula, the transition between the Miocene and the Pliocene ca. 5 MA was accompanied by a significant aridisation that resulted in a retreat of contemporary organisms to climatically more favourable coastal mountain refugia, such as the Dhofar region (Meister *et al*., 2007). Separation of the Dhofar lineage is estimated to have occurred around 4.4 MA and highly coincides with this aridisation event. On the other hand, the remaining *Asellia* populations may have found a refuge in the Hajar ranges of north-eastern Oman, as deduced from the high number of haplotypes found in this region and its orographic similarity with the Dhofar region. The phylogeographic pattern revealed from our analyses further suggests that the majority of contemporary populations of *Asellia* in Africa and the Middle East could have had their origins in this eastern Arabian refuge. The onset of Pleistocene climatic shifts at ca. 2 MA (deMenocal, 2004) probably opened a colonisation route from Arabia to Africa, as seen from the split between African and Middle Eastern lineages estimated at 1.8 MA. It seems plausible that this colonisation took place via a northern route through Sinai; no haplotypes of these two lineages were found in the Eritreo-Ethiopian or Somalian regions. These areas are rather well isolated from other African areas by pronounced mountain barriers, which probably limited the dispersal of local *Asellia* populations, resulting in the evolution of distinct evolutionary units, i.e. Socotro-Somalian and Eritreo-Ethiopian. Unfortunately, the phylogeography of the latter Eritreo-Ethiopian morphotype representing a separate species, *A. patrizii*, could not be inferred. However, it might be speculated that the ancestors of this species crossed the Red Sea during a period of lower sea level and became trapped in the Afar desert and adjacent regions.

All *Asellia* populations throughout Africa, west of the Great Rift zone, thus belong to the same evolutionary unit, which originated in the Arabian Peninsula. The phylogeographic pattern suggests that the two African lineages of *Asellia* independently colonised the continent. The West African lineage is known only from Mauritania, and very likely also from Senegal, according to the identity of ca. 100 bp fragments retrieved from ZFMK 76.243 and ZFMK 76.231 specimens from the Fété-Olé region (data not included, but see Appendix I for comparison). The identity of and the low divergence between the *Asellia* haplotypes from the northern part of the African distribution range totalling over 4000 km in latitudinal distance, as well as the star-like pattern confirming the presence of ancestral polymorphism, point towards a recent colonisation of North Africa from the eastern region (Egypt, Sudan). A similar phylogeographic pattern can be seen also in the Middle Eastern phylogroup, where the northern (i.e., Levantine and Iranian) and southern (Omani) populations show low genetic structuring. One exception is the haplotype OM3 from the Shisr oasis, which probably represents an ancient lineage that survived the Pleistocene in a geographically isolated area of the arid Omani highlands. Because of the close relationship of the sampled Iranian population with the other Middle Eastern populations, we may infer that at least Mesopotamia (including western Iran) was colonised via a continental route from the Arabian Peninsula. As the colonisation of the eastern parts of the Middle East was probably restrained by the Zagros range, further genetic data from south-eastern Iran and Pakistan are needed to support a plausible alternative for the immigration over the Strait of Hormuz during a lowered sea level in the Pleistocene.

Taxonomy

The opinion that there are only two species within the genus *Asellia*, monotypic *A. patrizii* and polytypic *A. tridens*, as suggested by De Beaux (1931), Kock (1969), Koopman (1993, 1994) and/or Simmons (2005), is not supported by the results of our analyses. The morphological analysis separated four exclusive groups of populations differing in body, skull and baculum size and in skull and baculum shape. The exclusivity of three of these morphotypes was supported by molecular genetic analysis which suggested that these populations are three evolutionarily separated units.

The fourth morphotype from Ethiopia and Eritrea, although not examined genetically, clearly represents a separate taxon. These very small bats do not overlap in metric characters with other morphotypes and they also possess a set of unique characters, viz. relatively large braincases and tympanic bullae, and laterally flattened bacula. Since the examined set of these specimens from north-eastern Africa also included the type series of *Asellia patrizii* De Beaux, 1931, originating from south-eastern Eritrea (Danakil region), this name fits this species. Hence, our results confirm the opinions of many previous authors (De Beaux, 1931; Allen, 1939; Harrison, 1965; Hayman and Hill, 1971; Koopman, 1993, 1994; Simmons, 2005). The species status of this form is also supported by its sympatric occurrence with another, large-sized morphotype (referred to as *A. tridens*) in central Ethiopia (see Hill and Morris, 1971, and Appendix I). *Asellia patrizii* is a species endemic to the Afar desert and limited adjacent areas, known only from central Ethiopia, SE and E Eritrea, and several islands on both sides of the southern part of the Red Sea (Largen *et al*., 1974; Moeschler *et al*., 1990).

The three remaining populations obviously represent separate taxa also. Most widespread and thus most diversified both metrically and genetically is the large-sized morphotype from northern areas of the genus range. This form occurs in a broad belt of dry habitats (including the harshest deserts) along the south-western margin of the Palaearctic, from the Maghreb and Gambia to Egypt and central Ethiopia in Africa and from central Syria and southern Yemen to south-western Afghanistan and western Pakistan in Asia. This morphotype is easily identifiable with the type species of the genus, *Asellia tridens* (Geoffroy, 1813), described from Upper Egypt. While morphological comparisons did not reveal remarkable differences between the examined sample sets concerning metric traits (contra e.g., Harrison, 1957; Kock, 1969; Owen and Qumsiyeh, 1987), the genetic comparison showed four subclades among the sampled African and Asian populations, which differed by 2.1–3.4% in the K2P genetic distance from each other. This finding does not concur with the existing opinions of intraspecific variation in this species. The last published reviews (Koopman, 1994; Simmons, 2005) suggested four subspecies within the species rank of *A. tridens*, defined according to metric data (cf. Owen and Qumsiyeh, 1987). While the subspecies from Somalia (*italosomalica*) is shown here as a separate morphotype (see below), the separation of other intraspecific taxa from West Africa/Maghreb (*diluta*), Sahara/Arabia (*tridens*) and the Levant/Iran (*murraiana*) was not supported by our results. The metric differences between populations, although present, were not significant enough to substantiate taxonomic division; the overlap of size ranges among the samples – when a large number of specimens was compared – was too extensive (see Figs. 2 and 3). On the other hand, the substantial genetic distance between two haplotype groups of the two continents suggests a possible taxonomic division between these geographic ranges. Moreover, such a separation just conforms to the taxonomic evaluation of another Saharo-Sindian faunal element, *Rhinopoma cystops* Thomas, 1903 (see Hulva *et al*., 2007). Although the phylogenetic positions of the West African and south-east Arabian populations were not fully resolved by our genetic analysis (but see Fig. 9), we tentatively assign the African populations of the large-sized *Asellia* morphotype to *A. tridens tridens* (Geoffroy, 1813), and the Asian ones to *A. t. murraiana* (Anderson, 1881); however, the taxonomic statuses of the populations from the Shisr oasis as well as from West Africa need further examinations. We consider the names *A. t. diluta* Andersen, 1918 and *A. t. pallida* Laurent, 1937, junior subjective synonyms of the nominotypical subspecies, based on geographic reasons – both descriptions originate from the Maghreb, inhabited by the North African haplogroup. The metric differences between some of the populations of *A. t. tridens*, as demonstrated by, for example, Harrison (1957), Kock (1969), and Gaisler *et al*. (1972), were most probably influenced by climatic differences between the regions of origin. However, these differences seem to have evolved relatively fast (see the close genetic similarity between '*tridens*' and '*murraiana*' sensu Kock, 1969, in North Africa) and do not represent differences between unique evolutionary units.

