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Systematic status of African populations of *Pipistrellus pipistrellus* complex (Chiroptera: Vespertilionidae), with a description of a new species from Cyrenaica, Libya

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The distribution of pipistrelles of the *Pipistrellus pipistrellus* complex (= *P. pipistrellus* s.l.) reaches only marginally the African continent. These bats are known only from a narrow belt of the Mediterranean zone in Maghreb and from NE Libya. We analysed museum specimens of African populations of *P. pipistrellus* s.l. using both morphologic and genetic techniques and compared them with Eurasian specimens of the complex. The African representatives of *P. pipistrellus* complex include two morphologically, genetically and geographically distinct populations. One of them inhabits the Mediterranean part of Cyrenaica, Libya. Belonging to the *P. pygmaeus* genetic lineage, these bats are represented by larger and more rusty coloured individuals with large massive rostrum and canines. In morphologic traits, this population differs significantly from all Western Palaearctic populations of the *P. pipistrellus* complex. These bats differ by about 6–7% in genetic distance from *P. pygmaeus* s. str. Within the *P. pygmaeus* lineage Libyan bats seem to be unique in their echolocation calls: the maximum energy of terminal frequencies was recorded at about 45 kHz. We consider the Libyan pipistrelles to represent a separate species, *Pipistrellus hanaki* sp. nov. Another distinct African pipistrelle population inhabits the Mediterranean parts of NW African countries, Morocco, Algeria and Tunisia. Individuals from the latter population are small and somewhat darker members of the *P. pipistrellus* genetic lineage, with relatively short and narrow mesial part of rostrum. Although both morphological and genetic differences between this population and Eurasian *P. pipistrellus* s. str. were found (genetic distance about 3–5%), they are probably not sufficient for the separation of this form at the specific level. However, the differences from European samples show rather not a cline character and therefore potential subspecific level of NW African *P. pipistrellus* has to be taken into consideration.

Key words: *Pipistrellus pipistrellus*, *Pipistrellus pygmaeus*, Africa, morphology, morphometry, cytochrome *b*, mitochondrial DNA, taxonomy

INTRODUCTION

Pipistrelle bats of the *Pipistrellus pipistrellus* complex (or *P. pipistrellus* sensu lato) are widely distributed in the western part of Eurasia, from Iberia, British Islands and southern Scandinavia through the Middle East up to Central Asia (Corbet, 1978; Koopman, 1994; Taake and Vierhaus, 2004). Distribution range reaches only the northernmost margin of the African continent, where these bats are known solely from the narrow belt of the Mediterranean zone in Maghreb and from NE Libya (Fig. 1).

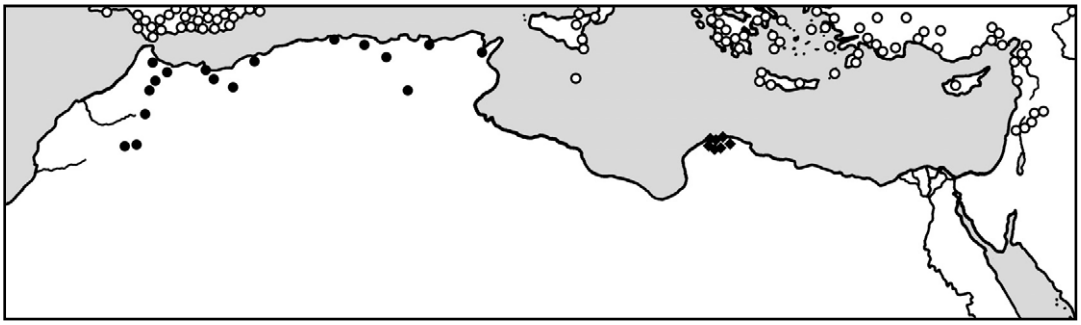

FIG. 1. Distribution of *Pipistrellus pipistrellus* s.l. in Africa. The closed symbols denote record sites of individual African taxa (circles — *P. pipistrellus*, diamonds — *P. hanaki* sp. nov.), open circles denote records of *P. pipistrellus* s.l. out of African continent

First African records of *P. pipistrellus* s.l. were published by Loche (1858, 1867) and Dobson (1880) from Algeria (Alger and Annaba). However, these data were doubted by Lataste (1885), and his conclusion was accepted by some subsequent authors (Rode, 1947; Hayman and Hill, 1971; Anciaux de Faveaux, 1976; Corbet, 1978). Therefore, first confirmed record of this bat in Africa was credited to Morales Agacino (1933, 1943), who found it in Morocco (the Rif Mts.). *Pipistrellus pipistrellus* s.l. is presently known from all Maghrebian countries (Morocco, Algeria, Tunisia), but only from their Mediterranean parts. According to Aulagnier and Thevenot (1986), *P. pipistrellus* s.l. is distributed in northern and northwestern Morocco in the Rif Mts., Moyen Atlas Mts. and the Beni Snassen region. Additionally, Fonderflick *et al*. (1998) and Z. Řehák (in Beneš and Hanák, 2003) found this bat also in the central Haut Atlas Mts. (Gorges du Todra). These records represented the southernmost known site of occurrence of *P. pipistrellus* s.l. in Africa. Kowalski and Rzebik-Kowalska (1991) summarised the distribution of this bat in Algeria, reporting on at least six sites from coastal zone and the Tell Atlas Mts. Only two verified records of *P. pipistrellus* s.l. come from Tunisia, both reported from the Jebel Ressas Mts. near Tunis (Vaughan *et al*., 1977).

Another African population of *P. pipistrellus* s.l. was discovered in Cyrenaica, Libya (Qumsiyeh and Schlitter, 1982; Hanák and Elgadi, 1984). Altogether, five sites were reported from relatively restricted area of the Mediterranean vegetation zone in Cyrenaica. This portion of the distribution is isolated by ca. 1,500 km of desert from both Maghrebian range in Tunisia and Middle Eastern range in Israel, and by ca. 800 and ca. 400 km of sea from the islands of Malta and Crete, respectively.

According to existing studies (Jones and van Parijs, 1993; Barrat *et al*., 1997; Mayer and von Helversen, 2001*a*, 2001*b*; Benda *et al*., 2003; von Helversen and Holderied, 2003), the *P. pipistrellus* complex is composed of two species, viz. *P. pipistrellus* (Schreber, 1774) and *P. pygmaeus* (Leach, 1825), in Europe and western Asia. The species status of the pygmy pipistrelle (also known as soprano pipistrelle or midge bat), *P. pygmaeus*, has been recognised just recently (Barrat *et al*., 1997). It was distinguished from its sibling species, the common pipistrelle *P. pipistrellus*, on the basis of differences in terminal frequencies of echolocation calls (Weid and von Helversen, 1987; Jones and van Parijs, 1993; Barlow and Jones, 1997). Differences were also found in their ecology (Kalko, 1994, 1995; Park *et al*., 1996; Barlow, 1997; Barlow and Jones, 1999) and in morphological

characters (Barlow *et al*., 1997; Häusler *et al*., 2000; Ziegler *et al*., 2001; von Helversen and Holderied, 2003). Although these authors mentioned several characters suitable for species identification (mainly wing and penial morphology), the most useful method for reliable determination of both species remains the genetic analysis along with the analysis of echolocation calls (Barrat *et al*., 1997; Mayer and von Helversen, 2001*b*; Benda *et al*., 2003; Hulva *et al*., 2004).

Pipistrellus pygmaeus occurs across a wide geographic range of Europe (Horáček *et al*., 2000; Mayer and von Helversen 2001*b*; von Helversen and Holderied, 2003; Vierhaus and Krapp, 2004). In most of its known distributional range it lives in sympatry with *P. pipistrellus* s. str., particularly in Great Britain, in central and south-eastern Europe (Mayer and von Helversen, 2001*b*; Hanák *et al*., 2001; Benda *et al*., 2003; Hulva *et al*., 2004); allopatric populations of *P. pygmaeus* were found in Sweden and eastern Europe. On the contrary, only *P. pipistrellus* was found in north-eastern Europe, in western France, as well as in the Middle East (Mayer and von Helversen, 2001*b*; Benda *et al*., 2003; Siivonen and Wermundsen, 2003).

Hulva *et al*. (2004) compared mitochondrial DNA (402 bp of cytochrome *b* gene) of *P. pipistrellus* s.l., which originated from most parts of the range of this complex. This analysis suggested a Mediterranean origin for the divergence of this complex. In the Asian part of its distribution range (Iran, Eastern Kazakhstan), only *P. pipistrellus* s. str. was recorded, which is in agreement with previous studies on geographically close populations (Mayer and von Helversen, 2001*b*; Benda *et al*., 2003). Genetic differences between these populations showed a gradual shift from the west to the east. The analysed samples of *P. pipistrellus* complex from northern Africa demonstrated their more or less extensive distinctness from other west Palaearctic individuals. While the pipistrelle bats from Morocco were more closely related to the European *P. pipistrellus* s. str. (with 3–5% of genetic difference), pipistrelles from Cyrenaica (Libya) were found to be more similar to *P. pygmaeus*, but with 6–7% of difference from corresponding European populations. These results suggest possible specific distinctness of African populations (cf. Hulva *et al*., 2004).

Although most authors did not mention any remarkable difference between Maghrebian pipistrelles and European ones (Panouse, 1951; Gaisler, 1983; Kowalski and Rzebik-Kowalska, 1991), Hanák and Elgadi (1984) observed some differences between bats from Cyrenaica and those from other populations bared on coloration and skull size. The present study aims to ascertain the systematic position of African populations of bats of the *P. pipistrellus* complex on the basis of a combination of morphological and genetic characters and to interpret their genetic differences from other Palaearctic populations from the taxonomic point of view.

Material and Methods

Morphological Analyses

In the morphological analysis, museum material of pipistrelle bats from northern Africa, the Middle East and central and south-eastern Europe was used. Examined specimens are listed in Appendix 1. For morphometric analysis, we primarily used the skull, tooth and wing data. From Europe only genetically identified specimens of *P. pipistrellus* and *P. pygmaeus* were used (with the exception of specimens from a colony of *P. pipistrellus*, from which not all individuals were identified genetically). From African and Middle Eastern localities all available specimens were used, but samples identified genetically were differentiated from non-identified individuals. Specimens were measured in a standard way using mechanical or optical callipers. Skull and dental

measurements were taken according to Barlow *et al*. (1997) and Benda *et al*. (2004), and dental measurements were taken including cingula of the respective teeth (see below). Bacula were extracted in 6% solution of KOH and coloured with alizarin red. From African and Middle Eastern individuals, echolocation calls were recorded by the heterodyne bat detector Petterson D 200. Statistical analyses of morphometric data were performed with the Statistica 6.0 software. Canonical discriminant analysis of skull, teeth and wing data were used to describe metric differences and/or the most variable characters among samples. Population samples were defined geographically and divided into genetically identified and not identified groups, respectively (see legends in Figs. 2–4). Because the Algerian specimens were prepared as dry skins, this sample was not used in the discriminant analysis of wing data.

Genetic Analyses

Partial sequences (402 bp, 5'end) of the cytochrome *b* gene were sequenced (Appendices 1 and 2; for the protocols see Hulva *et al*., 2004). The sequences were aligned by eye and the dataset was reduced to haplotypes. Phylogenetic analyses were performed in PAUP 4.0b10 (Swofford, 2001). For more details of phylogenetic anylyses see Hulva *et al*. (2004) where phylogenetics and phylogeography of *P. pipistrellus* complex was solved in details. This paper focuses on taxonomic consequences of resultant genetic patterns and exceeds it with morphological lines of evidence. Genetic distances (uncorrected) were computed as percent sequence divergence among haplotypes. Maximum parsimony and neighbor-joining methods were chosen to reconstruct phylogenetic relationships. 1,000 replicate bootstraps were run to obtain the nodal support. For hierarchical outgroup comparison we included *Pipistrellus kuhlii* (Kuhl, 1817) and *P. nathusii* (Keyserling et Blasius, 1839) (GenBank Accession Numbers AJ504444 and AJ504446, respectively — Hulva *et al*., 2004 and Stadelmann *et al*., 2004).

