

Morphometrics in the quantification of character state identity for the assessment of primary homology: an analysis of character variation of the genus *Artibeus* (Chiroptera: Phyllostomidae)

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The recognition of transformed homologues entails an evaluation of similarity of features among taxa and then an assessment of homology by character congruence. We approached the similarity test of character state identity with morphometric analyses of character variation to judge whether all variants from a taxonomic sample belong to the same phylogenetic state (taxic homology) or whether some variants are sufficiently different to recognize them as different states (transformational homology). This method seeks to recognize as many character states as the groups of taxon means that are revealed by an analysis of variance followed by a multiple range test. We exemplify this quantitative approach with an analysis of character variation for a phylogenetic study of eight *Artibeus* species and two outgroups. All 23 features analysed are taxonomically useful because at least two states could be defined. We argue that statistical tests are a consistent and non-arbitrary procedure for the similarity test in the assessment of character states when hypothesizing primary homologies. Cladistic analyses and descriptive indices of historical signals of previous molecular and morphological data, combined with our morphometric data, showed that morphometric characters contain cladistic structure and they are phylogenetically useful. © 2003 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2003, 80, 45–55.

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INTRODUCTION

At least two stages are present in any cladistic assessment of phylogenetic relationships. The first, character analysis, consists of all steps leading up to the elaboration of a data matrix. It includes examination of character variation and selection of character states (Brower & Schawaroch, 1996; Hawkins, Hughes & Scotland, 1997), which equates to hypothesizing primary homologies. The second stage, cladistic analysis, can be defined as the formal transformation of a data

matrix into a phylogenetic tree. Logical relations among primary homology statements (character congruence) are examined by means of cladistic parsimony as an optimality criterion (De Luna & Mishler, 1996). It consists of all search strategies of most-parsimonious networks, which are then polarized and subjected to evaluations of robustness or support (De Pinna, 1994). The result is a cladogram that can be used to propose phylogenetic relationships and classifications, and as an evolutionary scenario for biogeographical and other comparative biology approaches.

In analysis of character variation, the initial step is the empirical justification of primary homology.

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Empirical bases include similarity, conjunction, and discrete, heritable and independent variation. A hypothesis of taxic homology is a proposition that a shared similarity among organisms originated in a recent common ancestor. Observed similarity in a feature leads one to deduce that there has been no evolutionary change between the ancestor and organisms compared. On the other hand, a hypothesis of transformational homology is that a previous condition (plesiomorphy) was changed evolutionarily into another condition (apomorphy). A discrete difference among character states leads one to infer that there has been at least one evolutionary step between the ancestral and derived states. In this analytical stage, inferences about taxic and transformational homology are empirically justified from judgments of similarity or detected differences in character variation.

Topological correspondence and detailed similarity are initial criteria to postulate a conjecture of primary homology among organisms (Patterson, 1982; Roth, 1988; De Pinna, 1991). These empirical criteria consider three basic positional attributes of a feature: topography, geometry and relationships with other parts of the organism. After topographic identity has been established, the second step is character state identity (Brower & Schawaroch, 1996). In the case of nucleotide sequence data, both steps are undertaken during alignment of sequences. Positional similarity and correspondence alone are empirical criteria to postulate primary homology among nucleotides. Although sequence alignment is a challenging step (Hillis, 1994; Doyle & Davis, 1998), the states are defined without ambiguity as discrete nucleotides (A, G, C, T). This is in sharp contrast with the study of morphology, where determining topographical identity is mostly uncontroversial (factual, *sensu* De Pinna, 1991), while conjectures of character state identity are more complex (Brower & Schawaroch, 1996). This step requires a judgment of similarity for assessment of discrete states from patterns in character variation. In this paper we argue that morphometric analyses can be used in the quantification of character state identity for the assessment of primary homology.

The aim of phylogeny reconstruction is to discover discrete monophyletic groups, and discrete transformational states are necessary as evidence of lineage divergence. Although a distinction has been made between 'qualitative' and 'quantitative' characters, the need to distinguish character states is ubiquitous. When character variation is quantified there are no accepted procedures as to how to delimit character states (Stevens, 2000) and three epistemological positions have been taken. Some systematists have suggested that quantitative variation of characters should not be used in phylogenetic analysis because

there are no valid criteria for dividing quantitative data into discrete states (Pimentel & Riggins, 1987; Chappill, 1989; Farris, 1990). In fact, they claim that coding quantitative characters into states introduces artificial distinctions in cladistic analysis, even if distribution is discontinuous. In contrast, Felsenstein (1988) proposed to use all characters as raw continuous variables. In the middle, other systematists (with whom we agree) have suggested to disregard only those characters in which variation is overlapping. They have concluded that arguments against the use of quantitative data are not supported by systematic theory, and that problems to use them as characters reside basically in the methods by which states and codes are derived from quantitative variation (Thiele, 1993; Rae, 1998; Swiderski, Zelditch & Fink, 1998).

