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Variation and diagnostics of two close bat species from Ukraine: *Pipistrellus nathusii* and *P. pipistrellus (sensu lato)*

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ZAGORODNIUK I. Variation and diagnostics of two close bat species from Ukraine: Pipistrellus nathusii and P. pipistrellus (sensu lato). — The variability of morphometric and craniometric parameters of the common pipistrelle group, *Pipistrellus nathusii* and *P. pipistrellus*, is discussed. The variability of such metric characters as body weight (W), 4 standard (L, Ca, Pl, Au) and two additional external measurements (Tr, FA), as well as three skull measurements (CBL, CM3, Mand) important for the study of bats were studied. In the course of the study of collections, the re-identification of specimens was carried out based on "traditional" keys and on the studied metric features (in the light of sex and age), which allowed to re-identify 45 % of the collection specimens. All specimens with incorrect identification belong to *P. nathusii* (mostly young individuals), which were identified by the collectors as "*P. pipistrellus*".

Introduction

Pipistrellus is represented in the fauna of Ukraine and neighbouring countries by 4 species — *pipistrellus*, *nathusii*, *kuhlii*, and *savii* (Abelentsev & Popov 1956; Krochko 1994; Zagorodniuk & Tkach 1996). Two of them, *pipistrellus* (PIP) and *nathusii* (PIN), are widely distributed and ecologically similar species[2]. Their morphological similarity is significant (Strelkov 1963 etc.) and comparable with the level of similarity of many other pairs of closely related species, such as *Myotis myotis* + *blythii* or *Plecotus auritus* + *austriacus* (Zagorodniuk 1998). There are no special publications on these species from Ukraine, but some interesting data are presented in a review of Ukrainian bats by Abelentsev & Popov (1956).

This investigation was initiated after our survey of collected specimens of *Pipistrellus nathusii* in order to describe the seasonal dynamics of its geographical range in Ukraine. Our preliminary research showed that more than 50 % of known collected specimens of *Pipistrellus* must be re-identified and diagnostic keys should be revised. Therefore, the main goals of this study are: (1) to study the morphologi-

[2] Recently it was shown that ultrasonic signals of the common pipistrelle from Ukraine refer to the form "55 kHz", which is recognized as a separate species *P. pygmaeus*, as indicated in the previous issue of this bulletin (Limpens, 2000), so the material regarding *P. pipistrellus* should be designated as "*P. pipistrellus* (s. lato)» and most likely refer only to *P. pygmaeus*; all such materials are considered here as "*P. pipistrellus*» in broad sense.

cal variation of collected specimens, (2) to describe their diagnostic features, (3) to reveal general trends in morphological differentiation of related species.

Materials and methods

Bat collections of both of the central natural history museums of Ukraine were studied: National Museum of Natural History (NMNH, Kyiv) and State Museum of Natural History (SMNH, Lviv). Because of numerous mistakes in species identification in collections, all available specimens of "*nathusii*" and "*pipistrellus*" were studied and re-identified. The total number of studied specimens is 76 (specimens from the exhitions were not studied).

Two groups of characters were analysed: 5 non-metric (2 external and 3 dental features) and 10 metric characters (7 external and 3 skull measurements). The list of characters was compiled after analysis of keys to species distributed in Ukraine (Abelentsev & Popov 1956; Zagorodniuk *et al.* 1999) and neighbouring countries (Strelkov 1963; Kuziakin 1965; Pucek 1984; Woloszyn 1991).

The non-metric characters are as follows: (1) relative size of the second incisor compared to the first one, (2) level of reduction and place of the smallest upper premolar; (3) hiatus between the second and third lower incisors; (4) development of the fur on the upper side of tail membrane; (5) relative size of the thumb. Dental characters were studied using binocular microscope.

Metric characters of adult specimens are as follows: *W* — body weight, *L* — body length, *Ca* — tail length, *Pl* — hindfoot length, *Au* — ear length, *Tr* — tragus length, *Ra* — forearm length, *CBL* — condylobasal length of the skull, *CM3* — basal length of the upper tooth row, *Mand* — condylar length of the mandible.

Characters *Ra'*, *CBL*, *CM3*, and *Mand* are our original measurements by calliper; values of other characters are taken from the original labels of collection specimens. For each metric character, the mean value and standard deviation were calculated, and samples were compared using Mayr's coefficient of divergence estimated as $CD = (X_1–X_1) / ((SD_1+SD_2)/2)$ and DIF index (level of difference between separate characters).

Species names are abbreviated in the text as PIP (*P. pipistrellus* s. l.) and PIN (*P. nathusii*).

