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Comparative craniometrical analysis and distributional patterns of medium-sized horseshoe bats (Chiroptera: Rhinolophidae) in Bulgaria

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A b s t r a c t . Morphometric skull variation was studied in *Rhinolophus mehelyi*, *R. euryale* and *R. blasii* by means of 50 cranial, mandibular and dental measurements. In *R. euryale* differences between age groups (subadults, adults and old adults) were not significant. Poorly pronounced sexual dimorphism (male skulls were, on average, larger than those of females in some measurements) and low geographical variability within Bulgaria were observed in *R. euryale*. We propose a simple biplot (length of cheek teeth against the rostral posterior width), a stepwise discriminant analyses of some criteria and numerical keys that allow the clear determination of crania or their parts. A large additional sample of incomplete crania was determined on the basis of these keys which, in turn, allow data to be obtained on species distribution patterns. *Rhinolophus euryale* is the most frequent and probably the most abundant horseshoe bat species examined, and occurs throughout Bulgaria. *Rhinolophus mehelyi* is widespread but appears to be relatively rare. *Rhinolophus blasii* is confined mainly to the southern regions of Bulgaria, but may be abundant locally.

Key words: *Rhinolophus mehelyi*, *Rhinolophus euryale*, *Rhinolophus blasii*, cranium, mandible, teeth, multivariate analyses, discriminant criteria

Introduction

Three species of medium-sized horseshoe bats occur in Bulgaria: *Rhinolophus mehelyi* Matschie, 1901, *R. euryale* Blasius, 1853 and *R. blasii* Peters, 1866. Their morphological similarity sometimes leds to difficulties in species discrimination, especially when dealing with single specimens and without the aid of comparative material. As a rule, field determination is based on the shape of the noseleaf swellings – the lancet and connective process of the sella. However, bearing in mind that within-species variation includes specimens with intermediate shapes of these structures (M i l l e r 1912, D e B l a s e 1972, P a l m e i r i m 1990) determinations based on these characters may sometimes be dubious. On the other hand, these characters can only be observed in living or alcohol preserved specimens whereas, very often, the samples that must be identified consist only of badly preserved specimens (e.g. mummies from cave floors), isolated skulls (museum collections), remains in owl pellets, or even much more fragmentary material (e.g. subfossil or fossil remains). The determination of material such as this requires a more detailed knowledge of skull morphology. The morphological analyses presented here aim: (1) to find sets of cranial, mandibular and dental characters which enable unequivocal species determination of complete skulls or their fragments; (2) to reveal the major sources of variation within the species group under consideration; and (3) to evaluate the similarity between species within the context of geographic variation in character overlap. Moreover, the results of the study will permit acquisition of well determined samples of the three species, which in turn will allow their distribution in Bulgaria to be described in greater detail. The results obtained are especially important bearing in mind that the majority of the findings reported from Bulgaria are not substantiated by either concrete data or by reference to particular specimens.

Materials and Methods

In order to provide information in the above, the present study deals with the cranial and dental characters of a large sample of skulls of medium-sized horseshoe bats from Bulgaria and held in the collections of the National Museum of Natural History and the Institute of Zoology, Sofia. The bulk of the material consists of skulls without corresponding skins. The remaining specimens were found as mummies in the caves; their skulls, which were more or less fragmentary, were not included in initial statistical analyses because complete datasets could not be obtained from them. These were determined *a posteriori* according to the discriminant criteria specified through the morphological analysis of complete skulls, and then used to supplement information on species distributions within Bulgaria. A total of 163 skulls (or skull fragments) from 38 localities (Fig. 1, Table 1) was examined (including two specimens from northern Greece). According to the museum labels, 80 specimens had been determined as *R. euryale*, 23 as *R. mehelyi* 35 as *R. blasii*, 5 were designated as "cf. *euryale*" or "*euryale-mehelyi*" and 24 were without a species name on the specimen tag. Only 50 of the specimens available for study were sexed.

Each specimen was assigned to a relative age class on the basis of the degree of tooth wear. Skulls showing little or no wear were considered to be subadult, those with moderate wear (main cusps rounded) as adult, and those with pronounced abrasion (i.e. clear dentine fields) as old adults. The majority of the specimens belonged to the first and second age classes. A few clearly juvenile specimens were excluded from the analyses.

50 cranial and dental characters were measured. Some of the morphometric characters are widely used in bat taxonomy, others, such as tooth measurements, are suitable for comparison between recent and (sub-) fossil material, as well as for the identification of highly fragmented material from owl pellets. The other measurements were designed so as to take into account some possible specific differences between the species under study.

Individual teeth and tooth-rows were measured in occlusal view. These measurements and others (<5 mm) were taken with an eye-piece micrometer accurate to 0.01 mm. The remaining measurements were made with calipers accurate to the nearest 0.1 mm.

All 50 characters are illustrated in Fig. 2; abbreviations used in the figures, tables and text are given for each character: 1. greatest length of skull (GL); 2. greatest length of skull, including canines (GLC); 3. condylobasal length (CBL); 4. condylocanine length (CCL); 5. occipitonasal length (ONL); 6. anterior width of rostrum (WC^1-C^1); 7. posterior width of rostrum (WM^3-M^3); 8. zygomatic breadth (ZB); 9. interorbital width (IW); 10. mastoid breadth (MB); 11. height of the skull over the bullae (HBS); 12. length of C^1-M^3 (LC^1-M^3); 13. length of M^1-M^3 (LM^1-M^3); 14. length of C^1-P^4 (LC^1-P^4); 15. minimal distance between crowns of C^1 and P^4 ($MINC^1$-P^4); 16. length of upper canine (LC^1); 17. width of upper canine (WC^1); 18. length of P^3 (LP^3); 19. width of P^3 (WP^3); 20. length of P^4 (LP^4); 21. width of P^4 (WP^4); 22. length of M^1 (LM^1); 23. width of M^1 (WM^1); 24. length of M^2 (LM^2); 25. width of M^2 (WM^2); 26. length of M^3 (LM^3); 27. width of M^3 (WM^3); 28. length of mandible (LMD); 29. height of *processus coronoideus* (HPC); 30. height of horizontal branch of

Table 1. List of localities (see also Fig. 1) and number of specimens per species, according to the determinations based on craniometric analyses. A.-C. = latitudinal climatic zones in Bulgaria: A. = Moderate continental climate; B. = Transitory-continental climate; C. = Continental-Mediterranean climate. * = only mummies.