Methods used for coding character states from morphometric data fall into two broad categories: those that code gaps graphically, and those that judge discreteness based on statistical tests. The former uses a simple algorithm to generate gaps of a critical size to produce discrete codes for quantitative values that are continuous and overlapping. Means and ranges for each taxon are ordered throughout an axis. Taxon samples are classified in states only when the gap size among sample distributions is larger than certain reference value (Kitching *et al.*, 1998). Referring to the criteria used to establish the critical gap size, these methods are known as: simple gap coding (Mickevich & Johnson, 1976; Simon, 1983), generalized gap coding (Archie, 1985; Goldman, 1988), segment coding (Colles, 1980; Chappill, 1989), gap weighting (Thiele, 1993) and thin-plate spline (Zelditch, Fink & Swiderski, 1995). The second type of method uses statistic significance tests to recognize character states. Examples of these are the use of two-sample tests (like the Student's test; Farris, 1990; Rae, 1998) and analysis of variance followed by multiple range tests (Sosa & De Luna, 1998).

The main goal of this paper was to evaluate what morphometric analyses reveal about the similarity criterion of homology. We argue that results of statistical tests of character variation allow a consistent judgment of the similarity among variants in the establishment of character state identity. Furthermore, this quantitative approach is epistemologically compatible with the phylogenetic concept of homology, since it takes to a numerical level the first empirical criterion, the similarity test, to propose character states as primary homology hypotheses. We use as an example the morphometric variation in eight species of large *Artibeus*. We also evaluated the phylogenetic utility of morphometric characters discovered by examining several properties (consistency indices, data decisiveness, *g1* statistics) of the data and the trees inferred.

MATERIAL AND METHODS

CHARACTER ANALYSIS

The study was based on 557 individuals obtained from several museums (see Appendix). Only adult specimens aged by epyphyseal–diapyseal fusion, pelage colouration and reproductive conditions (Anthony, 1988) were considered. Specimens were assigned to eight species of large *Artibeus* as delimited in previous taxonomic works using qualitative differences in morphology (Handley, 1989; Lim & Wilson, 1993) for quantitative tests. We follow Davis (1984) and Lim (1997) in recognizing *A. intermedius* and *A. planirostris* as valid species, rather than subspecies, as maintained by Koopman (1993) and Handley (1987), respectively. *Uroderma bilobatum* and *Dermanura azteca* were included as outgroups based on previous molecular analysis (Van Den Bussche, Hudgeons & Baker, 1998). These ten taxa represent our operational taxonomic units (OTUs) in analyses of variance and in cladistic analysis.

Morphometric characters consisted of four external and 19 cranial distances. External dimensions were taken with a digital caliper (0.01 mm) on the right limb of each specimen. Cranial dimensions were recorded from digital images of skulls with ImagePro Plus software (Media Cybernetics, 1994). Pictures were digitized using a Mavica FD71 camera. All of these characters have been described and illustrated elsewhere (Kalko & Handley, 1994; Kennedy *et al.*, 2002). Therefore, they are only listed here: (1) forearm length; (2) metacarpal length of 3rd digit; (3) first phalange length of 3rd digit; (4) second phalange length of 3rd digit; (5) greatest length of skull; (6) greatest mastoid breadth; (7) brain case width; (8) greatest zygomatic width; (9) postorbital constriction; (10) width across canines; (11) length of rostrum; (12) least incisive foramen–palatine length; (13) least width across M2s; (14) width across M2s; (15) width of M1; (16) length of M1; (17) length of mandible; (18) angular process–coronoid process distance; (19) height of mandible on m1; (20) length of m1; (21) width of m1; (22) length of m2; (23) width of m2.