General characteristics of the specimens

In both studied collections, there are 76 specimens of *Pipistrellus* ex grex "*nathusii* + *pipistrellus*", 5 of which are stored SMNH and 71 in NMNH. Most specimens are represented by study-skins with prepared skulls, while 9 specimens in the collection of NMNH are fluid-preserved. According to initial identification (current designations in the catalogues*), 55 specimens are stored as "*Pipistrellus pipistrellus*" and 21 specimens are stored as "*P. nathusii*" (Table 1).

* In most cases, the specimens have the same primary identification on the original labels.

Contrary to primary identifications, 43 % of specimens were re-identified. Specimens of Nathusius' bat appears to be taxonomically homogenous and all *Pipistrellus* specimens stored as "*nathusii*" were identified as the same species. There is an opposite situation for specimens of "*pipistrellus*": 33 specimens of "*pipistrellus*" were re-identified as "*nathusii*" and 59 % of the total available specimens of "*pipistrellus*" were identified earlier incorrectly.

Thus, the real ratio between the two studied species in zoological collections contradicts to all traditional views (Abelentsev & Popov 1956; Zagorodniuk & Tkach 1996). According to initial data, there are 55 PIP and 21 PIN, while based on our revision there are 23 PIP and 53 PIN. Analysis of new data showed that all previous records of both species in the plain part of Ukraine should be attributed to Nathusius' bat, while Crimean records are *P. pipistrellus* only and records from the Carpathian region include both species (but mostly *P. pipistrellus*).

Table 1. The number of known collected specimens of *Pipistrellus pipistrellus* and *P. nathusii* in the two central natural history museums of Ukraine and results of their re-identification

Re-identification	Deposited as "*P. nathusii*"		Deposited as "*P. pipistrellus*"		Total
	NMNH	SMNH	NMNH	SMNH	both museums
P. nathusii	21 sp.	0 sp.	28 sp.	4 sp.	53 sp.
P. pipistrellus	0 sp.	0 sp.	22 sp.	1 sp.	23 sp.
total number	21 sp.	0 sp.	50 sp.	5 sp.	76 sp.
% of mistakes	0 %	0 %	56 %	80 %	43 %

Variation of the diagnostic characters

Variations of non-metric characters are relatively large. Diagnostically important characters are those metric features that show low variability within the specimens and noticeable differences between the average values. The traditional use of Student's t-test is not correct in assessing the diagnostic significance of characters, because this criterion evaluates differences between mean values rather than the level of divergence or overlap of character values. Mayr's divergence coefficient is more convenient (and similar in calculation technique), which normalises the difference between means due to the dispersion of characters.

Data are summarised in Table 2.

For comparison, Table 2 includes two criteria, CD and DIF. According to the data in Table 2, DIF shows the external features, while CD demonstrates the largest differences in craniometric characters + forearm length.

Three of the studied craniometric characters were highly significant: 1) mandible length (*Mand*) CD = 6.49, 2) condylobasal length (*CBL*) CD = 5.36, 3) basal length of the upper tooth row (*CM3*) CD = 3.48. According to these indices, the specimens do not overlap (see Table 2). Thus, for mandible measurements (*Mand*) the ranges are 7.7–8.6 and 9.2–9.7 mm (*P. pipistrellus* vs. *P. nathusii*). The length of the forearm (*Ra'*) showed a smaller value: CD = 3.15.

Table 2. Values of metric characters in two close species of *Pipistrellus* from Ukraine and their comparison using criteria CD and DIF

Metric	*P. pipistrellus*				*P. nathusii*				Comparison	
character	mean ± SD		min–max	n	mean ± SD		min–max	n	CD	DIF
Weight										
W	4.6 ± 0.75		3.3–5.7	15	7.7 ± 1.59		5.0–11.5	19	2.65	**67.4**
Body										
L	39.9 ± 3.05		34.5–44.0	18	48.9 ± 2.27		43.7–54.0	41	**3.38**	**22.6**
Ca	33.1 ± 1.58		29.0–35.5	19	36.8 ± 2.93		30.0–42.0	41	1.64	11.2
Pl	5.8 ± 0.89		4.5–7.0	18	7.0 ± 0.79		5.5–8.9	33	1.43	**20.7**
Au	10.3 ± 0.90		8.5–12.1	15	12.6 ± 1.11		9.9–14.0	31	2.29	**22.3**
Tr	4.8 ± 0.31		4.2–5.3	11	6.4 ± 0.91		5.0–8.0	15	2.62	**33.3**
Forearm										
Ra	30.8 ± 1.11		29.0–32.8	18	33.9 ± 1.12		31.5–36.0	38	2.78	10.1
Ra'	30.4 ± 0.98		29.0–32.3	21	33.2 ± 0.80		31.8–34.9	45	**3.15**	9.2
Skull										
CBL	11.2 ± 0.35		10.5–11.8	15	12.7 ± 0.21		12.4–13.2	27	**5.36**	13.4
CM3	4.0 ± 0.15		3.8–4.2	15	4.4 ± 0.08		4.3–4.5	27	**3.48**	10.0
Mand	8.2 ± 0.23		7.7–8.6	14	9.4 ± 0.14		9.2–9.7	24	**6.49**	14.6

