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IDENTIFICATION OF *MYOTIS BLYTHII* AND *M. MYOTIS* (CHIROPTERA, VESPERTILIONIDAE) FROM EASTERN EUROPE BASED ON THE MEASUREMENTS OF LOWER TEETH

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Identification of *Myotis blythii* and *M. myotis* (Chiroptera, Vespertilionidae) from Eastern Europe Based on the Measurements of Lower Teeth. Ghazali M. A. — Since the shape of lower teeth is similar in *Myotis* species, it was the size that was used for differentiation between them. Canonical variates analysis was used to determine the possibility of discrimination between *M. blythii* Tomes, 1857 and *M. myotis* Borkhausen, 1797 based on lower teeth. The lengths and widths of canines, premolars and molars were measured. More than 89% of the specimens were correctly classified in the models built using measurements of each tooth separately ("one-tooth" models). Better result was obtained for different combinations of the teeth. The largest and the most frequent in the fossils teeth (c inf., p/4, m/1, m/2, and m/3) were chosen for "several-teeth" models. Sets of three teeth maximize the discriminatory power almost to 100%.

Key words: canonical variate analysis, lower teeth, *Myotis blythii*, *M. myotis*, Eastern Europe.

Идентификация восточноевропейских *Myotis blythii* и *M. myotis* (Chiroptera, Vespertilionidae) по промерам нижних зубов. Гхазали М. А. — Качественные различия нижних зубов крупных ночниц недостаточно выражены, поэтому разделение видов следует проводить по размерам зубов. Дискриминацию между *M. blythii* Tomes, 1857 и *M. myotis* Borkhausen, 1797 проводили с помощью анализа канонических переменных по длинам и ширинам нижних зубов — клыков, премоляров, моляров. В результате более 89% особей было правильно классифицировано с помощью моделей, построенных на промерах одного зуба. При использовании нескольких зубов качество дискриминации значительно повышалось. Модели были созданы для разных сочетаний крупных и наиболее часто встречающихся в ископаемом виде зубов (c inf., p/4, m/1, m/2 и m/3). С помощью моделей, основанных на сочетании промеров трех зубов, правильно определяли все особи исследуемой совокупности.

Ключевые слова: анализ канонических переменных, нижние зубы, *Myotis blythii*, *M. myotis*, Восточная Европа.

Introduction

Teeth and jaw fragments are the most usual findings in the fossils. Descriptions of the species are often based on this material. Recent material gathered from the ground and from the bird pellets, is often very fragmented, consisting of fractured skulls, lower and upper jaws, and it is impossible to use common diagnostic measurements for identification of such a material. Besides, as shapes and sizes of teeth are closely associated with feeding of animals, it helps to predict overall size of the animal, and its diet.

Vespertilionid bats are insectivorous; their molars have well developed and W-shaped ectolophs (i. e. dilambdodont molars). Depending on the number of incisors and premolars dental formula varies from 30 to 38 teeth. In the normal state, *Myotis* have the full dental formula of 38 teeth. The dentition and shape of the feet has been used to distinguish among several subgenera in *Myotis* (Findley, 1972; Menu, 1987; Tate, 1941). However, the results of molecular studies (Ruedi, Mayer, 2001) rejected this separation and suggested that morphological similarity is the result of convergent evolution. Large body size, not sized feet, big skull and long jaws are the features of the subgenus *Myotis* s. str. represented by *Myotis myotis* (Borkhausen, 1797) and *Myotis blythii* (Tomes, 1857). They are widespread in the Palaearctic Region (Dzeverin, 1995; Strelkov, 1972), but the first species is distributed much further eastwards, and the second one is further northwards. A cline in size from west to south-east was observed for *M. myotis* (Benda, Horáček, 1995). Two subspecies are recognized; the larger one is *M. m. macrocephalicus* (Harrison and Lewis, 1961) from Lebanon and the smaller is *M. m. myotis* from Europe. Within *M. blythii*, the largest subspecies is *M. b. omari* (Thomas, 1906) from the Middle East, the smallest one is *M. b. blythii* from the Central Asia. The subspecies of an average size is *M. b. oxygnathus* (Monticelli, 1885) from Europe. The clinal variability is particular to each of the *M. blythii* subspecies (Benda, Horáček, 1995). According to estimations of the evolution rates of the skull measurements, variability of species and subspecies of *Myotis* s. str. occurred due to the size changes, rather than to the shape (Dzeverin, 2008). Shape analysis of the subspecies of *M. blythii* and *M. myotis* is concordant with that assumption (Evin, 2008).

