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MORPHOLOGICAL IDENTIFICATION OF THE SOPRANO PIPISTRELLE (*PIPISTRELLUS PYGMAEUS* LEACH, 1825) IN CROATIA

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ABSTRACT - After the discovery of two different phonic types within the common pipistrelle (*Pipistrellus pipistrellus*), mtDNA analysis confirmed the existence of two separate species named as common pipistrelle (*P. pipistrellus*) and soprano pipistrelle (*P. pygmaeus*). The discrimination of these two cryptic species using external characters and measures has proved to be somewhat problematic. We examined two colonies of soprano pipistrelle from Donji Miholjac, Croatia. As a result, only two characters proved to be of help for field identification: wing venation (89% of cases) and penis morphology and colour for males. The difference in length between the 2nd and 3rd phalanxes of the 3rd finger should be discarded as diagnostic trait between *P. pipistrellus* and *P. pygmaeus* in Croatia.

Key words: Vespertilionidae, *Pipistrellus*, soprano pipistrelle, morphology, identification, Croatia

RIASSUNTO - ***Identificazione su basi morfologiche del pipistrello pigmeo* (Pipistrellus pygmeaus, Leach, 1825) *in Croazia***. A seguito della descrizione di due differenti "tipi fonici" nel pipistrello nano (*Pipistrellus pipistrellus*) e della successiva conferma su basi genetiche dell'esistenza di due specie distinte, designate come pipistrello nano (*P. pipistrellus*) e pipistrello pigmeo (*P. pygmaeus*), la distinzione delle due specie in base a caratteristiche morfologiche esterne si è dimostrata un problema di difficile soluzione. Sulla base delle caratteristiche distintive e delle differenze biometriche proposte da altri Autori, sono state esaminate due colonie di pipistrello pigmeo a Donji Miholjac, in Croazia. I risultati ottenuti evidenziano che, tra tutti i potenziali caratteri sinora proposti, solo due risultano utili per un'identificazione diretta sul campo: la venatura delle ali, risultata utile alla discriminazione nell'89% degli esemplari analizzati, e la colorazione e morfologia del pene nei maschi. La presunta diversa lunghezza della seconda e terza falange del terzo dito è invece risultata inefficace per una corretta discriminazione tra *P. pipistrellus* e *P. pygmaeus* in Croazia.

Parole chiave: Vespertilionidae, *Pipistrellus*, pipistrello pigmeo, morfologia, identificazione, Croazia

INTRODUCTION

The common pipistrelle *Pipistrellus pipistrellus* (Schreber, 1774) is widely distributed all over Europe, from the Arctic Circle in the north to some regions of south-eastern Asia and northern Africa in the south. By

examining its echolocation calls, two different call types were detected, which suggested the existence of two cryptic species (Ahlén, 1981; Zingg, 1990). Nonetheless, at first they were regarded as two phonic types, one echolocating at 45 kHz and one emitting calls at a higher frequency (55 kHz). The separation process of the two cryptic species started with the description of the two echolocation types in Britain, where the common pipistrelle is by far the most abundant bat species (Jones and Parijs, 1993). Successively, differences in their morphology (Barlow *et al*., 1997; Barlow and Jones, 1999; Häussler *et al.,* 2000; Ziegler *et al.,* 2001; Sendor *et al.,* 2002), roosts (Park *et al.,* 1996; Oakeley and Jones, 1998), diet pattern and habitat use, song flight and social calls (Kalko, 1995; Barlow and Jones, 1997a; Barlow and Jones, 1997b; Oakeley and Jones, 1998) were described. Finally, confirming ecological studies, the analysis of mitochondrial DNA proved that the two cryptic forms were separated by high genetic distances and had to be regarded as different species (Barrat *et al.,* 1997). Jones and Barrat (1999) proposed the designation of neotypes, *P. pipistrellus* (common pipistrelle) for the 45 kHz phonic type, and *P. pygmaeus* (soprano pipistrelle) for the 55 kHz phonic type: these have been approved in the Bulletin of Zoological Nomenclature (Yalden *et al.,* 2000).

The external characters of the two species being quite similar, many attempts to find distinguishing morphological parameters have failed.

