

## Geometric morphometrics and cladistics: testing evolutionary relationships in mega- and microbats

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Traditionally, morphometric data have consisted of distances, angles, or ratios, and have been considered inappropriate for cladistic analyses. Recently, geometric morphometrics, based on homologous landmark point-coordinates, has provided a number of advantages over traditional morphometric data and methods, including the possibility that phylogenetically informative characters and character-states may be extracted and used in cladistic analyses. Using two data sets of 3-dimensional point coordinates collected from skulls of bats, we empirically evaluate this possibility. Partial warps were extracted from the point-coordinate matrix, and these were then re-coded by gap-coding, for use in the cladistic analyses. In the case of samples from *Eidolon helvum* populations (two mainland localities and four islands in the Gulf of Guinea), analyzing males and females separately, our analyses based on these data were unable to detect consistent phylogeographic patterns among the populations. In the case of samples from plecotine bat species, these analyses produced a consensus cladogram showing considerable concordance with an earlier cladistic analysis by us of this group. In both cases, our results reflect those of earlier studies (based on both morphologic and genetic data), suggesting that the data and analytic techniques described herein may have interesting utility in cladistic analyses.

*Key words:* geometric morphometrics, partial warps, gap-coding, phylogeny, Microchiroptera, Megachiroptera

### INTRODUCTION

It has been argued that continuous-state characters should be excluded from cladistic analysis mainly due to two reasons: (1) these data are inappropriate (not phylogenetically informative), and (2) the methods for their conversion into codes are arbitrary. Pimentel and Riggins (1987) stated that only character states derived from characters showing discrete variation can provide phylogenetic information, whereas continuous characters are transformational homologies not subject to test and, therefore invalid

for phylogenetic analysis. On the other hand, more recently Rae (1998: 221) concluded that “metric data [...] fulfill the sole criterion for inclusion in phylogenetic analysis, the presence of homologous character states, and thus cannot be excluded as a class of data.” This position is also supported by Thiele (1993), who noted that data scored for cladistic analyses may be quantitative or qualitative, continuous or discrete, and show overlapping or non-overlapping values between taxa.

Traditionally, morphometric research has relied on statistical analyses of distances,

angles or ratios to study quantitative variation among taxa. Recently, geometric techniques have been developed for the direct analysis of coordinates of homologous landmarks. This geometric morphometrics methodology has several advantages over the traditional morphometrics (*sensu* Marcus, 1993), e.g., by partitioning size from shape for separate analyses and by providing a graphic way of locating and comparing variability in different components of shape among studied groups (e.g., Rohlf and Marcus, 1993). Recently a number of arguments have been made in favor of regarding certain morphometric variables as putatively homologous characters and including them, sometimes along with other non-morphometric variables, in parsimony-based cladistic analyses. Zelditch *et al.* (1995; see also Fink and Zelditch, 1995 and Swiderski *et al.*, 1998; but cf. Adams and Rosenberg, 1995; Naylor, 1996; and Monteiro, 2000) have proposed the use of partial warp-based traits in phylogenetic analysis. These traits are derived from the shapes of objects under study, as defined by the selected number of  $x$ ,  $y$  (and  $z$  in 3-dimensional analysis) coordinates of homologous landmarks. The shapes are fitted to the references by stretching/compressing and shearing until complete identity of their landmark configurations is achieved. Eigenvectors of the resulting bending-energy matrix are defined as new shape variables, principal warps which yield another shape space with the origin defined by the reference. Projections of the shapes being compared onto principal warps yield partial warps, which warps are analogous to factor analysis projections, with the eigenvectors (principal warps) derived from a description of non-uniform differences between observed forms and some reference form (O'Higgins, 2000). Principal warps, together with the uniform component, also supply a basis for the space in which we compute

relative warps, which are the same as principal components (in particular, they are mutually orthogonal). Both partial and relative warps can be used in many multivariate statistical analyses as quantitative shape variables. MacLeod (2002) illustrated some of the weaknesses of partial warps using empirical and simulated examples. Unfortunately, the empirical trilobite example used by this author is not a good test case because, as noted by Wagner (2000), trilobite matrices are characterized by poor resolution of states.

The aim of our study was to re-evaluate the usefulness of methods applying partial warp analysis based on 3-dimensional information in recovering phylogenetically informative characters. This was tested by producing trees derived from classical (linear) parsimony of re-coded data, and squared-change parsimony and continuous maximum likelihood of continuous characters, and comparing them with a presumably known and well-established phylogeny of two groups of bats belonging to Megachiroptera and Microchiroptera.