No	Locality (Cave)	*R. euryale*	*R.blasii*	*R. mehelyi*	Total
A.					
1.	Magura*	21	-	-	21
2.	Suhi Petch	3	-	-	3
3.	Toschkova	-	-	2	2
4.	Svinska dupka	1	1	-	2
5.	Tatarkinjata	1	-	-	1
6.	Zadanenka	1	-	-	1
7.	Morovitsa	3	-	-	3
8.	Sedlarkata*	1	-	-	1
9.	Ljastovitsa*	1	-	-	1
10.	Parnitsi	1	1	1	3
11.	Mandra	1	-	-	1
12.	Kirov Vartop	2	-	-	2
13.	Nanin Kamak	5	-	5	10
14.	Uruschka Maara	2	-	-	2
15.	Futjovska	3	-	-	3
16.	Prilepna	1	-	-	1
17.	Emenska	11	-	-	11
18.	Orlova Tchuka	5	-	3	8
19.	Zorjuvitsa*	1	-	-	1
	Total (%)	64 (83.1)	2 (2.6)	11 (14.3)	77 (100)
B.					
20.	Jamkata	-	2	-	2
21.	Golak*	1	-	-	1
22.	Butchaschta	45	-	-	45
23.	Orlovata	1	-	-	1
24.	Prolazka	1	-	-	1
25.	Zandana	1	-	-	1
26.	Madara	1	-	-	1
	Total (%)	50 (96.1)	2 (3.9)	-	52 (100)
C.					
27.	Kresna	3	-	-	3
28.	Scharalijska	3	-	-	3
29.	Manuilova	3	-	-	3
30.	Karaguk	-	7	-	7
31.	Samara	1	1	-	2
32.	Aina Inii	3	3	1	7
33.	Manaf Kojuschu	-	-	1	1
34.	Zandana	-	1	-	1
35.	Rupata	-	1	-	1
36.	Drantchi Dupka	1	-	1	2
37.	Lesovo	-	-	1	1
38.	Karaul Tash	1	-	-	1
	N. Greece	-	1	1	2
	Total (%)	15 (44.1)	14 (41.2)	5 (14.7)	34 (100)

mandible under M_1, measured lingually (HMD_M_1); 31. the same, under M_2 (HMD_ M_2); 32. length of I_1-M_3 (LI_1-M_3); 33. length of C_1-M_3 (LC_1-M_3); 34. length of P_4-M_3 (LP_4-M_3); 35. length of M_1-M_3 (LM_1-M_3); 36. length of C_1-P_4 (LC_1-P_4); 37. length of P_2-P_4 (LP_2-P_4); 38. length of C_1 (LC_1); 39. width of C_1 (WC_1); 40. length of P_2 (LP_2); 41. width of P_2 (WP_2); 42. length of P_4 (LP_4); 43. width of P_4 (WP_4); 44. length of M_1 (LM_1); 45. width of M_1

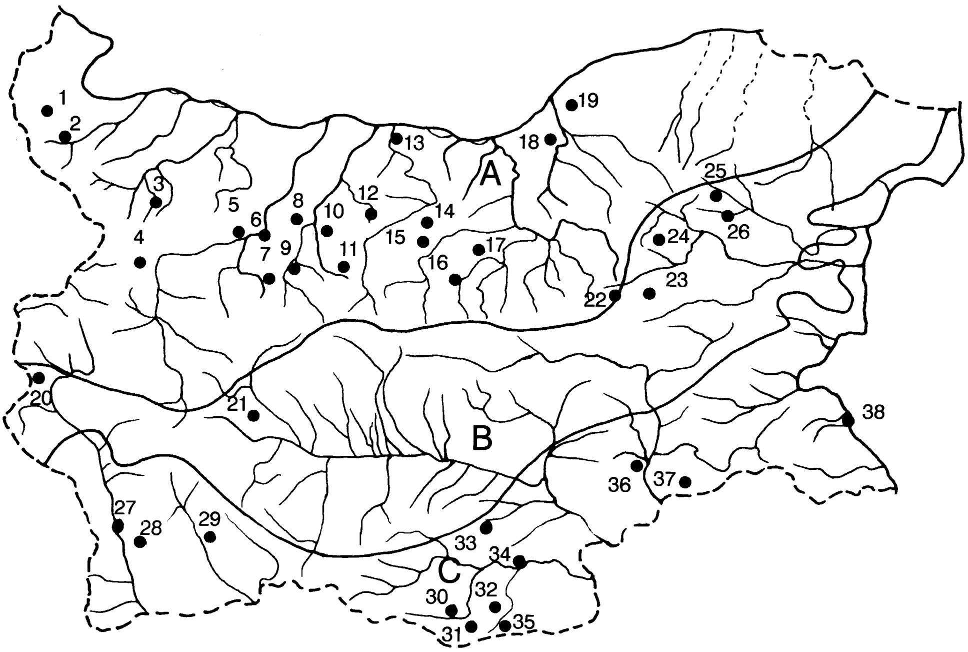

Fig.1. Map of Bulgaria showing the positions of the localities (1 - 38), (for more details see Table 1). A – C – latitudinal climatic zones: A. European (moderate) continental climate; B. Transitory-continental climate; C. Continental – Mediterranean climate.

(WM_1); 46. length of M_2 (LM_2); 47. width of M_2 (WM_2); 48. length of M_3 (LM_3); 49. trigonid width of M_3 ($trWM_3$); 50. talonid width M_3 ($taWM_3$). In addition, in order to better evaluate some of the diagnostic characters available in the literature, three composite variables were constructed: $SP_2 = LP_2 \times WP_2$; $SP_4 = LP_4 \times WP_4$ and $DIFSP_4\text{-}SP_2 = SP_4 - SP_2$. The first two represent the occlusal area of the respective premolars, whilst the third reflects the size difference between these two premolars.