Abbreviations

Cranial measurements (in mm): LCr — greatest length of skull; LCb — condylobasal length of skull; LaZ — zygomatic width; LaI — width of interorbital constriction; LaInf — rostral width between foramina infraorbitalia; LaN — neurocranium width; LaM — mastoidal width; ANc — neurocranium height; ACr — skull height (incl. tympanic bullae); I^1I^1 — rostral width between incisors (excl.); CC — rostral width between canines (incl.); P^4P^4 — rostral width between 2nd upper premolars, P^4 (incl.); M^3M^3 — rostral width between 3rd upper molars (incl.); I^1M^3 — length of upper tooth-row between 1st incisor and 3rd molar (incl.); CM^3 — length of upper tooth-row between canine and 3rd molar (incl.); M^1M^3 — length of upper molar-row (incl.); CP^4 — length of upper tooth-row between canine and 2nd premolar (incl.); LMd — mandible length; ACo — height of coronoid process; I_1M_3 — length of lower tooth-row between 1st incisor and 3rd molar (incl.); CM_3 — length of lower tooth-row between canine and 3rd molar (incl.); M_1M_3 — length of lower molar-row (incl.); CP_4 — length of lower tooth-row between canine and 2nd premolar (incl.).

Dental measurements (in mm): LI^1 — mesiodistal length of 1st upper incisor; LI^2 — mesiodistal length of 2nd upper incisor; LCs — mesiodistal length of upper canine; LaCs — palatolabial width of upper canine; LP^3 — mesiodistal length of 1st upper premolar; LP^4 — mesiodistal length of 2nd upper premolar; LaP^4 — palatolabial width of 2nd upper premolar; LM^1 — mesiodistal length of 1st upper molar (over parastyle and metastyle edges); LaM^1 — largest palatolabial width of 1st upper molar from parastyle; LM^3 — mesiodistal length of 3rd upper molar over metaconus; LaM^3 — palatolabial width of 3rd upper molar from parastyle; ACin — height of mesiopalatal cingular cusp of 2nd upper premolar (P^4); LI_1 — mesiodistal length of 1st lower incisor; LI_3 — mesiodistal length of 3rd lower incisor; LCi — mesiodistal length of lower canine; LP_3 — mesiodistal length of 1st lower premolar.

Wing measurements (in mm): LAt — forearm length (incl. wrist); Vp — length of the proximal phalanx of 5th finger; Vm — length of the medial phalanx of 5th finger; Vd — length of the distal phalanx of 5th finger; IVp — length of the proximal phalanx of 4th finger; IVm — length of the medial phalanx of 4th finger; IVd — length of the distal phalanx of 4th finger; IIIp — length of the proximal phalanx of 3rd finger; IIIm — length of the medial phalanx of 3rd finger; IIId — length of the distal phalanx of 3rd finger.

Collections: EBD — Biological Station Doñana, Seville, Spain; MHNG — Natural History Museum, Geneva, Switzerland; MUB — Department of Zoology and Ecology, Masaryk University, Brno, Czech Republic; NMP — National Museum (Natural History), Prague, Czech Republic; SMF — Senckenberg Museum, Frankfurt am Main, Germany; SMO — Silesian County Museum, Opava, Czech Republic; SMZ — South-Moravian Museum, Znojmo, Czech Republic.

Results

The simple comparison of biometric data of African samples shows clear difference between the Libyan and north-west African (Maghrebian) individuals. The Libyan population is represented by the largest individuals from the whole set of pipistrelle bats, while Maghrebian samples (from Morocco and Algeria) are composed by very small individuals. Libyan and Maghrebian bats differ distinctly in forearm length (LAt), skull lengths (LCr, LCb, LMd), almost all tooth-row lengths (I^1M^3, CM^3, M^1M^3, I_1M_3, CM_3, M_1M_3, CP_4), most of skull and rostral widths (LaM, I^1I^1, CC, P^4P^4, M^3M^3), two skull heights (ACr, ACo), and several tooth dimensions (LCs, LaCs, LM^1, LCi). In all mentioned measurements the values do not show any overlap (Table 1). In other values the two populations differ as well but with slight overlap of absolute values (LaInf, LaN, ANc, CP^4, LI^1, LI^2, LP^4, LaM^1, LM^3, LaM^3, LaI_1), including three wing measurements (IVp, Vd, Vp). Only one skull dimension (LaI), four dental measurements (LP^3, LaP^4, LaI_3, LP_3), and most wing measurements (Vm, IVm, IVd, IIIp, IIIm, IIId) do not differ significantly.

However, the Libyan sample differs in size also very clearly from other, non-African populations of pipistrelle bats, in some length and width of skull (LCr, LCb, LaM, M^3M^3, I^1M^3) and in tooth dimensions (LaCs, LM^1, LaM^1), but a slight overlap was observed in absolute values. However, the overlap is absent for a ratio of the rostral breadth over upper canines (CC), in dimensions of mandible (LMd, I_1M_3, CM_3), and in mesiodistal lengths of upper and lower canines (LCs, LCi). Bivariate comparisons shows that the Libyan sample has a unique skull shape: the rostrum is absolutely and relatively the largest and canines are most massive to all other samples (Figs. 2 and 3). The absolute

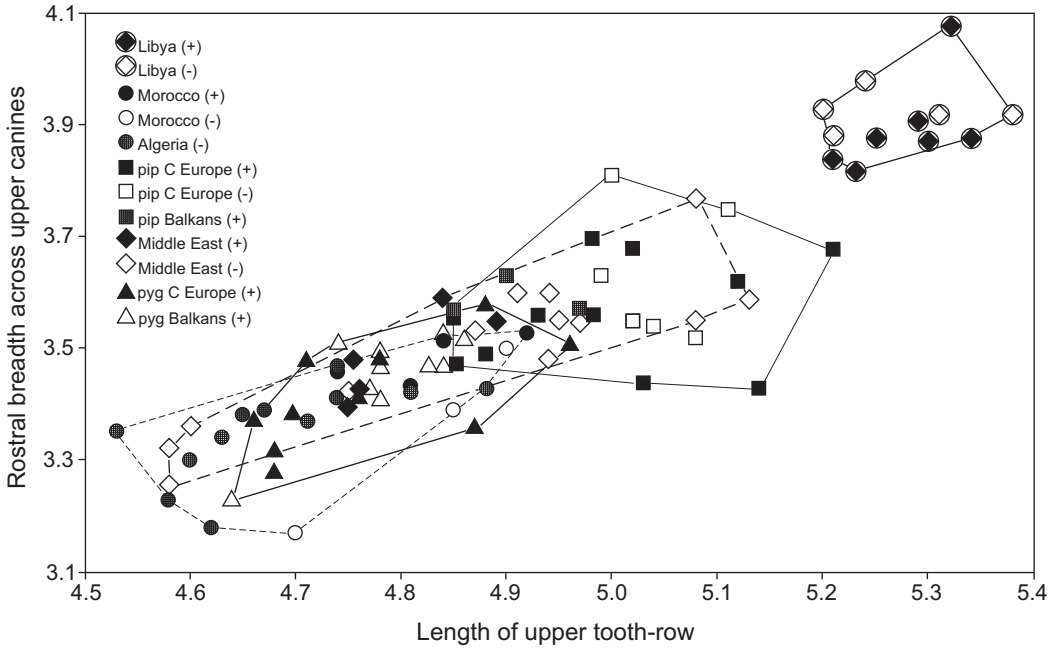

FIG. 2. Bivariate plot of western Palaearctic populations of *P. pipistrellus* s.l.: length of upper tooth-row (I^1M^3) versus rostral breadth across upper canines (CC). Explanations: pip — *P. pipistrellus* s. str.; pyg — *P. pygmaeus* s. str.; (+) — genetically identified specimens; (–) — specimens not identified genetically

TABLE 1. Biometric data of the examined samples. For abbreviations see Material and Methods

Feature	Libya					Morocco					Algeria				
	n	$\bar{x}$	Min	Max	SD	*n*	$\bar{x}$	Min	Max	SD	*n*	$\bar{x}$	Min	Max	SD
LAt	13	32.38	31.20	33.40	0.732	9	29.78	28.50	31.00	0.760	12	28.33	27.30	29.90	0.720
Vp	11	6.91	6.50	7.20	0.234	9	6.37	6.00	6.70	0.245	12	6.13	5.60	6.40	0.267
Vm	11	4.52	4.00	5.30	0.352	9	4.00	3.70	4.50	0.274	12	3.64	3.30	4.20	0.284
Vd	11	1.97	1.70	2.20	0.156	9	1.61	1.30	1.90	0.190	12	1.53	1.20	1.80	0.182
IVp	11	10.39	9.70	11.00	0.432	9	9.43	8.80	9.80	0.371	12	9.05	8.60	9.50	0.284
IVm	11	6.65	5.10	7.20	0.592	9	6.30	5.80	6.70	0.308	12	5.80	5.00	6.40	0.506
IVd	11	2.48	1.90	2.80	0.248	9	2.46	2.00	2.80	0.265	12	1.98	1.50	2.30	0.269
IIIp	11	10.82	10.20	11.30	0.296	9	10.46	9.70	11.10	0.500	12	9.59	8.90	10.20	0.432
IIIm	11	8.70	8.30	9.30	0.297	9	8.27	7.50	9.00	0.430	12	7.78	7.40	8.30	0.272
IIId	11	7.17	6.40	7.60	0.350	9	6.93	6.60	7.40	0.296	12	6.08	4.30	7.10	0.800
LCr	12	12.43	12.14	12.69	0.161	7	11.62	11.18	11.88	0.228	12	11.35	11.02	11.59	0.215
LCb	12	11.95	11.64	12.18	0.151	7	11.13	10.74	11.34	0.225	12	10.83	10.52	11.20	0.225
LaI	12	3.15	2.98	3.35	0.116	7	3.08	2.92	3.21	0.118	12	3.02	2.76	3.22	0.142
LaInf	12	3.70	3.50	3.90	0.134	7	3.44	3.20	3.55	0.122	12	3.36	3.22	3.48	0.084
LaN	12	6.32	6.12	6.51	0.100	7	5.96	5.77	6.12	0.132	12	5.89	5.68	6.15	0.138
LaM	12	7.17	7.02	7.39	0.132	7	6.57	6.36	6.78	0.138	12	6.44	6.27	6.66	0.127
ANc	12	4.45	4.36	4.63	0.072	7	4.18	4.03	4.41	0.118	12	4.10	4.02	4.18	0.063
ACr	12	5.75	5.58	5.86	0.089	6	5.38	5.25	5.55	0.108	12	5.33	5.17	5.52	0.099
I^1I^1	12	1.87	1.80	1.94	0.047	7	1.67	1.58	1.79	0.074	11	1.63	1.56	1.77	0.057
CC	12	3.91	3.82	4.08	0.069	7	3.43	3.17	3.53	0.125	12	3.36	3.18	3.47	0.084
P^4P^4	12	4.50	4.42	4.62	0.073	7	4.13	3.80	4.31	0.174	12	4.12	4.02	4.28	0.074
M^3M^3	12	5.14	4.98	5.31	0.091	7	4.74	4.51	4.93	0.144	12	4.69	4.50	4.82	0.097
I^1M^3	12	5.27	5.20	5.38	0.058	7	4.82	4.70	4.92	0.080	12	4.66	4.28	4.88	0.152
CM^3	12	4.46	4.35	4.53	0.060	7	4.09	3.96	4.19	0.091	12	3.97	3.83	4.08	0.079
M^1M^3	12	2.88	2.79	2.96	0.058	7	2.60	2.55	2.69	0.045	12	2.57	2.49	2.65	0.052
CP^4	12	1.86	1.76	1.94	0.047	7	1.69	1.60	1.77	0.064	12	1.65	1.56	1.71	0.047
LI^1	12	0.47	0.43	0.51	0.023	7	0.42	0.40	0.45	0.017	12	0.40	0.36	0.44	0.022
LI^2	12	0.39	0.37	0.46	0.025	7	0.34	0.30	0.38	0.025	12	0.34	0.30	0.39	0.023
LCs	12	0.99	0.95	1.04	0.027	7	0.81	0.75	0.87	0.041	12	0.79	0.72	0.88	0.039
LaCs	12	0.82	0.74	0.91	0.045	7	0.66	0.61	0.70	0.032	12	0.65	0.63	0.71	0.023
LP^3	12	0.41	0.34	0.47	0.037	7	0.35	0.32	0.39	0.024	12	0.34	0.30	0.39	0.025
LP^4	12	0.86	0.81	0.92	0.036	7	0.78	0.76	0.83	0.023	12	0.77	0.73	0.81	0.025
LaP^4	12	1.11	1.03	1.16	0.045	7	1.03	0.97	1.08	0.043	12	1.00	0.95	1.04	0.026
LM^1	12	1.18	1.13	1.26	0.040	7	1.03	1.01	1.06	0.021	12	1.05	0.99	1.12	0.042
LaM^1	12	1.38	1.34	1.42	0.031	7	1.22	1.09	1.35	0.084	12	1.19	1.15	1.24	0.031
LM^3	11	0.70	0.65	0.73	0.025	7	0.64	0.62	0.66	0.014	12	0.63	0.62	0.66	0.016
LaM^3	12	1.38	1.34	1.42	0.029	6	1.29	1.27	1.37	0.040	12	1.27	1.20	1.32	0.027
ACin	11	0.16	0.13	0.21	0.023	7	0.16	0.13	0.21	0.029	11	0.16	0.00	0.22	0.060
LMd	11	8.72	8.62	8.82	0.064	7	8.05	7.83	8.18	0.130	12	7.86	7.58	8.14	0.164
ACo	11	2.57	2.47	2.76	0.082	7	2.27	2.16	2.39	0.078	12	2.21	2.12	2.33	0.076
I_1M_3	11	5.53	5.48	5.59	0.042	7	5.06	4.91	5.18	0.102	12	4.91	4.68	5.08	0.123
CM_3	11	4.73	4.62	4.83	0.072	7	4.28	4.11	4.35	0.086	12	4.17	3.97	4.32	0.111
M_1M_3	11	3.09	2.99	3.16	0.055	7	2.78	2.68	2.89	0.073	12	2.76	2.71	2.93	0.061
CP_4	11	1.60	1.56	1.64	0.026	7	1.37	1.31	1.41	0.037	12	1.34	1.27	1.42	0.054
LaI_1	11	0.43	0.41	0.46	0.019	7	0.39	0.36	0.43	0.028	12	0.38	0.34	0.43	0.027
LaI_3	11	0.37	0.30	0.41	0.029	7	0.32	0.30	0.32	0.007	12	0.33	0.29	0.36	0.018
LCi	11	0.69	0.65	0.73	0.024	7	0.55	0.51	0.59	0.028	12	0.54	0.52	0.55	0.012
LP_3	11	0.45	0.41	0.50	0.028	7	0.42	0.40	0.45	0.016	12	0.41	0.39	0.44	0.017
I^1M^3/LCr	12	0.424	0.413	0.432	0.006	7	0.415	0.408	0.427	0.008	12	0.412	0.402	0.421	0.005
LaN/LCr	12	0.508	0.494	0.522	0.008	7	0.513	0.492	0.533	0.013	12	0.519	0.502	0.534	0.010
ANc/LCr	12	0.358	0.352	0.368	0.006	7	0.360	0.345	0.376	0.011	12	0.361	0.347	0.375	0.009
CC/LCr	12	0.315	0.305	0.327	0.006	7	0.295	0.284	0.305	0.008	12	0.296	0.283	0.304	0.006
CC/CM^3	12	0.876	0.843	0.913	0.018	7	0.837	0.801	0.861	0.023	12	0.846	0.811	0.862	0.013
ACo/LMd	12	0.295	0.280	0.315	0.010	7	0.282	0.275	0.294	0.006	12	0.281	0.267	0.296	0.009
LCs/CM^3	12	0.221	0.213	0.231	0.007	7	0.197	0.180	0.216	0.012	12	0.198	0.179	0.224	0.012
LCs/LaCs	12	1.208	1.057	1.380	0.087	7	1.231	1.164	1.277	0.038	12	1.204	1.015	1.377	0.084