## MATERIALS AND METHODS

### *Variables*

For testing interspecific relationships among fruit bats four taxa occurring in western Africa and with well-understood phylogenetic relationships were selected: *Eidolon helvum*, *Rousettus aegyptiacus*, *Myonycteris torquata* and *M. brachycephala*. In addition, the first species was also used in evaluation of evolutionary ties among its populations in the islands of the Gulf of Guinea. *Eidolon helvum* is the second largest fruit bat in Africa, with unique morphological (Andersen, 1912), ecological (Thomas, 1983), and reproductive (Bernard and Cumming, 1997) characteristics. Juste *et al.* (2000) reported that of four island populations examined (plus two mainland populations), the population from Annobón, the smallest and farthest, shows remarkable morphological and genetic differentiation, whereas the rest are similar phenetically and with low genetic distances among them.

A set of 20 homologous cranial landmarks (see Bookstein, 1991) was defined on bone sutures, foramina, and inflection points along the edges of cranial structures (Fig. 1) on the ventral view of skulls of the four species of fruit bats (Appendix). Dental landmarks were set on the bone (at the edge of the alveoli) to avoid variation due to differential tooth-wear. To facilitate repeatability, each landmark was gently marked in pencil on the surface of the bone, under 20x magnification, before being recorded. Three-dimensional coordinates of landmarks were digitized using a 3-D Reflex Microscope (Reflex Measurement Ltd., Butleigh, Somerset BA6 8SP, UK). This is a highly precise, non-contact instrument that uses a small light spot to digitize coordinates in any position within a magnified field. The microscope was periodically re-calibrated to ensure a linear scale error of less than 30  $\mu\text{m}$  over 100 mm in the  $x$ -axis. Landmarks were collected under a 20 $\times$  magnification lens from four different aspects, thus the skull was rotated three times to attain the complete data collection. Amount of error due to the new digitizing of the four reference landmarks was evaluated after each shift, and the whole transformation was discarded, and the digitizing process restarted, if the greatest error of any of the four reference points was larger than 0.1 mm in any direction. All these landmarks were recorded by

a single individual, and were taken without reference to prior values.

The same instrument was applied to gather 19 three-dimensional coordinates from the dorsal side of the skulls of microbats, including members of the tribe Plecotini sensu stricto (genera *Corynorhinus*, *Plecotus*, *Barbastella*, *Euderma*, *Idionycteris*) and some other taxa (*Otonycteris hemprichi*, *Antrozous pallidus*, and *Eptesicus fuscus*) within the subfamily Vespertilioninae, plus *Myotis lucifigus* of the subfamily Myotinae (sensu Hooper and Van Den Bussche, 2003), which was used as an outgroup (see Appendix). The plecotine bats represent the only supra-generic group within Chiroptera that is Holarctic in distribution, and there is considerable morphologic and karyotypic evidence supporting their monophyly (e.g., Frost and Timm, 1992; Tumlison and Douglas, 1992; Bogdanowicz *et al.*, 1998; see also Juste *et al.*, 2004). Also in this case all landmarks were recorded by a single individual.

### Analyses

Geometric coordinates were checked and visualized using Morphologika (O'Higgins and Jones, 1998). The average location of each landmark (if necessary) was obtained through a non-documented

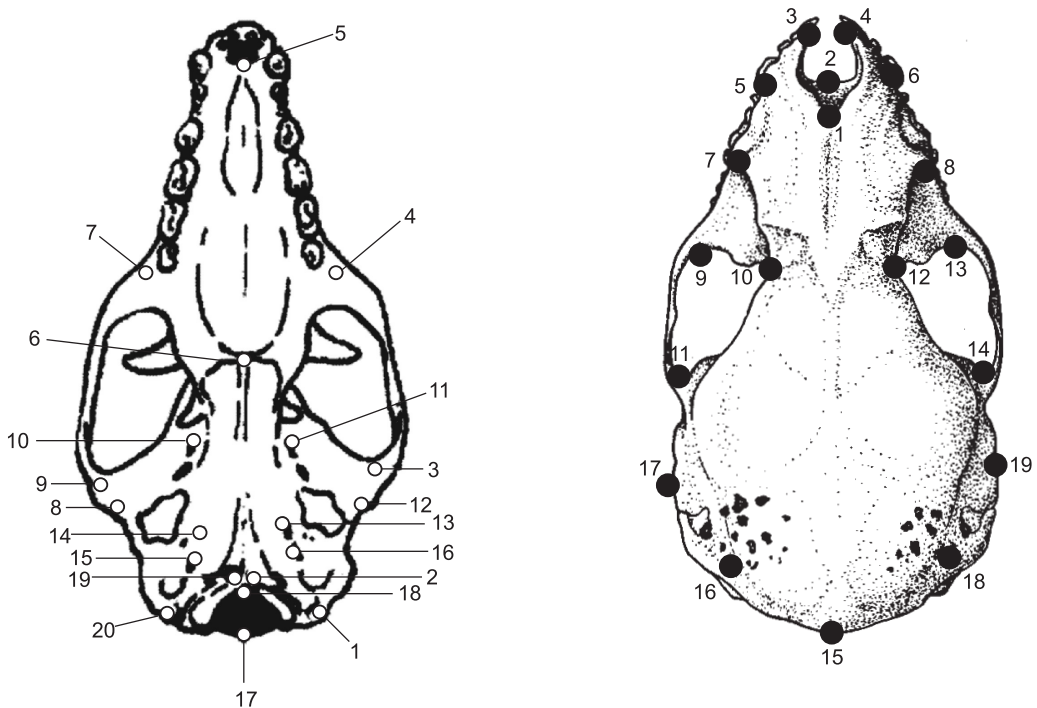


FIG. 1. Location of landmarks on the skulls of Megachiroptera (left) and Microchiroptera (right)