Given ten OTUs already distinguished on the basis of other characters, we addressed the following question: does a quantitative character provide additional evidence for the existence of monophyletic groups within this collection of OTUs? We used a standard inferential method designed to test if there is a statistically significant association of the quantitative feature (in this case a character state) with the a priori discrete groups (in this case species as OTUs). Methods of analysis of variance deal with such kinds of question. In particular, we are interested in the multiple comparison tests designed to discover which means can be divided into groups that are signifi-

cantly different from each other (Sokal & Rohlf, 1981).

We evaluated the differences between ten species' averages for each of the 23 morphometric characters with one-way analysis of variance (ANOVA). If differences among means were statistically significant ($P < 0.05$), then a multiple range test (MRT) was performed to identify homogeneous mean groups for each character. Confidence intervals (95% level) were used to evaluate the variance within and between species according to the Tukey method (Sokal & Rohlf, 1981). A multiple-comparison procedure takes all possible pairwise comparisons to determine which differences between species means are significant (Zar, 1998). This multiple comparison test is commonly performed after a multi-sample hypothesis of equal means is rejected by an analysis of variance. Thus, these tests are referred as 'a posteriori' tests (Sokal & Rohlf, 1981; Zar, 1998). All these tests were performed using Statistica version 6.0 (StatSoft, 1999).

Results of statistical analyses of character variation can be added to a data matrix for cladistic analyses. When species means are evaluated as significantly different and their confidence intervals do not overlap, several homogeneous groups are circumscribed. Therefore each group of means is coded as a different character state. Often, a multiple comparison test will yield ambiguous results in which sets of means overlap because the same sample mean is allocated into different homogeneous groups. In such cases, character state identity of a species remains ambiguous and should be coded as '?'. It is also possible that the test will fail to detect differences between any pair of means. One reason might be the number of taxonomic units being compared. Multiple comparison tests are sensitive to number of samples (means) and sample size (Zar, 1998).

CLADISTIC ANALYSES

Cladistic analyses were implemented to examine the phylogenetic utility of 23 morphometric characters as compared to previously published molecular and morphological data for the same taxa. Molecular data (cytochrome *b* sequences) were retrieved from GenBank for the two outgroups and only for seven of the eight species of large *Artibeus* that we studied (there were no sequences for *A. amplus*). All but one sequence were published by Van Den Bussche *et al.* (1998). The *A. jamaicensis* sequence was obtained from Pumo *et al.* (1998). Morphological data were obtained for only six *Artibeus* species from Marques-Aguilar, 1994). There were no data for *A. planirostris*, *A. intermedius* and our two outgroups. *Enchistenes hartii* and *D. gnomus* were added as outgroups following Marques-Aguilar (1994). The three data sets

(morphometric, molecular, morphological) were analysed separately to estimate several properties of the morphometric characters as an indication of performance (consistency indices, data decisiveness, $g1$ statistics). A combined analysis was possible only for nine taxa with our morphometric characters and available molecular data.

In all cases, exhaustive searches were implemented in PAUP* 4.0b8a (Swofford, 2000). Bootstrap support (Felsenstein, 1985) and Bremer support values (Bremer, 1994) were calculated as a measure of relative robustness of clades. Bootstrap values were calculated using an exhaustive search with 1000 replicates in PAUP*. Bremer values were generated using AutoDecay version 4.0.2 (Eriksson, 1999). An estimation of the historical signal in each data set was assessed by analysing the $g1$ statistic (Hillis, 1991). Data decisiveness (DD) values (Goloboff, 1991) were also calculated, providing an index that serves as a measure of the cladistic structure in the data.

RESULTS

CHARACTER ANALYSIS

All 23 morphometric characters showed taxonomically useful variation patterns. Analyses of variance revealed more variation between OTUs than within any of the ten species. The multiple range tests (MRT) detected at least two homogeneous groups of species means in the variation of all characters. These results are presented in Table 1.

CLADISTIC ANALYSIS

Results of separate analyses vary in terms of topology, degree of resolution, support and amount of homoplasy (Table 2). None of the trees generated by each data set are identical (Fig. 1). Only one node was shared among the analyses of morphometric and molecular data alone, and the combined analysis, although internal resolution is different. Levels of clade support also varies among data sets. Mean bootstrap values ranged from 85.3% for morphometric data to 52% for morphology, and $zTBS$ varied from 0.12 for morphometric and molecular data alone to 0.08 for morphology. Despite the higher number of informative characters, molecular data are characterized by the highest homoplasy (CI = 0.66, RI = 0.41), the lowest decisiveness and phylogenetic signal (DD = 0.27, $g1$ = -0.639). Morphology provided very little resolution; however, this data set had the highest value of $g1$ (-0.93). In contrast, morphometric data exhibited less homoplasy (CI = 0.97, RI = 0.94) and it was the most decisive (DD = 0.91).