Therefore, Europe is inhabited by two subspecies: *M. m. myotis* and *M. b. oxygnathus*, and European *M. blythii* and *M. myotis* are well studied. Different methods for discrimination between the two species were proposed (Arlettaz et al., 1997; Benda, 1996), but they refer to cranial traits.

Individual development of the species is another source of confusion in identification based on the skull. Differences between species are mostly arisen due to the different growth rates (Benda, Horáček, 1995). Intensive growth of the skull is observed in the first months, but the cessation occurs at different times. *M. blythii* growth terminates in the first year, and in *M. myotis* it stops at the age of 2–3 years. The increase of width measurements is similar in both species. This is why young *M. myotis* resembles adult *M. blythii* making difficulties for species identification. In that case teeth measurements may be much more reliable as their size does not change after the eruption, except for the wear. Thus, width and length measurements of teeth are the best for species discrimination as they are not related to age of animals.

The *Myotis* dentition was studied by Menu and Popelard (1987), Godawa Stormark (1998) and Rossina (2002), but the authors mostly observed qualitative data, not measurements. In the genus *Myotis*, species diagnoses are based on the presence of additional cusps (conules) and crests (lophs) on upper molars, on the shape of canines and premolars, and on the degree of teeth reduction. Lower teeth are more similar between different species, the additional elements are rare on them. Thus, the size (especially of the lower teeth) is to be considered as more important criterion in species identification.

M. blythii and *M. myotis* difference from the other *Myotis* bats is based on the large body and specialized traits of the dentition. Their upper molars lack conules and lophs, so the shapes of the masticatory surfaces are simple. The *Myotis* lower teeth are more similar, if compared with the upper ones. There are almost no additional elements on them. Examined species have no extra elements, and the separation of them can be performed only by the size of the teeth.

Since the measurements of the closely related species are overlapping, I used a multivariate approach. The method of discriminant analysis is applied to build models for species identification based on dental characters. The aim of this article is therefore demonstration of the possibility to differentiate two sibling species (*M. myotis* and *M. blythii*) based on the size of their lower teeth.

Material and methods

In total 102 lower jaws were studied: 29 of *M. myotis* and 73 of *M. blythii*. The specimens originated from the collections of the Zoological Museum of the Taras Shevchenko National University of Kyiv, Zoological Museum of the National Museum of Natural History of the National Academy of Sciences of Ukraine, Zoological Museum of Moscow University.

In Ukraine, *M. blythii* and *M. myotis* are sympatric in the Transcarpathian Region, and occur separately in Crimea (*M. blythii*) and Precarpathia (*M. myotis*). 22 *M. blythii* and 25 *M. myotis* from the Transcarpathian Region were studied. Others were from Precarpathia (4 *M. myotis*, Lviv and Ternopil reg.) and Crimean peninsula (51 *M. blythii*). As all of the subsamples originated from the similar environment, their morphological variation was presumed to be the result of the differences between species. I did not find significant differences between samples from different regions. So, all specimens of *M. myotis* and *M. blythii* with no regard to sex and geography were used for discriminant analysis.

All measurements are given in millimetres. They were made according to the descriptions and figures of Sigè (1968) and Ziegler (2003). The length (le) of the teeth were measured parallel to labial side, the width (br) parallel to mesial side. When measuring the width of the lower molar trigonid (Trd) and talonid (Tad), the entoconid must be exactly vertical in occlusal view. All measurements were made for left and right sides. 17 characters were measured from each side: length and width of canines (c inf.), premolars (p/2, p/3, p/4) and molars (m/1, m/2, m/3). The teeth were measured under the binocular microscope MBS–10 with ocular micrometer (ocular x8 and objective x2).