option in the program allowing to save the coordinates of the warped mean (P. O'Higgins, in litt.). The NTSYS-pc ver. 2.11T (Rohlf, 2000) package was used to calculate partial warps. The uniform component was estimated by sweeping the partial warps from the projections of the aligned coordinates into the tangent space and then using SVD to extract the non-singular dimensions (Rohlf and Bookstein, 2003). Partial warps (plus uniform components scores) were re-coded using the gap weighting method of Thiele (1993). A new character state  $X_{new}$  can be calculated with the following formula:

$$X_{new} = n * [(x - \min) / (\max - \min)],$$

where max and min are the maximum and minimum mean value of the character across all species, x is the mean value of the current taxon and n is the number of allowed character states.

Phylogenetic analyses were performed with PAST (Hammer *et al.*, 2001) and PHYLIP (Felsenstein, 2004b). The first taxon was always (but intentionally) treated as the outgroup. In the case of fruit bats the branch-and-bound algorithm was applied. For the Plecotini project we used heuristic search, with the subtree pruning and regrafting option. This algorithm is similar to the nearest neighbour interchange but with a more elaborate branch swapping scheme. The character optimization was based on the Wagner criterion, assuming that characters are reversible and ordered, meaning that 0- > 2 costs more than 0- > 1, but has the same cost as 2- > 0.

## RESULTS

### Fruit Bats

In the cladogram (Fig. 2A) representing interspecific relationships among these megabats *Rousettus aegyptiacus* figures as next most basal to the outgroup, followed by the *Myonycteris* clade, which contains *M. brachycephala* and *M. torquata*.

In the cladograms representing intraspecific relationships of the *E. helvum* populations, *Rousettus* is seen as basal, with a clade of the two *Myonycteris* species the next most basal (Fig. 2B and 2C). The relationships among the *Eidolon* populations are figured differently, depending on whether based on the male (tree of 936 steps, ensemble consistency index, CI = 0.76) or female shortest tree (1,002 steps, CI = 0.79) data. In the cladogram based on

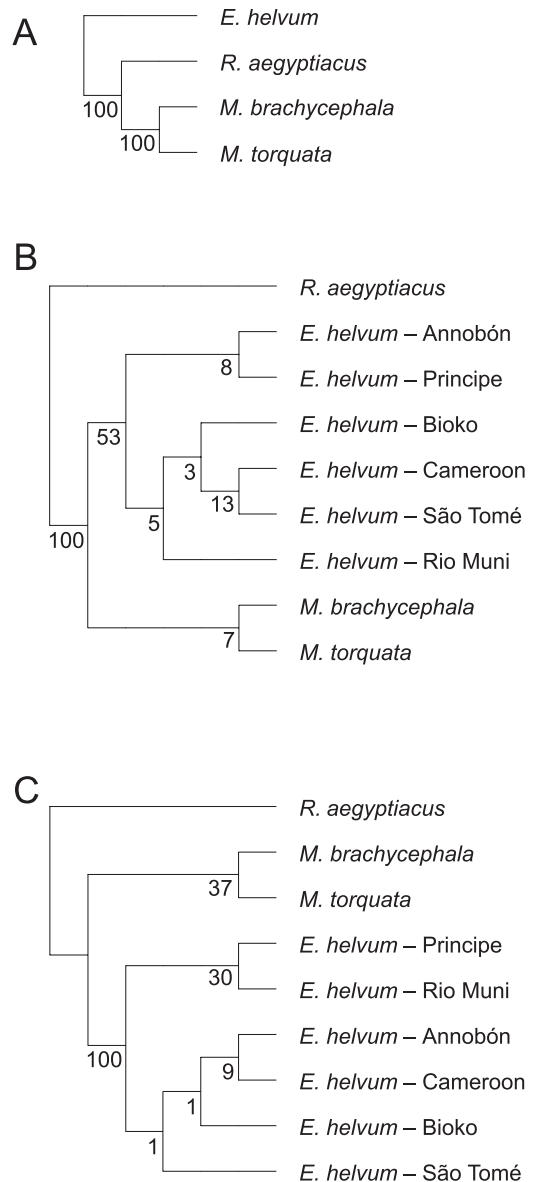


FIG. 2. Interspecific relationships (A) among four species of fruit bats derived from the maximum parsimony of re-coded partial warp scores (strict consensus tree). Interspecific relationships among populations of *E. helvum* are also shown (B, ♀♀; C, ♂♂). Numbers below branches indicate the bootstrap support values (percentage) for the same nodes selected in the topologies obtained under the evolutionary model of maximum parsimony (after 100 iterations and 500 reorderings). Please note that in each case only one possible orientation to the reference is taken into account