DISCUSSION

CHARACTER ANALYSIS

Our quantitative tests of character variation correspond to the first stage of cladistic analysis (i.e. the construction of the data matrix). Results of analysis of variance and multiple range tests distinguished at least two character states in all morphometric data analysed. According to cladistic theory, a potential taxonomic character is, necessarily, a system of at least two character states (De Luna & Mishler, 1996), thus, this collection of 23 characters can be considered as potentially useful for inferring the phylogeny of these taxa.

Nevertheless, there were some problems in assignment of state codes for some characters. When taxa are unambiguously classified into homogeneous groups it is clear how to code (e.g. characters 4, 8, 10). Problems arise when taxon means are placed in several homogeneous groups. Stevens (1991) suggested that if there is ambiguity in coding some terminal taxa, there is underestimation of character variation. MRT failed to detect unambiguous homogeneous groups for several cases (e.g. characters 9, 12, 20). This may be because of continuous and overlapping variation among taxa or due to a polymorphism within a taxon in those characters. That ambiguity could be solved with further analyses with means at a lower level of inclusion (see Sosa & De Luna, 1998).

Another problem also pointed out by Stevens (1991) is that variation in outgroup selection can affect delimitation of character states in the ingroup. He pointed out that in most cases details of outgroup variation is rarely given. We decided to include outgroup variation in the statistical analyses for assessing best results in character states coding, as done by Vrba *et al.* (1994). If outgroups are not included, our data would allow for a sharper distinction among states, but variation in outgroups generally did not cause problems with resolution of state identity.

A major concern in the phylogenetic interpretation of the validity of our morphometric characters is that some or most of them are statistically correlated with size. Indeed, the collection of species of *Artibeus* studied differ by size, among other features. One immediate conclusion is that such correlation affects all univariate analyses, therefore characters would not be independent, and not appropriate for phylogenetic inferences. This argument is flawed. Statistical correlation is not equivalent to logical dependence.

This is the same sort of argument that has been applied to eliminate any type of correlated characters. In the case of molecular data, some have argued that nucleotides in a sequence do not vary independently because biochemical and biophysical studies have showed that nucleotides often change in a concerted

Table 1. Species means, *F*-ratio and character codes assigned to OTUs. \bar{x} = mean; hg = homogeneous group. We coded every hg as a different character state. OTUs assigned to more than one hg were coded as '?'

Characters	Species										<i>F</i> -ratio
	<i>amplus</i>	<i>azteca</i>	<i>bilobatatum</i>	<i>fraterculus</i>	<i>hirsutus</i>	<i>jamaicensis</i>	<i>intermedius</i>	<i>lituratus</i>	<i>obscurus</i>	<i>planirostris</i>	
(1) Forearm length	\bar{x} f hg	43.95 b 2	42.09 a 1	55.62 c 3	53.19 d 4	57.63 d 4	64.08 e 5	68.35 f 6	57.58 d 4	63.73 e 5	753.9
(2) Metacarpal length of 3rd digit	\bar{x} f hg	41.85 a 1	41.76 a 1	52.05 c 3	49.04 b 2	53.41 d 4	60.22 e 5	64.53 f 6	54.58 d 4	59.53 e 5	461.5
(3) First phalanx length of 3rd digit	\bar{x} c hg	15.06 a 1	15.26 a 1	16.74 b 2	15.56 a 1	17.45 b 2	21.05 d 4	22.60 f 5	20.22 c 3	20.01 c 3	227.9
(4) Second phalanx length of 3rd digit	\bar{x} g hg	23.51 a 1	22.51 a 1	26.82 b 2	25.90 b 2	28.71 c 3	33.37 e 5	36.28 g 7	30.08 d 4	32.08 f 6	389.1
(5) Greatest length of skull	\bar{x} g hg	22.81 a 1	24.48 b 2	27.85 c 3	27.70 c 3	28.65 d 4	30.39 e 5	32.52 f 6	28.43 d 4	31.69 f 6	572.2
(6) Greatest mastiod breadth	\bar{x} f hg	11.50 b 2	11.11 a 1	14.58 c 3	14.22 c 3	14.90 d 4	16.50 e 5	17.13 f 6	14.79 d 4	16.66 f 6	719.9
(7) Brain case width	\bar{x} e hg	10.82 a 1	10.53 a 1	12.58 b 2	12.32 b 2	12.75 c 3	13.95 d 4	14.39 e 5	12.72 c 3	13.91 d 4	469.4
(8) Greatest zygomatic width	\bar{x} c hg	12.98 a 1	13.42 a 1	16.69 b 2	16.57 b 2	17.06 b 2	18.84 c 3	19.54 d 4	17.00 b 2	19.13 cd ?	547.1
(9) Postorbital constriction	\bar{x} f hg	6.10 a 1	6.19 a 1	7.21 cd ?	7.06 bcd ?	7.29 cd ?	7.04 c 3	7.38 d 4	6.81 b 2	8.03 e 5	201.2
(10) Width across canines	\bar{x} e hg	5.35 a 1	5.20 a 1	6.97 cd ?	6.40 b 2	6.87 c 3	7.59 d 4	7.85 e 5	7.10 c 3	7.73 d 4	342.4
(11) Length of rostrum	\bar{x} f hg	5.92 a 1	7.56 b 2	8.62 c 3	9.19 d 4	9.11 d 4	9.35 d 4	10.14 e 5	8.96 d 4	9.95 e 5	243.0
(12) Least incisive foramen-palatine length	\bar{x} g hg	7.99 a 1	10.64 b 2	10.65 b 2	10.87 c 3	10.84 c 3	11.06 cd ?	12.09 e 5	11.27 d 4	12.55 f 6	258.1

