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Notes on the distribution of *Pipistrellus pipistrellus* complex in the Eastern Mediterranean: First records of *P. pipistrellus* for Syria and of *P. pygmaeus* for Turkey

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Abstract. A genetic analysis (402 bp of cytochrome *b* gene, 5' end) of East-Mediterranean specimens of pipistrelle bats showed the occurrence of *Pipistrellus pygmaeus* in Turkish Thrace and *P. pipistrellus* in western Syria, respectively. No important difference in the examined gene sequences was found between *P. pygmaeus* from Turkey and those from other parts of its distributional range (the Balkans, central Europe); however, there was a difference of 0.75–1.25% between Syrian *P. pipistrellus* and central-European individuals.

***Pipistrellus pipistrellus*, *Pipistrellus pygmaeus*, the Balkans, Middle East, distribution**

Introduction

The *Pipistrellus pipistrellus* species complex is composed of two cryptic species (Horáček et al. 2000, Mayer & Helversen 2001a, b), viz., *P. pygmaeus* (Leach, 1825) and *P. pipistrellus* (Schreber, 1774). The species status of the pygmy pipistrelle (or midge bat), *P. pygmaeus*, has been recognised only recently (Barrat et al. 1997). From its sibling species, the common pipistrelle, *P. pipistrellus*, was distinguished on the basis of differences in terminal frequencies of echolocation calls (Weid & Helversen 1987, Jones & Parijs 1993), moreover, other differences were found in their ecology (Barlow 1997, Barlow & Jones 1999) and in morphological characters (Barlow et al. 1997, Häusler et al. 2000, Ziegler et al. 2001). Although these authors mentioned several characters suitable for the species identification, mainly the wing and penial morphology, the most useful method for reliable determination of both species of the *P. pipistrellus* complex remains the genetic analysis along with the analysis of echolocation calls (Mayer & Helversen 2001a).

Pipistrellus pygmaeus occurs across a wide geographic range of Europe (Horáček et al. 2000). In most of its known distributional range it lives in sympatry with *P. pipistrellus*, particularly in Great Britain, in central and south-eastern Europe (Mayer & Helversen 2001a); allopatric population of *P. pygmaeus* was found in Sweden and in southern Iberia. On the contrary, only *P. pipistrellus* was found in north-eastern Europe as well as in the Middle East, incl. Anatolia and Israel (Masing 1999, Mayer & Helversen 2001a, Siivonen & Wermundsen 2003).

In Turkey and Greece, bats of the *Pipistrellus pipistrellus* complex were found to rank among the most common bat forms, along with several species more typical of the Mediterranean, *Rhinolophus ferrumequinum*, *R. hipposideros*, *Myotis blythii*, and *Pipistrellus kuhlii* (Benda & Horáček 1998, Hanák et al. 2001). *P. pipistrellus* s. l. was found to be widespread in the whole territory of Turkey (altogether more than 70 record localities are known), although only a few records come from central and south-eastern Anatolia (Benda & Horáček 1998, Kryštufek & Vohralík 2001). In the Levant proper, bats of the *P. pipistrellus* complex were recorded only in the coastal montane regions; there are records from Hattay (Lehmann 1966), from Lebanon (Lewis & Harrison 1962), and from northern Israel (incl. Golan Highs; Mendelssohn & Yom-Tov 1999).

In most of mainland Greece, both species of the *P. pipistrellus* complex were found (Weid & Helversen 1987, Hanák et al. 2001, Mayer & Helversen 2001a, b). A new record of *P. pygmaeus* in Bulgaria, near Madžarovo in the Arda river valley, has been reported recently (Dietz et al. 2002), representing the easternmost record of this species in the Balkans. The only pipistrelle bats from Turkey which were genetically and/or acoustically analysed (coming from Izmir and Kizilcahamam in western Anatolia) were assigned to *P. pipistrellus* (Mayer & Helversen 2001a), like the bats from the islands of Crete and Samos. On Rhodes, both forms of the complex were found (Hanák et al. 2001, Mayer & Helversen 2001a), while only *P. pygmaeus* is known from the islands of Lesbos and Cyprus (Hanák et al. 2001).