female shape characteristics (Fig. 2B), populations from Annobón and Principe are sister taxa, forming a sister group to the remaining four populations studied. Within these four, Río Muni is basal, followed by Bioko, which is sister to the Cameroon/São Tome clade. In the male-based cladogram (Fig. 2C), Principe is again in the most basal group, but is sister to Río Muni. Together these two populations are sister to the remaining four, among which São Tome is most basal, followed by Bioko, which is sister to Cameroon and Annobón. Thus, there is little or no concordance

to be found between the interpopulational cladograms based on the female and male shape characteristics, and neither represents a recognizable geographic pattern.

### Microbats

The three shortest trees were 1,110 steps long and had an ensemble CI of 0.70. The strict consensus (Fig. 3A) reflects a high degree of concordance among the three cladograms, which are in agreement concerning the placement of the non-plecotine taxa

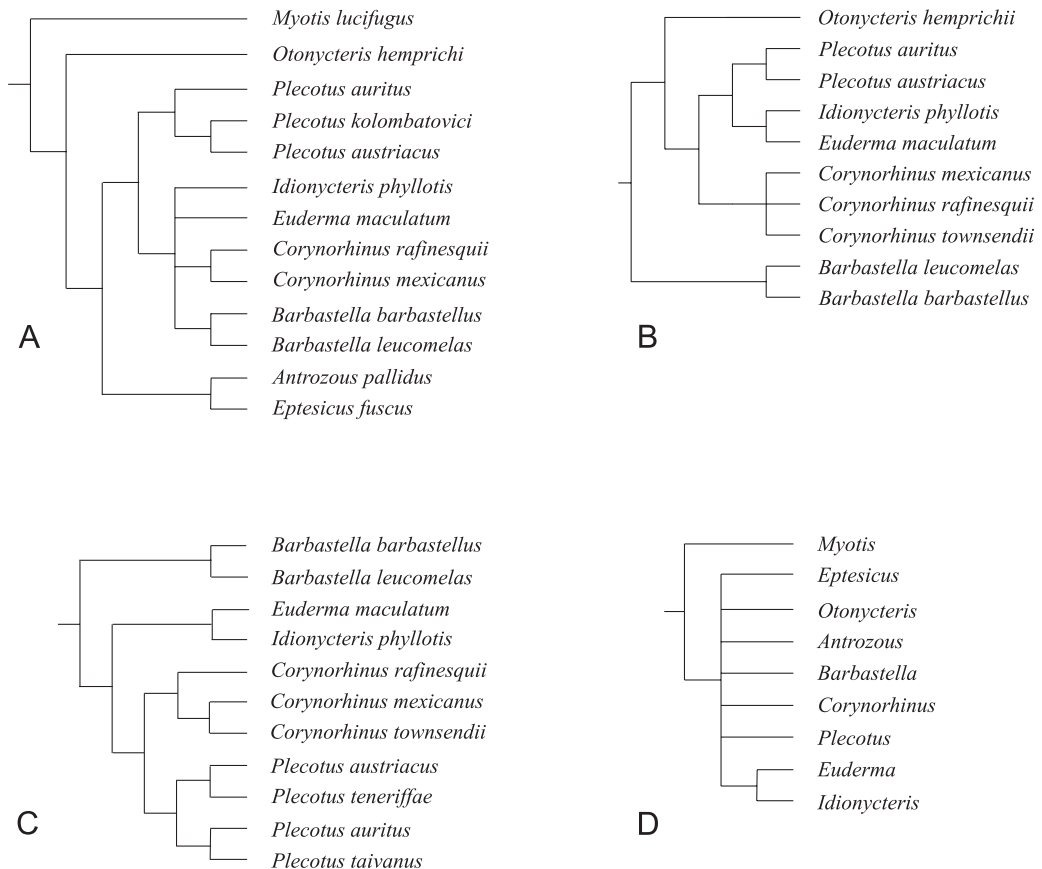


FIG. 3. Relationships among bats belonging to the tribe Plecotini: A — consensus of three most parsimonious trees from the analysis of re-coded partial warps; B — the most parsimonious tree based on morphological and karyotypic evidence (Bogdanowicz *et al.*, 1998); C — extracted super-tree of Plecotini (Jones *et al.*, 2002); D — cladogram for Plecotini based on ca. 2.6 kb pairs of mitochondrial DNA sequence (after Hoofer and Van Den Bussche (2003), but with only relationships supported strongly by either or both Bayesian and Parsimony analyses being depicted

*Otonycteris*, *Eptesicus*, and *Antrozous* (outgroups treated analytically as ingroups). The ‘correct’ arrangement of these taxa suggests that our characters: (a) carry phylogenetic information at this taxonomic level, and (b) are correctly polarized.