TABLE 1. Extended

Feature	Middle East					*P. pygmaeus* Europe					*P. pipistrellus* Europe				
	n	$\bar{x}$	Min	Max	SD	*n*	$\bar{x}$	Min	Max	SD	*n*	$\bar{x}$	Min	Max	SD
LAt	17	30.28	28.90	31.80	0.759	21	30.27	28.60	31.80	0.716	20	31.67	30.20	33.20	0.865
Vp	17	6.56	6.10	7.30	0.324	21	6.27	5.70	6.80	0.297	20	6.59	5.90	7.10	0.285
Vm	17	3.72	2.70	4.60	0.494	21	3.80	3.20	4.30	0.269	20	3.95	3.30	4.60	0.346
Vd	17	1.72	1.50	2.10	0.156	21	1.68	1.30	2.40	0.230	20	1.88	1.60	2.20	0.147
IVp	17	10.02	9.30	10.80	0.433	21	9.82	9.20	10.60	0.359	20	10.23	9.40	11.00	0.425
IVm	17	6.14	5.50	6.90	0.424	21	6.19	5.60	6.90	0.345	20	6.30	5.30	6.80	0.328
IVd	17	2.63	2.20	3.10	0.327	21	2.42	2.10	2.90	0.249	20	2.70	2.30	3.40	0.278
IIIp	17	10.47	9.80	11.30	0.380	21	10.20	9.60	10.80	0.353	20	10.80	10.00	11.50	0.388
IIIm	17	8.26	7.40	8.80	0.364	21	8.04	7.00	8.90	0.437	20	8.34	7.00	9.10	0.412
IIId	17	7.02	6.10	8.70	0.620	21	7.06	5.20	8.30	0.756	20	7.30	6.60	8.40	0.538
LCr	17	11.60	10.93	12.15	0.292	19	11.58	11.32	11.88	0.163	20	12.11	11.46	12.46	0.223
LCb	17	11.14	10.42	11.53	0.307	20	11.11	10.78	11.37	0.165	20	11.56	10.98	11.92	0.197
LaI	17	3.14	2.87	3.32	0.129	20	3.24	3.00	3.47	0.124	20	3.35	3.18	3.57	0.094
LaInf	17	3.48	3.00	3.70	0.166	20	3.42	3.29	3.63	0.102	20	3.58	3.26	3.84	0.130
LaN	17	5.99	5.62	6.29	0.188	20	6.13	5.92	6.27	0.098	20	6.35	6.05	6.61	0.162
LaM	17	6.55	5.97	6.92	0.213	20	6.70	6.48	7.02	0.113	20	6.81	6.62	7.05	0.137
ANc	17	4.20	4.02	4.37	0.115	20	4.31	4.07	4.58	0.140	20	4.37	4.08	4.55	0.118
ACr	17	5.46	5.21	5.62	0.120	19	5.48	5.24	5.75	0.142	18	5.66	5.37	5.94	0.148
I^1I^1	16	1.75	1.64	1.92	0.078	19	1.73	1.48	1.91	0.108	18	1.82	1.65	1.95	0.067
CC	17	3.50	3.25	3.77	0.126	20	3.44	3.23	3.58	0.090	17	3.59	3.43	3.81	0.109
P^4P^4	17	4.19	3.78	4.45	0.169	20	4.15	3.97	4.31	0.090	20	4.31	4.10	4.53	0.119
M^3M^3	17	4.76	4.32	5.04	0.172	20	4.77	4.62	4.98	0.098	18	4.90	4.68	5.09	0.128
I^1M^3	17	4.85	4.58	5.13	0.172	19	4.78	4.64	4.96	0.086	19	4.99	4.65	5.21	0.128
CM^3	17	4.13	3.78	4.37	0.149	19	4.05	3.95	4.29	0.083	17	4.25	4.05	4.42	0.096
M^1M^3	17	2.64	2.41	2.82	0.099	19	2.60	2.46	2.85	0.109	20	2.69	2.54	2.88	0.087
CP^4	17	1.78	1.63	1.93	0.071	20	1.75	1.66	1.88	0.067	19	1.80	1.68	1.99	0.070
LI^1	17	0.42	0.37	0.47	0.034	20	0.43	0.38	0.47	0.028	19	0.44	0.40	0.48	0.022
LI^2	17	0.36	0.26	0.40	0.032	20	0.33	0.28	0.41	0.031	19	0.35	0.27	0.39	0.027
LCs	17	0.85	0.79	0.89	0.035	20	0.80	0.75	0.89	0.040	18	0.84	0.76	0.91	0.040
LaCs	17	0.70	0.63	0.79	0.045	20	0.63	0.51	0.75	0.054	18	0.68	0.63	0.73	0.035
LP^3	17	0.38	0.35	0.44	0.023	20	0.35	0.31	0.40	0.020	19	0.39	0.31	0.45	0.033
LP^4	17	0.81	0.73	0.88	0.039	20	0.79	0.68	1.15	0.099	20	0.81	0.73	0.88	0.044
LaP^4	17	1.05	0.95	1.14	0.054	20	1.00	0.92	1.21	0.076	20	1.04	0.90	1.17	0.064
LM^1	17	1.07	1.02	1.14	0.040	20	1.06	0.97	1.19	0.053	20	1.09	0.99	1.13	0.036
LaM^1	17	1.24	1.16	1.31	0.046	20	1.20	1.09	1.37	0.078	20	1.22	1.15	1.36	0.048
LM^3	17	0.64	0.59	0.69	0.030	19	0.64	0.58	0.72	0.035	18	0.66	0.61	0.71	0.032
LaM^3	17	1.27	1.16	1.34	0.054	19	1.24	1.16	1.39	0.069	18	1.29	1.19	1.36	0.047
ACin	17	0.16	0.09	0.22	0.036	20	0.18	0.10	0.25	0.038	20	0.18	0.14	0.21	0.022
LMd	17	8.10	7.59	8.42	0.237	20	8.02	7.83	8.28	0.127	20	8.35	7.93	8.62	0.159
ACo	17	2.35	1.97	2.67	0.161	20	2.31	2.12	2.57	0.104	20	2.38	2.17	2.51	0.091
I_1M_3	16	5.15	4.83	5.42	0.169	20	5.07	4.89	5.28	0.099	18	5.27	5.05	5.43	0.104
CM_3	16	4.38	4.17	4.62	0.133	20	4.27	4.08	4.50	0.094	19	4.46	4.33	4.59	0.066
M_1M_3	17	2.84	2.66	3.00	0.092	19	2.80	2.66	3.10	0.116	20	2.89	2.74	3.11	0.096
CP_4	16	1.45	1.37	1.55	0.056	20	1.42	1.35	1.51	0.054	19	1.46	1.35	1.61	0.063
LaI^1	16	0.38	0.29	0.43	0.040	19	0.38	0.36	0.43	0.023	19	0.40	0.35	0.44	0.026
LaI^3	16	0.33	0.30	0.36	0.013	20	0.34	0.31	0.38	0.018	20	0.34	0.31	0.37	0.018
LCi	16	0.58	0.53	0.64	0.026	20	0.57	0.48	0.65	0.039	20	0.58	0.54	0.64	0.024
LP^3	17	0.45	0.40	0.54	0.034	20	0.45	0.38	0.49	0.028	20	0.46	0.40	0.50	0.024
I^1M^3/LCr	17	0.418	0.405	0.438	0.009	20	0.413	0.400	0.424	0.007	20	0.413	0.400	0.425	0.008
LaN/LCr	17	0.517	0.495	0.539	0.014	20	0.530	0.515	0.544	0.008	20	0.524	0.489	0.550	0.015
ANc/LCr	17	0.363	0.343	0.378	0.010	20	0.372	0.353	0.390	0.011	20	0.361	0.340	0.377	0.011
CC/LCr	17	0.301	0.288	0.310	0.006	20	0.297	0.278	0.306	0.007	20	0.296	0.276	0.313	0.010
CC/CM^3	17	0.848	0.816	0.881	0.019	20	0.848	0.810	0.881	0.018	20	0.845	0.790	0.901	0.026
ACo/LMd	17	0.290	0.254	0.330	0.016	20	0.287	0.270	0.316	0.012	20	0.285	0.256	0.304	0.011
LCs/CM^3	17	0.206	0.193	0.224	0.008	20	0.198	0.188	0.216	0.008	20	0.197	0.177	0.215	0.008
LCs/LaCs	17	1.223	1.095	1.357	0.065	20	1.284	1.125	1.469	0.093	20	1.227	1.090	1.333	0.051

rostral length, here expressed by length of upper tooth-row (I^1M^3), and the relative length of rostrum (I^1M^3/LCr) are the largest among all compared pipistrelle bats (Table 1). The absolute and relative widths of rostrum over upper canines (CC, CC/LCr and CC/CM^3; Table 1) are also the largest among examined pipistrelle bats.