The populations of medium-sized bats occurring in Socotra were found to be well separated from all remaining sample sets concerning genetic traits, differing by 12.3–13.7% in K2P genetic distance from the other *Asellia* populations examined. These bats showed unique traits in their bacular and skull morphology. Regarding the size and form of the skull, the Socotran bats conform to samples from Somalia, including the type series of *A. tridens italosomalica* De Beaux, 1931. This name is thus applicable for this well-defined taxon, which is, mainly due to its extraordinary genetic isolation, best considered as a separate species, *A. italosomalica*. The species is

endemic to a limited area of the lowland deserts in the southern and eastern parts of Somalia and the neighbouring Socotra archipelago (although only confirmed from its main island). The separate taxonomic position of Somalian populations was already suggested by De Beaux (1931) and Koopman (1975), and the metric differences between Socotran and Arabian samples were noted by Wranik *et al*. (1991) and Cesarini (2007); however, these authors determined the Somalian/ Socotran populations as being within the rank of *A. tridens*, and other authors even placed them within *A. t. tridens*.

The last distinct morphotype, with a similar size to *A. italosomalica*, was revealed by morphometric analysis from the samples originating from south-eastern Yemen and south-western Oman, the area terminologically simplified here as Dhofar. These bats possess gracile skulls with rather large tympanic bullae and very delicate bacula. Regarding genetic traits, they were found to differ by 5.3–5.8% in K2P genetic distance from the samples of *A. tridens* s.str. and by 12.4–12.8% from the Socotran *A. italosomalica*. These bats live in close parapatry to populations of *A. tridens*, with known distances between recorded localities being some 160 km across the desert in Yemen (Qatn–Ryan) and even 135 km in Oman (Shisr–Ain Jarziz — see Appendix I). These relatively small geographical and relatively large genetic distances suggest a lack of genetic flow between the two populations and a substantial isolation of the Dhofar bats. Thus, we consider a separate species level appropriate for these morphologically and genetically well-defined and geographically delimited populations. As only one specimen of this form was available for examination for a long period, an individual from the Cave of Sahaur, Oman (Pocock, 1935), Harrison (1957, 1964), Kock (1969) and Harrison and Bates (1991) considered this bat to be an extremely small representative of *A. tridens*. No available name exists within the synonymy of *Asellia* bats for this population and here we describe this population as a new species (see below).

To conclude, the taxonomy of *Asellia* is more complex than suggested by previous authors, as reviewed by Simmons (2005). Instead of two recognised species, we suggest the existence of four species within the genus; however, the largest part of the distribution range is inhabited by only one species, *A. tridens*. A trinity of smaller-sized species, *A. patrizii*, *A. italosomalica*, and *Asellia* sp. nov., occur in their restricted distribution ranges at the Afrotropic/Arabian transition.

Taxonomic Description

Asellia arabica sp. nov.

Synonymy

Asellia tridens (Geoffroy, 1813): Pockock, 1935: 442; Harrison, 1964: 98; Harrison, 1980: 390; Kingdon, 1990: 37; Harrison and Bates, 1991: 55.

Asellia tridens tridens (Geoffroy, 1813): Harrison, 1957: 5; Kock, 1969: 129; Nader, 1990: 340; Al-Jumaily, 1998: 483.

Asellia patrizii De Beaux, 1931: Al-Jumaily, 2004: 60.

Type material

Holotype: adult ♂ (NMP 92790 [S+A]), Hawf (Al Mahra Prov.), 14 October 2005, leg. P. Benda.

Paratypes (9): 3♂♂, 2♀♀ (NMP 92791, 92792–92794 [S+A], 92789 [A]), Hawf (Al Mahra Prov.), 14 and 15 October 2005, leg. P. Benda; – 2♂♂, 2♀♀ (NMP 92795, 92796, 92798 [S+A], 92797 [A]), Damqawt (Al Mahra Prov.), 16 October 2005, leg. P. Benda.

Type locality

Republic of Yemen, Province of Al Mahra, oasis of Hawf (easternmost edge of the country), 16°39'N, 53°03'E, 410 m a.s.l.

Description and diagnosis

Small bat and medium-sized to small representative of the genus *Asellia* Gray, 1838. It is in most

Fig. 10. Portrait of *A. arabica* sp. nov. from Ain Jarziz, Dhofar, SW Oman (photo by A. Reiter)

TABLE 3. Forearm and skull dimensions (in mm) of the examined holotype specimens of *Asellia*. See Abbreviations and Terminology for explanation of dimension abbreviations

Parameter	*tridens* MNHN 1986-1068	*diluta* BMNH 12.11.14.2.	*patrizii* MSNG 31313	*italosomalica* MSNG 30942	*arabica* sp. n. NMP 92790
LAt	49.10	52.40	39.00	43.40	46.50
LCr	18.47	18.76	15.02	17.08	16.81
LOc	–	18.48	14.88	16.97	16.52
LCc	16.33	16.43	13.24	14.85	14.71
LaZ	–	10.28	8.12	9.12	8.87
LaI	2.12	2.16	1.78	2.37	2.17
LaInf	6.60	5.66	4.80	5.52	5.19
LaN	7.48	7.08	6.18	7.02	6.69
LaM	–	8.54	7.43	8.08	7.86
ANc	5.60	5.81	4.53	5.08	4.82
ACr	6.97	7.48	6.15	–	6.43
LBT	–	2.98	2.62	–	2.81
CC	5.42	5.06	3.94	5.06	4.62
PP	6.38	6.32	4.68	5.83	5.39
M^3M^3	7.50	7.47	5.54	6.62	6.53
CM^3	6.83	6.84	5.33	6.19	6.02
M^1M^3	4.16	4.23	3.25	3.73	3.61
CP	3.03	2.89	2.17	2.67	2.51
LMd	–	12.33	9.89	11.49	10.93
ACo	–	3.67	2.81	3.57	3.34
I_1M_3	–	–	6.49	7.67	7.14
CM_3	–	7.42	5.73	6.82	6.51
M_1M_3	–	4.95	3.75	4.52	4.09
CP_4	–	2.66	2.46	2.82	2.34

respects very similar to the other species of the genus, including the structure of the nose leaf (Fig. 11). In body and skull size, *A. arabica* sp. nov. clearly differs from both the largest and the smallest species of the genus, *A. tridens* (Geoffroy, 1813) and *A. patrizii* De Beaux, 1931, respectively, but it partly overlaps with *A. italosomalica* De Beaux, 1931 (Table 1, Figs. 2 and 4). Forearm length 43.1–46.5 mm, condylocanine length of skull 14.2–15.3 mm, length of the upper tooth-row 5.7–6.1 mm. *Asellia arabica* sp. nov. has a relatively narrow skull (width across the zygomatic arches 8.7–9.3 mm; relative zygomatic width, LaZ/LCc, 0.589–0.622) and a relatively narrow and short rostrum (width of rostrum across canines 4.4–4.8 mm; relative width of rostrum across canines, CC/LCc, 0.297–0.324) in comparison to the other *Asellia* species; the skull and rostrum of *A. arabica* sp. nov. are, on average, the narrowest, and the rostrum is the shortest, relatively speaking. The braincase is low (height of braincase 4.7–5.1 mm; relative height of braincase, ANc/LCc, 0.320–0.342) and the tympanic bullae are rather large (largest horizontal diameter of bulla 2.6–2.9 mm; relative size of bulla, LBT/LCc 0.181–0.214).

The baculum of *A. arabica* sp. nov. is a tiny bone, 1.0–1.3 mm long, with a very narrow distal epiphysis, 0.1–0.2 mm wide (< 18% of the baculum length) and a rather narrow proximal epiphysis that is 0.3–0.4 mm wide (< 30% of the baculum length) (Fig. 6l–n).

The colouration of the dorsal pelage of *A. arabica* sp. nov. is beige or pale-brownish grey (Fig. 11) with yellowish or pale rusty tinges; the ventral pelage is somewhat paler than the dorsal colouration. The nose leaf is almost unpigmented to very pale brownish; often the anterior leaf is slightly pigmented while the posterior leaf and supplementary leaflets are unpigmented. The wing membranes are pale brownish-grey.

Dimensions of the holotype: see Table 3.