Within the plecotine taxa, the genus *Plecotus* is sister to all other taxa, with *P. austriacus* and *P. kolombatovici* sisters, with *P. auritus* a sister to that clade. The remaining taxa are figured as an unresolved polytomy among the genera *Euderma*, *Idionycteris*, *Corynorhinus* (2 species), and *Barbastella* (2 species). (The majority consensus of the three trees agrees with this, but shows *Euderma* and *Idionycteris* as sister species, and an unresolved trichotomy among this clade, *Corynorhinus*, and *Barbastella*).

## DISCUSSION

### *Interspecific and Intrapopulation Relationships of Fruit Bats*

The resulting phylogenetic hypothesis at the species level was consistent with the previously established arrangements of fruitbats based jointly on sequences derived from the mitochondrial cytochrome *b* and 16S rRNA genes of a wide representation of Megachiroptera (Álvarez *et al.*, 1999; Juste *et al.*, 1999; see also Juste *et al.*, 1997), despite outstanding differences in the evolutionary relationships among fruitbats suggested by molecular and ‘classical’ morphological data (Springer *et al.*, 1995; Kirsch and Lapointe 1997). On the other hand, it is not surprising because only a four-taxon hypothesis was tested using re-coded partial warps, including two taxa (*M. brachycephala* and *M. torquata*) belonging to one genus (Romagnoli and Springer, 2000).

The situation is much more complicated in the case of evolutionary affinities of a single species — *Eidolon helvum* — on the islands of the Gulf of Guinea (Central

Africa). An *Eidolon* ancestor probably reached mainland Africa independently from other fruitbats, and this colonization likely took place far earlier than the Late-Pliocene date (3 Myr) of its only fossil (Howell and Coppens, 1974) and maybe even earlier than the other African colonizations (Juste *et al.*, 1999). A recent phylogenetic study suggests that *Eidolon*’s origin may be closer to the typically Asian *Pteropus* group than to any extant African fruit bat (Juste *et al.*, 1999).

In ‘classical’ multivariate morphology, the populations of *E. helvum* from the islands of Bioko, Príncipe, and São Tomé do not show significant phenetic differentiation, although a trend towards a reduction of size is found in the latter two islands (Fig. 4). In terms of allozyme variation, the low genetic distances among these populations, as well as their values of Wright’s fixation indexes, suggest that gene flow has hampered differentiation on these islands (Juste *et al.*, 1999). Only the fourth insular population, from Annobón, was characterized by such remarkable morphological and genetic differentiation that it has been accorded the status of a separate subspecies: *E. helvum annobonensis*. Although we might expect better or more consistent resolution among these island populations based on re-coded partial warps, it appears that resolution of our approach mirrors that of more traditional approaches, and is unable to discern phylogeographic patterns among the *Eidolon* populations. Still, the partial warp analysis is able to tell apart the morphological differences specific of the females of the population from Annobón.

### *Interspecific Relationships within Plecotini*

A comparison of our strict consensus tree with the pertinent portion of the ‘supertree’ of Jones *et al.* (2002) is of considerable interest (see Fig. 3A and 3C), in that if the rooting is disregarded, the

topologies are concordant (albeit with differing resolution). Considering only the plecotine taxa, and configuring the topology of Jones *et al.* (2002) to reflect a primary dichotomy of *Plecotus* as sister to all other plecotine taxa (as in our consensus tree), their tree would figure *Corynorhinus* as sister to a clade containing *Barbastella* and *Euderma/Idionycteris*. Although our consensus tree figures these last three clades as an unresolved trichotomy, the topologies are concordant. Thus, the difference between our phylogenetic hypothesis for the plecotines, and that of Jones *et al.* (2002), is one of polarization, or more precisely, the ordering of character-state transformation series. In fact our consensus tree is more likely correct than Jones *et al.* (2002), because it is very difficult to postulate that the *Euderma/Idionycteris* clade is basal to a clade containing both *Plecotus* (Old

World) and *Corynorhinus* (New World). If we assume that the Plecotini originated in the Old World, their phylogeny requires either: (a) two dispersals from Old to New World, or (b) one dispersal from Old to New World, followed by a dispersal from New to Old World; whereas our phylogeny could be resolved to require only one dispersal from Old to New World (if we allow *Euderma* and *Idionycteris* to be sisters, as in our strict consensus tree, and further resolve the trichotomy to allow the *Euderma/Idionycteris* clade to be sister to *Corynorhinus*), which is reasonable on biogeographic grounds.