The peculiar position of the Libyan population confirmed the results of discriminant analyses for cranial (Fig. 4), dental and wing dimensions. The results of a discriminant analysis of the first two canonical variables of all cranial measurements (Fig. 4; most significant *F*-values in LaI, LaN, CC, and CP^4; 1st CV 49.66%, 2nd CV 22.59% of variance) as well as of all dental dimensions (most significant *F*-values in LI^2, LCs, LaCs, and LaP^4; 1st CV 61.70%, 2nd CV 11.14% of variance; not shown) very clearly separated the Libyan sample according the first canonical variable; this variable is likely correlated with the size of individuals. Results of a discriminant analysis of the first two canonical variables of all wing measurements (most significant *F*-values in LAt, Vm, IVp, and IIIp; 1st CV 50.71%, 2nd CV 20.31% of variance; not shown) show also a clear difference of the Libyan pipistrelle bats compared to other samples.

The Maghrebian population, comprised of Moroccan and Algerian samples, includes the smallest bats among all sampled individuals. Although the Maghrebian bats show an overlap with Eurasian populations in most of dimensions, they achieve lowest values in several skull and dental measurements (LaI, CC, I^1M^3, CM_3, CP_4, LCs, LaI_3) and in two wing dimensions (LAt, IVp). This comparison illustrates that the shorter and narrower mesial part of the rostrum is typical for the Maghrebian population. The Maghrebian samples show similar separate position on lowest margin of variation range in a number of size characters (LCr, LaN, LaM, P^4P^4, M^3M^3, CM^3, LMd, I_1M_3), together with the Middle Eastern sample.

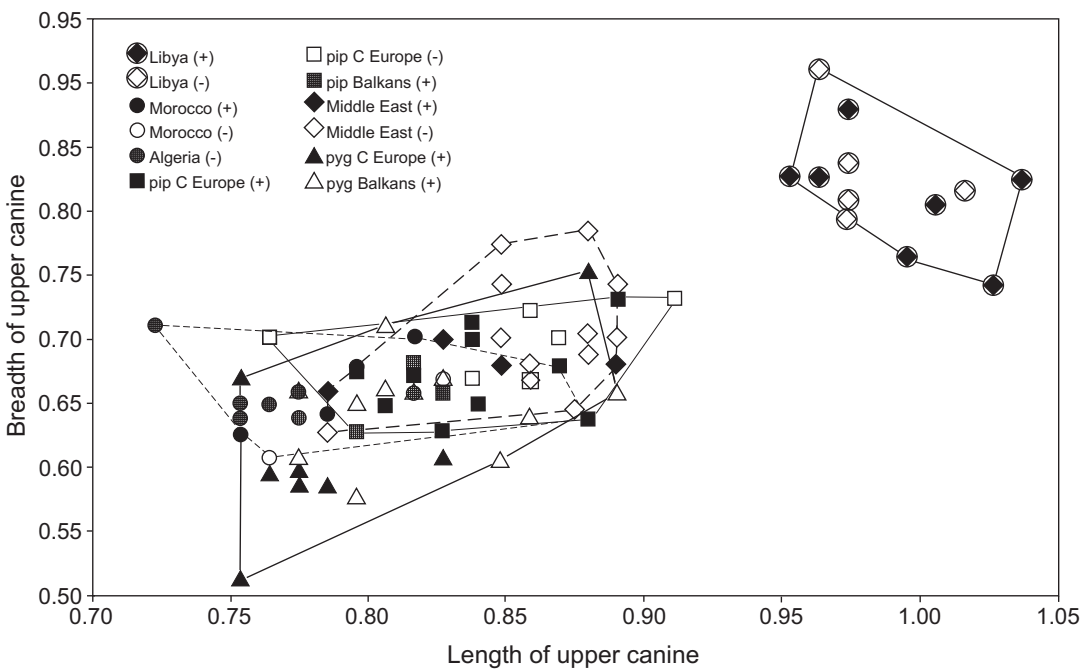

FIG. 3. Bivariate plot for western Palaearctic populations of *P. pipistrellus* s.l.: length of upper canine (LCs) versus breadth of upper canine (LaCs). For explanations see Fig. 2

These observations are confirmed by discriminant analyses of cranial and wing dimensions (Fig. 4, see above) which grouped Maghrebian individuals close to clusters of Eurasian pipistrelle bats. Discriminant analysis of skull characters separated pipistrelle bats from this lineage in two groups along the 2nd canonical axis (most significant for 2nd CV were LaN, ANc and ACr): the Central European and the Mediterranean one (Fig. 4). In the values of the 2nd CV, both African samples are close to the Balkan and Middle East populations in this respect, whereas the 1st CV very clearly divided African groups according to the skull size (see above).

Although the data on the external appearance of penis were obtained only from restricted numbers of adult males both from Morocco and Libya (see Appendix 1), the differences between the populations are evident (Fig. 5). Penes of the Moroccan specimens are very pale or almost unpigmented and have very distinct dorsomedial stripe on praeputium. Penes of the Libyan specimens have a simple praeputium without any distinct stripe on dorsal side and are pale greyish-brown with unpigmented hairs. Bacula were examined only for one adult male from Morocco and one adult male from Libya. Bacula of the Libyan and the Moroccan bats did not show any substantial difference from each other (Fig. 6). Both bones are ca. 1.8 mm long, with typical bifurcation on both epiphyses; small distinctions only can be observed in the dorsoventral angle between distal stick-like portion and proximal broader epiphysis, which is ca. 140° in the Libyan specimen and ca. 150° in the Moroccan specimen. Other slight difference is in the width of baculum diaphysis in the point of bifurcation of the proximal epiphysis: it is ca. 11.5% of the baculum length in the Libyan specimen, and ca. 8.0% in the Moroccan specimen. Thus, the observed minute bacular differences can be assesed rather as on the individual variation level.

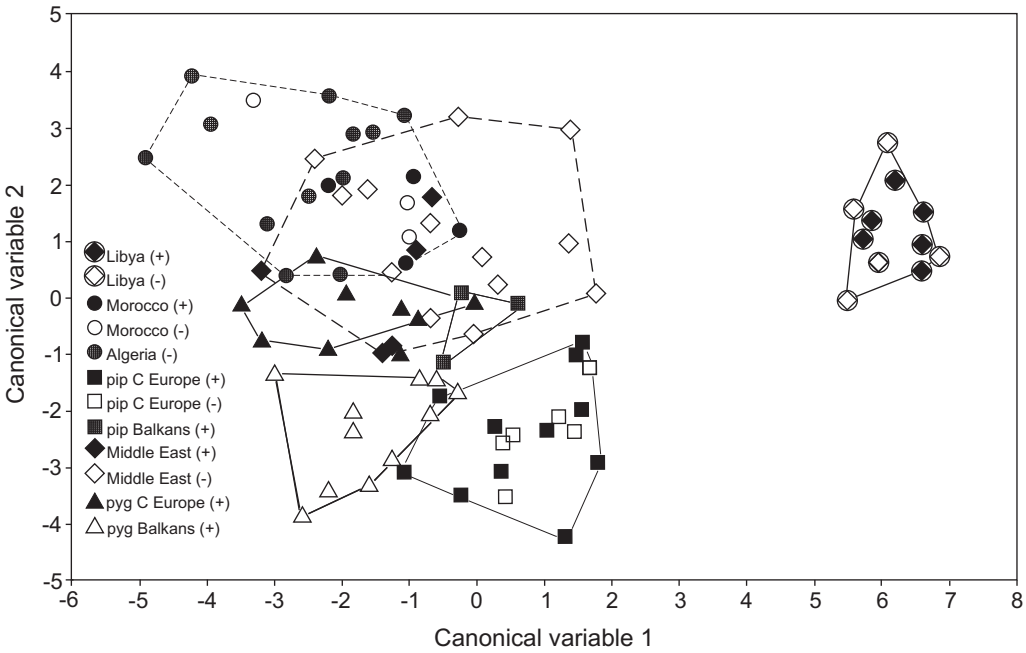

FIG. 4. Bivariate plot of the first two canonical axes of all cranial measurements in the western Palaearctic populations of *P. pipistrellus* s.l. (for details see text). For explanations see Fig. 2

FIG. 5. Penes of Libyan pipistrelle bat, *P. hanaki* sp. nov. (NMP 49902) (a) and of Moroccan specimen of *P. pipistrellus* (NMP 90069) (b). Frontolateral views

The pattern of wing veining (sensu von Helversen and Holderied, 2003) was studied in the African populations. In the Libyan sample, mostly the *P. pipistrellus* type of veining was observed (in nine individuals, i.e., 82% of the total sample), only minority of individuals showed the *P. pygmaeus* type (n = 2; 18%). The Moroccan bats showed largely *P. pipistrellus* type of wing veining (in six individuals, 67%), one individual was of *P. nathusii* type, one individual showed *P. pygmaeus* type, and one individual had combination of *P. pygmaeus* and *P. nathusii* types (the cell

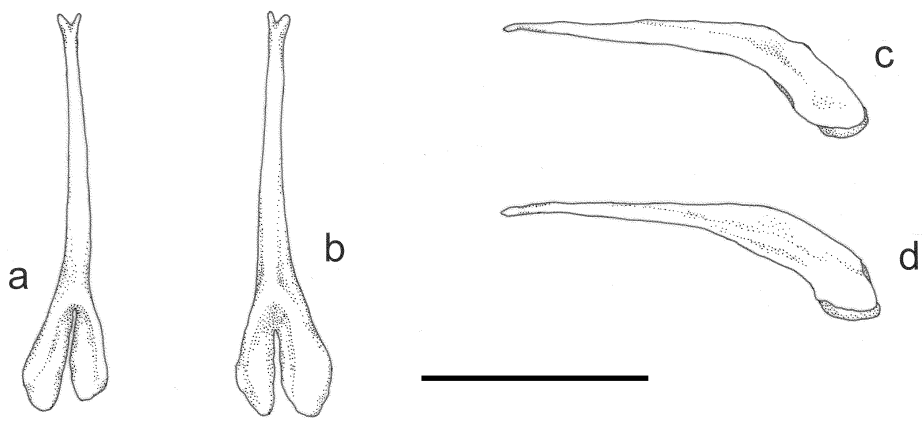

FIG. 6. Bacula of Libyan pipistrelle bat, *P. hanaki* sp. nov. (NMP 49902) (a, c) and Moroccan specimen of *P. pipistrellus* (NMP 90069) (b, d). Dorsal (a, b) and lateral (c, d) views. Scale bar = 1 mm

between elbow and the end of the fifth metacarpal was splited into two parts by a vein, while the cell between middle of forearm and the 5th metacarpal was without a crossing vein). All examined specimens of the Algerian sample (10) showed *P. pipistrellus* type of wing veining.

Bats from the African populations differ slightly in coloration. In both populations bats have sharply bicoloured hairs, both dorsal and ventral; basal parts of hairs are dark brown, distal parts pale brown (paler in ventral hairs). The Libyan pipistrelle bats have generally more rusty tinge of coloration, whereas individuals from Maghreb have dorsal side more brownish to greyish-brown, with larger portion of darker basal part of hairs (ca. 2/3–3/4 vs. ca. 1/2 in Libyan bats). Hairless parts of head and wings are dark brown in both populations. Paler or just white margins of plagiopatagia were observed in several individuals of Libyan as well as of Maghrebian populations.

Tentative registrations of echolocation calls by a heterodyne bat detector system in the field showed that individual bats examined from both Libyan and Moroccan populations as well as the pipistrelles from Middle East displayed approximately the same maximum energy of terminal frequency at about 45 kHz. Although these data are necessary to consider preliminar, their clear dissimilarity is evident with European samples of *P. pygmaeus*, which mostly show values over 50 kHz in the same way of field examination.

In the phylogenetic analysis (see also Table 2), the Libyan pipistrelle bats (two haplotypes acquired from seven specimens) is sister to *P. pygmaeus* s. str. samples from Europe (Fig. 7), however, with relatively deep divergence of 6–7% based on genetic distance (Table 3). Haplotypes of Libyan pipistrelle bats show unique base locations at 10 positions within the *P. pipistrellus* complex (Table 2): 21, 127, 186, 249, 306, 355 (c→t), 57, 69 (t→c), 300 (a→g), and 396 (a→c). The Magrebian (Moroccan) individuals (four haplotypes acquired from five specimens) cluster with *P. pipistrellus* s. str. from Eurasia with genetic distance of 3–5%. The Moroccan pipistrelle bats show unique base locations at five positions (Table 2): 27, 384 (c→t), 108 (a→g), 150 and 351 (t→c); all substitutions are transitions at the third codon position.