In combination with non-metric characters (e.g., the gap between the lower incisors is significant) and taking into account sex (females are larger) and age, species identification is not so problematic.

Differences in the two main metric characters that are important for species diagnostics (forearm length and mandible length) are shown in figs 1–2.

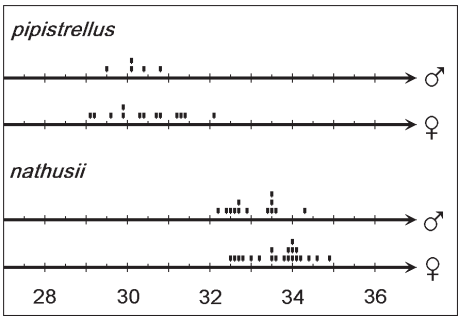

Fig. 1. Distribution of the forearm length in *Pipistrellus pipistrellus* and *P. nathusii* from Ukraine (measured on study skins, separately for females and males).

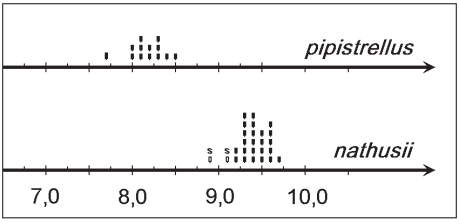

Fig. 2. Distribution of mandible length in two related species of *Pipistrellus*: *P. pipistrellus* (above) and *P. nathusii* (below).

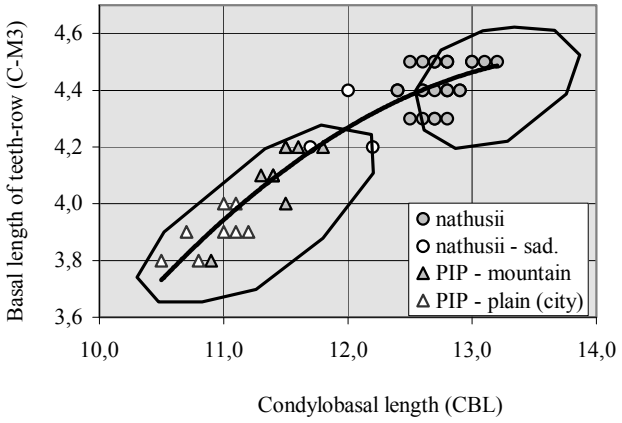

Fig. 3. Distribution of two diagnostically significant skull measurements in two related bat species, *Pipistrellus nathusii* and *P. pipistrellus* (s. lato).

The nature of morphological differences

The ontogenetic component in the variability of traits is expressive in the whole group of studied bats. In general, *Pipistrellus pipistrellus* is similar to subadult specimens of *P. nathusii*. Moreover, it is important to consider the sex of specimens, because males are always more juvenile: in both species, females are larger in all measurements than males, and these sex-related differences are similar to species differences. The comparison of species by metric characters shows that they form a continuous univariate series "♂ PIP → ♀ PIP → ♂ PIN → ♀ PIN". Due to the overlap of data in the middle pair of this series (♀ PIP vs ♂ PIN), effective identification could be provided by only taking into account sex and age. This feature was noticed a long time ago. Kessler (1851: p. 3) wrote:

> Кстати я долженъ здѣсь замѣтить, что мнѣ случалось имѣть кожановъ, которые по многимъ признакамъ занимали какъ-бы средину между двумя видами V. pipistrellus и V. Nathusii. Мнѣ даже казалось, что названіе V. Nathusii дано старымъ недѣлимымъ, а названіе V. pipistrellus молодымъ, однолѣтнимъ недѣлимымъ одного и того-же нераздѣльнаго вида, но до сихъ поръ не удалось мнѣ собрать достаточныхъ данныхъ, чтобы произнесть по этому дѣлу окончательное рѣшеніе.