The current knowledge of distribution of the individual species of the *P. pipistrellus* complex in the eastern Mediterranean is very scarce (except for Greece), based on examination of several individuals only made by Mayer & Helversen (2001a). Here we present results of species identification analyses of pipistrelle bats, collected during several field trips to the eastern Mediterranean and deposited in the collection of the National Museum, Prague, to contribute to a picture of distributional pattern of both species in the region.

Material and Methods

Material examined

All the examined specimens are deposited in the collection of Department of Zoology, National Museum (Natural History), Prague (NMP). The bats which were included into and were identified by genetic analysis described below are marked with an asterisk (*) in the list and in Tab. 2.

New material

Turkey (three specimens): **Velika Köprüsü** area (8 km SW Demirköy, Istranca Mts., Kirklareli Dist.; 41° 47' N, 33° 28' E; ca. 530 m. a. s. l.), 30 August 1996, two males (NMP 47946*, 47947 [S+A]), leg. M. Andreas, P. Benda & M. Uhrin (cf. Benda & Horáček 1998); the same site, 7 May 2001, adult male (NMP 90011*), leg. M. Andreas, P. Benda, A. Reiter & D. Weinfurtová; for locality description see also Kryštufek & Vohralík (2001: 64, Fig. 52).

Syria (15 specimens): **Baniyas** (Tartus Dist.; 35° 11' N, 35° 57' E, ca. 10 m a. s. l.), 31 May 2001, adult female (NMP 48902* [A]), leg. M. Andreas, P. Benda, A. Reiter & D. Weinfurtová; – **Maalula** (Dimashq Dist.; 33° 51' N, 36° 33' E, ca. 1770 m a. s. l.), 30 April 2001, four adult males (NMP 48981–48984 [A]), leg. J. Obuch; – **Kassab** (Al Lathiqiyeh Dist.; 35° 55' N, 35° 58' E, ca. 1700 m a. s. l.), border crossing-point, 3 July 1998, subadult female (NMP 48086 [S+A]), leg. M. Andreas, P. Benda & M. Uhrin; – **Rabi'ah** (Al Lathiqiyeh Dist.; 35° 49' N, 36° 2' E, ca. 620 m a. s. l.), 1 July 1998, two adult females (NMP 48084–48085 [S+A]), leg. M. Andreas, P. Benda & M. Uhrin; – **Ras al-Bassit** (Al Lathiqiyeh Dist.; 35° 51' N, 35° 51' E, ca. 10 m a. s. l.), 29 April 2001, adult female (NMP 49986 [S+A]), leg. R. Lučan; – **Sarghaya** (Dimashq Dist.; 33° 50' N, 36° 11' E, ca. 1330 m a. s. l.), 28 May 2001, adult male and female (NMP 48871, 48872* [A]), leg. M. Andreas, P. Benda, A. Reiter & D. Weinfurtová; – **Slinfeh** (Al Lathiqiyeh Dist.; 35° 36' N, 36° 13' E, ca. 1350 m a. s. l.), 29 June 1998, four adult males (NMP 48060–48062, 48063* [S+A]), leg. M. Andreas, P. Benda & M. Uhrin.

Comparative material

Pipistrellus pipistrellus (10 specimens): **Slovakia**: Tisovec (Rimavská Sobota Dist.; 48° 42' N, 19° 56' E), Slávča meadow, 22 and 24 July 2002, adult male and adult female (NMP 90014*, 90015* [S+A]), leg. P. Benda, E. Hapl

& M. Uhrin; – Zadiel (Košice-okolie Dist.; 48° 37' N, 20° 51' E), Erňa cave, 20 April 2003, two males and five females (NMP 90016–90022, 90017* [S+A]), leg. P. Benda, V. Hanák & M. Uhrin. – **Czech Rep**.: Brno (Lidická street) (Brno-město Dist.; 49° 13' N, 16° 36' E), 19 August 2002, juvenile female (NMP 90013* [A]), leg. Z. Řehák.