The following external characters have been proposed to differentiate between the two species:

1. the length of the 2nd and 3rd phalanxes of the 3rd finger - the 3rd phalanx of *P. pipistrellus* should be shorter than the 2nd, whilst their lengths should be equal in *P. pygmaeus* (Häussler *et al.,* 2000). Other parameters, such as ear length (shorter in *P. pygmaeus*), muzzle (shorter in *P. pygmaeus*) and nose width (wider in *P. pipistrellus*) are ineffective for species discrimination;
2. penis colour - orange in adult *P. pygmaeus* and dark gray to grayish brown in adult *P. pipistrellus*; in the latter the penis shows a contrasting pale median stripe which is absent in *P. pygmaeus* (Häussler *et al.,* 2000);
3. wing veneation - i.e. the aspect of the elastic fibers between the forearm and the 5th finger (Fig. 3), a character that has already been used for the discrimination of other species of the genus *Pipistrellus* (Vierhaus, 1996). In *P. pipstrellus* there is normally only one wing cell, connecting the first joint of the 5th finger with the elbow, which is not divided by a crossing elastic fiber; in *P. pygmaeus* also the next cell above (closer to the wrist) is not divided (von Helversen and Holderied, 2003).

Although the distribution range of *P. pygmaeus* is not fully known, it possibly occurs in sympatry with *P. pipistrellus* over much of Europe. *P. pygmaeus* prefers wetland and aquatic habitats, such as riverine forests and inland lakes, but it has also been found around more anthropogenic habitats, such as water reservoirs and parks (Vaughan *et al*., 1997; Oakeley and Jones, 1998; Russo and Jones, 2003). These habitat demands are closely

correlated with its diet that mainly consists of small Diptera (Chironomidae, Ceratopogonidae) (Barlow, 1997) performing mass swarming close to or over waterbodies. Our aims were to test the previously proposed diagnostic traits on a Croatian sample of *P. pygmaeus* and to collect data about its body measurements and their variation between sexes.

STUDY AREA AND METHODS

Pipistrelle bats were caught using mist nets at two large maternity colonies in Donji Miholjac, a small town on the River Drava, NE Croatia. Both netting locations were outside house lofts where the colonies used wooden roofs and tiles as typical locations for pipistrelle maternity roosts. A total of 35 males and 101 females were sampled from one colony during July 2002 and 2003, and 25 males and 30 females from the other one during July 2003. Using a Pettersson D 240x bat-detector adjusted to 55 kHz in heterodyne mode, all caught bats were identified as *P. pygmaeus*. Although bats were not individually marked, the mixing of individuals belonging to both cryptic species could be excluded according to previous observations carried out at the same sites, which had shown that the two sibling species do not make mixed maternity colonies (unpublished data), and available literature (Park *et al*., 1996; Sendor *et al*., 2002). Once only two female bats from one colony were identified as *P. nathusii* (Keyserling et Blasius, 1839) based on the length of the 5th finger and forearm.

By a digital caliper, the length of the forearm, 5th and 3rd fingers without wrist and ear were taken according to Schober and Grimberger (1998), whilst those of the 2nd and 3rd phalanxes of the 3rd finger after Häussler *et al*. (2000). Wing venation, following Helversen and Holderied (2003), and penis morphology and colour were noted. Wing venation was observed only during 2003, on a total of 91 animals: 11 males and 26 females from one colony and 24 males and 30 females from the other. For testing the differences between the 2nd and 3rd phalanxes of the 3rd finger we used a three-way analysis of variance (ANOVA) on pooled values. Mean length values for the two sexes were compared by the t test.

RESULTS

Significant difference between males and females of *P. pygmaeus* was observed for the length of the forearm, 5th and 3rd fingers, 2nd phalanx of the 3rd finger and ear, females showing larger values (Tab. 1).

Considering the animals from both colonies irrespective of sex, the difference in length between the mean values of the 2nd (L2) and 3rd (L3) phalanxes of the 3rd finger was statistically significant ($F_{1,37} = 162.8$, $P < 0.001$), with mean values of 8.07 mm and 7.31 mm respectively. Although females showed significantly higher values of L2 than males (Tab. 1), overall no difference was found between pooled values of L2 and L3 regarding sex ($F_{1,37} = 2.49$, $P = 0.11$) (Fig. 1). No significant difference was observed between L2 and L3 when con-

Table 1 - External measurements (mm) of *P. pygmaeus* from D. Miholjac, Croatia (t-test: *P ≤ 0.05; **P ≤ 0.001; L2 and L3: 2nd and 3rd phalanxes of the 3rd finger respectively; SD: standard deviation.

