LANDMARK-BASED SIZE AND SHAPE ANALYSIS IN SYSTEMATICS OF THE PLECOTINE BATS

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ABSTRACT

Morphometric divergence among the skulls of 10 species of plecotine bats (n = 105) was studied using x, y-coordinates of 11 homologous landmarks recorded from the left half dorsal view of each skull. Univariate and multivariate analyses of shape coordinates provided estimates of differences among taxa and between sexes. The differences in size among species were correlated with uniform shape differences. The centroid size to uniform factor allometry was more pronounced longitudinally (i.e., along the midline of the skull) than it was laterally. Significant shape differences among species were also detected in both uniform and non-uniform components. Non-uniform shape variation involved lateral rather than longitudinal displacement of landmarks on the skull. Sexual dimorphism was reflected by centroid size and was seen only in *Otonycteris hemprichi*, in which females were about 4.4% larger than males. The UPGMA phenograms of Mahalanobis D^2 of shape coordinates and of relative warp scores (in which the uniform shape component had been removed) revealed two consistent phenetic clusters. One was formed by *O. hemprichi*, with the remaining genera grouping in a second cluster. The relationships among genera in this second cluster varied depending on the phenogram generated. Nevertheless, the UPGMA phenogram derived from Mahalanobis D^2 computed on Bookstein shape coordinates (sexes combined) was entirely congruent with the current systematic hierarchy and phylogenetic hypothesis of the Plecotini sensu stricto recently put forward based on a parsimony analysis of 32 skin and skull characters. In our analysis, the most divergent species group was the monotypic *O. hemprichi*. A second group was formed by the genus *Barbastella*. The remaining clusters were composed of species of *Corynorhinus; Plecotus;* and a cluster containing *Idionycteris* and *Euderma*.

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INTRODUCTION

The tribe Plecotini (Chiroptera: Vespertilionidae) consists of 10 or 11 species of mainly "long-eared" bats, that are widely distributed throughout the Holarctic (e.g., Yoshiyuki, 1991; Frost and Timm, 1992; Tumlison and Douglas, 1992; Zima et al., 1992; Qumsiyeh and Bickham, 1993). Handley (1959) first examined the relationships among the tribe in detail and proposed the phylogeny (*Barbastella* (*Euderma Plecotus*)). His taxonomic arrangement included the genera *Barbastella*, *Euderma* and *Plecotus*, with the last containing three subgenera: *Plecotus* of the Old World, and *Corynorhinus* and *Idionycteris* of the New World. Subsequent works suggested that *Idionycteris* should be given full generic status (Williams et al., 1970; Nader and Hoffmeister, 1983) on the basis of a closer relationship between *Idionycteris* and *Euderma* (Stock, 1983). Yet, Handley's (1959) arrangement is still generally accepted (reviewed by Frost and Timm, 1992; Tumlison and Douglas, 1992; see also Menu, 1987; Topál, 1989). Recently, though, three different classifications of the Plecotini have been proposed based on cladistic treatments of morphological and chromosomal traits. Tumlison and Douglas (1992) examined 32 cranial and external characters. These data yielded the most parsimonious tree (*Barbastella* (*Corynorhinus* (*Plecotus* (*Idionycteris Euderma*)))). Frost and Timm (1992) used transformation series of 25 morphological and 11 karyological characters to propose the phylogeny (*Euderma* [including *Idionycteris*] (*Barbastella* (*Plecotus Corynorhinus*))). Studies of G-banded chromosomes (Zima et al., 1992; Qumsiyeh and Bickham, 1993; Volleth and Heller, 1994) indicated the phylogeny (*Euderma Idionycteris* (*Otonycteris Barbastella Plecotus Corynorhinus*)). Recent advances in geometric morphometrics provide systematists with potentially powerful tools for testing evolutionary patterns and processes (e.g., Tabachnick and Bookstein, 1990; Rohlf, 1993a; Rohlf and Marcus, 1993; Swiderski, 1993). These new techniques offer useful means to describe evolutionary shifts in the morphology of organisms and can provide new sets of characters with which to hypothesize systematic relationships. They also can show how anatomy, as depicted by the geometric relations between homologous landmarks, differs among populations and taxa in a way that may be related to their history (phylogeny) and ecology (e.g., Reyment, 1991; Loy et al., 1993). The aim of this study is twofold. First, we want to assess the amount of morphological variation and resolution seen among species within plecotine bats. This will be investigated by decomposing morphological variation into centroid size, uniform and nonuniform components (Bookstein, 1990, 1991), and distinguishing inter-taxon differences among forms. Second, we will evaluate some of the recently developed geometric methods as tools for evolutionary and phylogenetic inference by comparing our results with previously published arrangements proposed for the Plecotini.