Table 1. Continued

Characters	Species										F-ratio
	<i>amplus</i>	<i>azteca</i>	<i>bilobatum</i>	<i>fraterculus</i>	<i>hirsutus</i>	<i>jamaicensis</i>	<i>intermedius</i>	<i>lituratus</i>	<i>obscurus</i>	<i>planirostris</i>	
(13) Least width across M2s	\bar{x} hg de code	4.78 a 1 2	5.18 b 2	5.67 c 3	5.02 b 2	5.82 c 3	6.18 d 4	6.78 e 5	6.38 d 4	6.76 f 5	241.2
(14) Width across M2s	\bar{x} hg code	9.79 a 1	9.99 a 1	12.42 c 3	11.88 b 2	12.67 d 4	13.40 e 5	14.41 g 7	12.92 d 4	14.52 g 7	499.9
(15) Width of M1	\bar{x} hg code	2.93 b 2	2.70 a 1	3.96 cd ?	3.98 d 4	3.99 d 4	4.32 e 5	4.55 f 6	3.85 c 3	4.46 f 6	340.7
(16) Length of M1	\bar{x} hg code	2.00 a 1	1.94 a 1	2.59 b 2	2.55 b 2	2.69 d 3	2.91 e 4	3.03 f 5	2.74 d 3	3.18 g 6	148.4
(17) Length of mandible	\bar{x} hg code	14.31 a 1	16.36 b 2	18.51 c 3	18.17 c 3	19.31 d 4	20.93 e 5	22.75 g 7	19.35 d 4	22.22 f 6	660.6
(18) Angular process-coronoid process distance	\bar{x} hg code	5.89 a 1	6.01 a 1	7.81 b 2	7.98 b 2	8.31 c 3	10.04 e 5	10.86 e 5	8.18 c 3	9.61 d 4	547.3
(19) Height of mandible on m1	\bar{x} hg code	2.36 a 1	2.38 a 1	2.97 b 2	3.10 b 2	3.05 b 2	3.56 c 3	3.96 e 5	3.16 b 2	3.51 c 3	235.2
(20) Length of m1	\bar{x} hg code	2.21 a 1	2.11 a 1	3.12 c 3	2.98 b 2	3.18 c 3	3.31 d 4	3.60 f 6	3.24 de ?	3.58 ef ?	451.4
(21) Width of m1	\bar{x} hg code	1.42 a 1	1.59 b 2	2.08 c 3	2.10 c 3	2.22 c 3	2.14 c 3	2.31 d 4	2.11 c 3	2.52 e 5	196.8
(22) Length of m2	\bar{x} hg code	1.39 a 1	1.51 a 1	1.96 b 2	1.90 b 2	2.00 b 2	2.05 b 2	2.16 c 3	1.90 b 2	2.29 d 4	137.6
(23) Width of m2	\bar{x} hg code	2.00 b 2	1.89 a 1	2.48 c 3	2.40 c 3	2.47 c 3	2.81 d 4	2.88 d 4	2.46 c 3	2.78 d 4	225.1