Genetics: *Asellia arabica* sp. nov. showed unique base positions within the mitochondrial gene for cytochrome *b* (1140 bp) at 24 sites: 486, 636, 681, 696, 750 (A→G), 201, 507 (C→A), 1092 (C→G), 145, 282, 492, 504, 690, 915, 958, 963, 1020, 1044 (C→T), 981, 1080, 1093 (T→C), 510, 717 (A/T→C), and 498 (G/T→C). At two sites, 324 and 609, uniquely different positions of G instead of A (A→G, A→A/G, and A→G/A) were present

in three known haplotypes (DF1, DF2, DF3; see Appendix II). *Asellia arabica* sp. nov. was found to share identical unique base positions within the gene for cytochrome *b* with *A. tridens* (Geoffroy, 1813) at 88 sites: 18, 27, 105, 153, 237, 330, 357, 466, 582, 697, 709, 720, 807, 870, 933, 936, 945, 996, 1038, 1057, 1119 (A), 45, 57, 81, 84, 114, 165, 195, 204, 297, 315, 321, 329, 435, 438, 465, 477, 480, 519, 546, 564, 567, 589, 592, 618, 621, 624, 648, 687, 693, 699, 708, 713, 774, 789, 804, 831, 834, 840, 852, 858, 882, 888, 894, 916, 1126 (C), 129, 178, 372, 476, 591 (G), 66, 121, 126, 246, 285, 288, 345, 351, 399, 474, 528, 792, 897, 906, 999, 1077, and 1122 (T); and with *A. italosomalica* De Beaux, 1931 at 16 sites: 123, 864, 909, 984, 1023, 1116 (A), 174, 441, 447, 555, 663, 921, 1110 (C), 1017 (G), 684, and 828 (T).

Mitochondrial sequence of holotype and all examined paratypes (NMP 92789–92791, 92793, 92794, 92796–92798) — complete sequence of the mitochondrial gene for cytochrome *b* (GenBank Accession Number JF439015; haplotype DF1 — Appendix II), 5' end: atg acc aac atc cga aaa tcc cac cca cta ttc aaa att atc aac gac tca ttc atc gac cta cct gcc ccc tca agc atc tcc tcc tga tga aac ttt ggc tca cta cta ggc gta tgc tta gct gtg caa atc cta aca gga tta ttc cta gcc ata cac tac aca tcc gac aca gcc acc gcc ttc tat tcc gtc aca cac atc tgc cga gac gtt aat tat ggc tga atc cta cgc tac ctt cat gcc aac gga gca tcc ata ttc ttc atc tgc ctt ttt ctt cat gta ggc cga gga att tac tac ggc tcc tac aca ttc aca gaa aca tga aac att ggt att att cta ctt ttc gcc gtc atg gca aca gcc ttc ata ggc tac gtc ctt cca tga gga caa ata tcc ttc tga gga gca aca gtc atc acc aac ctc ctc tca gcc atc cca tac atc gga act agc ctc gta gag tga gtt tga ggc ggc ttt tca gtc gac aaa gcc acc ctc act cga ttc ttc gcc ctc cac ttc ctc ctc cca ttc atc atc gca gcc ata gta ata gtc cac ctg cta ttc cta cat gaa acg ggc tca aac aac ccc aca gga atc ccg tca gac ata gac ata atc ccc ttc cac cca tac tac acc atc aag gat atc ctt ggc ctg atc cta ata atc ata gca ctg cta tcc cta gtc cta ttc gca cca gac ctc ctg gga gac cca gac aac tac act ccc gca aac cca ctc aac act cca ccc cat atc aaa cca gag tga tat ttc cta ttt gcc tac gcc atc cta cgg tca atc cca aac aaa cta gga gga gta gta gcc ctc gtc ctc tca atc ctt atc cta gct gta atc cct cta ctc cac aca tca aaa caa cgc agc ata acc ttc cgc cca tta agt caa tgc tta ttc tga ctc cta gtc gcc gac cta ctt aca cta aca tga atc ggg ggt caa cct gta gaa cac cca ttc att att atc gga caa ata gcc tca atc cta tac ttt ctc atc atc cta gtg ctc ctc cca ctc gca agc atc gca gaa aat cac cta tta aaa tga aga.

Derivatio nominis

The name *arabica* (Arabian) reflects the area of occurrence of the new species, i.e. the southern part of Arabia.

Distribution

Coastal areas of southern Arabia stretching from the Hadramaut Province in south-eastern Yemen (ca. 49°E) to the Dhofar Province in south-western Oman (ca. 55°E), i.e., ca. 650 km of a narrow coastal strip.

Acknowledgements

We thank Paolo Agnelli (MZUF), Masaa Al-Jumaily and Abdul Karim Nasher (BCSU), Cécile Callou, Allowen Evin and Ronan Kirch (MNHN), Giuliano Doria (MSNG), Rainer Hutterer (ZFMK), Paula Jenkins and Louise Tomsett (BMNH), Dieter Kock and Gerhard Storch (SMF), Petr Koubek (IVB), Ján Obuch (JOC), Friederike Spitzenberger and Barbara Herzig-Straschil (NMW), and Bronisław W. Wołoszyn (ISEA) for providing us the access to the museum specimens under their care. We acknowledge grant supports of the Czech Science Foundation (# 206/09/0888) and Ministry of Culture of the Czech Republic (## MK00002327201, DE06P04OMG008).

Literature Cited

Al-Jumaily, M. M. 1998. Review of the mammals of the Republic of Yemen. Fauna of Arabia, 17: 477–502.

Al-Jumaily, M. M. 2004. Recent records of bats from Yemen including *Plecotus* cf. *austriacus* (Fischer, 1829) (Mammalia: Vespertilionidae) new to the country. Myotis, 41–42: 57–68.

Allen, G. M. 1939. A checklist of African mammals. Bulletin of the Museum of Comparative Zoölogy at Harvard College, 83: 1–763.

Anciaux de Faveaux, F. 1973. Distribution des chiropteres en Algerie, avec motes ecologiques et parasitologiques. Bulletin de la Société d'Histoire Naturelle d'Afrique du Nord, 67: 69–80.

Andersen, K. 1918. Diagnoses of new bats of the families Rhinolophidae and Megadermatidae. The Annals and Magazine of Natural History, Series 9, 2: 374–384.

Anderson, J. 1881. Catalogue of Mammalia in the Indian Museum, Calcutta. Part I. Primates, Prosimiae, Chiroptera, and Insectivora. Trustees of the Indian Museum, Calcutta, 223 pp.

Baker, R. J., and R. D. Bradley. 2006. Speciation in mammals and the genetic species concept. Journal of Mammalogy, 87: 643–662.

Bandelt, H.-J., P. Forster, and A. Röhl. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution, 16: 37–48.

Bates, P. J. J., and D. L. Harrison. 1997. Bats of the Indian Subcontinent. Harrison Zoological Museum, Sevenoaks, 258 pp.

Benda, P., A. Kiefer, V. Hanák, and M. Veith. 2004. Systematic status of African populations of long-eared bats, genus *Plecotus* (Mammalia: Chiroptera). Folia Zoologica, 53 (Monograph 1): 1–47.

Benda, P., M. Andreas, D. Kock, R. K. Lučan, P. Munclinger, P. Nová, J. Obuch, K. Ochman, A. Reiter, M. Uhrin, and D. Weinfurtová. 2006. Bats (Mammalia: Chiroptera) of the Eastern Mediterranean. Part 4. Bat fauna of Syria: distribution, systematics, ecology. Acta Societatis Zoologicae Bohemicae, 70: 1–329.

Beydoun, Z. R., and H. R. Bichan. 1970. The geology of Socotra Island, Gulf of Aden. Quarterly Journal of the Geological Society, London, 125: 413–446.

Borisenko, A. V., and I. Ya. Pavlinov. 1995. Podotryad Microchiroptera [Sub-Order Microchiroptera]. Pp. 72–120, *in* Mlekopitayushchie Evrazii. II. Non-Rodentia. Sistematiko-geograficheskiy spravochnik [Mammals of Eurasia. II. Non-Rodentia. Systematical-geographical review] (I. Ya. Pavlinov, A. V. Borisenko, S. V. Kruskop, and E. L. Yahontov, eds.). Sbornik Trudov Zoologicheskogo Muzeya MGU, 23: 1–334. [in Russian].

Bradley, R. D., and R. J. Baker. 2001. A test of the genetic species concept: cytochrome-*b* sequences and mammals. Journal of Mammalogy, 82: 960–973.