MATERIAL AND METHODS

In total 105 specimens (see Appendix) representing 10 species of the plecotine bats were examined. To avoid the effect of bilateral asymmetry, 11 landmarks were collected from the left half dorsal view of the skull of each specimen (Fig. 1). These landmarks are (1) center of posterior curvature of internasal opening; (2) proximal extremity of premaxilla; (3) point of maximum lateral curvature of maxilla at center of upper canine; (4) center of infraorbital foramen; (5) point of the maximum curvature (width) of the infraorbital plate; (6) point of postorbital constriction inside the orbit; (7) point where internal portion of the posterior zygoma contacts the cranium; (8) most posterior internal curvature of zygomatic arch; (9) most lateral point of the mastoid process; (10) most posterior point of interparietal; and (11) center of coronal suture.

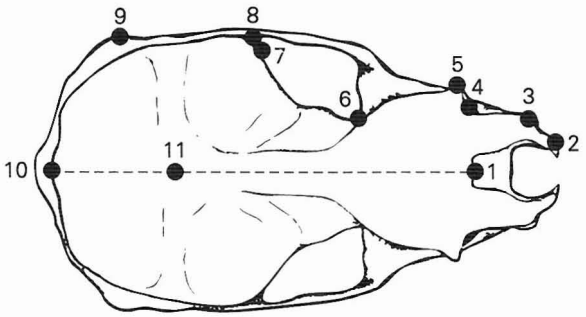

Figure 1. Anatomical positions of the 11 landmarks used in this study. Landmarks 1 and 10 were used as baseline end points.

Data were gathered using a COHU 4815 Series monochrome video camera equipped with a 60-300 mm macro zoom lens and using MorphoSys software (Meacham and Duncan, 1990). Format conversion of files from MorphoSys to other software packages was carried out using the FORCON program designed and written by D. E. Slice. Coordinates of the few missing landmarks were calculated based on the coordinates of existing landmarks (Richtsmeier et al., 1992). All specimens were translated and rotated using the SHAPETU transformation of the NTSYS-pc package (Rohlf, 1993b). This process places one designated landmark at the origin (coordinates 0,0) and a second along the x axis (coordinates x,0; $x >$ 0). To predict the coordinates of the missing landmark for a particular specimen, multiple regression was performed on the translated and rotated coordinates for each species separately.

A baseline was fixed to standardize both position and scale by selecting landmarks 1 and 10 (center of curvature of internasal opening and posterior point of the interparietal, respectively) situated along the midline of the skull (Fig. 1). All other landmarks were rotated and scaled to this baseline to produce Bookstein shape coordinates by means of the SAS IML program UNIGRAPH, written by L. F. Marcus (see Appendix III of volume for instructions for obtaining this and other programs from Stony Brook). A size variable expressed as centroid size was generated from the same landmarks before standardization in order to investigate size and shape covariance (Bookstein, 1986, 1991). Uniform x and y components were extracted from the shape coordinates based on Bookstein (1990) and by means of UNIGRAPH. Centroid size, baseline size, uniform x, and uniform y components were analyzed using a two-way analysis of variance (ANOVA unbalanced design) to test for effects of species, sex and species-sex interaction. When no differences between sexes or an interaction were detected, a one-way ANOVA was applied to test for among-species differences. The 95% probability ellipses for bivariate scattergrams were drawn using the SAS IML program ELLIPSE, written by L. F. Marcus. Sexual dimorphism as measured by centroid size was calculated using Storer's (1966) index: the difference between the female average and male average divided by the overall average and then multiplied by 100.

Differences in shape coordinates among species were tested using both univariate and multivariate methods. Multivariate analysis of variance (MANOVA) and canonical variate analysis were used to examine differences among taxa as described by the entire set of coordinates. Differences among species were summarized by unbiased Mahalanobis D^2 values by means of L. F. Marcus' SAS IML program DSQ. These values were then clustered by UPGMA by means of the NTSYS-pc program (Rohlf, 1993b). Significant differences among species means were tested using D^2 statistics and corresponding F-tests. Adjustments

in the level of significance for multiple comparisons were made using the Bonferroni inequality (Marcus, 1993). In order to find consensus configurations for each species, digitized coordinates were superimposed by using the Generalized Least Squares (GLS) option in the Procrustes superimposition software written by Slice (1992; see also Rohlf and Slice, 1990). The consensus configurations for each of the 10 species were then compared using relative warps (Thin-Plate Spline Relative Warp Analysis-TPSRW; Rohlf, 1993b, version October 1993). Relative warps were determined with respect to the mean configuration for all Plecotini based on an α, of 0 (Bookstein, 1991). Weights were based on deviations from the sample means. Relative warp scores were computed both retaining and removing the affine portion of the variation. Matrices of the squared average taxonomic distances were then produced and analyzed using the Mantel test in NTSYS-pc (Rohlf, 1993b). For each analysis, taxa were phenetically clustered using UPGMA. No significant differences between sexes were seen for shape coordinates or for the components of size and shape, in any of the species except for *Otonycteris hemprichi*, which showed slight sexual dimorphism in centroid size. Generally, this justified combining the data over sexes for each species and ignoring sex in further analyses. The IML analyses were done with the SAS version 5 (SAS, 1985) running under MS-DOS version 6.0.