We also note that the pertinent portion of the tree presented by Hoofer and Van Den Bussche (2003) is concordant with our consensus tree, though their data were unable to provide much resolution at this level, or even demonstrate that the Plecotini are monophyletic. They did agree, however, on

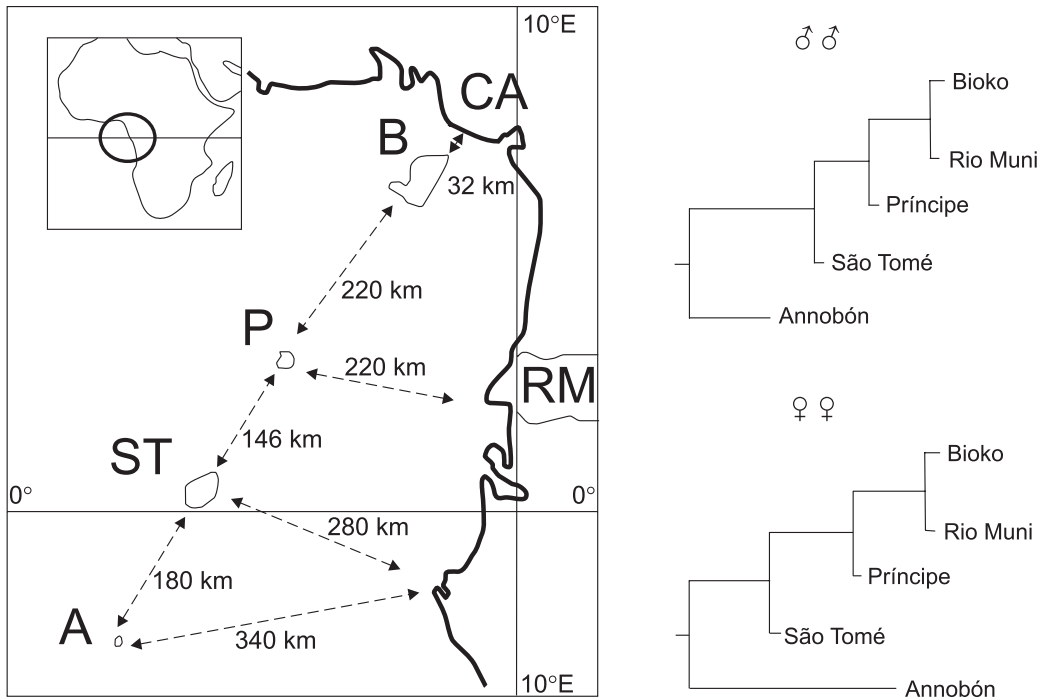


FIG. 4. The geographic situation in the Gulf of Guinea, Central West Africa (based on Juste *et al.*, 2000). The neighbour-joining topologies based on selected morphological characters, by sex, built on Mahalanobis  $D^2$  distances between populations of *E. helvum* from the Gulf of Guinea, according to Juste *et al.* (2000), are also presented

the sister-group relationship between *Euderma* and *Idionycteris*.

*Geometric Morphometrics, Partial Warps and Phylogeny*

In the present study, in the case of samples from plecotine bat species, these analyses produced a reasonable consensus cladogram showing considerable concordance with an earlier cladistic analysis by us of this group, and our results reflect those of earlier studies (based on both morphologic and genetic data; e.g., Frost and Timm, 1992, and Bogdanowicz *et al.*, 1998). The most fundamental problem in using traditional morphometric shape variables in a standard cladistic analysis is the fact that it requires the use of Manhattan distances (it is implied by the use of linear parsimony). Geometric morphometrics yields variables corresponding to an arbitrary rotation of shape space (depending in part on the reference configuration orientation). This arbitrariness does not matter in morphometrics because the multivariate methods used in morphometrics are invariant to rotation. Manhattan distances are not and thus one must select a particular rotation as being especially meaningful. Gap coding applied one variable at a time is also a problem because the results depend on the arbitrary rotation of the space (e.g., a different orientation of the reference configuration will yield different shape variables that will be coded differently by gap coding) (reviewed in MacLeod and Forey, 2002; see also Felsenstein, 2004a). The squared change parsimony does not have this problem so that would be quite compatible with morphometric data since the solution is invariant to rotation of the data. Continuous maximum likelihood methods are also compatible with morphometric data (F. J. Rohlf, in litt.). Nevertheless, at least as far as complex morphological structures are concerned, such models

have probably no biological meaning. Moreover, despite forcing continuous data into integer codes only the classical, linear parsimony of re-coded partials warps revealed considerable logical phylogenetic configuration for a 13-taxon example of Plecotini, contrary to the methods utilizing continuous data. It appears that a period of active experimentation with these methods is now needed to further explore their appropriateness and compatibility. An approach worth investigating in this context is presented by Bookstein (2000, 2002).