Discussion

In phylogenetic analysis, *P. pipistrellus* s. str. and *P. pygmaeus* s. str. lineages grouped into monophyletic clades, a pattern described in all previous genetic studies (Barrat *et al*., 1997; Mayer and von Helversen, 2001*b*; Benda *et al*., 2003; Hulva *et al*., 2004). However, African populations of pipistrelle bats do not follow this separation identically, but are separated into two distinct clades related to *P. pipistrellus* and *P. pygmaeus*, respectively. These groupings are supported by high bootstrap values (see Fig. 7; Hulva *et al*., 2004). In conclusion, African populations represent two genetically, geographically and also morphologically clearly separate units within the *P. pipistrellus* complex.

One distinct population inhabits the Mediterranean part of Libyan Cyrenaica. These bats belong to the *P. pygmaeus* lineage and are represented by large and more rusty coloured individuals with relatively large and broad rostrum and mesiodistally long and massive canines. This population differs significantly from all west Palaearctic populations of *P. pipistrellus* complex in skull morphology, including other African populations. This dissimilarity was noted already by Hanák and Elgadi (1984). Within the *P. pygmaeus* lineage, the Libyan population shows unique echolocation calls, the maximum energy of terminal frequencies was evidenced being at about 45 kHz.

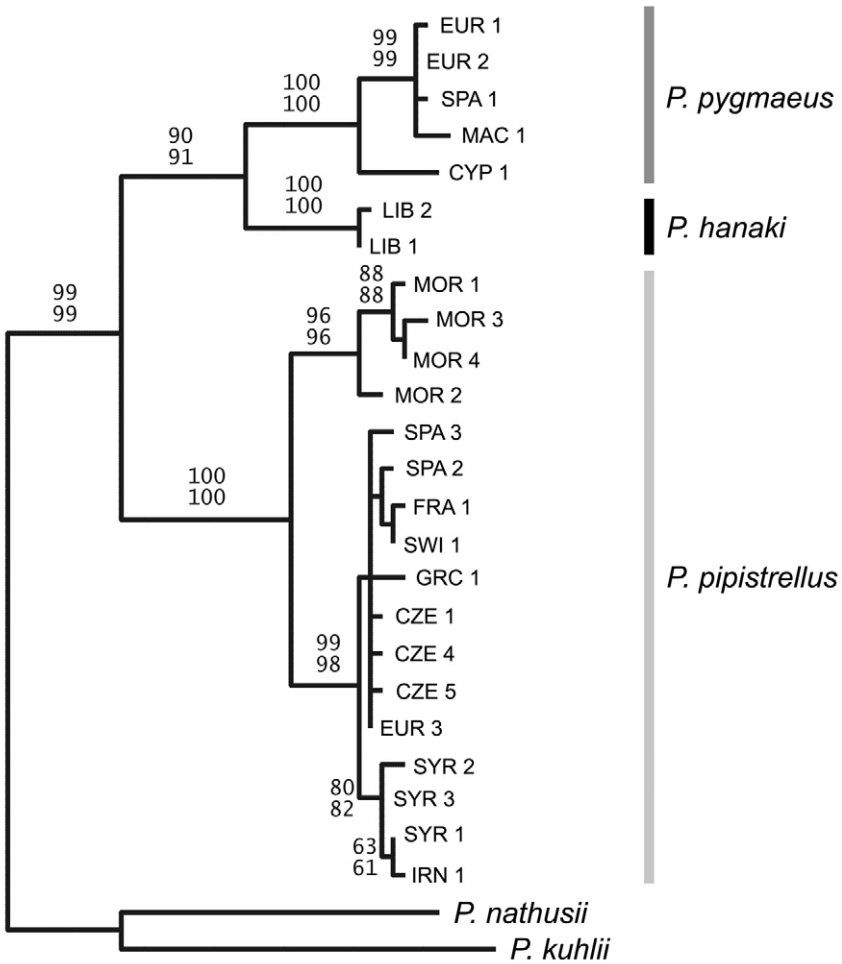

FIG. 7. Equal-weight parsimony tree for analysed haplotypes of bats from *P. pipistrellus* complex. The tree has a length of 185 mutations, consistency index (CI) excluding uninformative characters = 0.68, retention index = 0.89, rescaled CI = 0.68. Bootstrap supports with 1,000 replicates are given for maximum parsimony (upper value) and neighbor joining (lower value). For haplotype acronyms see Appendix 2

But it is invariably more than 50 kHz in all European populations of *P. pygmaeus* (Barrat *et al*., 1997; Mayer and von Helversen, 2001*b*) and thus it represents the autapomorphy of *P. pygmaeus* s. str. However, this comparison is based on insufficient data obtained from tentative field registrations, therefore, it must be confirmed in subsequent analyses. The distribution of wing veining types in the Libyan population indicates synapomorphic state of this character rather with *P. pipistrellus* clade than with *P. pygmaeus* lineage. The derived state of this character in *P. pygmaeus* s. str. also corroborates its separation from Libyan pipistrelle bats. Moreover, the genetic distance of 6–7% between Libyan bats and its sister form, European *P. pygmaeus* s. str., coincides with category indicative of conspecific populations or valid species within genetic species concept in bats (see e.g., Bradey and Baker, 2001). Formation of such phylogenetic gap is conditioned by considerable period of reproductive isolation (2.1–1.8 Myr according to molecular clock analyses; Hulva *et al*., 2004), which is

TABLE 2. Polymorphic sites identified in the partial cytochrome *b* sequenced in *P. pipistrellus* complex. Dot indicates a same nucleotide compared to the first reference sequence. For haplotype acronyms see Appendix 2

Species	Haplotype	1111111111111111111111112222222222222222222222333333333333333333334 1222223344456666667890000112222234455667788990011122234445667788899000011244555667789 90 8014570925770136792132689271367921506584836581406935843495476905847034625728125395846 72
Ppyg	EUR 1	GCCTCCATTCGTCCAAGTACGACATGCCAGCGAACTTCGTGACTCCCCTATTACTACCAACTCTAAAATCTTGTTATATCAAGCATA
Ppyg	EUR 2	.....C.....
Ppyg	SPA 1	.....CC.....
Ppyg	MAC 1	.A..T.G.....C.....
Ppyg	CYP 1	.....GA.GT..T.....C.....C.....C...GTCA...G.....
Phan	LIB 2	A.T....CC..C.....C.TA.T.....ATA.....AC.CT.....CT...TC.....G.AC.A.....C...A.C..
Phan	LIB 1	A.T....CC..C.....C.TA.T.....ATA.....AC.CT.....CT...TC.....G..C.A.....C...A.C..
Ppip	MOR 1	A..C.TG.CTA...G....TAG.G..ATGA.A.GTC..AC.C.CTT...G...TCC..T.TC...G..C..CA.C.C..TG.AT.C.
Ppip	MOR 2	A..C.TG.CTA.....TAG.G...TGA.A.GTC..AC.C.CTT...G.CGTCC..T.TC.C.GC.C..CA.C.C..TG.AT.C.
Ppip	MOR 3	A..C.TG.CTA...G....TAG.G...TGA.A.GTC..AC.C.CTT...G...TCC..T.T....GG.C..CA.C.C..TG.AT.CG
Ppip	MOR 4	A..C.TG.CTA...G....TAG.G...TGA.A.GTC..AC.C.CTT...G...TCC..T.T....G..C..CA.C.C..TG.AT.C.
Ppip	IRN 1	A..C..GCCTA.T.....TAG..C..TGA.AGGT...AC.C.CTT.T.G.C.T.C..T.TC.CGG..C..CA.CG.G.TGGA.CC.
Ppip	SYR 1	A..C..GCCTA.T.....TAG..C..TGA.AGGT...AC.C.CTT.T.G.C.TCC..T.TC.CGG..C..CA.CG.G.TGGA.CC.
Ppip	SYR 2	A..C..GCCTA.T.....TAG..C..TGA.AGGT...ACAC.CTT.T.G...TCC..T.TC.CGG..C..CA.CG...TGGA.CC.
Ppip	SYR 3	A..C..GCCTA.T.....TAG..C..TGA.AGGT...AC.C.CTT.T.G.C.TCC..T.TC.CGG..C..CA.CG...TGGA.CC.
Ppip	GRC 1	A..C..GCCTA.T.....TAG.....TGA.AGGT...A..C.CTTTT.G.C.TCC.TT.TC.CGG..C..CACC....TGGA.CC.
Ppip	CZE 1	A..C..GCCTA.T.....TAG.....TGA.AGGT...AC.C.CTT...G.C.TCC..T.TC.CGG..C..CACC....TGGA.CC.
Ppip	CZE 4	A..C..GCCTA.T.....TAG...A.TGA.AGGT...AC.C.CTT.T.G.C.TCC..T.TC.CGG..C..CACC....TGGA.CC.
Ppip	CZE 5	A..C..GCCTA.T.....TAG.....TGA.AGGT..TAC.C.CTT.T.G.C.TCC..T.TC.CGG..C..CACC....TGGA.CC.
Ppip	EUR 3	A..C..GCCTA.T.....TAG.....TGA.AGGT...AC.C.CTT.T.G.C.TCC..T.TC.CGG..C..CACC....TGGA.CC.
Ppip	FRA 1	A..C..GCCTA.TT.....TA.....TGA.AGGT.C.AC.C.CTT.T.G.C.TCC..T.TC.CGG..C..CACC....TGGA.CC.
Ppip	SWI 1	A..C..GCCTA.TT.....TAG.....TGA.AGGT.C.AC.C.CTT.T.G.C.TCC..T.TC.CGG..C..CACC....TGGA.CC.
Ppip	SPA 2	A..C..GCCTA.TT.....TAG.....TGA.AGGT...AC.C.CTTTT.G.C.TCC..T.TC.CGG..C..CACC....TGGA.CC.
Ppip	SPA 3	A..C..GCCTA.T.....TAG.....TGA.AGGT...AC.C.CTT.T.GCC.TCC..T.TC.CGG..C..CACC....TG.A.CC.

major criterion of most species concepts. The allopatric distribution plus the position in the phylogenetic tree indicates separate status from the cladistic point of view. To sum up, because the morphological differences between the Libyan population and all other western Palaearctic pipistrelle bats are substantial and incomparable to the situation of other populations and species in the *P. pipistrellus* complex in Europe and the Middle East (Barlow *et al*., 1997; Barlow et Jones, 1999; Häussler *et al*., 2000; Benda *et al*., 2003; etc.), and because of the genetic exclusiveness and reproductive isolation of these groups, we conclude that the Libyan pipistrelle bats represent a separate species as suggested by Hulva *et al*. (2004). Combination of morphological, biogeographic and genetic lines of evidence, resulting in defining of Libyan bats as a phenetic cluster as well as a historical entity, corroborates this statement in terms of both pattern and process categories of species concepts (see e.g., Rines, 1994).

Another distinct African population, which belongs to the *P. pipistrellus* clade, inhabits Mediterranean parts of the northwest African countries, Morocco, Algeria and Tunisia, i.e. the Maghreb. In the examined material, this form is represented by limited samples from Morocco and Algeria, while only Moroccan bats were examined genetically. Algerian individuals fall well within the range of morphological variation range for the Moroccan samples. Thus, the Maghrebian region is occupied by very small and rather darker pipistrelle bats with relatively shorter and narrower mesial part of rostrum. Due to its morphological and genetic differences from Eurasian *P. pipistrellus* s. str., the Maghrebian population undoubtedly has unique position, which indicates that it also had unique evolutionary history. Its genetic distinctness from European samples (incl. Iberian samples) is 3.5–5.5% and tends to fit the conditions of phenetic species or subspecies concept. Hulva *et al*. (2004) found that the Moroccan population is ancestral and very distinct within the *P. pipistrellus* lineage. However, this position would complicate the application of modern monophyletic species concepts — the species comprising Moroccan populations without Eurasian ones would be a paraphyletic clade. This aspect would illustrate difficulties of applying theoretical taxonomical concepts on real genealogies, as has been referred for example in ring species (e.g., Wake, 1997; Irwin *et al*., 2001). Moreover, although the morphological and metric characters of Maghrebian population seem to be very uniform, the fact that only south Moroccan specimens (from the Moyen Atlas Mts. to the south) could be genetically examined, leaves certain questions open. Genetic continuum of pipistrelle bats from southern Iberia via northern Morocco (the Rif Mts.) cannot be excluded. In conclusion, further sampling and genetic data will be necessary to decide whether the current taxonomic classification of the Maghrebian population into *P. pipistrellus* s. str. is appropriate.