Pipistrellus pygmaeus (14 specimens): **Greece** (see also Hanák et al. 2001): Anthiro (Kardítsa Dist.; 39° 12' N, 21° 45' E), 31 August 2001, adult male (NMP 49030* [S+A]), leg. P. Benda; – Artíki (Messinía Dist.; 37° 16' N, 21° 47' E), 25 August 2001, subadult male (NMP 49021* [S+A]), leg. P. Benda; – Dimítra (Grevená Dist.; 39° 57' N, 21° 41' E), 1 Sept. 2001, subadult male and subadult female (NMP 49040, 49041* [S+A]), leg. P. Benda; – Kombotádes (Fthiótida Dist.; 38° 52' N, 22° 21' E), 9 Sept. 1996, two adult males and five adult females (NMP 48716–48722 [S+A]), leg. M. Andreas, P. Benda & M. Uhrin; – Simopoulo (Ilía Dist.; 37° 51' N, 21° 32' E), 23 August 2001, adult male (NMP 49016* [S+A]), leg. P. Benda; – Spárti (Lakonía Dist.; 37° 5' N, 22° 26' E), 16 Sept. 1996, adult male (NMP 48738* [S+A]), leg. P. Benda & M. Uhrin. – **Czech Rep**.: Střížovice (Jindřichův Hradec Dist.; 49° 8' N, 15° 8' E), Malý Ratmírov, 2 July 2002, adult female (NMP 90023* [A]), leg. V. Hanák.

Genetic analysis

The genomic DNA was extracted from alcohol preserved tissue samples (pectoral muscle and/or wing membrane) by proteinase K lysis and PCl extraction in PLG tubes (Eppendorf). The part of cytochrome *b* gene (402 bp, 5' end) was amplified by PCR with predenaturation (94 °C, 3 min), 40 cycles of annealing (45 °C, 1 min), extension (72 °C, 1 min) and denaturation (94 °C, 1 min) followed by final annealing (45 °C, 1 min) and extension (72 °C, 4 min). 25 µl reaction mixtures contained 1x Taq buffer (Promega), 2.5 mM $MgCl_2$, 200 µM each dNTP, 0.5 µM primers (MVZ04 and MVZ05; Smith & Patton 1991), 1u of Taq polymerase (Promega) and 100 ng of template. Amplicons were excised from agarose gel, purified with a QIAquick Gel Extraction Kit (Qiagen) and sequenced by ABI PRISM Terminator kit (with the same primers as PCR) and automated DNA sequencer (PE310). Chromatograms were edited with Chromas (McCarthy 1996), resultant sequences were aligned by Clustal W (Thompson et al. 1994) and compared with both published and own genetic and acoustic data.

Results and Discussion

The obtained cytochrome *b* sequences exhibit a low level of guanidine residues content (A = 0.2983, C = 0.2477, freq G = 0.1633, T = 0.2907), a phenomenon characteristic also for other mammalian mtDNA (Irwin et al. 1991). The data fit best the HKY model of sequence evolution with TS/TV ratio of 13.39 (Modeltest 3.06; Posada 1998).

Two analysed bats from Turkey belong to *Pipistrellus pygmaeus*. The individual NMP 90011 matches exactly the *P. pygmaeus* haplotypes from central Europe (Czech Rep., NMP 90013) and the Balkans (Greece, NMP 48738, NMP 49016, NMP 49021, NMP 49030, NMP 49041). The other Turkish *P. pygmaeus* (NMP 47946) differs in 1 position (C → A at position 244) (Fig. 1). Surprisingly, this mutation occurs at the first codon position and is not synonymous – after translation there is Leu in spec. NMP 90011 and Met in NMP 47946 at amino acid position 82.