Cesarini, D. 2007. Observations on the Chiroptera of Socotra Island, Republic of Yemen. Atti della Società italiana di Scienze naturali e del Museo Civico di Storia naturale di Milano, 148: 135–147.

Corbet, G. B. 1978. The mammals of the Palaearctic Region: a taxonomic review. British Museum (Natural History), London, 314 pp.

De Beaux, O. 1931. Spedizione del barone Raimondo Franchetti in Dancalia. Mammiferi. Annali del Museo Civico di Storia Naturale di Genova, 55: 183–217.

DeBlase, A. F. 1980. The bats of Iran: systematics, distribution, ecology. Fieldiana: Zoology (N.S.), 4: xvii + 424 pp.

deMenocal, P. B. 2004. African climate change and faunal evolution during the Pliocene–Pleistocene. Earth and Planetary Science Letters, 220: 3–24.

Ellerman, J. R., and T. C. S. Morrison-Scott. 1951. Checklist of Palaearctic and Indian Mammals 1758 to 1946. British Museum (Natural History), London, 810 pp.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. Journal of Molecular Evolution, 17: 368–376.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution, 39: 783–791.

Fleitmann, D., A. Matter, S. J. Burns, A. Al-Subbary, and M. A. Al-Aowah. 2004. Geology and Quaternary climate history of Socotra. Fauna of Arabia, 20: 27–43.

Gaisler, J., G. Madkour, and J. Pelikán. 1972. On the bats (Chiroptera) of Egypt. Acta Scientiarum Naturalium Academiae Scientiarum Bohemoslovacae Brno (NS), 6(8): 1–40.

Geoffroy Saint-Hilaire, E. 1813. Sur une genre de chauves-souris sous le nom de Rhinolophes. Annales du Museum d'Histoire Naturelle, Paris, 20: 254–266.

Gray, J. E. 1838. A revision of the genera of bats (Vespertilionidae), and the description of some new genera and species. Magazine of Zoology and Botany, 2: 483–505.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series, 41: 95–98.

Harrison, D. L. 1956. A key to the identification of the bats (Chiroptera) of the Arabian peninsula. Proceedings of the Zoological Society of London, 127: 447–452.

Harrison, D. L. 1957. Some systematic notes on the trident bats (*Asellia tridens* E. Geoffroy) of Arabia. Mammalia, 21: 1–8.

Harrison, D. L. 1964. The mammals of Arabia. Volume I. Introduction, Insectivora, Chiroptera, Primates. Ernest Benn Ltd., London, 192 pp.

Harrison, D. L. 1965. Israel South Red Sea Expedition, 1962, reports No. 5. Remarks on some trident leaf-nosed bats, (genus *Asellia* Gray, 1838), obtained by the Israel South Red Sea Expedition, 1962. Sea Fisheries Research Station Haifa, Bulletin, 38: 3–5.

Harrison, D. L. 1980. The mammals obtained in Dhofar by the 1977 Oman Flora and Fauna Survey. Journal of Oman Studies, Special Report, 2: 387–397.

Harrison, D. L., and P. J. J. Bates. 1991. The mammals of Arabia, 2nd edition. Harrison Zoological Museum, Sevenoaks, 354 pp.

Hayman, R. W., and J. E. Hill. 1971. Part 2. Order Chiroptera. Pp. 1–73, *in* The mammals of Africa. An identification manual (J. Meester and H. W. Setzer, eds.). Smithsonian Institution Press, Washington D.C., 73 pp.

Hill, J. E., and P. Morris. 1971. Bats from Ethiopia collected by the Great Abbai Expedition 1968. Bulletin of the British Museum (Natural History), Zoology, 21: 27–49.

Horáček, I., V. Hanák, and J. Gaisler. 2000. Bats of the Palearctic region: a taxonomic and biogeographic review. Pp. 11–157, *in* Proceedings of the VIIIth European Bat Research Symposium. Volume I. Approaches to biogeography and ecology of bats (B. W. Wołoszyn, ed.). Chiropterological Information Center, Institute of Systematics and Evolution of Animals PAS, Kraków, 280 pp.

Huang, X. Q. 1992. A contig assembly program based on sensitive detection of fragment overlaps. Genomics, 14: 18–25.

Huelsenbeck, J. P., and K. A. Crandall. 1997. Phylogeny estimation and hypothesis testing using maximum likelihood. Annual Review in Ecology and Systematics, 28: 437–466.

Hulva, P., I. Horáček, and P. Benda. 2007. Molecules, morphometrics and new fossils provide an integrated view of the evolutionary history of Rhinopomatidae (Mammalia: Chiroptera). BMC Evolutionary Biology, 7: 165.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 16: 111–120.

Kingdon, J. 1990. Arabian mammals. A natural history. Academic Press, London, vii + 279 pp.

Koch-Weser, S. 1984. Fledermäuse aus Obervolta, W-Afrika (Mammalia: Chiroptera). Senckenbergiana Biologica, 64: 255–311.

Kock, D. 1969. Die Fledermaus-Fauna des Sudan (Mammalia, Chiroptera). Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft, 521: 1–238.

Kock, D., L. Barnett, J. Fahr, and C. Emms. 2002. On a collection of bats (Mammalia: Chiroptera) from The Gambia. Acta Chiropterologica, 4: 77–97.

Koopman, K. F. 1975. Bats of the Sudan. Bulletin of the American Museum of Natural History, 154: 353–443.

Koopman, K. F. 1993. Order Chiroptera. Pp. 137–241, *in* Mammal species of the World. A taxonomic and geographic reference (D. E. Wilson and D. M. Reeder, eds.). Smithsonian Institution Press, Washington D.C., 1206 pp.

Koopman, K. F. 1994. Chiroptera: Systematics. Pp. 1–217, *in* Handbuch der Zoologie. Band VIII. Mammalia. Teilband 60 (J. Niethammer, H. Schliemann, and D. Starck, eds.). Walter de Gruyter, Berlin and New York, vii + 224 pp.

Kowalski, K., and B. Rzebik-Kowalska. 1991. Mammals of Algeria. Zakład Narodowy im. Ossolińskich — Wydawnictwo, Kraków, 370 pp.

Largen, M. J., D. Kock, and D. W. Yalden. 1974. Catalogue of the mammals of Ethiopia. 1. Chiroptera. Monitore Zoologico Italiano (N.S.), 16: 221–298.

Laurent, P. 1937. Une forme nouvelle du genre „*Asellia*: *A. tridens pallida*" subsp. nov. Mammalia, 1: 111–116.

Laurent, P. 1942. La chauve souris trident, d'Etienne Geoffroy Saint Hilaire (*Asellia tridens* E. Geofroy 1813), description complémentaire. Bulletin du Muséum National d'Historie Naturelle, 2e Série, 14: 43–48.

Legendre, S. 1982. Hipposideridae (Mammalia: Chiroptera) from the Mediterranean Middle and Late Neogene, and evolution of the genera *Hipposideros* and *Asellia*. Journal of Vertebrate Paleontology, 2: 372–385.

Meister, J., N. Kilian, and C. Oberprieler. 2007. Genetic structure of *Euclea schimperi* (Ebenaceae) populations in monsoonal fog oases of the southern Arabian Peninsula. Nordic Journal of Botany, 25: 217–226.

Moeschler, P., I. A. Nader, and P. Gaucher. 1990. First record of *Asellia patrizii* De Beaux, 1931 (Chiroptera: Hipposideridae) in Saudi Arabia. Mammalia, 54: 654–655.

Nader, I. A. 1990. Checklist of the mammals of Arabia. Fauna of Saudi Arabia, 11: 329–381.

Nader, I. A., and D. Kock. 1983. Notes on some bats from the Near East (Mammalia: Chiroptera). Zeitschrift für Säugetierkunde, 48: 1–9.

Owen, R. D., and M. B. Qumsiyeh. 1987. The subspecies problem in the Trident leaf-nosed bat, *Asellia tridens*: homomorphism in widely separated populations. Zeitschrift für Säugetierkunde, 52: 329–337.