The two African populations show good congruence with their sister lineages in external penial characters: Libyan pipistrelle bats have penes similar to those of *P. pygmaeus* s. str. described from Central Europe, while Maghrebian ones to those of *P. pipistrellus* s. str. from Central Europe (Häussler *et al*., 2000; Ziegler *et al*., 2001; von Helversen and Holderied, 2003). However, the examined bacula were almost identical in both African forms and in concordance with the bacula of bats from other studied populations of the complex (Topál, 1958; Lanza, 1959; Hill and Harrison, 1987). Ziegler *et al*. (2001) described some differences in shape between the bacula of *P. pipistrellus* and *P. pygmaeus*, and infinitesimal differences of this type were also observed in African specimens. However,

TABLE 3. Uncorrected p-distances among haplotypes of *Pipistrellus pipistrellus* complex

No.	Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	13
1	EUR1													
2	EUR2	0.002												
3	SPA1	0.005	0.002											
4	MAC1	0.010	0.007	0.010										
5	CYP1	0.032	0.030	0.032	0.037									
6	LIB2	0.067	0.065	0.067	0.072	0.070								
7	LIB1	0.065	0.062	0.065	0.070	0.067	0.002							
8	MOR1	0.109	0.107	0.104	0.110	0.117	0.107	0.104						
9	MOR2	0.114	0.112	0.109	0.115	0.119	0.112	0.109	0.015					
10	MOR3	0.109	0.107	0.104	0.110	0.117	0.112	0.109	0.010	0.017				
11	MOR4	0.104	0.102	0.100	0.105	0.117	0.107	0.104	0.005	0.015	0.005			
12	IRN1	0.119	0.117	0.119	0.120	0.127	0.107	0.104	0.050	0.045	0.055	0.050		
13	SYR1	0.122	0.119	0.117	0.122	0.129	0.109	0.107	0.047	0.042	0.052	0.047	0.002	
14	SYR2	0.119	0.117	0.114	0.120	0.127	0.107	0.104	0.045	0.045	0.050	0.045	0.010	0.007
15	SYR3	0.119	0.117	0.114	0.120	0.127	0.107	0.104	0.045	0.040	0.050	0.045	0.005	0.002
16	GRC1	0.119	0.117	0.114	0.120	0.132	0.112	0.109	0.050	0.045	0.055	0.050	0.020	0.017
17	CZE1	0.114	0.112	0.109	0.115	0.122	0.102	0.100	0.040	0.035	0.045	0.040	0.015	0.012
18	CZE4	0.119	0.117	0.114	0.120	0.127	0.107	0.104	0.045	0.040	0.050	0.045	0.015	0.012
19	CZE5	0.119	0.117	0.114	0.120	0.127	0.107	0.104	0.045	0.040	0.050	0.045	0.015	0.012
20	EUR3	0.117	0.114	0.112	0.117	0.124	0.104	0.102	0.042	0.037	0.047	0.042	0.012	0.010
21	FRA1	0.119	0.117	0.114	0.120	0.127	0.107	0.104	0.050	0.045	0.055	0.050	0.020	0.017
22	SWI1	0.122	0.119	0.117	0.122	0.129	0.109	0.107	0.047	0.042	0.052	0.047	0.017	0.015
23	SPA2	0.122	0.119	0.117	0.122	0.129	0.109	0.107	0.047	0.042	0.052	0.047	0.017	0.015
24	SPA3	0.117	0.114	0.112	0.117	0.124	0.104	0.102	0.042	0.037	0.047	0.042	0.017	0.015
25	*P. nathusii*	0.162	0.160	0.157	0.168	0.162	0.152	0.150	0.157	0.152	0.157	0.157	0.167	0.165
26	*P. kuhlii*	0.154	0.154	0.157	0.160	0.162	0.154	0.152	0.162	0.164	0.169	0.164	0.162	0.164

these differences can tentatively be considered as individual variation. Within the *P. pipistrellus* complex, the penial characters seem apparently to be rather conservative.

TAXONOMY

Pipistrellus hanaki
Hulva and Benda, sp. nov.

Synonymy

Pipistrellus pipistrellus: Hůrka, 1982: 87; Qumsiyeh and Schlitter, 1982: 385; Corbet, 1984: 10 [partim]; Hanák and Gaisler, 1983: 585; Hanák and Elgadi, 1984: 173; Le Berre, 1990: 96; Nowak, 1994: 193 [partim]; Amr and Qumsiyeh, 1993: 44; Horáček *et al*., 2000: 127–128 [partim].

Pipistrellus sp. I: Hulva *et al*., 2004: 1025–1030.

Type Material

Holotype

Adult ♀ (NMP 49897), specimen in alcohol, skull extracted; 19 May 2002, Wadi al Kuf, SW of Al Bayda, Cyrenaica, Libya, leg. P. Benda, V. Hanák, M. Andreas, A. Reiter, and M. Uhrin.

Paratypes

Three adult ♀♀ (NMP 49894–49896), specimens in alcohol, skulls extracted; the same date, locality and collectors as for the holotype specimen. One subadult ♂ (NMP 49902), specimen in alcohol, two adult ♀♀ (NMP 49903, 49904), specimens in alcohol, skulls extracted; Libya, Cyrenaica, Wadi Al Kuf, central part, 8 km SW of Massah, small cave, 20 May 2002, leg. P. Benda, V. Hanák, M. Andreas, A. Reiter, and M. Uhrin. One adult ♂, two adult ♀♀ (NMP

TABLE 3. Extended

No.	Haplotype	14	15	16	17	18	19	20	21	22	23	24	25	26
1	EUR1													
2	EUR2													
3	SPA1													
4	MAC1													
5	CYP1													
6	LIB2													
7	LIB1													
8	MOR1													
9	MOR2													
10	MOR3													
11	MOR4													
12	IRN1													
13	SYR1													
14	SYR2													
15	SYR3	0.005												
16	GRC1	0.020	0.015											
17	CZE1	0.015	0.010	0.010										
18	CZE4	0.015	0.010	0.010	0.005									
19	CZE5	0.015	0.010	0.010	0.005	0.005								
20	EUR3	0.012	0.007	0.007	0.002	0.002	0.002							
21	FRA1	0.020	0.015	0.015	0.010	0.010	0.010	0.007						
22	SWI1	0.017	0.012	0.012	0.007	0.007	0.007	0.005	0.002					
23	SPA2	0.017	0.012	0.007	0.007	0.007	0.007	0.005	0.007	0.005				
24	SPA3	0.017	0.012	0.012	0.007	0.007	0.007	0.005	0.012	0.010	0.010			
25	*P. nathusii*	0.167	0.162	0.162	0.162	0.167	0.162	0.165	0.167	0.170	0.165	0.160		
26	*P. kuhlii*	0.162	0.162	0.172	0.162	0.167	0.167	0.164	0.167	0.169	0.169	0.164	0.147	

49890–49892), specimens in alcohol, skulls extracted; Libya, Cyrenaica, ‘Arqub Ash Shafshaf, ca. 3 km S of Al Hilal, 18 May 2002, leg. P. Benda, V. Hanák, M. Andreas, A. Reiter, and M. Uhrin.

Type locality

Libya, Cyrenaica, upper part of the Wadi Al Kuf (the Jabal Akhdar Mts.), ca. 5 km southwest of Al Bayda, Al Jabal Al Akhdar Dist., 32°44’N, 21°41’E; ca. 495 m a.s.l.

Etymology

Patronymic; named in honour of Prof. Vladimír Hanák (Prague, Czech Republic) who significantly contributed to the knowledge of the Palaearctic bat fauna. Prof. V. Hanák co-discovered the existence of *P. pipistrellus*-like bats in Cyrenaica, Libya, and was the first who pointed out the possible difference of this population from other bats of the *P. pipistrellus* complex.

Description and Diagnosis

Small vespertilionid bat, but largest known member of the *Pipistrellus pipistrellus* group. Forearm is rather long (LAt 31.2–33.4), skull is large (LCr 12.1–12.7) (Fig. 8). Rostral part of skull is relatively long (I^1M^3/LCr 0.42–0.43) and quite broad, relatively and absolutely widest in the *P. pipistrellus* group (CC 3.8–4.1; CC/LCr 0.30–0.33; CC/CM^3 0.84–0.91). Braincase is relatively narrow (LaN/LCr 0.49–0.52) and rather low (ANc/LCr 0.35–0.37). Mandible and the madibular tooth-row are long (LMd 8.6–8.8; I_1M_3 5.5–5.6), both dimensions are largest in the group. Coronoid process of mandible is rather high (ACo 2.5–2.8; ACo/LMd

0.28–0.31). Upper incisors are mesiodistally long (LI^1 0.43–0.51, LI^2 0.37–0.46). Crowns of upper canines are mesiodistally absolutely and relatively very long (LCn 0.95–1.04; LCs/CM^3 0.21–0.23, $\bar{x}$ = 0.22), longest from the whole *P. pipistrellus* group, and relatively narrow (LCn/LaCn 1.06–1.38, $\bar{x}$ = 1.21). First upper premolars (P^3) are large, their crowns are mesiodistally long (LP^3 0.34–0.47), they are located palatally from the tooth-row, being almost completely hidden by canine in the view from the lateral side (Figs. 8 and 9). Second upper premolars (P^4) bear a cusp on the mesiopalatal edge of cingulum (ACin 0.13–0.21). Molars are large, first upper molars are very large (LM^1 1.13–1.26, LaM^1 1.34–1.42).

The glans penis is simple, without long medial stripe on dorsal side of praeputium, the skin of the whole penis is pale greyish-brown with unpigmented hairs. The dorsomedial praeputial band is noticeable only as a short stripe on the most distal part of the glans penis, just above the urethral orifice (see Fig. 5). Penis resembles the same organ in *P. pygmaeus* in general appearance (Ziegler *et al*., 2001: Fig. 1 right, Fig. 2c, e, f; von Helversen and Holderied, 2003: Abb. 3 right). The baculum is a long thin bone bifurcated on both epiphyses, of the same appearance as in other populations of the

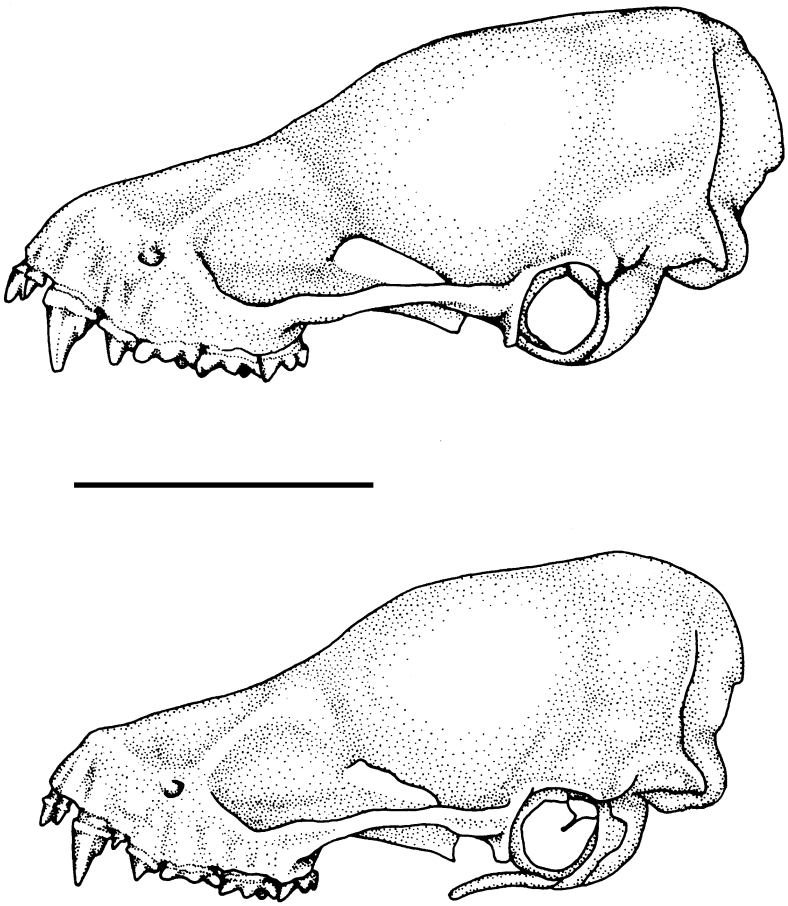

FIG. 8. Skulls of *P. hanaki* sp. nov (NMP 49884; above) and *P. pipistrellus* from Morocco (NMP 90074; below). Scale bar = 5 mm

group (Fig. 6; see also Topál, 1958; Lanza, 1959; Hill and Harrison, 1987; Ziegler *et al*., 2001; etc.). In the examined specimen it was 1.8 mm long, its proximal epiphysis was 0.4 mm wide, and its distal epihysis was 0.1 mm wide. The venation of wing is most often of the *P. pipistrellus* type (von Helversen and Holderied, 2003: Abb. 4 middle), rarely of the *P. pygmaeus* type (von Helversen and Holderied, 2003: Abb. 4 below).