RESULTS

Sexual Dimorphism

Results of the two-way ANOVA indicated that none of the Bookstein shape coordinates exhibit statistically significant sexual variation and only two (x_2 and x_6) show significant

Table 1. Two-way ANOVA testing sexual dimorphism against taxon variation for 18 shape coordinates and one-way ANOVA with sexes combined. Mean squares (x 10^4) from SAS Type III GLM procedure for unbalanced designs

	Two-way ANOVA				One-way ANOVA	
Coordinates	Species	Sex	Interaction	Error	Species	Error
x_2	43.93***	3.20	4.22**	1.38	58.99***	1.69
y_2	4.23***	0.23	0.81	1.13	5.01***	1.09
x_3	63.43***	2.53	2.39	1.25	79.51***	1.38
y_3	8.08***	0.02	0.34	0.58	10.22***	0.55
x_4	41.80***	0.31	2.25	1.30	56.67***	1.38
y_4	26.66***	0.03	0.70	0.72	28.88***	0.71
x_5	19.54***	0.50	1.11	1.29	24.91***	1.27
y_5	12.52***	0.00	0.79	0.90	14.33***	0.88
x_6	28.23***	0.97	2.32*	1.12	35.15***	1.24
y_6	14.78***	0.17	0.75	0.57	18.51***	0.58
x_7	73.42***	0.70	2.34	1.86	82.23***	1.89
y_7	29.22***	0.02	2.35	2.38	36.69***	2.36
x_8	53.84***	1.44	2.18	1.65	65.31***	1.70
y_8	10.98***	0.57	0.81	1.96	13.66***	1.83
x_9	71.88***	0.87	3.29	3.84	74.24***	3.75
y_9	44.47***	0.54	1.49	1.70	51.26***	1.67
x_{11}	13.78***	4.07	2.45	3.27	14.28***	3.18
y_{11}	0.76	0.02	0.81	0.63	0.72	0.64
df	9	1	9	85	9	95

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

Table 2. Summary of two-way ANOVA testing sexual dimorphism against species variation both with and without *O. hemprichi* ($n = 5$) for centroid size, baseline size, uniform x, and uniform y components and one-way ANOVA combining sexes. The F-values from SAS Type III GLM procedure for unbalanced designs

	Two-way ANOVA			One-way ANOVA
Variable	Species	Sex	Interaction	Species
O. hemprichi included				
Centroid size	546.50***	5.61*	1.95	516.65***
Baseline size	410.04***	3.20	1.81	411.07***
Uniform x	36.53***	0.10	1.54	42.41***
Uniform y	15.12***	0.07	0.74	19.35***
O. hemprichi removed				
Centroid size	219.65***	0.86	0.90	247.59***
Baseline size	198.45***	0.13	0.73	236.69***
Uniform x	33.05***	0.00	1.63	38.32***
Uniform y	12.97***	0.06	0.81	16.86***

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

interaction (Table 1). Neither significant sexual differences (Hotelling–Lawley Trace [HLT] = 0.197; df 18, 68; $p = 0.75$) nor interaction between sexes and taxa (HLT = 2.343; df 162, 596; $p = 0.62$) were found by MANOVA. This suggests that sexual and species variation are, in general, independent of each other in the morphospace studied.

Sexual dimorphism is exhibited only for centroid size (Table 2). Females are usually bigger than males but the differences are small and do not exceed 2.3%. The one exception