Palumbi, S. R. 1989. Rates of molecular evolution and the function of nucleotide positions free to vary. Journal of Molecular Evolution, 29: 180–187.

Pocock, R. I. 1935. The mammals collected in S.E. Arabia by Mr. Bertram Thomas and Mr. H. St. J. Philby. The Annals and Magazine of Natural History, Series 10, 15: 441–467.

Posada, D., and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics, 14: 817–818.

Posada, D., and K. A. Crandall. 2001. Intraspecific gene genealogies: trees grafting into networks. Trends in Ecology and Evolution, 16: 37–45.

Qumsiyeh, M. B. 1985. The bats of Egypt. Special Publications, Museum of Texas Tech University, 23: 7–102.

Rambaut, A., and N. C. Grassly. 1997. Seq-Gen: an application for the Monte Carlo simulation of DNA sequence evolution along phylogenetic trees. Computer Applied Bioscience, 13: 235–238.

Remy, J. A., J.-Y. Crochet, B. Sigé, J. Sudre, L. De Bonis, M. Vianey-Liaud, M. Godinot, J.-L. Hartenberger, B. Lange-Badré, and B. Comte. 1987. Biochronologie des phosphorites du Quercy: mise à jour des listes faunistiques en nouveaux gisements de mammiferes fossiles. Münchener Geowissenschaftliche Abhandlungen, Reihe A, Geologie und Palaeontologie, 10: 169–188.

Ronquist, F. R., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics, 19: 1572–1574.

Sakai, T., Y. Kikkawa, K. Tsuchiya, M. Harada, M. Kanoe, M. Yoshiyuki, and H. Yonekawa. 2003. Molecular phylogeny of Japanese Rhinolophidae based on variations in the complete sequence of the mitochondrial cytochrome *b* gene. Genes and Genetic Systems, 78: 179–189.

Samuel, M. A., N. Harbury, R. Bott, and A. M. Thabet. 1997. Field observations from the Socotran platform: their interpretation and correlation to Southern Oman. Marine Petrology and Geology, 4: 661–673.

Simmons, N. B. 2005. Order Chiroptera. Pp. 312–529, *in* Mammal species of the World: a taxonomic and geographic reference, 3rd edition (D. E. Wilson and D. M. Reeder, eds.). The Johns Hopkins University Press, Baltimore, xxxviii + 743 pp.

Tate, G. H. H. 1941. Results of the Archbold Expeditions. No. 36. Remarks on some Old World leaf-nosed bats. American Museum Novitates, 1140: 1–11.

Tavaré, S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. American Mathematical Society: Lectures on Mathematics in the Life Sciences, 17: 57–86.

Wranik, W., A. Feiler, and N. Obadi. 1991. Notes on some bats from the southern part of Yemen (Mammalia, Chiroptera). Zoologische Abhandlungen, Staatliches Museum für Tierkunde Dresden, 46: 207–213.

Received 22 July 2010, accepted 23 March 2011

Appendix I

List od the specimens examined in morphological analysis

Asellia arabica sp. nov. — **Oman** (6): 2 ♂♂, 2 ♀♀ (NMP 92721–92724 [S+A]), Ain Jarziz (Dhofar Prov.), 27 October 2009, leg. P. Benda, A. Reiter and M. Uhrin; – 1 ♀ (NMP 92753 [A]), Ain Tabruq (Dhofar Prov.), 29 October 2009, leg. A. Reiter; – 1 ind. (BMNH 34.8.4.1 [S+B]), Cave of Sahaur, Qara Mts., SE Arabia, date unlisted, leg. B. S. Thomas. – **Yemen** (14): 2 ♂♂, 2 ♀♀ (NMP 92795, 92796, 92798 [S+A], 92797 [A]), Damqawt (Al Mahra Prov.), 16 October 2005, leg. P. Benda; – 4 ♂♂, 2 ♀♀ (NMP 92790, 92791, 92792–92794 [S+A], 92789 [A]), Hawf (Al Mahra Prov.), 14 and 15 October 2005, leg. P. Benda; – 1 ♂ (BCSU 013 [B]), Ryan (Hadramaut Prov.), July 1995, leg. A. Basmaidi; – 2 ♂♂, 1 ♀ (BCSU 128, 136, 195 [S+B]), Shikhawi (Hadramaut Prov.), 12 March 2000, leg. A. K. Nasher.

Asellia italosomalica De Beaux, 1931 — **Somalia** (16): 1 ♀ (MSNG 32582 [S+B]), Bender-Cassim, January–February 1932, leg. I. Zanetti; – 1 ♀ (MZUF 8277 [S+A]), Callis, 20 October 1973, leg. Granchi and B. Lanza; – 2 ♂♂, 2 ♀♀ (MSNG 12232a–d [S+B], incl. the paratype of *A. tridens italosomalica* De Beaux, 1931), Dolo, May–July 1911, leg. C. Citerni; – 2 ♂♂ (MZUF 9940, 9941 [S+A]), Mahas, 12 February 1977, leg. A. Simonetta; – 1 ♀ (MSNG 30942 [S+B], holotype of *A. tridens italosomalica* De Beaux, 1931), Oddur, 1929, leg. N. Mosconi Bronzi; – 1 ♀ (MZUF 9942 [S+A]), Pozzi di

Mahas, 11 April 1977, leg. A. Simonetta; – 1 ♂, 1 ♀ (MZUF 6291 [S+B], 6305 [S+A]), Run, 15 and 18 August 1969, leg. B. Lanza; – 2 ♂♂, 2 ♀♀ (MZUF 13099, 13100 [S+A], 15728, 15734 [S+A]), Showli Berdi, 15 March 1984 and 15 November 1985, leg. L. Chellazi and Messana. – **Yemen**, **Socotra** (15): 10 ♀♀ (BCSU pb2718, 2719, 2721, BMNH 54.1010., NMP 90571–90575 [S+A], BCSU pb2720, NMP 90570 [A]), Kam, date unlisted, leg. G. B. Popov, 5 May 2004, leg. P. Benda and A. Reiter; – 4 ♂♂ (BCSU pb2749, NMP 90590, 90592 [S+A], NMP 90591 [A]), Mazaaba, 14 May 2004, leg. P. Benda and A. Reiter; – 1 ♂ (NMP 90579 [S+A]), Suq, 7 May 2004, leg. P. Benda and A. Reiter.

Asellia patrizii De Beaux, 1931 — **Eritrea** (8): 3 ♂♂, 1 ♀ (MSNG 33203, 33204 [S+B], 31315a, 31315b [S+A], incl. three paratypes of *A. patrizii* De Beaux, 1931), Assab, July 1893, leg. G. Pestalozza, October 1906, leg. P. Felter; – 1 ♂, 1 ♀ (MSNG 31313, 31314 [S+A], incl. the holotype of *A. patrizii* De Beaux, 1931), Gaarre, Dancalia, December 1928, leg. S. Patrizi; – 2 ♀♀ (BMNH 70.2288., 70.2289. [S]), Nocra Island, Dahlak Islands, 27 December 1969, leg. M. J. Largen. – **Ethiopia** (8): 1 ♂, 1 ♀ (SMF 44998, 44999 [S]), "an der Bahnlinie westl. zw. Metahara und Lake Basaka, Awash River Gebiet", 11 September 1973, leg. H. Rupp; – 1 ♀ (SMF 52377 [S+B]), W of Metahara, Shoa, 28 December 1970, ded. M. Largen; – 2 ♂♂ (MZUF 6546, 6547 [S+B]), Awash NP, 4.5 km from Metahara, 12 April 1971, leg. M. L. Azzaroli and B. Lanza; – 1 ♂, 1 ♀ (MZUF 6545 [A], 6548 [S+A]), Dint. Metahara, Mte. Fantalle, 14 April 1971, leg. M. L. Azzaroli and B. Lanza; – 1 ♂ (BMNH 70.475. [S]), North bank of river, Awash River Valley, Awash NP, Shoa, 25 September 1968, leg. P. Morris.