Initially, in order to summarise the morphological variation in the data, Principal Component Analysis (PCA) (performed on a covariance matrix; varimax normalised rotation) was used. The resulting diagrams (plots of the character loadings and specimen scores) allow interpretation of the principal axes extracted and reveal groups of morphologically similar specimens which, in turn, permit the ordination patterns to be related to the taxonomic structure of the material available and provided a check on the initial identifications. Subsequently, Stepwise Discriminant Function Analyses were performed in order to provide accurate discriminant keys. The groups resulting from PCA were chosen as reference samples. Undetermined material and specimens which appeared to be wrongly determined were entered individually as 'unknown' and were not used to compute the discriminant functions. By varying the threshold F-values in the option „F to enter-F to remove" a minimum number of characters allowing 100 % correct determination was selected for each discriminant function. In *R. euryale* the effect of age, sex and climate on cranial measurements was tested by one-way MANOVA. The multivariate analyses were performed using the software package STATISTICA from StatSoft Inc® .

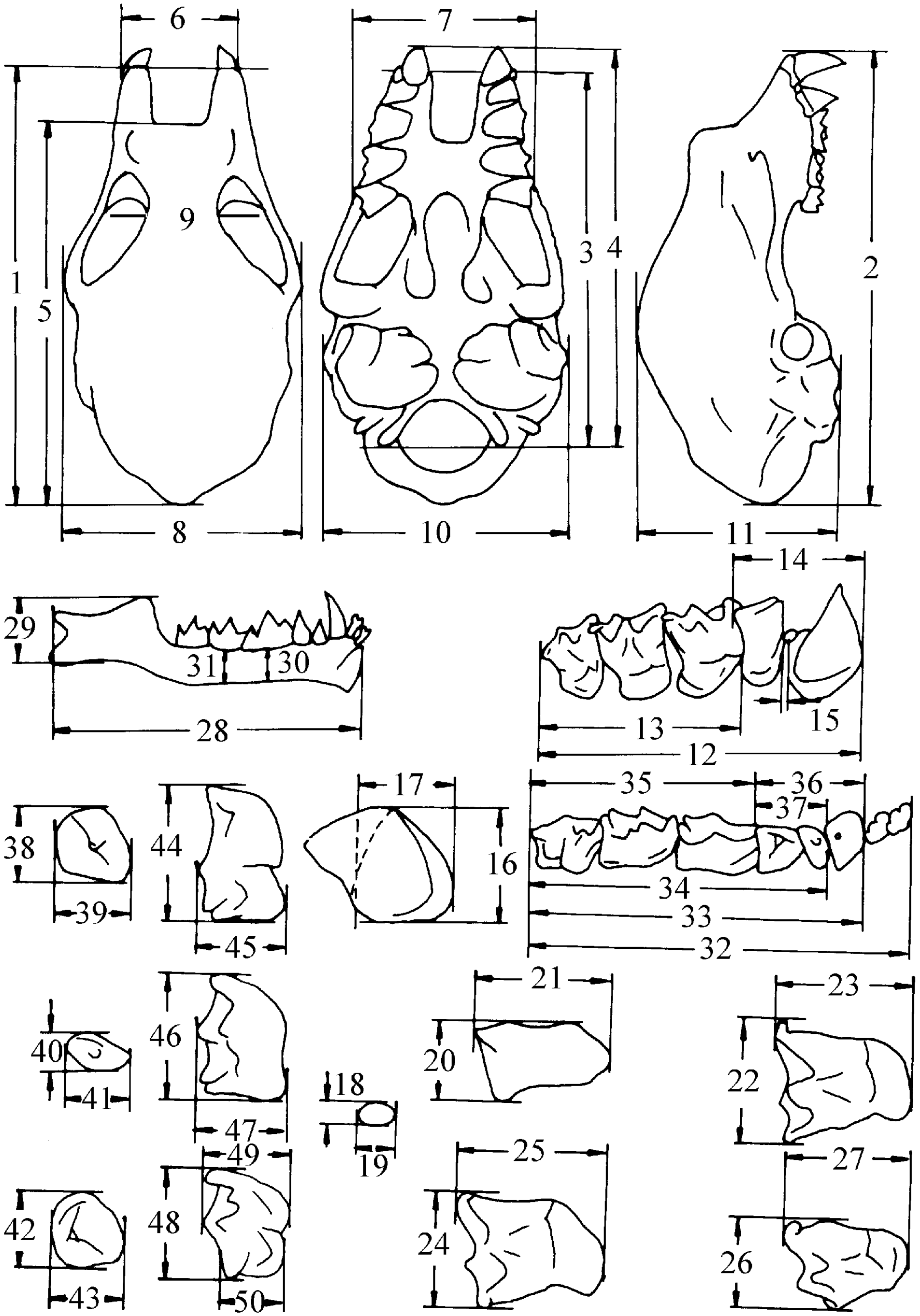

Fig. 2. The limits of each measurement on the skull, mandible and individual teeth. Numbers correspond with measurements and abbreviations described in text.

Results

M o r p h o l o g y

General morphological pattern (PCA). Three major groups of specimens were evident in the reduced subspace of the first two principal components (PC) (Fig. 3a). The first PC appears