Table 2. Tree statistics for separated and combined analyses. chars = characters, PI = phylogenetically informative, MPT = most-parsimonious trees, L = length, CI = consistency index, RI = retention index, DD = data decisiveness, MBT = mean bootstrap values, z TBS = standardized total Bremer support. MBTs are based on all bootstrap values on each tree, including those with <50%. z TBS values were computed by summing all Bremer Support values across all nodes on each tree and divided by the minimum possible number of steps for each data set

Data set	Total OTUs	Total chars	PI chars	MPT	L	CI	RI	g1	DD	MBT	z TBS
Morphometric	10	23	21	1	104	0.97	0.94	-0.65	0.91	85.3	0.12
Morphology	7	31	16	7	43	0.75	0.31	-0.93	0.68	52.1	0.08
Molecular	9	1140	152	1	497	0.66	0.41	-0.63	0.27	73.5	0.14
Combined*	9	1163	173	2	604	0.70	0.44	-0.76	0.31	78.0	0.09

*including only morphometric and molecular data.

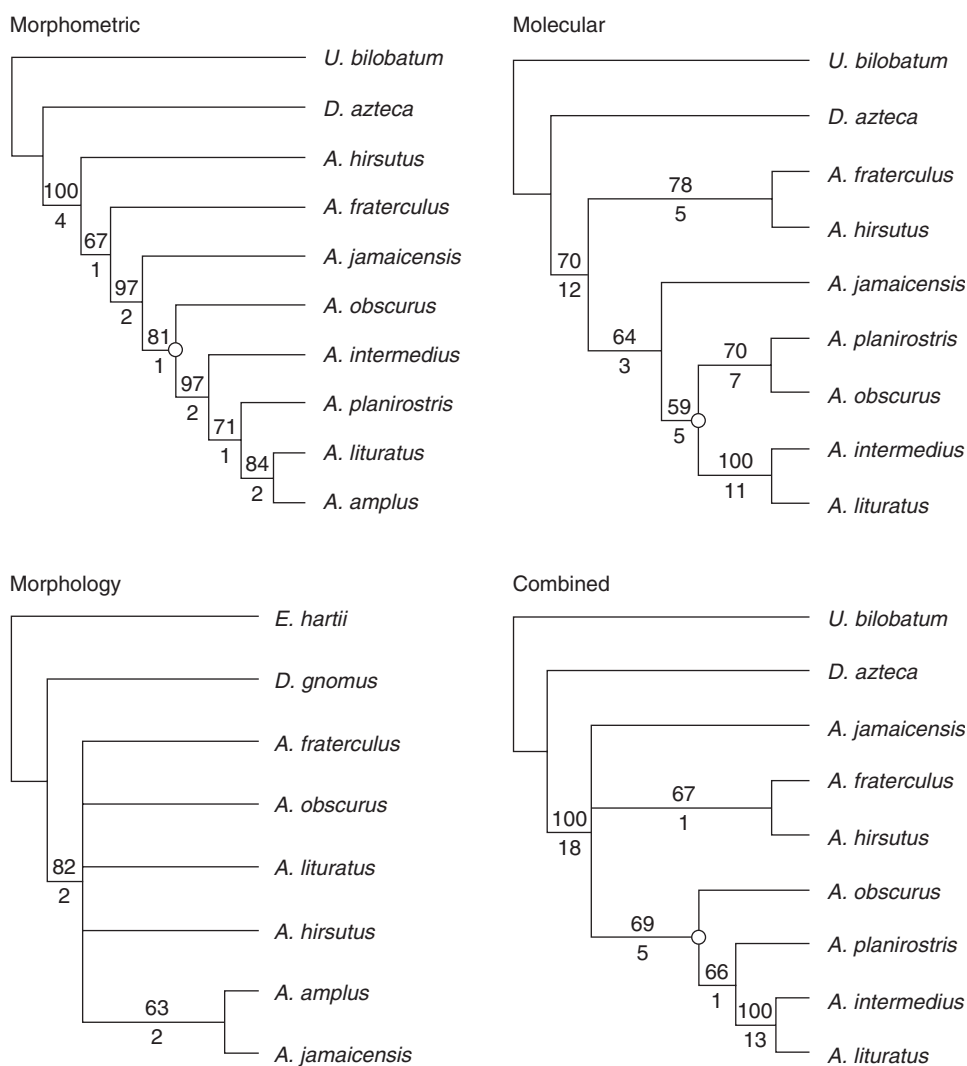


Figure 1. Cladograms recovered from separate analysis of each data set and from the morphometric and molecular data combined. Tree statistics are presented in Table 2. A circle represents a node recovered from morphometric and molecular data alone, and from the combined analyses. Bootstrap proportions are provided above branches, Bremer support values below branches.