Asellia tridens (Geoffroy, 1813) — **Afghanistan** (10): 9 inds. (ZFMK 96.022, 96.023 [S]), cave nr Kuh-i-Duzd, nr Dilaram, 32°11'N, 63°27'E, 1965, leg. J. Niethammer and D. Meyer-Oehme; – 1 ♀ (SMF 39167 [S]), Ebn-e-Yamin, Farah, 2 September 1965, leg. D. Meyer-Oehme. – **Algeria** (20): 9 ♂♂, 2 ♀♀ (ISEA 9523, 9524, 9527, 9528, 9530, 9532, 9533, IVB A477 [S+B], ISEA 9534 [S], 9526, 9531 [B]), Ain Ouarka, 20 May 1982, leg. K. Kowalski, B. Rzebik-Kowalska and J. Gaisler; – 2 ♂♂, 1 ♀ (ISEA 9522, 9534, 9535 [S+B]), Benni Abbes, 15 May 1981, 19 July 1983, leg. K. Kowalski and B. Rzebik-Kowalska; – 1 ind. (BMNH 94.7.12.1. [S]), Biskra, date and collector unlisted; – 1 ♂ (ISEA 9536 [S+B]), Brezina, 23 July 1983, leg. K. Kowalski and B. Rzebik-Kowalska; – 3 ♂♂, 1 ♀ (BMNH 12.11.14.1., 12.11.14.2., MSNG 33629a, b [S+B], incl. the holotype of *A. tridens diluta* Andersen, 1918), El Golea, 16 May 1912, leg. W. Rotschild and E. Hartert, April 1938, leg. P. Laurent. – **Egypt** (47): 2 ♀♀ (MSNG 33202a, b [S+B]), Abu Simbel, 27 January 1904, leg. E. A. D'Albertis; – 8 ♂♂, 2 ♀♀ (NMP 92585–92592 [S+A], 92593, 92594 [A]), Aswan, 24 January 2010, leg. P. Benda, I. Horáček and R. Lučan; – 2 ♂♂ (IVB 22, 27 [S+B]), Dandara, temple, 27 April 1969, leg. J. Gaisler; – 1 ♂ (MNHN 1986-1068 [S+B]), Egypte [= Thebes], leg. E. Geoffroy Saint-Hilaire (lectotype of *Rhinolophus tridens* Geoffroy, 1813); – 4 ♂♂, 2 ♀♀ (MSNG 33201a–d, 6994, 6995 [S+B]), Garbu Suan, in faccia ad Assuan, February 1907, leg. E. A. D'Albertis; – 4 ♂♂ (MSNG 33200a–d [S+B]), Sakkarah (Cairo), May 1906, leg. F. W. Innes Bey; – 3 inds. (NMP 90351, 90352, 90354 [S+A]), Shali, Siwa oasis, 12 April 2002, leg. P. Munclinger and P. Nová; – 17 ♂♂, 2 inds. (IVB 30, 31, 33–36, 38–43, 45–51 [S+B]), Thebes, tombs, 30 April 1969, leg. J. Gaisler. – **Ethiopia**: 3 inds. (BMNH 70.472. –70.474. [S]), from cave floor, Awash NP, Shoa, 28 September 1968, leg. P. Morris. – **Gambia** (3): 1 ♂ (SMF 82480 [S]), Georgetown [=Janjangbureh], MacCarthy Island, 20 April 1995, leg. D. Kock; 1 ♂ (SMF 92154 [S]), Janjangbureh, 22 January 2001, leg. L. Barnett and C. Emms; – 1 ♀ (BMNH 49.476. [S+B]), 10 mi SE of Kontaur, 24 August 1948, leg. A. P. Buxton. – **Iran** (26): 5 ♂♂, 10 ♀♀, 6 inds. (NMP 48173–48187 [S+A], JOC unnumbered [S+Sk]), Choqa Zanbil, 15 October 1998, leg. P. Benda, J. Obuch and M. Uhrin; – 1 ♂ (BMNH 77.824. [S+B]), Chehar-Zanbil, Dezful, SW Iran, 19 May 1964, leg. R. Szbokar; – 1 ind. (BMNH 77.827. [S+B]), Iranshahr, Baluchistan, 12 March 1975, ded. E. Etemad; – 1 ♂ (BMNH 77.826. [S+B]), Kahnuge, S of Kerman, 3 March 1971, ded. E. Etemad; – 1 ♀ (BMNH 77.825. [S+B]), Rudan Minab, SW of Iranshah, South coast of Iran, 10 March 1966, leg. E. Etemad; – 1 ind. (BMNH 6.1.2.1. [S]), Seistan, date and collector unlisted. – **Iraq** (8): 8 ♀♀ (NMW 21994, 21996–22002 [S+A]), Mosul, 5 December 1910, leg. S. Hassoun. – **Israel** (1): 1 ♀ (SMF 18987 [S]), Jaffa, ded. Z. Lev. – **Libya** (4): 1 ♀ (NMP 48317 [S+A]), Ghat, 3 October 1999, leg. P. Benda; – 1 ind. (MSNG 54783 [A]), Serdeles (Tripolitania), March 1934, leg. G. Scortecci; – 1 ♀ (MSNG 32180 [S+B]), Ubari, October 1932, leg. L. Cipriani; – 1 ♀ (MSNG 33222 [S+B]), Zuila, September 1933, leg. E. Zavattari. – **Mauritania** (9): 1 ♀ (MZUF 20732 [A]), Ouadane, 23 November 2002, leg. P. Agnelli; – 1 ♂ (NMP 93647 [S+A]), Tarjit, 21 October 2010, leg. P. Benda, A. Reiter and M. Uhrin; – 7 ♀♀ (NMP 93639–93641, 93643–93645 [S+A], 93642 [A]), Tin Labbé, 19 October 2010, leg. P. Benda, A. Reiter and M. Uhrin. – **Morocco** (3): 3 ♀♀ (NMP pb3842–3844 [S+A]), Tassetift, 22 April 2008, leg. P. Benda, J. Červený, A. Konečný and P. Vallo. – **Oman** (21): 2 ♂♂, 1 ♀ (NMP 92627, 92628 [S+A], 92629 [A]), Al Ghubrah, 18 October 2009, leg. P. Benda, A. Reiter and M. Uhrin; – 2 ♀♀ (NMP 92761, 92762 [S+A]), Al Mintirib, 1 November 2009, leg. P. Benda, A. Reiter and M. Uhrin; – 4 ♂♂ (NMP 90770–92772 [S+A], 92773 [A]), Muqal, 1 November 2009, leg. P. Benda, A. Reiter and M. Uhrin; – 5 ♂♂, 7 ♀♀ (NMP 92683–92692, 92694 [S+A], 92693 [A]), Shisr, 24 October 2009, leg. P. Benda, A. Reiter and M. Uhrin. – **Pakistan** (6): 1 ind. (BMNH 85.8.1.368. [S+B]), Mekran Coast, date unlisted, leg. Capt. C. J. Bringham; – 1 ♂ (BMNH 25.7.3.1. [S+B]), Nawab Issakhans Tomb, Tatta, Sind, 22 October 1922, leg. C. McCann; – 4 ♀♀ (BMNH 19.11.7.7.–19.11.7.10. [S+B]), Panjgur, Baluchistan, 7–13 June 1918, leg. E. J. Hotson.– **Saudi Arabia** (8): 2 ♂♂, 1 ♀ (BMNH 25.4.3.1.–25.4.3.3. [S+B]), Hufuf, C. Arabia, 4–12 December 1923, leg. R. E. Cheesman; – 1 ♂ (BMNH 1950.33. [S+B]), Jedda, 20 October 1949, leg. A. C. Trott; – 4 ♀♀ (SMF 80445–80448 [S]), Massullamiyah Cliff, N of al-Jubail, al-Sinaiyah, Eastern Prov., 28 May 1992, D. Kock and I. A. Nader. – **Senegal** (4): 3 ♂♂, 1 ♀ (ZFMK 76.29–76.31 [S+B], 76.28 [B]), Tatki, 20 km E Fété-Olé, 21 November 1975, leg. W. Boehme *et al*. – **Sudan** (33): 2 ♂♂, 2 ♀♀ (ZFMK 97.001–97.003, 97.005 [S+Sk+B]), Djebel, ca. 30 km NW of Khartoum, 18 March 1986, leg. J. Niethammer; – 2 ♂♂, 7 ♀♀, 6 inds. (IVB 1–9 [S+B], NMP 90344–90349 [S+A]), Jebel el Azraq, 18 February 1966, leg. P. Štys; – 4 ♂♂ (NMP 93664–93666 [S+A], 93667 [A]), Dongola Alajuz, 7 December 2010, leg. P. Benda and J. Šmíd; – 3 ♂♂, 3 ♀♀ (NMP 93651–93653, 93655–93657 [S+A]), Karima, Jebel Barkal, 6 December 2010, leg. P. Benda and J. Šmíd; – 1 ♂ (NMP 93680 [S+A]), Masoud, Bayudah Desert, 13 December 2010, leg. P. Benda and J. Šmíd; – 1 ♂, 2 ♀♀ (NMP 93670, 93671 [S+A], 93672 [A]), Quikkah, 9 December 2010, leg. P. Benda and J. Šmíd. – **Syria** (44): 1 ♂, 15 ♀♀ (NMP 48019–48021, 48024–48027 [S+A], 48022, 48023 [S+B], SMF 55478 [S+A], 74105–74110 [S+Sk+B]), Halabiyyeh, 15–17 August 1978, leg. R. Kinzelbach, 1 June 1989, leg. D. Kock, 17 June 1998, leg. M. Andreas, P. Benda and M. Uhrin; – 1 ♂ (NMP 48813 [S+A]), Qala'at ar Rahba, 17 May 2001, leg. M. Andreas, P. Benda, A. Reiter and D. Weinfurtová; – 27 ♂♂ (SMF 60365, 73414–73418, 74099 [S+Sk+B], 734111, 73419 [S+Sk], 73407–73410, 73412, 73413 [S+A], NMP 47926 [A],