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## APPENDIX

Specimens used in the present study. Acronyms: AMNH: American Museum of Natural History, New York, USA; Acronyms: CM — Croatian Natural History Museum, Zagreb, Croatia; CMNH — Carnegie Museum of Natural History, Pittsburgh, USA; EBD — Estación Biológica de Doñana, Spain; HNHM — Hungarian Museum of Natural History, Budapest, Hungary; HZM — Harrison Institute, Sevenoaks, United Kingdom; MRI — Mammal Research Institute, Polish Academy of Science, Białowieża, Poland; MSB — Museum of Southwestern Biology, Albuquerque, New Mexico, USA; ROM — Royal Ontario Museum, Toronto, Canada; TTU — Texas Tech University, Lubbock, USA; ZFMK — Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany. Letters after species number refer to geographic location in the Gulf of Guinea: a — Annobón; b — Bioko; ca — Cameroon; p — Principe; rm — Rfo Muni; st — São Tome

### Megachiroptera:

*Eidolon helvum*, ♀ ♀ ( $n = 44$ ): CMNH40990 — ca; EBD17386 — st; EBD17387 — st; EBD17388 — st; EBD17520 — st; EBD17540 — st; EBD17598 — a; EBD17600 — a; EBD17603 — a; EBD18488 — a; EBD18825 — b; EBD18826 — b; EBD18827 — b; EBD18840 — b; EBD18874 — a; EBD18875 — a; EBD18876 — a; EBD18897 — rm; EBD18898 — rm;

EBD18899 — b; EBD18901 — b; EBD18907 — a; EBD18913 — st; EBD19044 — b; EBD19064 — b; EBD19217 — rm; zBD19218 — rm; EBD19224 — rm; EBD19225 — rm; EBD19226 — rm; EBD19227 — rm; EBD19238 — rm; EBD20348 — st; EBD20358 — st; EBD20362 — st; EBD20448 — st; EBD8818 — rm; EBD8820 — rm; ROM50865 — ca ROM54940 — ca; ROM55626 — ca; ROM55627 — ca; ZFMK73368 —

ca; ZFMK74302 – ca; ♂♂ ( $n = 68$ ): EBD17382 – p; EBD17389 – st; EBD17471 – st; EBD17519 – p; EBD17538 – p; EBD17539 – p; EBD17542 – st; EBD17595 – rm; EBD17597 – a; EBD17599 – a; EBD17601 – a; EBD17602 – a; EBD18199 – p; EBD18204 – st; EBD18212 – st; EBD18487 – a; EBD18489 – a; EBD18492 – a; EBD18494 – a; EBD18828 – b; EBD18829 – b; EBD18833 – b; EBD18834 – p; EBD18836 – p; EBD18837 – p; EBD18844 – rm; EBD18869 – p; EBD18879 – b; EBD18900 – b; EBD18906 – a; EBD18915 – st; EBD18917 – p; EBD18958 – a; EBD19020 – rm; EBD19037 – b; EBD19038 – rm; EBD19039 – rm; EBD19053 – b; EBD19054 – b; EBD19065 – b; EBD19214 – rm; EBD19215 – rm; EBD19216 – rm; EBD19220 – rm; EBD19221 – rm; EBD19222 – rm; EBD19239 – rm; EBD19247 – rm; EBD20344 – st; EBD20345 – p; EBD20346 – p; EBD20347 – p; EBD20349 – st; EBD20350 – p; EBD20351 – p; EBD20352 – p; ; EBD20355 – st; EBD20357 – p; ; EBD20359 – p; EBD20364 – p; EBD8819 – rm; EBD8821 – rm; HZM21.4835 – ca; ROM39044 – ca; ROM39058 – ca; ZFMK64328 – b; ZFMK64331 – b; ZFMK64333 – b.

*Myonycteris brachycephala*, ♀♀ ( $n = 9$ ): EBD 18934, 18935, 17410, 17524, 17470, 18904, 17477, 17468, 18936; ♂♂ ( $n = 8$ ): EBD17413, 22284, 17469, 17411, 17526, 18937, 19066, 37413.

*Myonycteris torquata*, ♀♀ ( $n = 42$ ): AMNH 236237, 236239, 236240, 236246, 236247, 236249, 236254, 236255, 236256, 240999, 241000, 241001, 241002, 241003, 241004; CMNH107996, 40951, 40957; EBD13767, 15046, 15057, 15061, 15065, 15066, 15110, 19058, 22486, 22488, 22489, 22493, 22501, 22503; ROM39393, 43356, 57148, 69001; TTM17954; TTU17952, 3938, 3939; USNM241112; ZFMK61621; ♂♂ ( $n = 53$ ): AMNH236236, 236242, 236243, 236245, 236250, 236251, 236252, 236253; BM139122; CMNH40949, 40950, 40952, 40953, 40954, 40955, 40956, 58253; EBD13888, 15011, 15012, 15013, 15054, 15055, 15056, 15058, 15059, 15060, 15062, 15063, 15727, 17715, 19057, 20487, 20488, 20489, 22487, 22490, 22495, 22497, 22498, 22499, 22504, 22505, 22506, 22507, 22508, 22509, 22510; SNH511901; TTU3937, 3940; ZFMK61623, 69609.