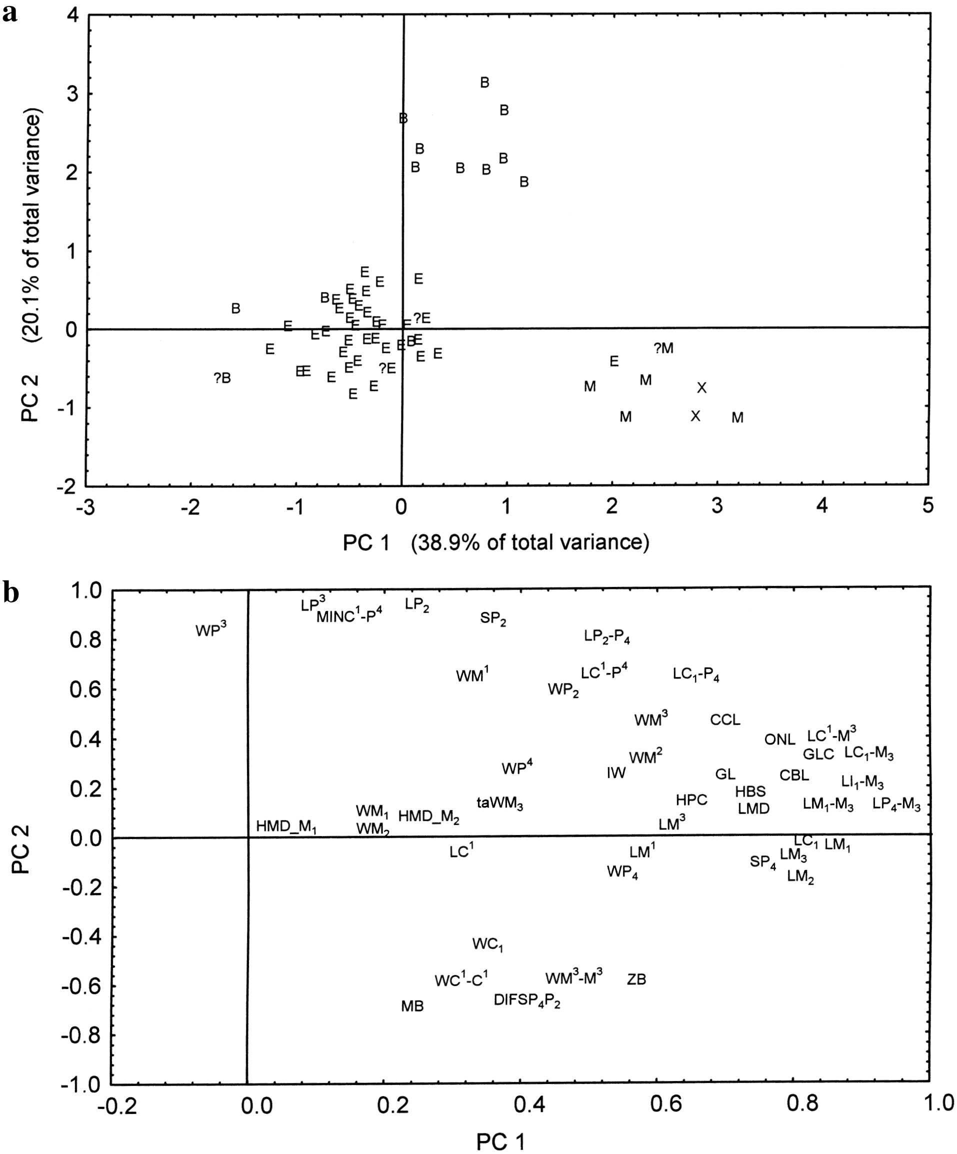

Fig. 3. Plots resulting from a principal component analysis of unstandardised craniometric data of all available complete skulls of medium-sized horseshoe bats, included in the study. **a.** plot of specimen scores; **b.** plot of character loadings (for abbreviations – see text); the symbols represent the determinations on the museum tags: E – *Rh. euryale*, B – *Rh. blasii*, M – *Rh. mehelyi*, X – undetermined, ? – dubious original determination ("cf. *euryale*" or "*euryale-mehelyi*").

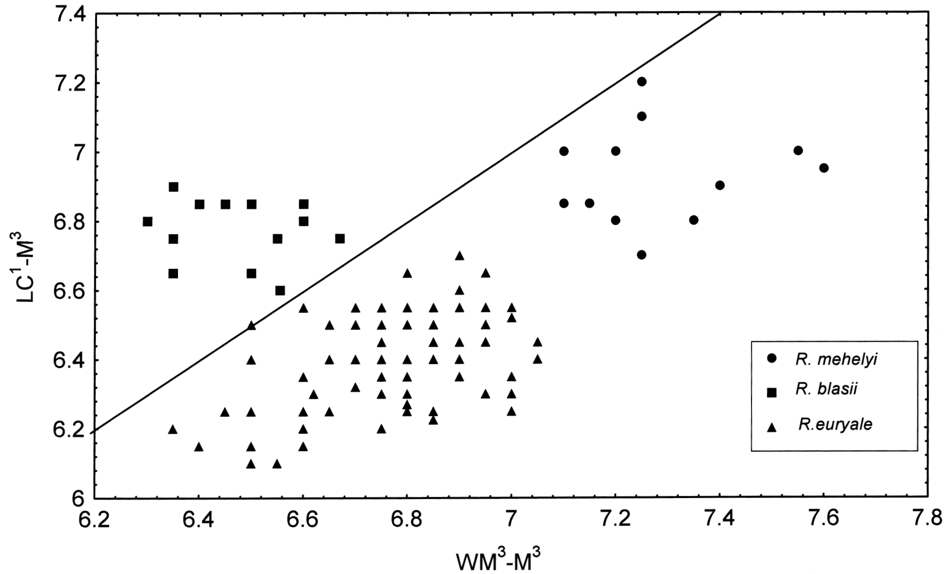

Fig. 4. A graph showing the posterior width (mm) of rostrum between outer crowns of M3's (WM^3-M^3) versus crown length (mm) of C^1-M^3 (LC^1-M^3) for all available crania of medium-sized horseshoe bats. The line represents equal widths to lengths.

to be a size factor. The scatter diagram (Fig. 3b) suggests that most characters have positive loadings on the first PC and that these are of greatest value in the separation of specimens determined as *R. mehelyi* from these attributed to *R. euryale*. As is common in PCA, the second PC describes the shape of the skull. The specimens with highly positive scores on this axis, initially determined as *R. blasii* (Fig. 3a), appear to have rather narrow skulls and elongated anterior parts of their toothrows or individual premolars (Fig. 3b, Table 3). In

Table 2. Classification functions resulting from stepwise discriminant function analyses (A. - C.) of craniometric data for three medium-sized *Rhinolophus* species from Bulgaria. A. = based on all craniometric features, by species and sex, * females (*R. mehelyi* N = 4, *R. blasii* N = 10, *R. euryale* N = 13), ** males (*R. mehelyi* N = 3, *R. blasii* N = 4, *R. euryale* N = 13); B. = based on rostral characters, by species; C. = based on mandibular characters, by species (for B. and C., *R. mehelyi* N = 15, *R. blasii* N = 16, *R. euryale* N = 86). For abbreviations of variables see text.