Repeated measurements were done to determine measurement error. Two-time measurements were conducted for 20 jaws of *M. blythii*. Four jaws of *M. myotis* were measured three times. I used the index ME2 (Palmer, Strobeck, 2003) to estimate the measurement error. The maximum error was 0.06 mm, with an average of 0.04 mm.

The following statistical descriptors were computed: number of specimens (N), mean (M), minimal and maximal values (Min-Max), standard deviation (SD) and coefficient of variation (CV). Normality of the variables was examined with Shapiro-Wilks' test. Nonparametric tests were used for comparison between groups (Mann-Whitney (U) and Wilcoxon (W) statistics). Significance level p was determined by the transformation of these statistics into normally distributed values (Z).

Assessment of the measurements' overlap was conducted according to Kendall and Stuart (1976). This method is independent from distribution. The abbreviations OL to attribute the overlap between groups by one measurement and OL1 for the overlap by several of measurements were used throughout the tables below.

Canonical analysis is a convenient method for two-group classification as only one function is computed (Afifi, Azen, 1982). The functions were built from the right-side measurements. Canonical models were established for all measurements of one tooth ("one-tooth" models), and for groups of the teeth ("several-teeth" models). A priori classification probabilities were proportional to the group size. Chi-square test (χ^2) with specific degrees of freedom (df), Wilks' Λ coefficients and percent of total correct classification (%) were calculated for each model.

Results

Most of the measurements did not follow normal distribution. That is why I used nonparametric statistics for comparisons. Violations of normality assumptions are usually not fatal for discrimination between groups (Hammer, Harper, 2006).

Most of the left and right traits of both species did not differ (Wilcoxon test, $p > 0.05$). Differences in c inf. le and br, and m/1 le were comparable to the measurement error. Left-right mean differences for these measurements did not exceed 0.04 mm. Thus, left and right measurements are considered to be equivalent and the models can be used for either left or right teeth. Almost all mean values of the measurements differed significantly in *M. blythii* and *M. myotis* (Mann-Whitney test, p < 0. 001), except m/3 Tad (U = 902. 0; Z = 0.969; p = 0.33). For descriptive statistics see table. 1.

Regional comparisons were performed only for *M. blythii*. I found different ($p < 0.05$) m/1 le (U = 397.0; Z = 1.972), m/2 le (U = 360.5; Z = 2.317), m/1 Trd (U = 393.0; Z = 2.020), m/1 Tad (U = 314.0; Z = 2. 963) and m/3 Tad (U = 279.5; Z = −3.227). As the between-region differences did not exceed 0.07 mm, I attributed this result to the measurement error. I also compared species within the Western Ukraine and between Crimea and the Western Ukraine regions. Both groups differed

Table 1. Descriptive statistics and overlap percentage of teeth measurements of *M. blythii* and *M. myotis* (for abbreviations, see the Material and methods)

Таблица 1. Описательная статистика и процент перекрывания промеров зубов *M. blythii* и *M. myotis* (сокращения указаны в рубрике Материал и методы)