All the analysed Syrian bats were identified as *P. pipistrellus*. They represent three haplotypes with genetic distance of about 1% from central-European haplotypes (e.g. Czech Rep., NMP 90023; Slovakia, NMP 90017), see Tab. 1 and Fig. 1. The variability among Syrian haplotypes was detected at positions 178 (G → A in NMP 48063, 1st codon position, Ala-Thr after translation in position 60), 225 (C → T in NMP 48063, 3rd codon position) and 352 (A → G in NMP 48872, 1st codon position, Ile → Val after translation in position 118).

The lack of phylogeographic structure in our *P. pygmaeus* mtDNA data suggests a very recent genetic exchange within studied regions. It corresponds with the data presented by Mayer & Helversen (2001a) who found only one nucleotide substitution difference between a Swedish individual and a Greek one, and two substitution difference between Swedish and Spanish bats. On the other hand, Syrian *P. pipistrellus* exhibits deeper separation from the central-European population. Surprisingly, the majority of mutations (3 out of 4) detected within both cryptic species

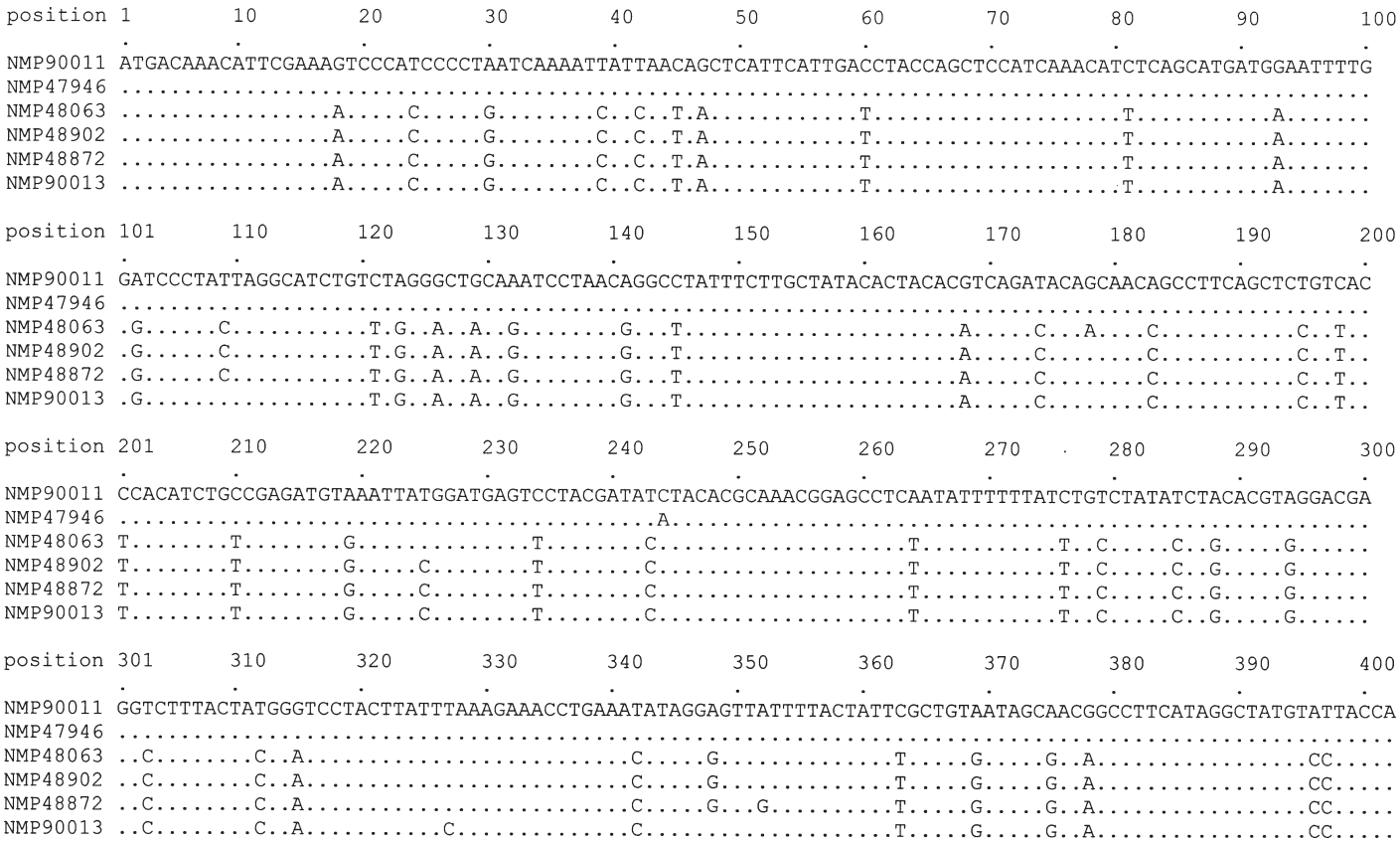

Fig. 1. Aligned nucleotide sequences used in this study (mt DNA, 402 bp 5' end of cytochrome *b* gene). For the list of specimens see Tab. 1 and Material examined.