Analysis	Variables	*R. mehelyi*	*R. blasii*	*R. euryale*
A.	LC^1-M^3	622.82* / 626.86**	617.86* / 621.68**	566.99* / 573.66**
	ZW	260.16 / 260.49	213.26 / 209.42	247.19 / 248.44
	LP_2-P_3	-303.74 / 310.03	-208.21 / -199.14	-292.30 / -296.20
	Constant	-3239.09 / -3256.95	-2906.22 / -2913.90	-2758.05 / -2806.29
B.	LP^3	148.44	313.95	177.85
	LC^1-M^3	381.60	378.42	351.73
	WM^3-M^3	206.70	173.03	192.46
	Constant	-2099.62	-1910.22	-1807.61
C.	LM_1-M_3	802.92	743.71	736.19
	LP_2	-161.45	-5.61	-122.52
	WC_1	352.39	221.57	321.42
	Constant	-2106.29	-1816.49	-1784.13

Table 3. Cranial, mandibular and dental measurements (mm) of three medium sized *Rhinolophus* species from Bulgaria. N = sample size; Range = observed range; X = mean; SD = standard deviation; CV = coefficient of variability (%); for other abbreviations - see text.

Measurement	*Rhinolophus euryale* N = 80				*Rhinolophus blasii* N = 17				*Rhinolophus mehelyi* N = 14			
	Range	X	SD	CV	Range	X	SD	CV	Range	X	SD	CV
GL	17.40 - 18.90	18.31	0.277	1.5	18.20 - 19.20	18.76	0.127	0.7	18.60 - 19.60	19.26	0.079	0.4
GLC	18.10 - 19.40	18.90	0.275	1.4	19.00 - 19.90	19.53	0.102	0.5	19.40 - 20.30	19.96	0.094	0.5
CBL	15.40 - 16.80	16.11	0.290	1.8	16.10 - 17.00	16.58	0.113	0.7	16.30 - 17.60	16.98	0.124	0.7
CCL	16.00 - 17.05	16.66	0.227	1.4	16.60 - 17.50	17.11	0.105	0.6	16.95 - 17.80	17.45	0.094	0.5
ONL	15.20 - 16.40	15.98	0.234	1.5	16.00 - 16.80	16.52	0.099	0.6	16.20 - 17.00	16.72	0.084	0.5
WC^1-C^1	4.30 - 5.20	4.82	0.164	3.4	4.00 - 4.90	4.46	0.093	2.1	4.80 - 5.40	5.12	0.065	1.3
WM^3-M^3	6.35 - 7.05	6.77	0.141	2.1	5.60 - 6.67	6.42	0.106	1.6	7.10 - 7.60	7.28	0.061	0.8
ZB	9.40 - 10.20	9.82	0.163	1.7	9.00 - 9.70	9.40	0.073	0.8	10.20 - 11.00	10.55	0.082	0.8
MB	9.40 - 10.10	9.85	0.146	1.5	9.20 - 9.70	9.46	0.069	0.7	9.80 - 10.40	10.12	0.053	0.5
HBS	7.90 - 8.80	8.25	0.219	2.6	8.30 - 8.90	8.54	0.076	0.9	8.40 - 9.20	8.87	0.066	0.7
LC^1-M^3	6.10 - 6.70	6.40	0.133	2.1	6.60 - 6.90	6.76	0.040	0.6	6.70 - 7.20	6.93	0.052	0.7
LP^4-M^3	4.00 - 4.90	4.73	0.121	2.6	4.70 - 5.05	4.89	0.036	0.7	5.00 - 5.35	5.13	0.037	0.7
LM^1-M^3	3.70 - 4.10	3.91	0.083	2.1	3.80 - 4.10	3.96	0.041	1.0	4.10 - 4.40	4.23	0.037	0.9
LC^1-P^4	2.50 - 3.10	2.78	0.106	3.8	2.85 - 3.20	3.00	0.037	1.2	2.67 - 3.05	2.89	0.035	1.2
$MINC^1$-P^4	0.00 - 0.27	0.14	0.060	42.9	0.32 - 0.55	0.41	0.031	7.6	0.07 - 0.25	0.14	0.023	16.4
LC^1	1.00 - 1.67	1.52	0.085	5.6	1.40 - 1.60	1.51	0.023	1.5	1.45 - 1.75	1.57	0.031	2.0
WC^1	0.90 - 1.35	1.11	0.056	5.0	0.97 -- 1.22	1.11	0.030	2.7	1.10 - 1.32	1.24	0.024	1.9
LP^3	0.25 - 0.40	0.32	0.029	9.1	0.41 - 0.57	0.46	0.019	4.1	0.27 - 0.37	0.29	0.028	9.6
WP^3	0.30 - 0.52	0.42	0.040	9.5	0.42 - 0.60	0.52	0.025	4.8	0.30 - 0.42	0.37	0.012	3.2
LP^4	0.90 - 1.17	1.05	0.053	5.0	1.02 - 1.30	1.15	0.028	2.4	1.02 - 1.20	1.09	0.019	1.7
WP^4	1.40 - 1.90	1.73	0.079	4.6	1.65 - 1.92	1.79	0.039	2.2	1.62 - 1.97	1.80	0.043	2.4
LM^1	1.42 - 1.67	1.58	0.049	3.1	1.50 - 1.67	1.58	0.022	1.4	1.62 - 1.70	1.66	0.007	0.4
WM^1	1.57 - 2.07	1.86	0.088	4.7	1.92 - 2.20	2.04	0.034	1.7	1.72 - 2.00	1.90	0.021	1.1
LM^2	1.35 - 1.52	1.43	0.034	2.4	1.42 - 1.52	1.45	0.013	0.9	1.50 - 1.62	1.56	0.015	1.0
WM^2	1.60 - 1.86	1.74	0.064	3.7	1.75 - 1.95	1.81	0.021	1.2	1.70 - 2.07	1.85	0.027	1.5
LM^3	0.92 - 1.17	1.02	0.050	4.9	0.96 - 1.15	1.05	0.033	3.4	1.05 - 1.25	1.13	0.020	1.8
WM^3	1.40 - 1.62	1.54	0.048	3.1	1.50 - 1.65	1.57	0.070	4.5	1.57 - 1.70	1.63	0.017	1.1
LMD	11.50 - 12.60	12.03	0.114	0.9	12.00 - 12.70	12.26	0.069	0.6	12.30 - 13.20	12.79	0.088	0.7
HPC	2.40 - 3.00	2.69	0.105	3.9	2.60 - 3.00	2.81	0.044	1.6	2.77 - 3.25	3.01	0.053	1.8
HMD_M_1	1.32 - 1.70	1.56	0.091	5.8	1.44 - 1.70	1.55	0.026	1.7	1.50 - 1.67	1.58	0.018	1.1
HMD_M_2	1.30 - 1.87	1.55	0.086	5.5	1.47 - 1.70	1.56	0.024	1.5	1.47 - 1.72	1.61	0.024	1.5
$LI1$-M_3	7.30 - 7.92	7.67	0.135	1.8	7.70 - 8.10	7.87	0.045	0.6	7.85 - 8.50	8.10	0.040	0.5