Variable	*M. blythii*					*M. myotis*					OL, %	OL1, %
	N	M	Min−Max	SD	CV	N	M	Min−Max	SD	CV		
c inf. le	65	1.14	0.80−1.35	0.095	8.4	29	1.28	1.05−1.50	0.108	8.4	90.4	41.7
c inf. br	64	1.23	1.10−1.35	0.057	4.6	29	1.36	0.85−1.50	0.117	8.6	88.2	
p/2 le	73	0.88	0.75−1.00	0.056	6.4	29	1.06	0.90−1.15	0.066	6.3	43.1	15.8
p/2 br	72	0.89	0.70−1.05	0.059	6.6	29	1.07	0.95−1.20	0.054	5.1	37.6	
p/3 le	72	0.71	0.55−0.85	0.058	8.2	29	0.82	0.65−1.40	0.131	15.9	90.1	25.9
p/3 br	71	0.85	0.75−1.00	0.051	6.0	29	1.06	0.85−1.20	0.067	6.4	52.0	
p/4 le	71	1.21	0.85−1.35	0.074	6.1	29	1.48	1.35−1.65	0.074	5.0	6.0	3.0
p/4 br	72	0.95	0.80−1.10	0.059	6.3	29	1.16	1.05−1.25	0.053	4.6	14.9	
m/1 le	73	2.02	1.85−2.25	0.083	4.1	29	2.24	2.05−2.35	0.071	3.2	52.0	15.7
m/1 Trd	73	1.24	1.10−1.50	0.092	7.4	29	1.45	1.25−1.75	0.101	7.0	62.7	
m/1 Tad	73	1.46	1.30−1.70	0.078	5.3	29	1.67	1.50−2.00	0.095	5.7	51.0	
m/2 le	72	2.05	1.90−2.25	0.078	3.8	29	2.35	2.20−2.50	0.086	3.7	12.9	4.3
m/2 Trd	72	1.39	1.25−1.60	0.092	6.6	29	1.61	1.40−2.00	0.124	7.7	58.4	
m/2 Tad	72	1.50	1.35−1.65	0.071	4.7	29	1.72	1.60−2.05	0.096	5.6	17.8	
m/3 le	72	1.82	1.70−2.00	0.068	3.7	29	2.03	1.75−2.25	0.102	5.0	84.2	15.6
m/3 Trd	72	1.27	1.10−1.45	0.070	5.6	29	1.49	1.30−1.85	0.104	7.0	46.5	
m/3 Tad	71	0.83	0.70−1.00	0.080	9.7	29	0.81	0.60−0.95	0.080	9.9	97.0	

($p < 0.001$) in almost all traits, except m/3 Tad ($p > 0.82$) in the last case. As the inter-population variability in *M. blythii* was not high, I may conclude that the distributional occurrences of species do not affect the models.

In total, 14 models were designed. Eight of them are "one-tooth" models. Others are "two-teeth" and "three-teeth" models. As the talonid of m/3 did not differ between species, I performed two models for this tooth — including and excluding the talonid breadth. To design "several-teeth" models I selected the best preserved in fossil teeth. So the following combinations were chosen: c inf. — p/4, p/4-m/1, m/1-m/2, m/2-m/3, p/4-m/1-m/2, and m/1-m/2-m/3. Discrimination coefficients and constants, and models' quality analysis are given in the tabl. 2, 3.

In contrast to some other *Myotis* bats, lower canines of *M. blythii* and *M. myotis* do not have cingular cusplet (Godawa Stormark, 1998). The base of the tooth is oval, the crown is high, and the principal cusp is vertically oriented on the anterior part of the tooth (Menu, 1985: type A2). Overlap of the canine's measurements was high (tabl. 1). Despite the significant difference between means of the canine lengths, the magnitude of overlap showed the low importance of this measurement in the discrimination of the species. The width of the tooth contributed more to the discrimination. The total percent of the correctly classified specimens was the least among all of the models.

In comparison to the upper small premolars, lower ones are less reduced. They are not displaced from the tooth-row. Their bases are oval, and the tips are almost at the

Table 2. Description of the "one-tooth" models

Таблица 2. Описание моделей, построенных на основе промеров одного зуба

Model number	Tooth	Coefficients and constant				Models' quality			
		le	br		constant	Wilks' Λ	χ^2	df	%
			Trd	Tad					
1	c inf.	6.628	8.991		-19.655	0.518	59.265	2	89.25
2	p/2	12.075	12.802		-24.268	0.192	161.734	2	97.03
3	p/3	3.482	16.797		-18.623	0.236	140.178	2	96.00
4	p/4	9.531	11.781		-25.216	0.155	180.719	2	99.00
5	m/1	8.645	3.087	4.382	-29.433	0.293	120.931	3	96.08
6	m/2	9.980	0.734	7.031	-34.372	0.180	167.040	3	100.00
7	m/3	8.441	9.628	-6.364	-24.339	0.229	142.388	3	97.00
8	m/3	8.248	8.374	—	-27.443	0.274	127.051	2	96.04