Obr. 1. Nukleotidové sekvence použité v této studii (mtDNA, 402 bp 5' konec genu pro cytochrom *b*). Upřesnění exemplářů viz Tab. 1 a Material examined.

Tab. 1. Genetic distances (in %) among haplotypes of studied pipistrelle bats
Tab. 1. Genetické vzdálenosti (v %) mezi haplotypy studovaných netopýrů skupiny *P. pipistrellus*

haplotype	no. coll.	1	2	3	4	5	6
1 *P. pygmaeus*, Turkey	NMP 90011	–					
2 *P. pygmaeus*, Turkey	NMP 47946	0.00249	–				
3 *P. pipistrellus*, Syria	NMP 48063	0.11692	0.11940	–			
4 *P. pipistrellus*, Syria	NMP 48902	0.11692	0.11940	0.00498	–		
5 *P. pipistrellus*, Syria	NMP 48872	0.11940	0.12189	0.00746	0.00249	–	
6 *P. pipistrellus*, Czech Rep.	NMP 90013	0.11443	0.11692	0.01244	0.00746	0.00995	–

occurred at 1st codon positions. This contrasts with a typical pattern (connected with degeneration of genetic code) of divergence between these two species – 47 variable nucleotide positions (11.7% sequence divergence) – 4 first codon positions, 1 second codon position, 42 third codon position).

Our record of *P. pygmaeus* in Turkish Thrace is the first finding of this species in the country (Fig. 2); until now, only two records of this complex identified to species were mentioned from