The coloration of the pelage is generally pale brown to rusty brown, moderately paler and distinctly more rusty than in other forms of the group. Dorsal hairs are ca. 5 mm long, their distal half is hazelnut brown, the proximal half is dark chestnut brown. The ventral side is overall brown, moderately paler than dorsal side. Ventral hairs are ca. 5 mm long, their distal parts are umber, proximal two thirds are dark chestnut brown. Face, wing membranes, ears and tragi are dark brown, around eyes and on ear bases slightly paler. Plagiopatagium, uropatagium and mainly epiblema bear a paler up to translucent margin. The tail end projects 0.5–1.0 mm from the uropatagium (see also Table 1). Echolocation calls have a maximum energy of terminal frequencies at about 45 kHz.

Partial cytochrome b sequence of the holotype

402 bp, 5'end, haplotype LIB2 (Tables 2 and 3, Fig. 7; NCBI Accession Number AY316334): 5' atg aca aac att cga aaa tct cat ccc cta atc aaa atc atc aac agc tca ttc atc gac cta cca gcc cca tca aac att tca gca tga tga aat ttt gga tcc tta tta ggc atc tgt cta gga tta caa atc cta aca ggc cta ttt ctt gct ata cac tac aca tca gac aca gca acc gct ttc agc tct gtc acc cac atc tgc cga gat gta aat tat gga tga gtc cta cga tat cta cat gca aac gga gcc tca ata ttt ttt att tgc cta tat cta cac gta gga cgg ggt atc tac tat

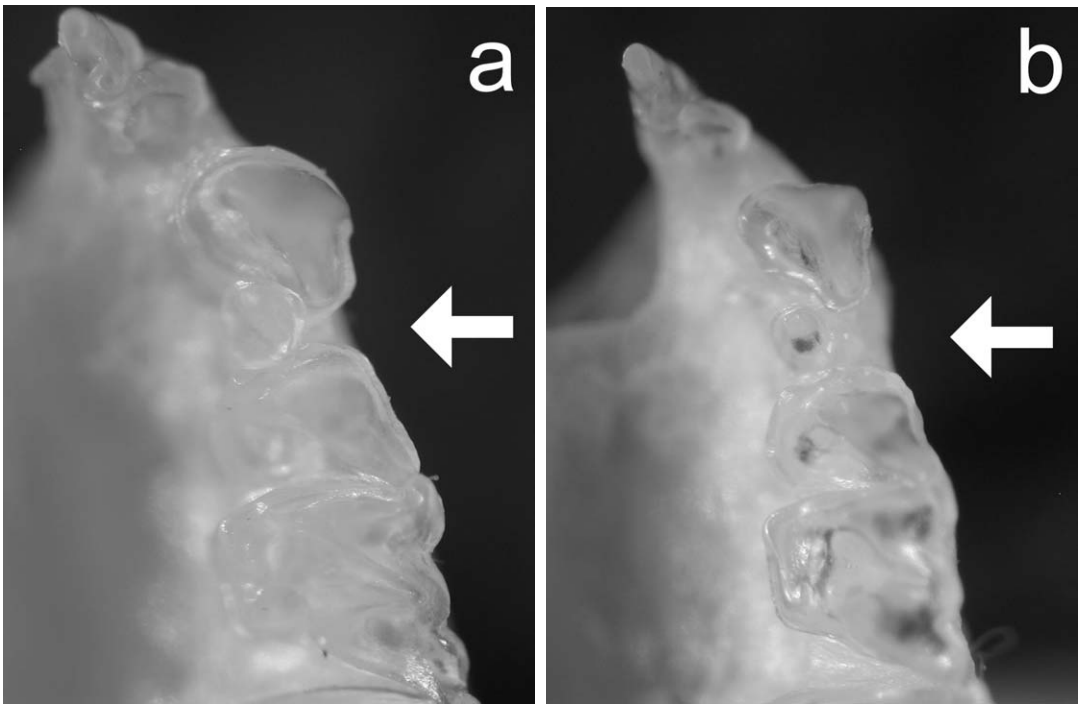

Fig. 9. Occlusal views on the upper tooth-rows of *P. hanaki* sp. nov. (NMP 49884) (a) and *P. pipistrellus* from Morocco (NMP 90074) (b)

gga tcc tac tta ttt aaa gaa acc tga aat ata gga gtt att cta cta ttc gct gta ata gca aca gcc ttc ata ggc tat gtc tta cca 3'.

Dimensions of the holotype

External measurements (in mm): body length 43, tail length 39, LAt 32.5, ear length 12.3, tragus length 5.4, body mass 4.7 g. Cranial measurements (in mm): LCr 12.47, LCb 12.17, LaZ 8.09, LaI 3.14, LaInf 3.89, LaN 6.51, LaM 7.39, ANc 4.52, ACr 5.86, I^1I^1 1.92, CC 4.08, P^4P^4 4.56, M^3M^3 5.19, I^1M^3 5.32, CM^3 4.53, M^1M^3 2.93, CP^4 1.92, LMd 8.77, ACo 2.76, I_1M_3 5.58, CM_3 4.76, M_1M_3 3.16, CP_4 1.62. Dental measurements (in mm): LI^1 0.48, LI^2 0.37, LCs 1.01, LaCs 0.81, LP^3 0.43, LP^4 0.90, LaP^4 1.14, LM^1 1.19, LaM^1 1.38, LM^3 0.73, LaM^3 1.41, ACin 0.15, LI_1 0.41, LI_3 0.37, LCi 0.70, LP_3 0.48. Wing measurements (in mm): Vp 6.8, Vm 4.8, Vd 2.1, IVp 10.5, IVm 7.2, IVd 2.6, IIIp 10.7, IIIm 8.7, IIId 7.2.

Differential diagnosis

Pipistrellus hanaki sp. nov. markedly differs from all other species of the *P. pipistrellus* complex from western Palaearctic in 402 bp 5'end cytochrome *b* sequences (Table 2); uncorrected genetic distance value lies between 6.2 and 11.2% (Table 3).

Pipistrellus hanaki sp. nov. differs significantly from the other two species, *P. pipistrellus* s. str. and *P. pygmaeus* s. str., by larger skull and teeth. Mandible is long in *P. hanaki* sp. nov. (LMd > 8.61 mm), in other species it measures up to 8.62 mm (Table 1). The mandibular tooth-row of *P. hanaki* sp. nov. is absolutely longer (I_1M_3 > 5.47) than in other species of the group (I_1M_3 < 5.44). Rostrum is broader in *P. hanaki* sp. nov. than in other species, breadth over upper canines is larger than 3.81 mm (CC 3.82–4.08; Table 1 and Fig. 8), in the other two species it is under 3.82 mm. Canines of *P. hanaki* sp. nov. are larger than in other species, the crowns of upper canines mediodistally are long at least 0.95 mm (0.95–1.04; Table 1, Figs. 3 and 9) while in other species the crowns of upper canines are long up to 0.91 mm (incl.). The crowns of lower canines of *P. hanaki* sp. nov. are mediodistally long at least 0.65 mm, in other species this length is less than 0.65 mm (Table 1). Upper canines of *P. hanaki* sp. nov. are also relatively larger than in other species of the group, from the lateral view the canine in most cases almost covers the first upper premolar (P^3); in other species P^3 is often well visible from lateral (buccal) aspect.

Pipistrellus hanaki sp. nov. differs from *P. pipistrellus* by the external appearance of penis, glans penis does not bear a pale dorsomedial stripe on praeputium in the former species. According to present data, *P. hanaki* sp. nov. differs from *P. pygmaeus* by lower terminal frequency of echolocation calls which is about 45 kHz in the former species.

Distribution

Pipistrellus hanaki sp. nov. occurs in the belt of Mediterranean climate and vegetation in northern Cyrenaica, Libya. The known distribution range covers a restricted area of ca. 3,000–5,000 km^2 of mountainous and coastal vegetation between Qasr Libiya in the west and Al Qubbah in the east (ca. 32°30'–32°50'N, 21°30'–22°20'E). This bat was recorded on at least eight sites in northern Cyrenaica, incl. Wadi al Kuf, 8 km SW of Massah (Qumsiyeh and Schlitter, 1982; Amr and Qumsiyeh 1993; own records), 8 km SSE of Haniya (Qumsiyeh and Schlitter, 1982; Amr and Qumsiyeh, 1993), 5 km SW of Al Abraq (Hanák and Elgadi, 1984; Qumsiyeh and Schlitter, 1982), 4 km S of Beida (Qumsiyeh and Schlitter, 1982), Wadi al Kuf, 5 km SW of Al Bayda (Hanák and Elgadi, 1984; Hůrka 1982; own records), Wadi Al Minshiyah, 'Arqub Ash Shafshaf

and the estuary of Wadi Al Kuf (own records). This very restricted distribution range would imply extreme conservation priorities; thus, we suggest the IUCN category vulnerable, subcategories A2c, D2 (sensu Hutson *et al*., 2001).

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APPENDIX 1

List of material examined in the morphological analyses. Abbreviations: S — skull, A — alcohol specimen, B — dry skin (balg); ind. – sex undetermined, for other abbreviations see Material and Methods

Pipistrellus pipistrellus (Schreber, 1774)

MOROCCO (9 specimens): 1♂ (NMP 90033 [S+A]), Oum er Rbia River, 5 km SW Bekrite (ca. 40 km S Azrou), 28 August 2003, leg. P. Benda; – 1♂ (NMP 90069 [S+A]), Gorges du Dadès, 5 km NW Aït-Ali (ca. 20 km N Boumalne Dadès), 2 Sept. 2003, leg. P. Benda; – 2♂♂, 3♀♀ (NMP 90073–90077 [S+A]), Gorges du Todra, 5 km SW Tamtattouchte (ca. 30 km N by road of Tinerhir), 3 September 2003, leg. P. Benda; – 1♂, 1♀ (SMO 6486, 6487 [S+B]), Gorges du Todra, 5 km N Tinerhir, 27 June 1993, leg. Z. Řehák (cf. Beneš and Hanák, 2003).

ALGERIA (12; cf. Gaisler, 1983; 1984): 5♂♂, 3♀♀ (MUB A-042, A-060, A-100, A-181, A-182, A-199, A-256, A-417 [S+B]), Setif, citadel, 20 and 30 May, 7 Oct., and 16 Dec. 1981, 10 January and 16 May 1982, 6 April 1983, leg. J. Gaisler; – 1♂ (MUB A-526 [S+B]), Kef Larous, Aures, 9 August 1983, leg. J. Gaisler; – 2♂♂, 1♀ (MUB A-534, 535, 536 [S+B]), Yakouren, Tizi Ozou, 11 August 1983, leg. J. Gaisler.

FRANCE (1): 1♂ (MHNG 1813.035 [S+A]), Collonge sous Salève (Haute-Savoie), 16 August 2001, leg. M. Chanson.

Appendix 1. Continued

SWITZERLAND (1): 1♂ (MHNG 1882.055 [S+A]), Genève, Bernex, 4 August 2003, leg. M. Bussard.

CZECH REPUBLIC (6): 1♀ (NMP 90013 [S+A]), Brno, Lidická ul., 19 August 2000, leg. Z. Řehák (cf. Benda *et al*., 2003); – 1♂ (NMP 90134 [S+A]), Velký Jindřichov u Benešova nad Černou, 31 July 2001, leg. V. Hanák; – 2♀♀ (SMZ 6695, 6696 [S+A]), Čížov, Ledové sluje caves, 15–16 August 2001, leg. A. Reiter (cf. Reiter *et al*., 2003); – 1♀ (SMZ 6688 [S+A]), Boskovštejn, 10 July 2001, leg. A. Reiter (cf. Reiter *et al*., 2003); – 1♂ (SMZ 6692 [S+A]), Onšov, 18 August 2001, leg. A. Reiter (cf. Reiter *et al*., 2003).