48037, 48038, 48040–48047 [S+A], 48039 [S+B]), Tadmor, Afqa cave, 11 March 1979, leg. R. Kinzelbach, 20 September 1988, leg. D. Kock, 17 May 1989, leg. D. Kock, 30 April 1995, leg. M. Kaftan, 23 June 1998, leg. M. Andreas, P. Benda and M. Uhrin. – **Tchad** (2): 1 ♂, 1 ♀ (BMNH 64.240., 64.241. [S+B]), Zouar, Tibesti, Sahara, 1735 m, 9 and 16 October 1955, leg. I. G. Gibson. – **Tunisia** (24): 24 ♂♂ (IVB 1.2.75–1.2.98 [S+B]), El Hammá, nr. Tozeur, autumn 1973, leg. Pavlík. – **Yemen** (19): 1 ♂ (BCSU 312 [S+B]), Bayt Al-Faqech (Al-Hodeida Prov.), October 2002, leg. A. As-Sabri; – 2 ♂♂ (MSNG 33196a, b [S+B]), Mokkha, 1891, leg. G. Pestalozza; – 3 ♂♂, 8 inds. (BMNH 95.6.1.11.–95.6.1.14., 99.11.6.93., 99.11.6.94., 99.11.6.97. [S], BMNH 99.3.14.25., MSNG 33197a, b, 33198 [S+B]), Aden, 1883, 1892, 1895, 1899, leg. Ruspoli, V. Ragazzi, R. O. Grant, J. W. Yerbury, A. B. Percival and W. Dodson; – 1 ♂ (MSNG 33199 [S+B]), near Aden, 1893, leg. A. Pogliani; – 2 ♀♀, 1 ind. (BMNH 95.6.1.50., 95.6.1.51., 99.11.6.12. [S+B]), Lahej, 9 and 28 March 1895, 9 May 1899, leg. J. W. Yerbury, A. B. Percival and W. Dodson; – 1 ♂ (BMNH 57.426. [B]), Qatn, E. Aden Prot., Arabia, 2000', 5 June 1956, leg. D. J. Greathead.

Appendix II

List of the specimens examined in molecular genetic analysis. Sequence (NA2) of the specimen NMP 90351 from Egypt was published already by Benda and Vallo (2009)

Sample	Haplotype	Accession #	Species	State	Site	Coordinates
NMP 48020	ME1	JF439008	*A. tridens*	Syria	Halabiyyeh	35°41'N, 39°50'E
NMP 48027	ME2	JF439009	*A. tridens*	Syria	Halabiyyeh	35°41'N, 39°50'E
NMP 48037	ME2		*A. tridens*	Syria	Tadmor	34°33'N, 38°17'E
NMP 48041	ME2		*A. tridens*	Syria	Tadmor	34°33'N, 38°17'E
NMP 48813	ME2		*A. tridens*	Syria	Qala'at ar Rahba	35°00'N, 40°25'E
NMP 48174	ME2		*A. tridens*	Iran	Choqazanbil	32°01'N, 48°32'E
NMP 48173	ME3	JF439010	*A. tridens*	Iran	Choqazanbil	32°01'N, 48°32'E
NMP 48317	NA1	JF438999	*A. tridens*	Libya	Ghat	24°56'N, 10°10'E
NMP pb3842	NA1		*A. tridens*	Morocco	Tassetift	30°23'N, 06°52'W
NMP pb3844	NA1		*A. tridens*	Morocco	Tassetift	30°23'N, 06°52'W
NMP pb3843	NA2		*A. tridens*	Morocco	Tassetift	30°23'N, 06°52'W
NMP 90351	NA2	FJ457617	*A. tridens*	Egypt	Siwa, Shali	29°12'N, 25°31'E
NMP 92590	NA2		*A. tridens*	Egypt	Aswan	24°07'N, 32°54'E
NMP 93670	NA2		*A. tridens*	Sudan	Quikkah	20°40'N, 30°20'E
NMP 93671	NA2		*A. tridens*	Sudan	Quikkah	20°40'N, 30°20'E
NMP 93665	NA2		*A. tridens*	Sudan	Dongola Alajuz	18°13'N, 30°45'E
NMP 93652	NA2		*A. tridens*	Sudan	Karima, Jebel Barkal	18°32'N, 31°50'E
NMP 92586	NA3	JF439000	*A. tridens*	Egypt	Aswan	24°07'N, 32°54'E
NMP 90354	NA4	JF439001	*A. tridens*	Egypt	Siwa, Shali	29°12'N, 25°31'E
NMP 93651	NA5	JF439002	*A. tridens*	Sudan	Karima, Jebel Barkal	18°32'N, 31°50'E
NMP 93656	NA5		*A. tridens*	Sudan	Karima, Jebel Barkal	18°32'N, 31°50'E
NMP 93655	NA6	JF439003	*A. tridens*	Sudan	Karima, Jebel Barkal	18°32'N, 31°50'E
NMP 93664	NA7	JF439004	*A. tridens*	Sudan	Dongola Alajuz	18°13'N, 30°45'E
NMP 92627	OM1	JF439011	*A. tridens*	Oman	Al Ghubrah	23°19'N, 57°42'E
NMP 92629	OM2	JF439012	*A. tridens*	Oman	Al Ghubrah	23°19'N, 57°42'E
NMP 92688	OM3	JF439013	*A. tridens*	Oman	Shisr, Wubar	18°15'N, 53°39'E
NMP 92694	OM3		*A. tridens*	Oman	Shisr, Wubar	18°15'N, 53°39'E
NMP 92762	OM4	JF439014	*A. tridens*	Oman	Al Mintirib	22°26'N, 58°48'E
NMP 92771	OM4		*A. tridens*	Oman	Muqal	22°37'N, 59°06'E
MZUF 20732	WA1	JF439005	*A. tridens*	Mauritania	Ouadane	20°56'N, 17°37'W
NMP 93647	WA2	JF439006	*A. tridens*	Mauritania	Tarjit	20°15'N, 13°05'W
NMP 93639	WA2		*A. tridens*	Mauritania	Tin Labbé	20°58'N, 11°40'W
NMP 93641	WA2		*A. tridens*	Mauritania	Tin Labbé	20°58'N, 11°40'W
NMP 93644	WA2		*A. tridens*	Mauritania	Tin Labbé	20°58'N, 11°40'W
NMP 93640	WA3	JF439007	*A. tridens*	Mauritania	Tin Labbé	20°58'N, 11°40'W
BCSU pb2719	SC1	JF439018	*A. italosomalica*	Yemen	Socotra, Kam	12°40'N, 54°07'E
NMP 90570	SC2	JF439019	*A. italosomalica*	Yemen	Socotra, Kam	12°40'N, 54°07'E
NMP 90592	SC3	JF439020	*A. italosomalica*	Yemen	Socotra, Mazaaba	12°29'N, 54°02'E
NMP 92789	DF1	JF439015	*Aselia* sp. n.	Yemen	Hauf	16°39'N, 53°03'E
NMP 92790	DF1		*Aselia* sp. n.	Yemen	Hauf	16°39'N, 53°03'E
NMP 92791	DF1		*Aselia* sp. n.	Yemen	Hauf	16°39'N, 53°03'E
NMP 92793	DF1		*Aselia* sp. n.	Yemen	Hauf	16°39'N, 53°03'E
NMP 92794	DF1		*Aselia* sp. n.	Yemen	Hauf	16°39'N, 53°03'E
NMP 92796	DF1		*Aselia* sp. n.	Yemen	Damqawt	16°35'N, 52°50'E
NMP 92797	DF1		*Aselia* sp. n.	Yemen	Damqawt	16°35'N, 52°50'E
NMP 92798	DF1		*Aselia* sp. n.	Yemen	Damqawt	16°35'N, 52°50'E
NMP 92723	DF1		*Aselia* sp. n.	Oman	Ain Jarziz	17°06'N, 54°05'E
NMP 92722	DF2	JF439016	*Aselia* sp. n.	Oman	Ain Jarziz	17°06'N, 54°05'E
NMP 92753	DF3	JF439017	*Aselia* sp. n.	Oman	Ain Tabruq	17°06'N, 54°20'E