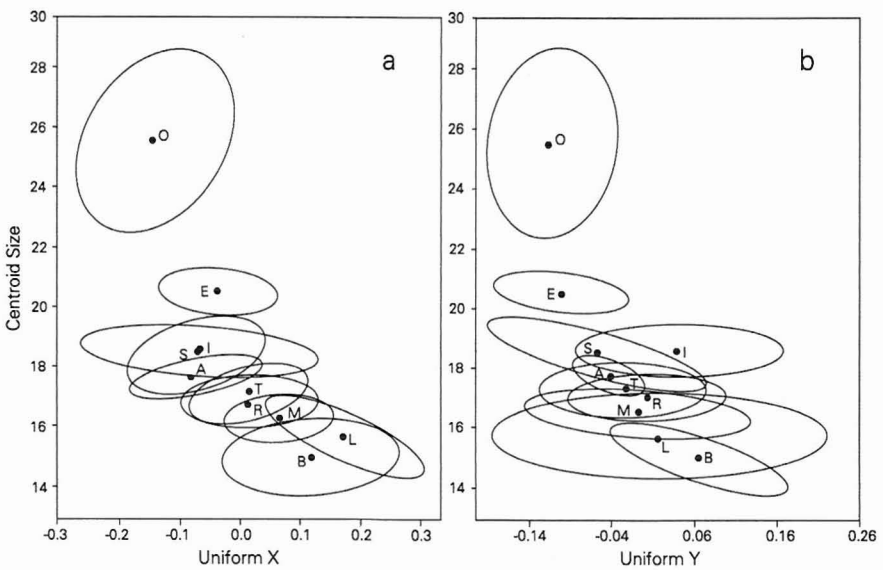

Figure 2. Relationships shown by projections of centroids and 95% probability ellipses of bivariate means between centroid size and (a) uniform x component and (b) uniform y component for 10 species of plecotine bats. Abbreviations used: A–*Plecotus auritus*; B–*Barbastella barbastellus*; E–*Euderma maculatum*; I–*Idionycteris phyllotis*; L–*Barbastella leucomelas*; M–*Corynorhinus mexicanus*; O–*Otonycteris hemprichi*; R–*Corynorhinus rafinesquii*; S–*Plecotus austriacus*; and T–*Corynorhinus townsendii*.

is for females of *O. hemprichi* which are approximately 4.4% larger than males. When this species was removed from the analysis, no significant sex effect was detected. No interaction of centroid size between species and sexes was detected by the ANOVA. Baseline size and the x and y components of the uniform factor are similar in females and males (Table 2).

Interspecific Relationships

Size and Uniform Shape Variation. Comparison of size and uniform shape components reveals a pattern of differences in which *O. hemprichi* is characterized by much larger centroid size as compared with the other taxa of Plecotini (Fig. 2). Next in size is *Euderma maculatum*. The 95% probability ellipses of these two species do not overlap and they form well-differentiated groups. The remaining bats represent a more homogeneous assemblage, with the genera *Plecotus* and *Corynorhinus* showing marked similarity.

Differences in uniform shape are highly correlated with variation of centroid size. Correlations indicate that 48.0 and 39.1% of the total variation of the centroid size is explained by either uniform x ($F = 95.01$; df 1, 103; $p < 0.001$; Fig. 2a) or uniform y ($F = 66.09$; df 1, 103; $p < 0.001$; Fig. 2b) variable, respectively. The pooled effect of these components accounts for as much as 61.8% of the total variance of the centroid size ($F = 82.42$; df 2, 102; $p < 0.001$). The uniform shape components along the x and y axes are significantly intercorrelated, with an $r = 0.415$ and $p < 0.001$. The 95%-probability ellipses around centroids of these components (not shown) indicate largely overlapping bivariate distributions among species. The differences, however, are still highly significant and might at least define groups of similar taxa.

Bookstein Shape Coordinates Space. Highly significant differences among species were found for 17 of the 18 (except y_{11}) standardized shape coordinates (Table 1). These differences are also highly significant when all coordinates are examined simultaneously (HLT = 48.71; df 162, 596; $p < 0.001$). Mahalanobis D^2 is always large and the F-values are significant ($p < 0.001$) for each pairwise comparison (Table 3). The Bonferroni multiple range test adjusted for the number of groups shows no overlap between taxa for an overall 0.05 significance level (see Loy et al., 1993) and with 100% correct assignment for all samples.

Table 3. Unbiased Mahalanobis D^2 (above diagonal) and their corresponding F-values (below) computed among 10 species of Plecotini based on Bookstein shape coordinates. Females and males combined. All values significant at 0.001 level. Abbreviations used: A—*Plecotus auritus*; B—*Barbastella barbastellus*; E—*Euderma maculatum*; I—*Idionycteris phyllotis*; L—*Barbastella leucomelas*; M—*Corynorhinus mexicanus*; O—*Otonycteris hemprichi*; R—*Corynorhinus rafinesquii*; S—*Plecotus austriacus*; and T—*Corynorhinus townsendii*

Species	A	B	E	I	L	M	O	R	S	T
A		120	58	60	146	55	315	66	29	54
B	66.8		128	106	14	54	256	79	131	99
E	29.2	76.1		31	143	73	232	76	42	54
I	32.7	70.4	18.5		135	70	251	61	37	51
L	58.0	7.2	59.8	60.4		66	282	89	169	109
M	35.9	45.3	50.6	55.7	34.6		283	20	66	13
O	111.0	104.0	85.7	99.0	88.6	127.0		282	247	285
R	41.2	62.6	50.5	46.1	45.2	20.3	123.0		55	20
S	15.7	82.1	23.6	22.9	73.1	48.9	94.5	39.3		47
T	28.1	62.2	30.0	31.1	47.6	10.8	109.0	14.8	27.5	