manner as a result of structural or functional interactions (Wollenberg & Atchley, 2000). In the case of morphological data, the same argument appears when interpreting functional constraints, such as pollination syndromes. Several characters vary in response to the same function, and since they are correlated, it is prescribed that they should be excluded from cladistic analyses (Armbruster, 1993).

Character association attributed to genetic, ontogenetic or ecological constraints is a different kind of interaction among characters than the logical correlation tested by character congruence in parsimony analysis (Deleporte, 1993; Luckow & Bruneau, 1997). The same applies to the nature of statistical correlation. In other words, statistical or functional correlation among characters is not equivalent to non-independence as phylogenetic characters. Consequently, the exclusion of statistically correlated characters from a phylogenetic analysis is unwarranted. As discussed at length by Kluge & Wolf (1993) and Kluge (1997), characters in cladistic analysis are considered independent if they provide separate pieces of evidence that can refute or corroborate phylogenetic relationships, i.e. if they are discrete homology statements. Thus, for statistical correlation and historical dependence to be equivalent, one would have to assume that all character states affected by wherever factor or constraint changed simultaneously as a single historical event. Even if size-dependent, our morphometric characters do not behave as if a column was being replicated with all changes optimized on a single branch. Every column in the data matrix (Table 1) shows an heterogeneous character state distribution, therefore providing primary homology statements that are logically independent.

HOMOLOGY AND CHARACTER STATE RECOGNITION

Transformation of morphometric data into useful information (e.g. taxonomic characters) to infer phylogenetic relationships among any taxa is a controversial and complex issue. Several methods are available for assessing the problem, but most have been criticized because coding character states is arbitrary and often depends on how graphic data are presented (Gift & Stevens, 1997). How can we decide which method is the most consistent to translate quantitative character variation into discrete states? We think that an evaluation and consequent selection of a method must be based on phylogenetic systematic theory that applies to character selection and character state identity, such as the homology concept and the empirical criteria to develop primary homology hypotheses (Patterson, 1982; De Pinna, 1991; Brower & Schawaroch, 1996). Under cladistic theory, homology is conceptualized as correspondence among two or more

characteristics of taxa caused by continuity of information (Van Valen, 1982; Roth, 1994). The recognition of transformed homologies involves an evaluation of the topographic similarities and character state identity between the taxa studied (Brower & Schawaroch, 1996).

The first step in evaluating similarity of features is to describe and group the observed variability in order to infer if distinct character states (e.g. hypothesized historical changes) can be recognized. If characters studied are quantitative, probability concepts and statistical procedures can be used to evaluate whether taxa involved in the analysis are 'the same' or 'different' for a feature (Jardin, 1969; Rae, 1998). Observations of each taxon are summarized in estimated parameters of distribution and central tendency (in a statistical sense). The decisions of which distributions (and associated central tendencies) are different or not should be made by evaluating variation levels within and between taxa included in the analysis (Rae, 1998; Swiderski *et al.*, 1998). A character can be considered as potentially useful if it shows greater variance between groups than within (De Luna & Mishler, 1996). Because of that, our statistic tests (e.g. ANOVA and MRT) provide a consistent and non-arbitrary method to infer character state identity in morphological features. Since morphometric similarity is not phylogenetic homology, such quantitative judgment of the existence of character states are still hypotheses of primary homology, which need to be tested for congruence.

PHYLOGENETIC UTILITY OF MORPHOMETRIC CHARACTERS

It has been questioned if morphometric characters contain historical signal (Felsenstein, 1988; Farris, 1990). This claim is not exclusive to this type of data only, but also applies to any character that potentially could be used in a cladistic analysis. The basic question is: do these data help to resolve the relationships? Phylogenetic hypothesis for large *Artibeus* based on other characters (molecular, morphological) are different in topology and resolution as compared to cladograms recovered with our morphometric data. However, the interaction of morphometric and molecular data in the simultaneous analysis indicates character congruence. This synergistic effect of data combination increases the branch support values of those branches that are found in a separate analysis of molecular data. For example, the clade that includes *A. obscurus*, *A. intermedius*, *A. lituratus* and *A. planirostris* has a bootstrap value of 59, but this goes up to 69 in the combined analysis (Fig. 1).