Table 3. Continued.

Measurement	*Rhinolophus euryale* N = 80				*Rhinolophus blasii* N = 17				*Rhinolophus mehelyi* N = 14			
	Range	X	SD	CV	Range	X	SD	CV	Range	X	SD	CV
LC_1-M_3	6.55 - 7.05	6.83	0.125	1.8	6.90 - 7.25	7.10	0.036	0.5	7.20 - 7.55	7.31	0.035	0.5
LP_4-M_3	5.10 - 5.60	5.40	0.096	1.8	5.30 - 5.65	5.47	0.045	0.8	5.65 - 6.05	5.79	0.038	0.7
LM_1-M_3	4.30 - 4.70	4.53	0.078	1.7	4.50 - 4.75	4.62	0.024	0.5	4.80 - 5.10	4.90	0.034	0.7
LC_1-P_4	2.10 - 2.55	2.34	0.091	3.9	2.55 - 2.65	2.60	0.017	0.6	2.35 - 2.65	2.55	0.030	1.2
LP_2-P_4	1.35 - 1.70	1.55	0.079	5.1	1.75 - 1.92	1.84	0.019	1.0	1.60 - 1.75	1.68	0.022	1.3
LC_1	0.77 - 0.95	0.87	0.040	4.6	0.80 - 0.97	0.90	0.015	1.7	0.95 - 1.15	1.04	0.020	1.9
WC_1	0.85 - 1.05	0.95	0.037	3.9	0.82 - 0.95	0.88	0.015	1.7	0.95 - 1.12	1.02	0.017	1.7
LP_2	0.57 - 0.77	0.66	0.044	6.7	0.77 - 0.88	0.83	0.014	1.7	0.60 - 0.77	0.67	0.016	2.4
WP_2	0.56 - 0.77	0.67	0.051	7.6	0.71 - 0.81	0.76	0.013	1.7	0.67 - 0.80	0.74	0.014	1.9
LP_4	0.80 - 1.00	0.90	0.037	4.1	0.88 - 1.02	0.93	0.013	1.4	0.92 - 1.12	1.04	0.023	2.2
WP_4	0.67 - 0.92	0.81	0.050	6.2	0.70 - 0.95	0.80	0.023	2.9	0.85 - 1.05	0.95	0.022	2.3
LM_1	1.57 - 1.82	1.69	0.042	2.5	1.65 - 1.80	1.72	0.015	0.9	1.80 - 1.94	1.85	0.015	0.8
WM_1	0.85 - 1.20	1.02	0.077	7.5	0.87 - 1.17	1.03	0.027	2.6	1.00 - 1.25	1.09	0.030	2.7
LM_2	1.45 - 1.67	1.56	0.035	2.2	1.50 - 1.65	1.56	0.013	0.8	1.62 - 1.80	1.70	0.022	1.3
WM_2	0.92 - 1.22	1.04	0.069	6.6	0.92 - 1.20	1.04	0.029	2.8	1.02 - 1.25	1.11	0.024	2.2
LM_3	1.32 - 1.52	1.41	0.046	3.3	1.36 - 1.53	1.42	0.019	1.3	1.50 - 1.67	1.57	0.018	1.1
$trWM_3$	0.80 - 1.15	0.95	0.091	9.6	0.82 - 1.08	0.93	0.032	3.4	0.87 - 1.10	1.00	0.026	2.6
$taWM_3$	0.63 - 0.92	0.76	0.067	8.8	0.71 - 0.90	0.79	0.025	3.2	0.77 - 1.02	0.89	0.026	2.9

contrast, the specimens having negative scores along PC2 must have relatively wide skulls, massive lower canines and a great difference between the large lower premolars (Fig. 3b). The specimens initially assigned to *R. mehelyi* and *R. euryale* score along the negative half of this axis (Fig. 3a). Thus, it is evident that the shape factor synthesises the difference between *R. blasii* on one hand and *R. euryale* and *R. mehelyi* on the other (Fig. 3a). Thus, the specimen clusters shown by PCA represent the three study species. In this context, it was clear that some of the original determinations were incorrect (Fig. 3a).

Discriminant criteria. It may be expected that a pairwise combination of measurements based on the species' cranial characteristics could be used to construct a simple scatter diagram suitable for rapid species determinations. According to preliminary empirical tests, the two characters that most clearly distinguished amongst species were LC^1-M^3 and WM^3-M^3. From the resulting bivariate plot (Fig. 4) it is evident that most of the specimens could be related to one of the three species unequivocally.

The next stage of analysis aimed to provide more accurate discrimination keys, thus permitting the unambiguous determination of all specimens. Initially, Stepwise Discriminant Function Analysis was performed on subsamples consisting of sexed specimens. Only the first two discriminant functions had eigenvalues greater than one and were found to be statistically significant. As it is evident from Table 2A, the resulting classification coefficents document only the differences between species and not between sexes.