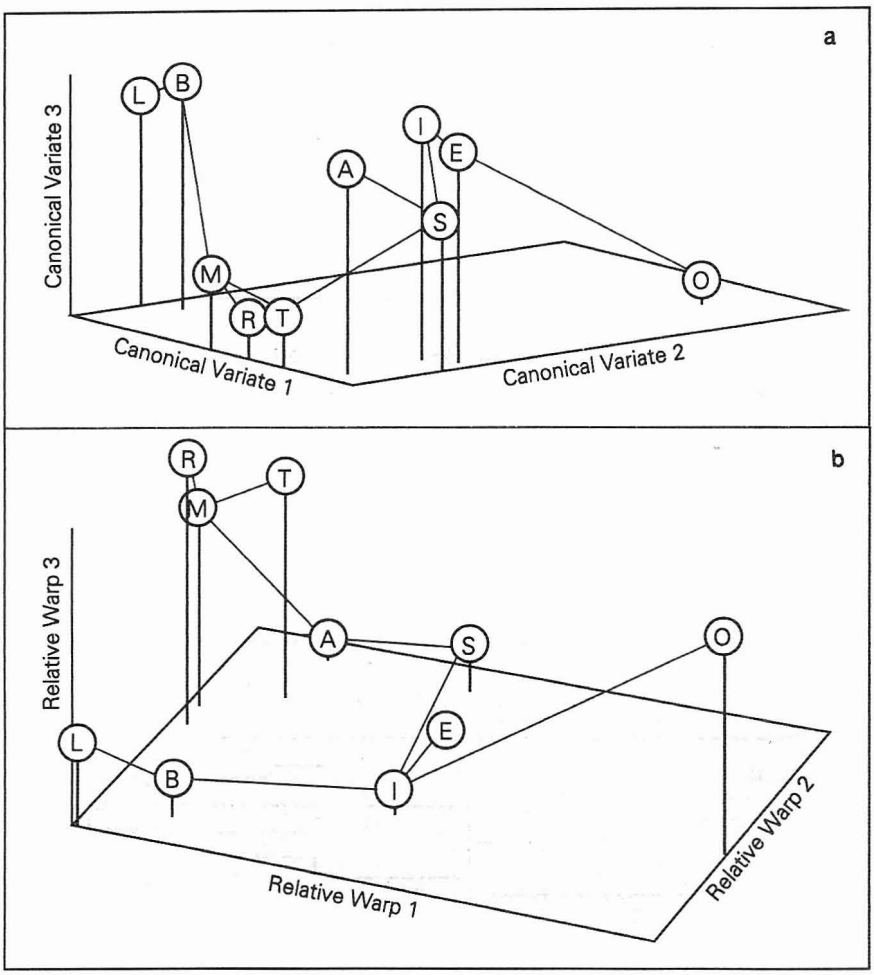

Figure 3. (a) Distribution of canonical–variate centroids of 10 species of plecotine bats in three-dimensional space. The first three canonical–variate axes account for 35.3, 32.0 and 12.8% of the total sample variation, respectively. (b) Relative warp analysis (uniform factor removed) of 11 pairs of *x*, *y*-coordinates from the dorsal view of the skull showing projections of 10 species of Plecotini onto the first three relative warps. The first, second, and third axes account for 42.5, 25.7 and 14.1% of the total variance. Both plots are shown with minimum spanning trees superimposed. Species abbreviations as in Fig. 2.

A canonical variate analysis based on the Bookstein shape coordinates reveals five possible groupings that consist of: (1) *Otonycteris,* (2) *Euderma*, *Idionycteris,* (3) *Plecotus,* (4) *Corynorhinus,* and (5) *Barbastella* (Fig. 3a). Similar patterns are seen both when a minimum spanning tree is superimposed onto the plot for the first three canonical variates and when a UPGMA phenogram is computed from canonical variate scores (Fig. 4a).

Otonycteris hemprichi is the most distant from all other taxa. *Euderma maculatum* has as its nearest neighbor I. *phyllotis*, and they are connected to the group *P. austriacus* and *P. auritus*. *Corynorhinus townsendii*, *C. rafinesquii* and *C. mexicanus* are intermediate between the genera *Plecotus* and *Barbastella*.