Appendix III

Polymorphic sites identified in the complete gene for cytochrome *b* (1,140 bp) sequenced in *Asellia*

```
    ..........1111111111111111112222222222222233333333333333333344444
    .123456668800122222344556779990011234788889001222344555578913344
    98795706914564013696453954802514695760256873651490581457249458 17

ME1 TAATCCCTTCCACCCTGTGCACAGCTGTTCCCCTCATTCTCTCATCCACATTTTTAGCTACCTT
ME2 ....................................................................
ME3 ..........................C.........................................
OM1 ..........................C.......................C.................
OM2 ....................................................................
OM3 ............T.....G.......C.....T.....A.............C..........
OM4 ....................................................................
NA1 C.....T.C...T.............C.....AT..C.....GC.........CC..T.G....
NA2 C.....T.C...T.............C.....AT..C.....G.........CC..T.G....
NA3 C.....T.C...T.............C.....AT..C.....G.........CC..T.G....
NA4 C.....T.C...T.............C.....TAT..C.....G.........CC..T.G....
NA5 C.....T.C...T.............C.C.....TAT..C.....G.........CC..T.G....
NA6 C.....T.C...T.....A.......C.....TAT..C.....G.........CC..T.G....
NA7 C.....T.C...T.............C.....TAT..C.....GC........CC..T.G....
WA1 ...C.....................A.........AT.....................CC.........
WA2 ...C.....................A.........AT.....................CC.........
WA3 ...C.....................A.........AT.....................CC.......C.
DF1 C.......C.......A....T.A.C....A...T..CT................C.......CC
DF2 C.......C.......A....T.A.C....A...T..CT........G......C.......CC
DF3 C.......C.......A....T.A.C....A...T..CT........G......C.......CC
SC1 CGC.TT.CCTAG.A.CACA...GATCA..T.T.C.GC..C.CA..AT.TGCCCCCTA.C.TTCC
SC2 CGC.TT.CCTAG.A.CACAT..GATCA..T.T.C.GC..C.CA..AT.TGCCCCCTA.C.TTCC
SC3 CGC.TT.CCTAG.A.CACAT..GATCA..T.T.C.GC..C.CA..AT.TGCCCCCTA.C.TTCC

    44444444444455555555555555555555566666666666666666677777777777777
    56677775888990011224566678889999900122346888999999000112234557788
    95645670692847095865147025912478398146831470367896893702570314 69

ME1 CCATAGCCAACGCCTCTTCGTCCGACCGCACCCACCCACTACCCCAATCACACAACCCAGTCCC
ME2 ....................................................................
ME3 ....................................................................
OM1 ................C......A........T.....................................
OM2 ....G...............................................................
OM3 T..............C..A...A.A....TTT......C..............T.......
OM4 ....................................................................
NA1 ..............C...C..A.....GT.T.........................AC.T.
NA2 ..............C...C..A.....GT.T.........................AC.T.
NA3 ..............C...C..A.....GT.T............C..........AC.T.
NA4 ..............C...C..A.....GT.T.........................AC.T.
NA5 ..............C...C..A.....GT.T.........................AC.T.
NA6 ..............C...C..A.....GT.T.........................AC.T.
NA7 ..............C...C..A.....GT.T.........................AC.T.
WA1 .........G......C......A.....T.T..............G...T.T.T......
WA2 .........G......C......A.....T.T..............G...T.T.T......
WA3 .........G......C......A.....T.T..............G...T.T.T......
DF1 .........G.TCTAC.C..CC..A.........TG...G.CGT.T.G.......G....GA....
DF2 .........G.TCTAC.C..CC..A.........TG...G.CGT.T.G.......G....GA....
DF3 .........G.TCTAC.C..CC..A.........T....G.CGT.T.G.......G....GA....
SC1 .TGC.CTA.G.T..ATCCTC.TTAGTTATG....TTA.TC.TT.G.G.A.TTGTC....ACT.T
SC2 .TGC.CTA.G.T..ATCCTC.TTAGTTATG....TTA.TC.TT.G.G.A.TTGTC....ACT.T
SC3 .TGC.CTA.G.T..ATCCTC.TTAGTTATG....TTA.TC.TT.G.G.A.TTGTC....ACT.T
```

APPENDIX III. Continued

```
    ........................................1111111111111111111111111
    7888888888888888888899999999999999999990000000000000000000111111111
    9000123334455677889900111233445567889990112234555677899911112222
    2147981470628409284769256136564830140698470384678947002303692469

ME1 TCCACCCCCCACCCACCCCTTGCCCTAAAATCCTTGAATACACGACAATCCTTGCTTCTATACC
ME2 ........................................................................
ME3 ........................................................................
OM1 ..............T.....................................C.....................
OM2 ........................................................................
OM3 .T..T...T.G.....................C..T.........G.....................
OM4 ............................................................T.......
NA1 ....T.....G...........T.......C.....C.............T...A.......G.T
NA2 ....T.....G...........T.............C.............T...A.......G.T
NA3 ....T.....G...........T.............C.............T...A.......G.T
NA4 ....T.....G...........T.............C.............T...A.......G.T
NA5 ....T.....G...........T.............C.............T...A.......G.T
NA6 ....T.....G...........T.............C.............T...A.......G.T
NA7 ....T.....G...........T.............C.............T...A.......G.T
WA1 ..........G..........A.......G......C.............C..A.......G.T
WA2 ..........G..........A.......G......C...T.........C..A.......G.T
WA3 ..........G..........A.......G......C...T.........C..A.......G.T
DF1 .T..TT....G..A.......A.T.C....CTT.CAC....GTA.T....C.C.GCC.A....T
DF2 .T..TT....G..A.......A.T.C....CTT.CAC....GTA.T....C.C.GCC.A....T
DF3 .T..TT....G..A.......A.T.C....CTT.CAC....GTA.T....C.C.GCC.A....T
SC1 C.TGTTTT.TGTTAT.ATTCCAT.ACGGG.A....ATCC..G.AC..C..CC....C.AGC.TT
SC2 C.TGTTTT.TGTTAT.ATTCCAT.ACGGG.A....ATCC..G.AC..C..CC....C.AGC.TT
SC3 C.TGTTTT.TGTTAT.ATTCCAT.ACGGG.A....ATCCG.G.AC..C..CC....C.AGC.TT
```