SLOVAKIA (9; cf. Benda *et al*., 2003): 1♂, 1♀ (NMP 90014, 90015 [S+A]), Tisovec, Slávča, 22–24 July 2002, leg. P. Benda, E. Hapl and M. Uhrin; – 2♂♂, 5♀♀ (NMP 90016–90022 [S+A]), Zadiel, Erňa cave, 20 April 2003, leg. P. Benda, V. Hanák and M. Uhrin.

BULGARIA (1): 1♂ (NMP 50439 [S+A]), Gara Lakatnik, Suhata peštera cave, 16 Dec. 2002, leg. P. Benda, T. Ivanova and M. Uhrin.

GREECE (2; cf. Hanák *et al*., 2001): 1♂ (MHNG 1807.052 [S+A]), Pili, Prespa Lake, 13 July 2000, leg. M. Ruedi; – 1♂ (MHNG 1807.055 [S+A]), Corycian Grotta, Mt. Parnass, 31 July 2000, leg. M. Ruedi.

SYRIA (14; cf. Benda *et al*., 2003): 4♂♂ (NMP 48060–48063 [S+A]), Slinfeh, 29 June 1998, leg. M. Andreas, P. Benda and M. Uhrin; – 2♀♀ (NMP 48084, 48085 [S+A]), Rabi'ah, 1 July 1998, leg. M. Andreas, P. Benda and M. Uhrin; – 1♂, 1♀♀ (NMP 48871, 48872 [S+A]), Sarghaya, 28 May 2001, leg. M. Andreas, P. Benda, A. Reiter, and D. Weinfurtová; – 1♀ (NMP 48902 [S+A]), Baniyas, 31 May 2001, leg. M. Andreas, P. Benda, A. Reiter, and D. Weinfurtová; – 4♂♂ (NMP 48981–48984 [S+A]), Maalula, 30 April 2001, leg. J. Obuch; – 1♀ (NMP 49986 [S+A]), Ras al-Bassit, 29 April 2001, leg. R. Lučan.

IRAN (4): 1♂ (SMF 46397 [S+B]), Rafsanjan, 1972; – 1♂, 1♀ (NMP 48112, 48113 [S+A]), Yazd, Towers of Silence, 4 May 1997, leg. P. Benda; – 1♀ (NMP 48155 [S+A]), Lenje Abad, 20 km S Dorud, 9 Oct. 1998, leg. P. Benda and M. Uhrin.

Pipistrellus pygmaeus (Leach, 1825)

CZECH REPUBLIC (6 specimens): 2♀♀ (NMP 90023, 90136 [S+A]), Malý Ratmírov, 2 July 2002, leg. V. Hanák (cf. Benda *et al*., 2003); – 1♀ (NMP 90135 [S+A]), Kolence u Třeboně, Čertova šlápota, 1 August 2002, leg. V. Hanák; – 1♀ (NMP 90137 [S+A]), Třeboň, Opatovický mlýn, 29 July 2002, leg. V. Hanák; – 1♂ (NMP 90138 [S+A]), Mláka u Třeboně, Krávovna, 5 August 2002, leg. V. Hanák; – 1♀ (NMP 90139 [S+A]), Lednice, 22 June 2001, leg. Z. Řehák.

SWITZERLAND (6): 2♂♂ (MHNG 1828.016, 1828.017 [S+A]), Genève, 21 and 30 May 2002; – 1♂ (MHNG 1826.019 [S+A]), Genève, Vandoeuvres, 12 Sept. 2001, leg. M. Ruedi; – 2♂♂ (MHNG 1828.064, 1828.065 [S+A]), Genève, Cologny, 1 and 3 Oct. 2000, leg. T. Sandoz; – 1♂ (MHNG 1882.045 [S+A]), Genève, Aïre, Crêt-des-îles, 10 May 2003, leg. Bärtschi.

GREECE (6; cf. Hanák *et al*., 2001): 1♂ (NMP 49016 [S+A]), Simopoulo, 23 August 2001, leg. P. Benda; – 1♂ (NMP 49021 [S+A]), Artíki, 25 August 2001, leg. P. Benda; – 1♂ (NMP 49030 [S+A]), Anthiro, 31 August 2001, leg. P. Benda; – 1♀ (NMP 49041 [S+A]), Dímitra, 1 Sept. 2001, leg. P. Benda; – 1♂ (NMP 49038 [S+A]), Spárti, 16 Sept. 1996, leg. P. Benda and M. Uhrin; – 1♂ (MHNG 1807.059 [S+A]), Rendina, 28 July 2000, leg. M. Ruedi.

TURKEY (2; cf. Benda *et al*., 2003): 2♂♂ (NMP 47946, 90011 [S+A]), Velika Köprüsü, 30 August 1996 and 7 May 2001, leg. M. Andreas, P. Benda, A. Reiter, M. Uhrin, and D. Weinfurtová.

CYPRUS (1; cf. Hanák *et al*., 2001): 1♂ (MHNG 1807.090 [S+A]), Kryos River, Trodos Mt., 5 Sept. 2000, leg. M. Ruedi.

Pipistrellus hanaki Hulva and Benda, sp. nov.

CYRENAICA, LIBYA (13 specimens): 1♂, 2♀♀ (NMP 49890–49892 [S+A]), 'Arqub Ash Shafshaf (above a permanent creek), 18 May 2002, leg. M. Andreas, P. Benda, V. Hanák, A. Reiter, and M. Uhrin; – 2♀♀ (NMP 90158, 90159 [S]), Wadi Al Kuf, 5 km SW Al Bayda (above a small river), 29 April 1980, leg. V. Hanák and K. Hůrka (cf. Hanák and Elgadi 1984, Hůrka 1982); – 4♀♀ (NMP 49894–49897 [S+A]), Wadi Al Kuf, 5 km SW Al Bayda (above a small river), 19 May 2002, leg. M. Andreas, P. Benda, V. Hanák, A. Reiter, and M. Uhrin; – 1♂, 2♀♀ (NMP 49902 [A], 49903, 49904 [S+A]), Wadi Al Kuf, central part, 8 km SW Massah, small cave, 20 May 2002, leg. M. Andreas, P. Benda, V. Hanák, A. Reiter, and M. Uhrin; – 1♀ (NMP 49884 [S+A]), Wadi Al Minshiyah (under a bridge), 17 May 2002, leg. M. Andreas, P. Benda, V. Hanák, A. Reiter, and M. Uhrin.

Appendix 2

List of material used in the genetic analysis. For other data of specimens see Appendix 1 (with exception of the Iberian individuals of which only DNA samples were used). The sequences of which accession numbers are marked with an asterisk (*) we took from GenBank (all reported by Stadelmann *et al.*, 2004)

Species	Haplotype	Country	Site	Accession No.	Voucher
P. pygmaeus	EUR 1	Turkey	Kirklareli, Velika Koprüsü	AY426087	NMP 47946
P. pygmaeus	EUR 1	Greece	Thessaloniki, Rendina	AY663796	MHNG 1807.060
P. pygmaeus	EUR 1	Spain	Tarragona	AY582277	biopsy (J. Juste *et al.*)
P. pygmaeus	EUR 2	Czech Rep.	Třeboň, Opatovický mlýn	AY316325	NMP 90137
P. pygmaeus	EUR 2	Czech Rep.	Jindř. Hradec, Malý Ratmírov (*n* = 2)	AY316323, AY316324	NMP 90136, 90023
P. pygmaeus	EUR 2	Czech Rep.	Třeboň, Krávovna	AY316326	NMP 90138
P. pygmaeus	EUR 2	Czech Rep.	Třeboň, Kolence	AY316322	NMP 90135
P. pygmaeus	EUR 2	Czech Rep.	Břeclav, Lednice	AY316319	NMP 90139
P. pygmaeus	EUR 2	Greece	Karditsa, Anthiro	AY316330	NMP 49030
P. pygmaeus	EUR 2	Greece	Lakonía, Spárti	AY426088	NMP 48738
P. pygmaeus	EUR 2	Greece	Messinía, Artiki	AY316327	NMP 49021
P. pygmaeus	EUR 2	Greece	Ilía, Simopoulo	AY316329	NMP 49016
P. pygmaeus	EUR 2	Greece	Grevená, Dimítra	AY316331	NMP 49041
P. pygmaeus	EUR 2	Turkey	Kirklareli, Velika Koprüsü	AY316328	NMP 90011
P. pygmaeus	EUR 2	Switzerland	Genève, Aïre, Crêt-des-îles	AY663797	MHNG 1882.045
P. pygmaeus	EUR 2	Switzerland	Genève	AY663798	MHNG 1828.017
P. pygmaeus	EUR 2	Spain	Sevilla	AY582280	biopsy (J. Juste *et al.*)
P. pygmaeus	EUR 2	Spain	Logroño	AY582281	biopsy (J. Juste *et al.*)
P. pygmaeus	SPA 1	Spain	Malaga	AY582282	biopsy (J. Juste *et al.*)
P. pygmaeus	MAC 1	Greece	Thessaloniki, Rendina	AJ504441*	MHNG 1807.059
P. pygmaeus	CYP 1	Cyprus	Trodos Mts, Kryos River	AJ504442*	MHNG 1807.090
P. hanaki sp. nov.	LIB 1	Libya	Cyrenaica, Wadi al Minshiyah	AY316333	NMP 49884
P. hanaki sp. nov.	LIB 1	Libya	Cyrenaica, Arqub ash Shafshaf (*n* = 2)	AY426091, AY426092	NMP 49891, 49892
P. hanaki sp. nov.	LIB 2	Libya	Cyrenaica, Wadi al Kuf (*n* = 4)	AY426089, AY426090	NMP 49894, 49903
				AY316332, AY316334	NMP 49904, 49897
P. pipistrellus	MOR 1	Morocco	Tinerhir, Gorges du Todra	AY426093	SMO 6486
P. pipistrellus	MOR 2	Morocco	Tinerhir, Gorges du Todra	AY426094	SMO 6487
P. pipistrellus	MOR 3	Morocco	Azrou, Bekrite	AY582283	NMP 90033
P. pipistrellus	MOR 4	Morocco	Boumalne Dadès, Gorges du Dadès	AY582284	NMP 90069
P. pipistrellus	MOR 4	Morocco	Tinerhir, Gorges du Todra	AY582285	NMP 90074
P. pipistrellus	IRN 1	Iran	Yazd, Towers of Silence	AY316335	NMP 48113
P. pipistrellus	SYR 1	Syria	Dimashq, Sarghaya	AY316337	NMP 48872

APPENDIX 2. Continued

Species	Haplotype	Country	Site	Accession No.	Voucher
P. pipistrellus	SYR 2	Syria	Al Lathiqiyeh, Slinfeh	AY316336	NMP 48063
P. pipistrellus	SYR 3	Syria	Tartus, Banyas	AY426096	NMP 48902
P. pipistrellus	EUR 3	Czech Rep.	Brno	AY316344	NMP 90013
P. pipistrellus	EUR 3	Czech Rep.	Jevišovice, Boskovštejn	AY316342	SMZ 6688
P. pipistrellus	EUR 3	Czech Rep.	Vranov n. Dyjí, Čížov	AY316341	SMZ 6696
P. pipistrellus	EUR 3	Slovakia	Tisovec, Slávča (n = 2)	AY426098, AY426099	NMP 90014, 90015
P. pipistrellus	EUR 3	Slovakia	Košice, Zadiel, Erňa cave	AY426100	NMP 90017
P. pipistrellus	CZE 1	Czech Rep.	Český Krumlov, Velký Jindřichov	AY316347	NMP 90134
P. pipistrellus	CZE 4	Czech Rep.	Vranov n. Dyjí, Onšov	AY316343	SMZ 6692
P. pipistrellus	CZE 5	Czech Rep.	Vranov n. Dyjí, Čížov	AY316340	SMZ 6695
P. pipistrellus	GRC 1	Greece	Mt. Parnass, Corycian Grotta	AJ504443*, AY663799	MHNG 1807.055
P. pipistrellus	FRA 1	France	Haute-Savoie, Collonge sous Salève	AY663800	MHNG 1813.035
P. pipistrellus	SWI 1	Switzerland	Genève, Bernex	AY663801	MHNG 1882.055
P. pipistrellus	SPA 2	Spain	Logroño	AY582292	biopsy (J. Juste *et al.*)
P. pipistrellus	SPA 3	Spain	Cádiz (n = 2)	AY582293, AY582294	EBD C-2290, C.I.-613
P. nathusii		Switzerland	Vaud, Lausanne	AJ504446*	MHNG 1806.010
P. kuhlii		Greece	Kilkís, Kilkís	AJ504444*	MHNG 1807.054