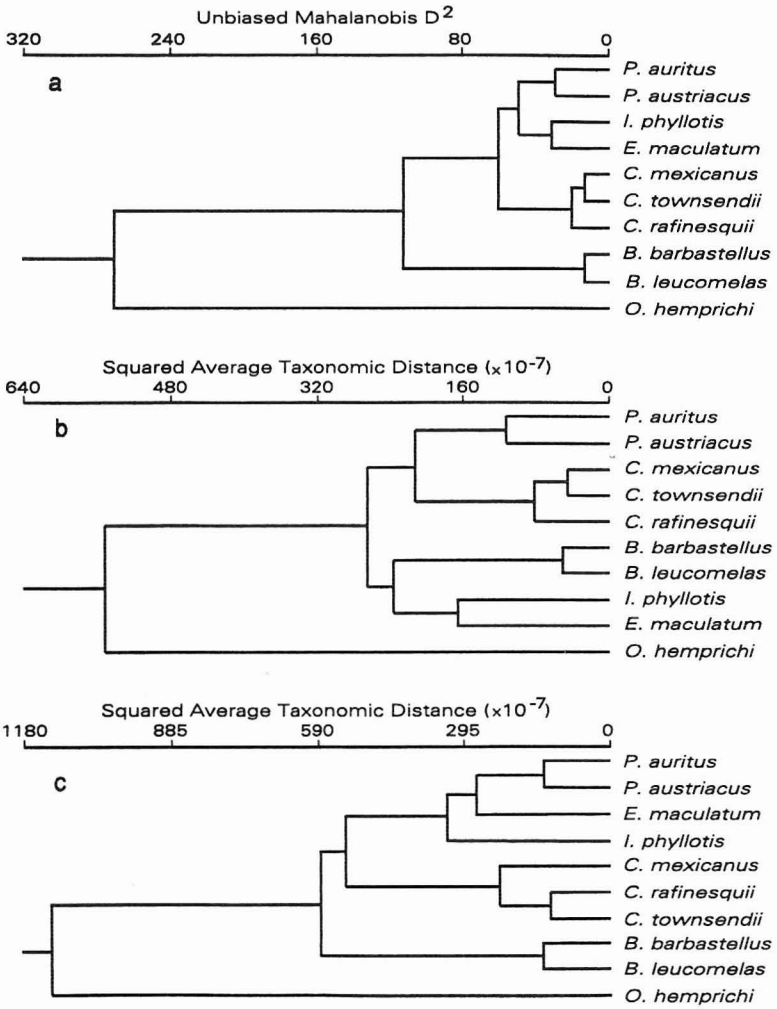

Figure 4. UPGMA phenograms of 10 taxa of Plecotini (a) derived from unbiased Mahalanobis D^2 computed from all 18 Bookstein coordinates; (b) computed on squared average taxonomic distances in relative warp space with the uniform factor removed; and (c) based on the matrix of squared average taxonomic distances computed from relative warp scores with the uniform factor retained. Their cophenetic values are 0.970, 0.859 and 0.834, respectively.

Relative Warps. Most of the variation among species is distributed along the first relative warp axis which accounts for 42.5% of the total variance; *B. leucomelas* is at the extreme left and *O. hemprichi* at the extreme right (Fig. 3b). Displacement vectors for this warp (Fig. 5) suggest that the most obvious difference between these two taxa is a displacement of landmarks 2, 3, 4, 5 and 9 in the direction of the decreased rostrum and mastoid region of *O. hemprichi*. The displacements in relative warp 2 (25.7% of total variance) imply that landmarks 1, 10 and 11 along the midline of the skull are more rostrally positioned (making the distance between internasal opening and supraoccipital larger) in *Plecotus* spp. than they are in *Barbastella* spp. The third relative warp axis (14.1% of total variance) emphasizes the differences between *I. phyllotis* and *P. auritus*, and *Corynorhinus* spp. and *Otonycteris hemprichi* relative to the lengthening and widening rostrum and narrowing of the braincase for the genera *Corynorhinus* and *Otonycteris*.

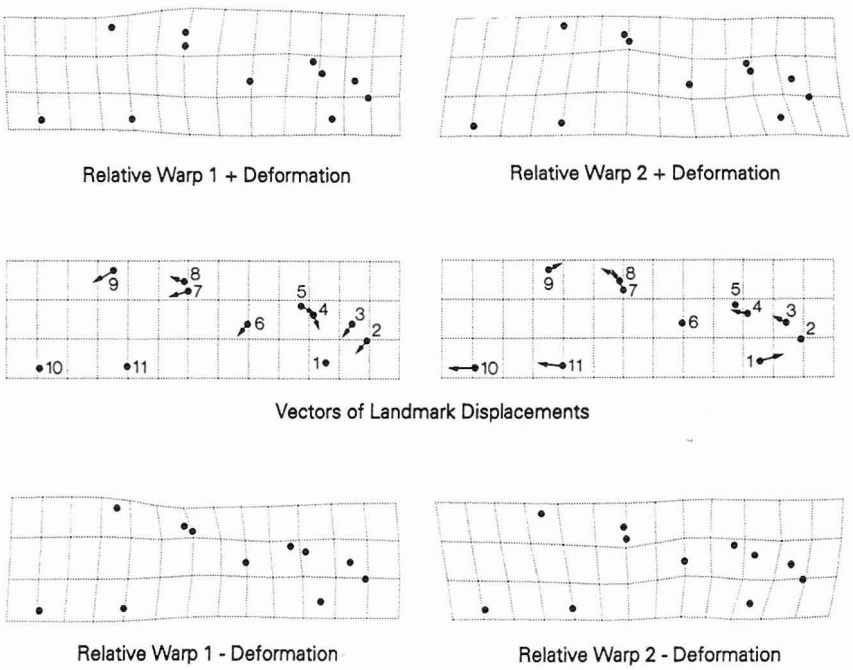

Figure 5. Displacement vectors and deformation grids for the first and second relative warps computed for 10 species of Plecotini based on the mean configuration as the reference and $\alpha = 0$. Landmarks numbered as in Fig. 1.