Possible heterogeneity of Pipistrellus pipistrellus

The author found no significant differences between the two pipistrelle samples and believes that all available materials should be assigned to the two mentioned species, *Pipistrellus nathusii* and *Pipistrellus pipistrellus* s. l. The latter is likely to be identified as *P. pygmaeus*, given that the first detector tests in Ukraine showed the presence of *P. pygmaeus* (ultrasonic signals at 55 kHz: Limpens, 2000).

It should be noted that most of the re-identifications of pipistrelles, which concerned the misidentifications of young *P. nathusii* as "*P. pipistrellus*", refers to specimens that originate from the northern part of Ukraine. This gives rise to a mys-

tery: re-identification of all northern (within Ukraine) "*P. pipistrellus*" as *P. nathusii* suggests that "*P. pipistrellus*" was absent 50–70 years ago throughout the northern part of Ukraine in general. However, *P. pygmaeus* was discovered namely in the north (Chernihiv Oblast), which raises the question: why it did not appear in the collections earlier, especially since it was found in a synanthropic location (colony in a country club). Therefore, the answer to this question should be sought in the changes of fauna and biological invasions.

Diagnostic characters and their stability

In all cases, metric characters are hightly significant for species diagnostics and demonstrate good hiatus. Taking into account that the most important character in primary study of both field materials and collected specimens is forearm length, the author tested this metric character in old specimens and showed that metric features decrease in time. Moreover, this "drying" is proportional to the value of characters, and individuals with larger characters become clearly smaller (Fig. 4).

Thus, long-term storage of specimens affects the value of diagnostic characters reaching 1 mm of 30–35 mm in forearm length, i. e. about 3 %. This is a significant value because the hiatus between the species is the same.

Species abundance and geographical ranges

Results of re-identification completely changed our previous view on species abundance. *Pipistrellus pipistrellus* appears to be a relatively rare and narrowly distributed bat species in contrast to earlier considerations. Collection materials show that the geographical range of this species is restricted to the Carpathian region (from Zakarpattia to Lviv Oblasts) and to the Crimea (up to Askania-Nova).

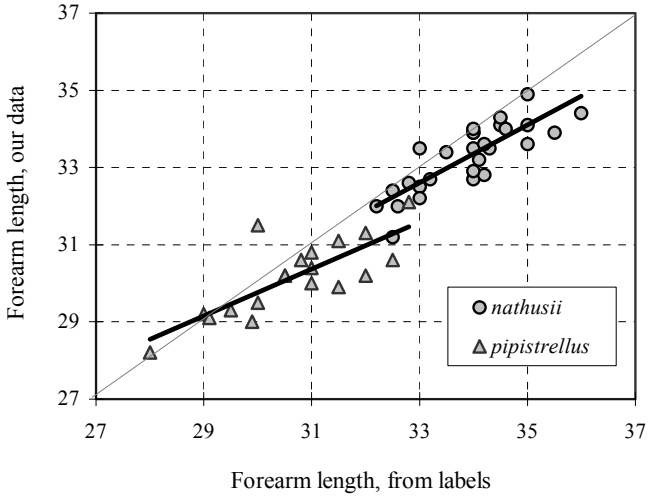

Fig. 4. Correlation between initial (fresh materials) and secondary (study skins) measurements of the forearm length in two *Pipistrellus* species from Ukraine.

A more detailed picture of geographical ranges of the two studied species is presented in Negoda & Zagorodniuk 2001.

Thus, most of the previous records of the pipistrelle bat, at least based on the known collected specimens, should be recognised as erroneous. Modern records of *P. pipistrellus* in Podolia (V. Tyshchenko, pers. comm.), Kyiv (L. Godlewska, pers. comm.), and Sumy Oblasts (Gavrys *et al.* 1997) can be classified as follows: 1) the species is present in most part of Ukraine, but its abundance is low, and these records are not confirmed by collected materials, 2) the species expanded its range during the last 2–3 decades after the period when the known museum specimens were collected. In any case, previous descriptions of the pipistrelle bat in the main part of Ukraine by Abelentsev and Popov (1956) are incorrect.

Conclusions

To sum up the results of collection analysis we can conclude:

1. Many researchers have regular problems with species identification. About 50–70 % of available collected specimens were re-identified during this research. The latter is one of the reasons that both traditional and modern views on distribution and migratory status of these two species are too preliminary and need to be revised. The main reason for erroneous definitions is the fact that young *P. nathusii* were accepted for "*P. pipistrellus*".
2. All collection materials of *P. pipistrellus* (after re-identifications of some specimens as young *P. nathusii*) are a homogeneous sample that cannot be divided into two "small" species; but taking into account the field identifications of "lesser" pipistrelle as "*P. pygmaeus*", the author suggests that collection specimens of "*P. pipistrellus*" can be preliminarily referred as "*P. pygmaeus*".
3. In both species, females are larger in all measurements than males, and these differences are similar to species differences. Comparison of species by metrics shows that they form a continuous series: ♂ PIP — ♀ PIP — ♂ PIN — ♀ PIN. Due to the overlap of data between sexes of different species (♀ PIP vs ♂ PIN), effective identification can be provided considering sex-related variation.
4. Analysis of changes of charactres in collection specimens during their long-term storage showed that metric features decrease over time. This decrease is proportional to the value of characters and in individuals (as well as species) with larger characters the effect of "drying" is greater, while the studied characters are almost unchanged in smaller individuals.