*Rousettus aegyptiacus*, ♀♀ ( $n = 96$ ): AMNH 240988, 240990; CMNH58254; EBD13869, 15152, 15191, 15680, 17352, 17396, 17405, 17406, 17496, 17497, 17533, 17534, 17535, 18203, 18207, 18241, 18250, 18525, 18550, 18557, 18558, 18559, 18841, 18856, 18857, 18858, 18859, 18860, 18861, 18863, 18864, 18865, 18866, 18873, 18918, 18938, 18939, 18952, 19021, 19029, 19030, 19031, 19032, 19033,

19049, 19050, 19052, 19056, 19059, 19267, 19268, 19270, 19276, 19277, 19278, 20178, 20179, 20181, 20183, 20185, 20188, 20190, 20191, 20193, 20196, 20197, 22293, 22307, 22313, 223311; ROM43348, 46756, 55663, 55698, 55699, 55700, 55701, 55702, 55704, 55707, 55732, 55733, 55735, 55737, 56227; TTM17963; TTU17956, 17962; ZFMK444084, 64312, 64313, 64315, 64319; ♂♂ ( $n = 97$ ): AMNH 240977, 240989, 240996, 318299; CMNH3928; EBD 13679, 15192, 15462, 15463, 17397, 17398, 17521, 17531, 17536, 17597, 17597, 17599, 17601, 17602, 17673, 18208, 18209, 18213, 18242, 18252, 18487, 18489, 18492, 18494, 18830, 18832, 18850, 18851, 18854, 18855, 18865, 18867, 18868, 18870, 18880, 18881, 18891, 18902, 18902, 18906, 18921, 18953, 18954, 18958, 18976, 18978, 18979, 19006, 19007, 19034, 19051, 19060, 19061, 19074, 19269, 19271, 19272, 19273, 19279, 19280, 20180, 20184, 20186, 20195, 20663, 22294, 22308, 22309, 22310, 22312; HZM504098; ROM46755, 55693, 55694, 55885, 56207, 56226, 56250, 56251, 56255, 58309, 58340; TTM17958; TTU3923, 3924, 17961; ZFMK64309, 64310, 64314, 64316, 64317, 64318.

#### Microchiroptera:

*Antrozous pallidus*, ♀♀ ( $n = 3$ ): ROM67106, 67107, 67111; ♂♂ ( $n = 3$ ): ROM67110, 67121, 78631.

*Barbastellus barbastellus*, ♀♀ ( $n = 3$ ): HNHM 53.29.1, 58.65.1, 2000.43.4; ♂♂ ( $n = 4$ ): HNHM 57.56.1., 57.110.1, 70.18.1; MRI85469.

*Barbastella leucomelas*, ♂♂ ( $n = 1$ ): HNHM 2743.3.

*Corynorhinus mexicanus*, ♂♂ ( $n = 2$ ): AMNH 203933, 203934.

*Corynorhinus rafinesquii*, ♀♀ ( $n = 3$ ): AMNH 142010, 142003, 166892; ♂♂ ( $n = 3$ ): AMNH 142005, 142011, 142006.

*Eptesicus fuscus*, ♀♀ ( $n = 3$ ): ROM41705, 43176, 46037; ♂♂ ( $n = 3$ ): ROM19388, 24448, 54179.

*Euderma maculatum*, ♀♀ ( $n = 1$ ): MSB96066.

*Idionycteris phyllotis*, ♀♀ ( $n = 1$ ): AMNH 178893; ♂♂ ( $n = 1$ ): AMNH185341.

*Myotis lucifugus*, ♀♀ ( $n = 3$ ): ROM43605, 88947, 88951; ♂♂ ( $n = 3$ ): ROM40122, 43595, 43594.

*Otonycteris hemprichi*, ♀♀ ( $n = 1$ ): HZM1122.

*Plecotus auritus*, ♀♀ ( $n = 3$ ): MRI10638, 49960, 57090; ♂♂ ( $n = 3$ ): MRI85094, 91305, 91306.

*Plecotus austriacus*, ♀♀ ( $n = 3$ ): MRI130/91303, 7868, 96981; ♂♂ ( $n = 3$ ): MRI12410, 38248, 96982.

*Plecotus kolombatovici*, ♀♀ ( $n = 3$ ): CM3004, 3006, 3008; ♂♂ ( $n = 2$ ): CM2152, 3054.