		males				females		
Length	N	Mean	Min-max	SD	N	Mean	Min-max	SD
Forearm**	60	29.81	28.15-31.80	0.70	131	30.91	27.90-32.60	0.78
5th finger**	59	37.03	35.00-39.50	1.12	131	38.16	36.00-41.00	0.96
3rd finger**	58	49.65	41.00-54.00	2.50	130	51.85	46.00-58.00	1.85
L2**	60	7.97	6.79-9.40	0.51	130	8.30	6.99-9.65	0.54
L3	60	7.29	6.40-8.20	0.42	130	7.40	5.96-9.00	0.54
L2-L3*	60	0.68	-0.29-1.80	0.53	130	0.90	-0.60-2.60	0.64
Ear*	59	8.45	7.50-9.50	0.50	129	8.67	7.00-11.00	0.60

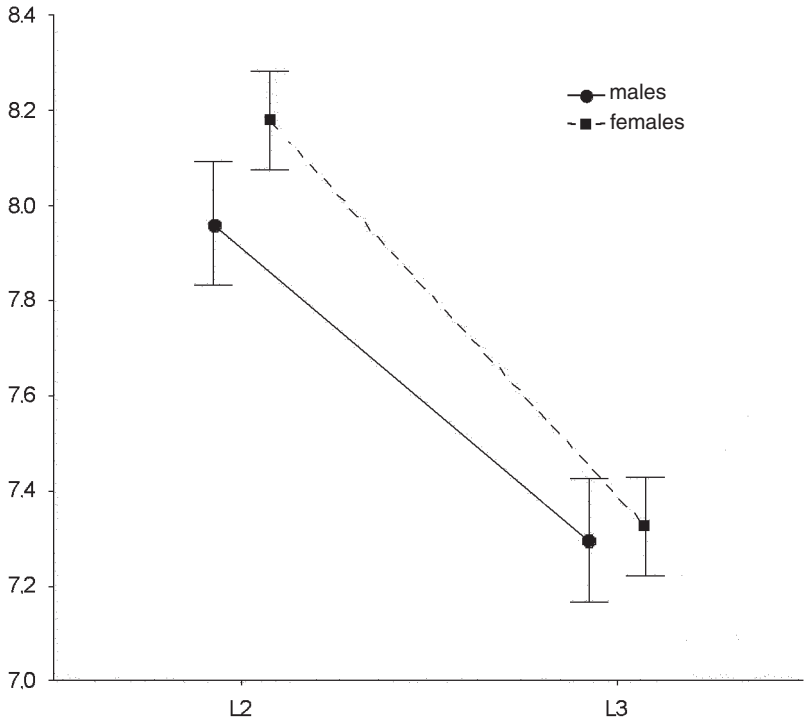


Figure 1 - Effect of sex on pooled values (mm) of L2 and L3: 2nd and 3rd phalanxes of the 3rd finger respectively. Vertical bars denote 0.95 confidence intervals.