The plot of the first three relative warps with the minimum spanning tree superimposed produces five different groupings of species (Fig. 3b), the most divergent of which is the monotypic *O. hemprichi*. The second grouping includes *I. phyllotis* and *E. maculatum*. *Plecotus austriacus* and *P. auritus* form another cluster, which is intermediate between *I. phyllotis* and *Corynorhinus* spp. *Idionycteris phyllotis* is connected to *Barbastella barbastellus* which clusters together with *B. leucomelas*. The relative warp loadings suggest complex and localized patterns of deformations. Relative warp 1 indicates displacement of landmarks 2 and 9 towards the center of the neurocranium relative to the displacement of landmarks 4, 5 and 8 in the opposite direction. Relative warp 2 indicates an expansion of the region between internasal opening and the coronal suture (landmarks 1 and 11). The longest vectors for relative warp 3 imply the major modifications in the zygomatic and rostral regions of the skull (landmarks 4, 7 and 8). None of the relative warps examined is significantly intercorrelated with centroid size ($r = 0.261$–0.498, df 8, $p > 0.05$). The relationships obtained from cluster analysis of relative warp scores were similar to those seen for the phenogram of canonical variates scores, with one exception (Fig. 4b). *Idionycteris* and *Euderma* are associated with *Barbastella*, whereas in the previous analysis the first two genera had their closest affinities with the genus *Plecotus*. In both cases, however, the distances between species means are congruent with the current systematic hierarchy; i.e., interspecific distances are smaller than intergeneric ones. However, inclusion of the affine eigenvectors in the computation of relative warp scores affects these relationships. Although the matrices of nonaffine and affine relative warps scores are significantly correlated (Mantel test, $r = 0.89$, $p < 0.001$), the inclusion of affine eigenvectors in computations results in a partial contradiction of the previously generated "natural" generic groupings. This is especially visible in the UPGMA phenogram (Fig. 4c).

DISCUSSION

In this study, interpretation of morphological variation within the plecotine bats is evaluated by decomposing landmark variation into centroid size and uniform and nonuniform shape components. All these variables are significantly different among taxa and express well-defined morphometric divergence within the tribe. The differences in size among species are closely linked to only linear (uniform) shape modifications. More linear deformations were detected longitudinally than laterally. Nonlinear shape variation was independent of centroid size and mainly involved lateral displacement of landmarks associated with the widening of different regions of the skull. Significant sexual dimorphism was indicated by centroid size in only *O. hemprichi*.

One of the key questions in the taxonomy of Plecotini concerns the systematic position of *O. hemprichi*. Handley (1959) and Tumlison and Douglas (1992) did not consider this species in their analyses, probably because Miller (1907) had suggested that *Otonycteris* is only superficially similar to the plecotine bats. The bacular classification of Plecotini, including *Rhogeessa* (along with *Baeodon*), *Nycticeius* and *Otonycteris* (Hill and Harrison, 1987), was rejected due to its subjectivity (Frost and Timm, 1992) and karyological incongruence (reviewed by Horácek, 1991). In the G-band structure of chromosomes, only *Otonycteris* exhibits the highly specific banding pattern typical of *Plecotus*, *Corynorhinus*, and *Barbastella* (Zima et al. 1992; Qumsiyeh and Bickham, 1993). In our analyses, the highly specialized *O. hemprichi* (see Horácek, 1991) is the most divergent taxon of the plecotines. This implies, in contrast to the chromosomal findings, that it may belong to a different ancestral stock.

One of the central aims of this study was to evaluate landmark-based methods as practical tools for systematic inference. The UPGMA phenograms for Bookstein shape coordinates and relative warp scores (computed excluding uniform shape variation) depict an arrangement of species within their respective generic groups that is consistent with cut-off levels for genus recognition. This result is very clear in spite of the low number of specimens examined (Figs. 4a and 4b). This indicates that landmark-based morphometrics effectively capture the information necessary to distinguish genera. Retention of the affine eigenvectors in the computation of relative warp scores increased intertaxon distances and affected these relationships. This was especially evident in UPGMA phenograms (Fig. 4c), which are subject to distortion of distances between major clusters (Sneath and Sokal, 1973).

Phenetic distances computed on Bookstein shape coordinates and relative warp scores (uniform shape change removed) showed two similarities. Each confirmed that *O. hemprichi* is morphologically the most divergent of all Plecotini and *Barbastella* is separated from *Plecotus* and *Corynorhinus*. The main differences in phenograms concerned the relative position of the genera *Idionycteris* and *Euderma*, which either grouped with *Plecotus* or with *Barbastella*. Comparison of these phenograms with recently published systematic arrangements of Plecotini sensu stricto (lacking *Otonycteris*) indicates that the phenogram derived from the analysis of Bookstein coordinates matches perfectly with the cladistic classification proposed by Tumlison and Douglas (1992). On the other hand, the phenogram computed from relative warp scores partly corresponds to two of the four morphology-only most parsimonious trees illustrated by Frost and Timm (1992: Figs. 2.3 and 2.4).