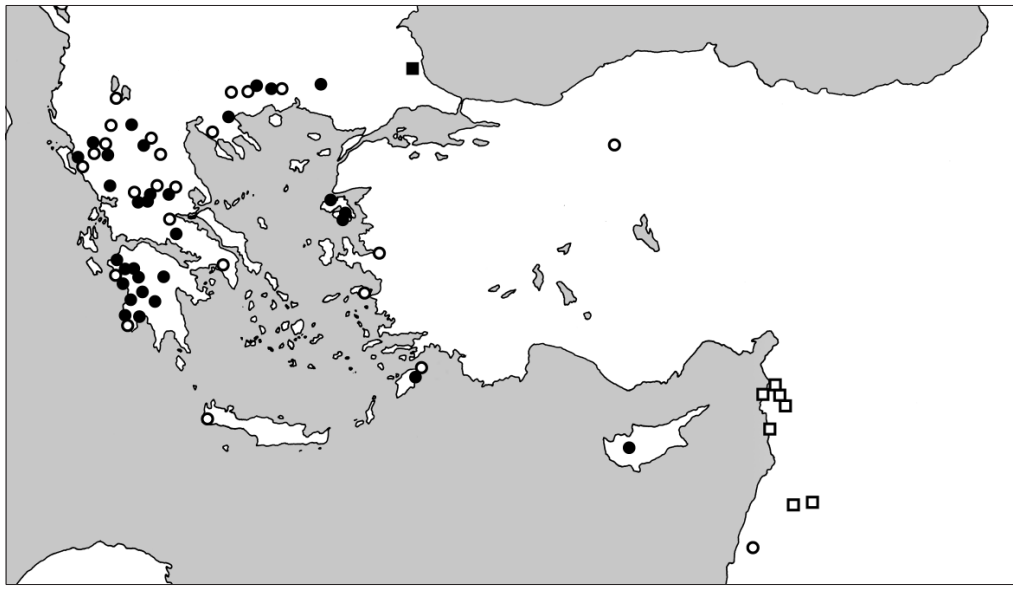

Fig. 2. Records of *Pipistrellus pygmaeus* (full symbols) and *P. pipistrellus* (open symbols) in the Eastern Mediterranean. Circles denote records of both forms after Hanák et al. (2001), Mayer & Helversen (2001a), and Dietz et al. (2002). The squares denote sites of records of *P. pipistrellus* in Syria and of *P. pygmaeus* in Turkey.
Obr. 2. Nálezy netopýra nejmenšího (*Pipistrellus pygmaeus*) – plné symboly, a netopýra hvízdavého (*P. pipistrellus*) – prázdné symboly, ve východním Středomoří. Kroužky označují nálezové lokality obou forem podle Hanáka et al. (2001), Mayera & Helversena (2001a) a Dietze et al. (2002). Čtverce představují lokality nálezů netopýra hvízdavého v Syrii a netopýra nejmenšího v Turecku.

Turkey and they both belonged to *P. pipistrellus* (Mayer & Helversen 2001a). On the other hand, this record is not surprising from the biogeographical point of view, since *P. pygmaeus* is a relatively commonly found bat in the southern Balkans (Hanák et al. 2001, Mayer & Helversen 2001a), and the nearest record comes from ca. 200 km westward situated Madžarovo in southern Bulgaria (Dietz et al. 2002). Thus, the occurrence of *P. pygmaeus* has been confirmed from the whole Balkan peninsula s. str. (Fig. 2).

The genetic analysis confirmed the occurrence of *P. pipistrellus* in Syria at three sites. Our records of echolocation calls of pipistrelle bats in Syria (using the heterodyne ultrasonic detector only) indicated only the presence of bats of the 45 kHz phonic type. It is close to the situation known in *P. pipistrellus* in Europe, where maximum energy of the species' echolocation call is at around 45 kHz (Weid & Helversen 1987, Zingg 1990, Jones & Parijs 1993, Mayer & Helversen 2001a, etc.). Thus, there are the first records of this species and of the species complex, respectively, from the territory of Syria (Kumerloeve 1975, Harrison & Bates 1991). Nevertheless, the presence of *P. pipistrellus* in western Syria is in accordance with the previous data by Mayer & Helversen (2001a) who found this species in Israel and western Anatolia (Fig. 2). Data by Mendelssohn