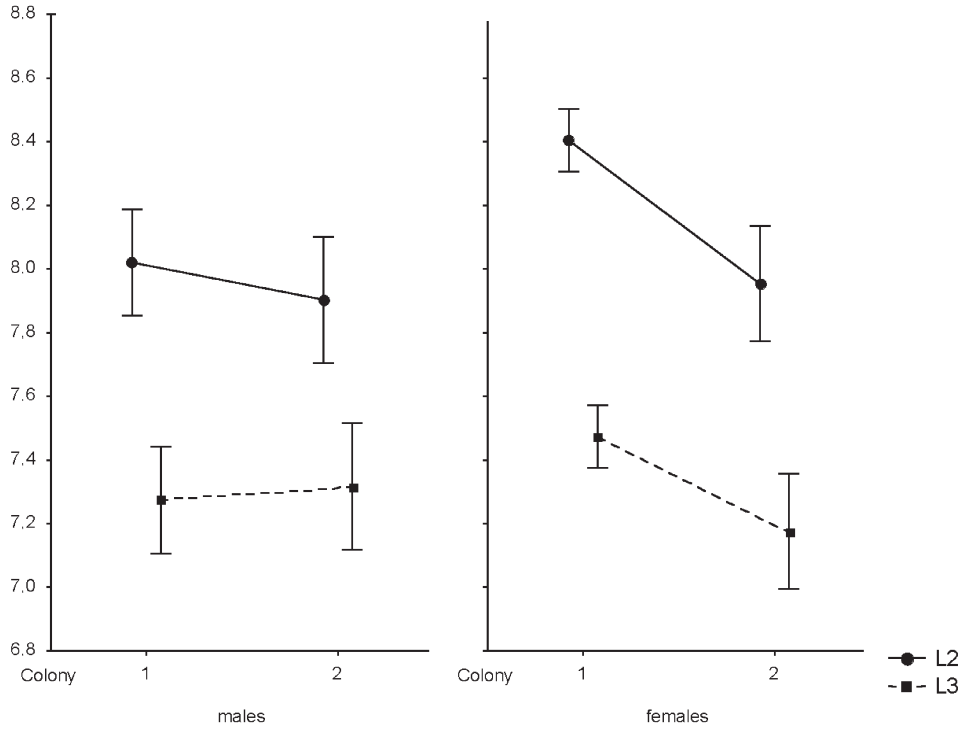


Figure 2 Result of three-way analysis of variance on pooled values (mm) of L2 and L3 per sex in two colonies. Vertical bars denote 0.95 confidence intervals.

sidering the interaction sex per colony ($F_{1,37} = 0.001$, $P = 0.974$) (Fig. 2).

The "typical" wing venation proposed by von Helversen and Holderied (2003) for discriminating *P. pygmaeus* from other *Pipistrellus* species (Fig. 3) was observed in a total of 81 individuals (89%). Other animals exhibited none of the patterns proposed for the designation of the genus.

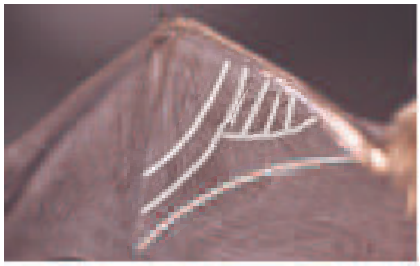
The penis showed the typical orange colour with a somewhat paler terminal papilla and a clearly visible median stripe which was never longer than 1/3 of the total penis length. A few males exhibited a somewhat less intensive orange colour of the penis.

DISCUSSION

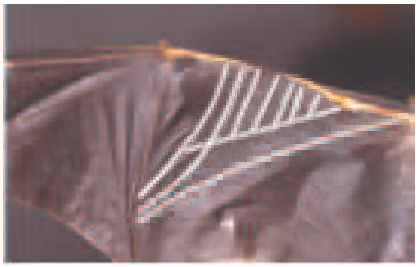
The length of the forearm and 5th finger agreed with those reported by Häussler *et al.* (2000) for both males and females, with a somewhat larger range of values which was a consequence of our larger sample.

Our results clearly showed that the morphological discrimination between the two cryptic species of pipistrelles based on the difference in length between the 2nd and 3rd phalanxes of the 3rd finger is inappropriate because of their too wide variation and overlap.

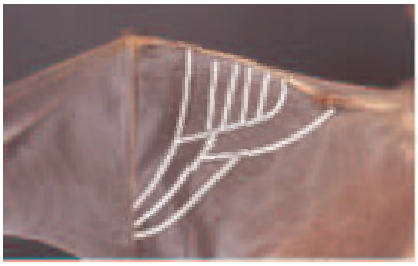
Similar results were obtained by Sendor *et al.* (2002) when the same



P. pygmaeus



P. pipistrellus/kuhlii



P. nathusii

Figure 3 - Wing venation proposed for designation of *Pipistrellus* species.

hypothesis was tested on a large sample of *P. pipistrellus:* 30.4% of the observed animals showed none or opposite length difference between phalanxes. Further investigations are needed to confirm our results in the sympatric distribution range of both species.

Wing venation proved to be a somewhat more reliable identification feature and easier to use in the field irrespective to the sex and age of animals. Animals which showed "negative" for the typical *pygmaeus* venation form also showed no match with the other two patterns of venation shown by pipistrelle bats, whose identification based on this feature should also be tested in the future. These "unidentifiable" animals could be the result of some kind of injury or malformation of the wing membrane which lead to different distortions in wing venation.

Penis morphology and colour agreed with the results of Häussler *et al.* (2000) and can be used as discrimination characters between males of *P. pygmaeus* and *P. pipistrellus*. This possibility should be confirmed by experienced bat researchers. No data are available on possible penis colour variation between young and adult males.

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