On the basis of this result, some discriminant analyses based on all the complete skulls available were performed, with the objective of defining suitable keys for the determination of fragmentary material. For this purpose skulls and mandibles were treated separately; in the former only measurements related to the rostral part were considered. The classification functions obtained are shown on Table 2B, C. On the basis of these criteria a large number of fragmentary crania, most originating from cave floor mummies, were determined (see Table 1).

As a result of the above analyses, standard statistics were calculated for each morphometric variable for each species (Table 3).

Variability of *Rhinolophus euryale*. This species, being the most abundant and widely distributed of the three, allowed intraspecific variability to be studied in greater detail.

The age variation within cranial measurements was tested (one-way MANOVA) in a large subsample from locality No. 22. The effect of age was not significant. As juvenile specimens were not included in the analyses, it is clear that the growth of the skull of *R. euryale* is completed during the first year of life. The same can be supposed for the other two species, taking into account their generally similar sizes. The result obtained validates the elimination of age variation as a factor which affecting the morphometric patterns observed.

Despite the low level of sexual dimorphism observed (Fig. 5) the relatively large samples of males (N=13) and females (N=13) of *R. euryale* permitted more detailed analysis. The one-way MANOVA revealed nine characters showing statistically significant differences between the sexes: HMD_M_1, HMD_M_2, WP_2, SP_2, LC^1, GL, WC^1-C^1, ONL, GLC (F for characters varies between 3.01 and 15.36 with $0.001 < P < 0.05$). In all cases the males show greater values.

As concerns spatial variability, in order to obtain adequate samples for statistical analysis, specimens were assigned to three geographical samples representing the main latitudinal climatic zones in Bulgaria (Fig. 1). It should be noted that the sample from zone B consists only of specimens from locality 22 (Table 1). Since the sex of a large proportion of the specimens was not known, measurements showing significant sexual dimorphism (see above) were omitted from the analysis – thus reducing the potential influence of differences in male:female ratio between samples. Twenty one characters showed statistically

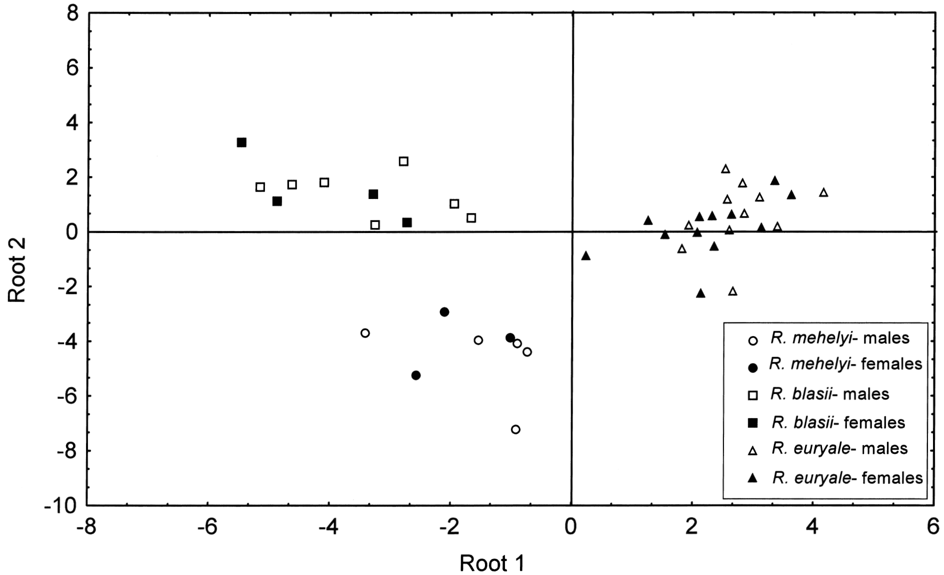

Fig. 5. Scatterplot of discriminant scores obtained by Stepwise Discriminant Analysis based on all measurements for subsamples consisting of sexed specimens of the three species.

significant differences between climatic zones: LC_1-M_3, LP_4-M_3, LP_2-P_3, LC_1, WC_1, LP_2, LP_4, $DIFSP_4$-P_2, WM_1, WM_2, LM_3, $taWM_3$, LC^1-M^3, LP^4-M^3, LM^1-M^3, LC^1-P^4, LP^4, LM^2, LM^3, WM^3, HBS (Wilk's lambda = 0.045, Rao's R = 2.60, $P < 0.001$; F for characters varying between 2.74 and 9.21 with $0.001 < P < 0.05$). With the exception of the height of the skull (HBS), these characters represent measurements connected with the teeth and toothrow lengths. Inspection of the means shows that the values for zone B are greater in all cases, but the magnitude of these differences is rather low.

Discussion

Distributional patterns of medium-sized *Rhinolophus* in Bulgaria

Literature-derived data on the distribution of the three *Rhinolophus* spp. in Bulgaria have been summarised recently by Pandurska (1995), in which the maps presented do not show any geographical variation. Our data (Table 1), based on a detailed analysis of voucher specimens, even though founded on a smaller number of localities, permits us to refine our knowledge of the distribution of the three horseshoe bat species in Bulgaria. *Rhinolophus blasii* is associated mainly with warmer parts of the country with a pronounced Mediterranean climatic influence, within which it appears to be rather common. *Rhinolophus mehelyi*, although relatively rare, occurs throughout the country. *Rhinolophus euryale* is the most widespread species, and probably is the most abundant medium-sized horseshoe bat in Bulgaria. However, it appears that *R. euryale* becomes rarer towards the south, a trend opposite to that seen in *R. blasii*. Table 1 suggests that it is probable that in southern Bulgaria horseshoe bats have comparable frequencies (all three species) and abundances (*R. euryale* and *R. blasii* only).

Morphological variability

Sexual dimorphism. The analyses reveal the occurrence of slight, but statistically significant, differences between the sexes in *R. euryale*: all characters are larger in males. Sexual dimorphism in skull measurements has already been reported in this species (Dinale 1971), so these observations are consistent with extant data on sexual dimorphism in other bat species which show that, with regard to cranial measurements, males tend to be larger (Benda 1994). However, in some species, e.g. genus *Myotis*, breadth measurements show more pronounced sexual dimorphism than do those based on length (Bogdanowicz 1990, Benda 1994).