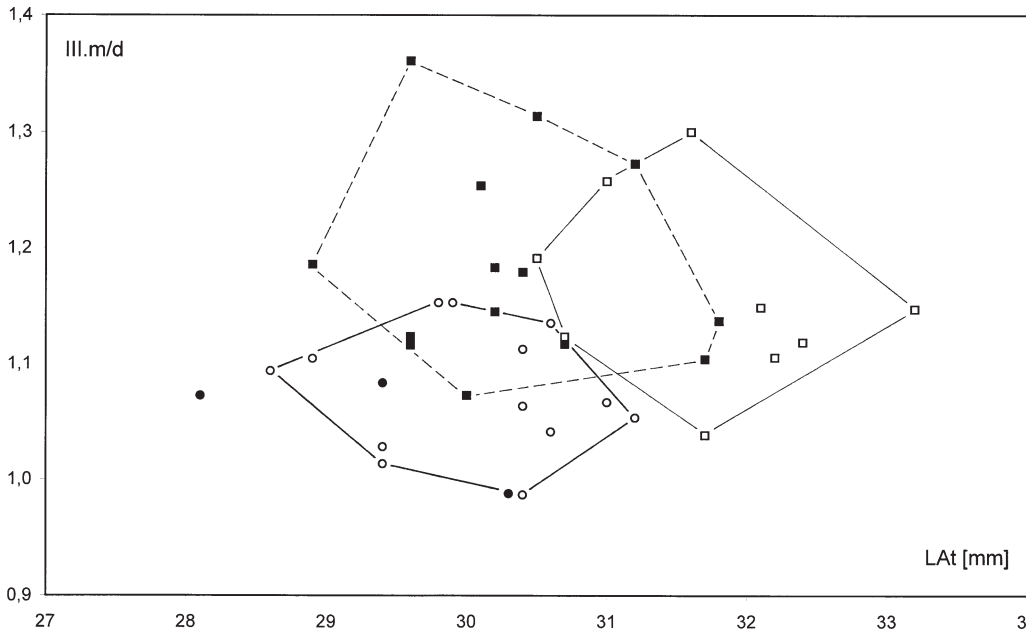

Fig. 3. Comparison of forearm length (LAt) versus ratio between lengths of medial and distal phalanges of the third finger (IIIm/d) of *P. pygmaeus* from Greece (open circles) and Turkey (closed circles) and of *P. pipistrellus* from Central Europe (open squares) and Syria (closed squares).
Obr. 3. Srovnání délky předloktí (LAt) s poměrem délky středního a posledního článku třetího prstu (IIIm/d) netopýra nejmenšího (*P. pygmaeus*) z Řecka (prázdné kroužky) a Turecka (plné kroužky) a netopýra hvízdavého (*P. pipistrellus*) ze střední Evropy (prázdné čtverce) a Syrie (plné čtverce).

Tab. 2. Wing measurements (the lengths of forearm, LAt, and of phalanges of IIIrd–Vth fingers) of *P. pygmaeus* from Turkey and of *P. pipistrellus* from Syria
Tab. 2. Křídelní rozměry (délka předloktí, LAt, a délky článků 3. až 5. prstu) netopýra nejmenšího (*P. pygmaeus*) z Turecka a netopýra hvízdavého (*P. pipistrellus*) ze Syrie

coll. no.	site / lokalita	sex, age	LAt	IIIp	IIIm	IIId	IVp	IVm	IVd	Vp	Vm	Vd
P. pygmaeus, Turkey												
NMP 47946*	Velika Köprüsü	m sad.	30.3	10.5	8.1	8.2	10.1	6.5	2.9	6.5	3.8	1.8
NMP 47947	Velika Köprüsü	f juv.	28.1	9.4	7.4	6.9	9.0	5.5	2.1	6.4	3.6	1.6
NMP 90011*	Velika Köprüsü	m ad.	29.4	9.9	7.8	7.2	9.3	6.4	2.9	6.4	3.6	1.8
P. pipistrellus, Syria												
NMP 48060	Slinfeh	m ad.	30.2	10.3	8.4	7.1	10.0	6.1	2.4	6.2	3.4	1.6
NMP 48061	Slinfeh	m ad.	30.2	10.4	7.9	6.9	9.6	6.0	2.2	6.5	4.0	1.8
NMP 48062	Slinfeh	m ad.	31.7	11.3	8.5	7.7	10.8	6.9	3.0	7.3	4.5	1.9
NMP 48063*	Slinfeh	m ad.	30.0	10.1	7.4	6.9	9.7	5.7	2.6	6.4	3.3	1.8
NMP 48084	Rabi'ah	f ad.	28.9	10.7	8.3	7.0	9.5	6.8	2.2	6.3	4.0	1.7
NMP 48085	Rabi'ah	f ad.	29.6	10.4	8.3	6.1	10.3	5.5	2.4	6.8	3.0	1.8
NMP 48871	Sarghaya	f ad.	31.8	11.0	8.3	7.3	10.4	6.6	2.9	6.2	3.8	1.8
NMP 48872*	Sarghaya	m ad.	30.4	10.3	7.9	6.7	10.2	5.9	3.0	6.4	3.6	1.9
NMP 48902*	Baniyas	f ad.	30.7	10.6	8.6	7.7	10.2	6.0	2.4	6.6	3.7	1.7
NMP 48981	Maalula	m ad.	29.6	10.4	8.2	7.3	9.5	6.1	2.5	6.3	4.0	1.6
NMP 48982	Maalula	m ad.	30.1	11.0	8.4	6.7	10.3	5.9	2.7	6.8	3.4	1.7
NMP 48983	Maalula	m ad.	31.2	10.5	8.4	6.6	10.3	6.1	2.2	6.9	3.7	2.1
NMP 48984	Maalula	m sad.	29.6	9.9	7.7	6.9	9.3	5.5	2.4	6.6	3.4	1.6
NMP 48986	Ras al Bassit	f ad.	30.5	10.5	8.8	6.7	10.3	6.2	2.6	6.4	4.1	1.5