There are some intrinsic and extrinsic reasons that suggest that the phenogram from Bookstein shape coordinates (Fig. 4a) should be treated as a working hypothesis of systematic relationships among Plecotini. First, its goodness of fit to the original data set, expressed as a cophenetic value, is higher (0.970) than that seen for the phenogram of relative warp scores (0.859) and the obtained relationships duplicate those suggested by a three-dimensional plot with the minimum spanning tree superimposed (see Fig. 3). Second, ancestral

character states in Frost and Timm's (1992) analyses were inferred from examination of an outgroup represented by several species of only one genus, *Myotis*. This approach excludes the possibility of detecting characters that are derived in all *Myotis* taxa (cf. Tumlison and Douglas, 1992). Third, Frost and Timm's (1992) morphological analyses resulted in several trees of similar length (and therefore with comparable amounts of implied homoplasy) but with considerable variation in topology. This suggests that their data show only marginally preferential support for their chosen tree. These authors opted for the tree (*Euderma* [including *Idionycteris*] (*Barbastella* (*Plecotus Corynorhinus*))) because it was supported by both morphological and karyological data.

Both phenetic and cladistic findings based on morphology are in disagreement with certain aspects of the chromosomal data. The karyological evidence implies that the genera *Otonycteris*, *Plecotus*, *Corynorhinus* and *Barbastella* form one lineage of Plecotini and *Euderma* and *Idionycteris* form the other (Zima et al. 1992; Qumsiyeh and Bickham, 1993; see also Volleth and Heller, 1994). Both morphological (e.g., Tumlison and Douglas, 1992; this study) and chromosomal (Qumsiyeh and Bickham, 1993; Volleth and Heller, 1994) analyses support a close relationship between *Idionycteris* and *Euderma*, but they differ in their support for other relationships. This may be because the karyological tree includes only centric fusion events as synapomorphies. Such conditions may produce misleading phylogenies if the possibility of fissions are not also considered (Qumsiyeh, 1989).

Several problems are also associated with the use of morphometric methods in estimating systematic relationships (e.g., Wiley, 1981; Bookstein et al., 1985; Felsenstein, 1988) and in equating the relative degree similarity with recency of common ancestry (MacLeod and Kitchell, 1990; Bookstein et al., 1985). The distribution of taxa in morphospace can, however, corroborate a phylogenetic hypothesis if monophyletic groups are localized in the morphospace (Bookstein et al., 1985). Further analyses of additional populations of Plecotini will show the extent to which the generic groups identified here remain distinct and whether this phylogenetic hypothesis is being confounded by morphological convergence or our inability to detect accurately the synapomorphic patterns within morphological data.

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APPENDIX

Specimens Examined

Museum acronyms for specimens used in this study are defined in the acknowledgments section. Numbers in parentheses after species name indicate the number of examined males and females, respectively. *Plecotus auritus* (3, 5)—MGU1281; MGU2351; MGU4194; MGU29103; MGU104736; MGU105481–3; *P. austriacus*—USNM476627; USNM476629; USNM476632-4; USNM470707-11; *Barbastella barbastellus* (2, 10)—MNW11516; NMW11586; NMW12028-9; AMNH212187-9; USNM142583; USNM347421; HZM7.606; MCZ37000-1; *B. leucomelas* (3, 3)—AMNH16307; AMNH44562; FMNH34768; FMNH82737-8; HZM2.4415; *Euderma maculatum* (5, 4)—MSB6235; MSB9610; MSB17285; MSB23376-7; MSB24999-25000; MSB27715; MSB37724; *Idionycteris phyllotis* (4, 7)—MSB9474; MSB9518-9; MSB9578; MSB9612-3; MSB11084; MSB11635; MSB13013; MSB13847; MSB14833; *Corynorhinus mexicanus* (7, 11)—KU29848-60; KU29888; KU29890; KU29915; KU29918; KU29923; *C. rafinesquii* (8, 8)—LSUMZ54; LSUMZ6258; LSUMZ6263; LSUMZ8733; LSUMZ9305; LSUMZ1395-6; LSUMZ19793; LSUMZ20390-1; LSUMZ20393; LSUMZ20396; LSUMZ21927; LSUMZ23793; LSUMZ25225; LSUMZ28462; *C. townsendii* (5, 5)—TTU6149; TTU6464; TTU9161; TTU17232; TTU19960-1; TTU23277; TTU34549; TTU43684-5; *Otonycteris hemprichi* (2, 3)—MBQ1154; MBQ1170; MBQ1190; MBQ1201; MBQ1226.