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References

Abelentsev V. I., Popov B. M. 1956. Order of handwinging, or the bats — Chiroptera. In: *Fauna of Ukraine*. Vol. **1**: Mammals; issue 1. AS Ukr. SSR Publ. House, Kyiv, 229–446. (In Ukrainian)

Gavris, G., Y. Kuzmenko, A. Mishta, I. Kotzerzhynska. 2007. *Fauna of Vertebrate Animals of National Nature Park Desnyansko-Starohutsky*. Kozatsky Wal, Sumy, 1–131. (In Ukrainian)

Kessler, K. F. 1851. *Mammals. Trudy of Commission ... to Description of the Provinces of Kyiv School District.* Kyiv, 1–88. (Series: Natural History of the Provinces of Kyiv Educational District. Vol. 1: Zoology). (In Russian)

Krochko Ju. I., 1994. Bats of Ukraine. *Tezy dopovidej [=Abstracts] of 48th Scientific Conference of Uzhgorod State University. (Ser. Biol.)*. Uzghorod, 27–28.

Kuziakin A. P. 1965. Order Bats. Ordo Chiroptera. *In: Bobrinsky N. A., Kuznetsov B. A.,* Kuziakin A. P. Keys to Mammals of USSR. Prosveschenie, Moskva, 79–116. (+ maps in Annex: 15–27).

Limpens, H. 2000. Reports about detector seminars in East Europe. Ukraine. *Novitates Theriologicae*, **2**: 28–28. (In Russian)

Negoda V., Zagorodniuk I., 2001. Migratory status of *Pipistrellus nathusii* and *Pipistrellus pipistrellus* in Ukraine. *Novitates Theriologicae*, **3**: 25–27.

Pucek, Z. (red.). 1984. *Klucz do oznaczania ssakow Polski.* Panstw. Wydaw. Nauk., Warszawa, 1–388.

Strelkov P. P. 1963. Order Chiroptera — Bats. *In: Mammals of the USSR fauna. Part 1.* USSR Acad. Sci. Press, Moskva, Leningrad, 122–218. (In Russian)

Wołoszyn, B. W. 1991. *Jaki to nietoperz? Polowy klucz do oznaczania nietoperzy krajowych*. Krakowskie Wydawnictwo Zool., Krakow, 1–56.

Zagorodniuk I. V., Tkach V. V. 1996. The present state of fauna and the historical changes of abundance of the bats (Chiroptera) in the territory of Ukraine. *Reports of the NAS of Ukraine*, No. 5: 136–142. (In Ukrainian)

Zagorodniuk I. 1998. Polyspecies of bats from the Eastern Europe and their diagnostics. *European Bat Night '98 in Ukraine*. Kyiv, 56–65. (Proceedings of the Theriological School; Vol. 1). (In Ukrainian)

Zagorodniuk I., Postawa T., Wołoszyn B. W. 1999. A field key to bats from the underground roosts of Eastern Europe. Platan Publ. House, Krakow, Kyiv, 1–43. (In Ukrainian)

Резюме

Загороднюк І. Мінливість та діагностика двох близьких видів кажанів з України: Pipistrellus nathusii та P. pipistrellus (sensu lato). — Розглянуто мінливість морфометричних та краніометричних ознак нетопирів групи *Pipistrellus nathusii* та *P. pipistrellus*. Досліджено мінливість таких показників, як маса тіла (W), 4 стандартних екстер'єрних виміри (L, Ca, Pl, Au) та два додаткові екстер'єрні виміри важливі для вивчення кажанів (Tr, FA), три виміри черепа (CBL, CM3, Mand). У процессі вивчення колекцій проведено реідентифікацію зразків з урахуванням «традиційних» ключів та досліджених метричних ознак (з урахуванням статі й віку), що дозволило реідентифікувати 45 % колекційних зразків. Всі зразки з помилками визначення — це зразки *P. nathusii* (переважно молоді особини), що були ідентифіковані колекторами як «*P. pipistrellus*».