Geographical variability. Data available in the literature (Dinale 1971) concerning spatial variability in *R. euryale* indicate the occurrence of slight, but well pronounced, differences in some cranial measurements between more or less closely situated localities. The above analyses show differences of a similar magnitude between climatically defined samples. The most obvious difference in this respect concerns the larger size of the dentition and in some cranial measurements in specimens from the transitional zone (zone B). This sample is composed mainly of specimens from locality No. 22 (near Kotel), which is situated in a mountainous landscape at about 700 m a.s.l.; and it may be supposed that some environmental peculiarities may contribute to this result. Climatic data indicate that the population from locality No. 22 lives in a cooler, more humid climate than do the other *R. euryale* populations from lowland areas.

Bulgarian populations within the context of geographical variability. Comparisons with data on *R. euryale* from Portugal (Palmeirim 1990), southern France (König & König 1961), Italy (Dinale 1971), Transcaucasia and Kopet Dagh (Kuzyakin 1950), the Near East (DeBlase 1972, 1980, Felten et al. 1977) and other parts of the Balkan Peninsula and central Europe (Hanák 1964) show that the largest morphometric character values are in bats from Bulgarian samples. In the majority of cases the differences are statistically significant. Although it can be supposed that at least some of the differences detected may be due to observer bias or local environmental conditions, the consistent trend in all comparisons indicates that, in general, the Bulgarian study sample shows somewhat larger values for the cranial morphometric traits under consideration.

Similar regularities, although not so unequivocal, appear when considering *R. blasii*. Bulgarian specimens appear to be larger when compared with specimens from Dalmatia (Đulić 1961), Macedonia (Đulić 1966), Serbia and Montenegro (Paunović & Stamenković 1998), Albania (Hanák 1964) and western Anatolia, Iran and Afghanistan (Felten et al. 1977, DeBlase 1980). According to Felten et al. (1977) Mediterranean populations should be referred to the nominate subspecies, whilst those from Iran and Afghanistan represent a large subspecies, *R. b. meyeroehmi* Felten, 1977. Comparison with this subspecies shows that the form from Bulgaria is larger, at least according to the majority of cranial measurements available for comparison (except for LC^1-M^3). On the other hand, our specimens are nearly equal with those from North Africa (Aellen 1955, Aellen & Strinati 1970).

In contrast to the aforementioned species, *R. mehelyi* does not show any pronounced geographical variability. In terms of cranial measurements, the study material is nearly identical with samples reported from Portugal (Palmeirim 1990), Spain and France (Strinati & Aellen 1958) and western Asia Minor (Felten et al. 1977). The southern and eastern populations are somewhat smaller (DeBlase 1972, Baker et al.

1974, Hanák & Elgadi 1984) but the differences are not always well expressed (Kahmann 1958).

The size differences observed in *R. euryale* and *R. blasii*, although slight, suggest the existence of a clinal west-east size increase. Such a cline also appears in other bats with Ponto-mediterranen distributions, notably *Rhinolophus ferrumequinum* (DePaz 1995, Kryštufek 1993), *Myotis blythii*, *Myotis myotis* and *Myotis nattereri* (Benda & Horáček 1995) . In some degree these differences may be related to Bergmann's rule, bearing in mind that the climate in the central part of the Balkan Peninsula (and especially in the mountainous regions; Fig. 1) is cooler than that in typically Mediterranean areas. The influence of other factors may also contribute to the pattern observed (Bogdanowicz 1990, Kryštufek 1993).

Identification criteria. The skull and dentition of *R. euryale* are similar than those of *R. mehelyi*. Although the first species is smaller on average, there is some overlap in the majority of skull measurements (Frick & Felten 1952, Strinati & Aellen 1958, Strinati 1959, Etemad 1963, DeBlase 1972, Felten et al. 1977). The data available show that some characters such as zygomatic width and mandibular and upper tooth row (LC^1-M^3) lengths are useful characters for separating the species (Strinati 1959, Etemad 1963, DeBlase 1972, 1980, Felten et al. 1977). However, the diagnostic usefulness of these characters is not always incontestable as the craniometric overlap between the species varies geographically (Palmeirim 1990). Moreover, the occurrence of a third species of similar size in Bulgaria (*R. blasii*) further obscures the picture (Table 3). The same is true when trying to separate these species on the basis of scatter plots of mandibular length against the height of the coronoid process (Ruprecht 1987). DeBlase (1972) presents a comprehensive review of the diagnostic characters of *R. euryale* and *R. mehelyi*. The results of present study suggest that, of the cranial measurement-based characters used in distinguishing these species, some require correction when applied to Bulgarian material (Table 3).

As regards *R. blasii*, according to Miller (1912) a useful diagnostic trait is the absence of a marked contrast between the sizes of the anterior and posterior lower premolars. Additionally, Kuzyakin (1950) pointed out that the large space between these teeth is occupied by a relatively large P_3, situated near to the tooth-row axis. In contrast, the other two species have a small P_3 which is shifted labially. Our results substantiate these differences, however, the data in Table 3 indicate that although the characters describing these differences have their largest average values in *R. blasii*, they also overlap amongst the three species.

According to Kuzyakin (1950) three other features of *R. blasii* deserve attention: the large distance between the crowns of C^1 and P^4, the narrow skull (especially in its nasal part) and the pronounced concavity of the dorsal outline of the posterior part of the brain case. The first two peculiarities are well pronounced in our material, but again there is a wide overlap between species (Fig. 3b, Table 3). A shallow concavity on the dorsal profile of the brain capsule may also be observed in *R. euryale*, and sometimes it is not easy to separate the species on the basis of this feature.

As shown by the above, the morphological peculiarities of skulls revealed by our analyses, correspond fairly well to the species characteristics already available in the literature. However, these peculiarities taken in isolation cannot distinguish unequivocally between the three species. Biplots or, better still, discriminant functions, permit a clearer separation.

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