& Yom-Tov (1999) indicate rather the occurrence of *P. pipistrellus* s. str. in Upper Galilee, Israel, as they mention observations of bats with maximum energy of their calls at 48 kHz.

Hitherto described findings of pipistrelle bats from the mainland Middle East show the only confirmed occurrence of *P. pipistrellus* (Fig. 2). Nevertheless, the previous records of *P. pygmaeus* from the islands of Lesbos, Rhodes, and Cyprus as well as our record from Thrace indicate the possible distribution of this species at least in the coastal regions of western and south-western Anatolia.

The genetic separation of Syrian samples from central-European population of *P. pipistrellus* is probably caused by geographic isolations during the history of the glaciation events in western Palaearctic. The simple comparison of wing dimensions of Syrian *P. pipistrellus* with those of limited material of European pipistrelles (Fig. 3) also suggests moderate distinctness of Syrian bats from the European ones: in forearm length the Syrian bats are closer to *P. pygmaeus*, but in the ratio between lengths of medial and distal phalanges of the third finger (IIIm/d; Tab. 2) they are similar to European *P. pipistrellus*. Nevertheless, the latter character is more important for distinguishing of the species living in sympatry in central Europe (Häusler et al. 2000, Zöphel et al. 2002). Traditionally, the Levantine population of *P. pipistrellus* s. l. was assigned to the nominotypical form (Lewis & Harrison 1962, Corbet 1978, Harrison & Bates 1991) or to *P. p. aladdin* Thomas, 1905 (Albayrak 1987). Our preliminary data support rather the latter opinion, i. e., more independent position of the Syrian population, but the present finds need a broader comparison in the geographic context of all Palaearctic populations by both genetic and morphologic approaches.

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Souhrn

Poznámky k rozšíření komplexu *Pipistrellus pipistrellus* ve východním Středomoří: první nálezy netopýra hvízdavého (*P. pipistrellus*) v Syrii a netopýra nejmenšího (*P. pygmaeus*) v Turecku. Na základě genetické analysy částečné sekvence mitochondriálního genu cytochrom *b* byl prokázán výskyt netopýra nejmenšího (*P. pygmaeus*) v turecké Thrakii a netopýra hvízdavého (*P. pipistrellus*) v západní (středomořské) části Syrie. Jedná se o první nálezy uvedených druhů na území těchto států, v případě syrských nálezů se jedná o vůbec první nálezy druhového komplexu v této zemi. Mezi haplotypy netopýra nejmenšího z Turecka a ostatních částí areálu (zbytek Balkánského poloostrova, střední Evropa) nebyly nalezeny žádné významné rozdíly. Syrští jedinci netopýra hvízdavého se odlišují v 0,75–1,25 % zkoumané sekvence od středoevropských populací.

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