Table 3. Description of the "several-teeth" models

Таблица 3. Описание моделей, построенных на основе промеров нескольких зубов

Model number	Tooth	Coefficients and constant				Models' quality			
		le	br		constant	Wilks' Λ	χ^2	df	%
			Trd	Tad					
9	c inf.	0.828	1.081		-28.632	0.133	177.348	4	98.91
	p/4	11.516	10.150						
10	p/4	8.810	7.565		-34.513	0.125	198.540	5	98.00
	m/1	4.640	3.154	0.374					
11	m/1	2.493	2.339	-5.515	-35.912	0.172	169.215	6	100.00
	m/2	8.901	0.885	9.431					
12	m/2	8.544	-1.519	6.033	-32.795	0.156	174.683	6	100.00
	m/3	0.747	5.932	-3.739					
13	p/4	7.011	7.026		-38.317	0.107	208.211	8	100.00
	m/1	1.940	3.594	-4.078					
	m/2	5.288	-1.086	5.539					
14	m/1	2.760	1.391	-5.310	-34.691	0.148	176.681	9	100.00
	m/2	7.474	-0.784	8.352					
	m/3	0.670	6.017	-3.735					

midpoint of the crown. The length and the width of the third premolar are smaller than those of the second one in the both species ($p < 0.001$). The relative size of p/3 (le/br ratio) differed between species ($p < 0.001$). The length was 78.2% (min-max range: 61.9–133.3%) of the width in *M. myotis*, which was less than in *M. blythii* (84.0%, 61.1–100.0%). Relative size of p/2 was similar in both species. Its width and length were developed to an equal extent in both species. The discriminatory power was higher for p/2 model.

The lower big premolar is trapezoid in the occlusal view. The only cusp is well developed, and occupies the anterior part of the tooth (Menu, 1985). Measurements of the tooth are least overlapped in the two species, thus discrimination power is one of the best in "one-tooth" models.

M. myotis has more massive molars in the absolute size. In both species, m/2 is the biggest among them. The talonid was distinctly wider than the trigonid in the first and the second molars. In m/3 the talonid was reduced, and Tad/Trd ratio was 65.4% (53.6–79.2%) in *M. blythii* and 54.3% (41.4–65.5%) in *M. myotis* (difference is significant, $p < 0.001$). Molars' canonical functions showed good discriminatory power. The best discrimination was for m/2 model. The least one was for m/3 models. Although no differences were found in the talonid width of m/3, it is better to use all three measurements in the discrimination. Inclusion of m/3 Tad increased the percent of correct classification (cf. models 7 and 8, tabl. 3).

Different combinations of these teeth are more effective in the discrimination. The best discrimination was observed in the models which included m/2 measurements (tabl. 4).

Discussion

The dentition of *M. myotis* is less plesiomorphic than that of *M. blythii*. The main trend in the evolution of bats is the simplification of dentition: reduction in size, loss of the additional elements. On the lower tooth-row, size reduction usually affects p/3 and the talonid of m/3. The ratio of the talonid and the trigonid width of m/3 was higher in *M. blythii*, resulting in the bigger relative size of the talonid in this species. This ratio was even bigger (73.3–74.8%) in the Pliocene *M. blythii longicanninus* (Popov, 2004) from Muselievo (Bulgaria) (Popov, 2004). Measurements of p/3 were relatively bigger in *M. blythii*, too. But vestigial structures are not reliable for discrimination models, their variability is increased, their development is less stable (Dzeverin, 2007) and allows different abnormal effects (for upper premolars in *M. blythii*: Ghazali, 2008).

Comparatively high overlap in the teeth measurements of *M. blythii* and *M. myotis* can hamper the identification (tabl. 1). Canonical functions can discriminate more than 89% of the cases (tabl. 2, 3). Thus, *M. blythii* and *M. myotis* can be well separated with the measurements of the lower teeth, especially when several teeth are combined in the discriminant models. The critical value for separation of the species is zero. The probability of correct classification increases with the increasing of absolute value of canonical function (Afifi, Azen, 1982). Thus, if the canonical values are close to zero, the "several-teeth" models should be applied.

The application of the models is limited to Europe. As the canonical functions were calculated for the Ukrainian bats, they are good for discrimination between subspecies *M. m. myotis* and *M. b. oxygnathus*. Because of the noted variability of the species (Benda, Horáиek, 1995; Dzeverin, 1995; Strelkov, 1972) odontometric analysis of the species from other regions should be done in the future.

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