Several approaches have been developed to test whether the data at hand do in fact contain phyloge-

netic signal or mostly random noise (Kitching *et al.*, 1998). Indices such as DD, g1 and zTBS were calculated as an assessment of usefulness of morphometric characters produced by our approach. The results showed that by virtually any measure used (Table 2), the phylogenetic utility of the morphometric data is superior to morphological and molecular data when analysed separately. Morphometric data exhibit less homoplasy, and provide greater support than morphological and molecular data alone. In the combined analyses, we additionally evaluated the relative contribution of each data to the relationships through the partitioned Bremer support (PBS; Baker & DeSalle, 1997). PBS scores for each node were summed, and standardized by the minimum possible number of steps for each data partition to control for differences in size (Baker, Wilkinson & DeSalle, 2001). Standardized score for the morphometric partition (0.06) is similar to that for molecular matrix (0.08). Therefore, PBS values provide additional insight into the utility of our morphometric data.

CONCLUSIONS

Assessment of homology in the study of morphology consists of statements of topographical identity and conjectures of character state identity. Statistical analyses can be used in the judgment of similarity for character state identity. In this paper we have illustrated how morphometric analyses can be used in assessment of discrete states from patterns in character variation for the establishment of primary homology. ANOVA followed by MRT represents an epistemologically robust approach to recognize character states, since it is grounded in a phylogenetic concept of homology. These statistical tests are specifically aimed to evaluate if features are the same or not for the assessment of character state identity among taxa. Although problems can emerge in its implementation when taxa are ambiguously classified in two or more homogeneous groups, such tests provide a non-arbitrary quantitative method for the judgment of similarity in the recognition of discrete states from patterns of morphological variation.

Our cladistic analysis of *Artibeus* species suggests that morphometric characters help to resolve the relationships of these bats and increase branch support values. Descriptive indices of historical signal showed that morphometric data contain cladistic structure and they are phylogenetically useful. These results argue for the inclusion of morphometric data into cladistic analysis. Application of this method should allow objective coding of morphological quantitative traits into discrete character states, which might be suitable for phylogenetic analyses.

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APPENDIX: SPECIMENS EXAMINED

The 557 specimens included in the morphometric analysis are listed below. Collection acronyms are as follows: CNMA, Colección Nacional de Mamíferos, Instituto de Biología, Universidad Nacional Autónoma de México; IPN, Colección de Cordados Terrestres, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México; CVULA, Colección de Vertebrados, Universidad de Los Andes, Venezuela; ICN, Colección Zoológica, Instituto de Ciencias Naturales, Universidad Nacional de Colombia; MVZ, Museum of Vertebrate Zoology, University of California, Berkeley; FMNH, Field Museum of Natural History, Chicago.

D. azteca ($N = 34$): Mexico, CNMA 7151, 8479, 19673–19682, 38284, 38285, 38656–38659, 39132–39134, 39136, 39137, 39139–39142, 39151–39156, 41620.

U. bilobatum ($N = 34$): Mexico, CNMA 6361, 19228–19230, 19232, 20153–20162, 20401, 20403–20406, 22268–22270, 22272, 22273, 22676–22828, 22936–22939, 24455, 30335.

A. amplus ($N = 36$): Colombia, ICN 7921, 7922, 7924, 7925, 10639, 10642, 10650, 10652, 10656, 10658, 10664, 10682, 10687, 10688; Venezuela, CVULA 2068, 2069, 2999–3002, 3004–3006, 3017, 3018, 3079, 3080, 3111–3113, 3117, 3123, 3124, 5916, 5917, 6085.

A. hirsutus ($N = 51$): Mexico, FMNH 141791, 141792, 150612, 150613, 150615–150618; IPN, 21623, 22696–

22698, 22700–22702, 23355, 23356, 23366, 23369, 24246, 24247, 24251, 24252, 24255, 26336, 26342–26344, 26347, 26426–26428, 34822–34825, 35250, 36991, 36992, 39827–39830, 40072–40079.

A. jamaicensis ($N = 143$): Belize, FMNH 106805, 120824–120826, 120881; Colombia, FMNH 72121, 72122, 122071, 122072; ICN 9012, 9020, 9022, 9023, 9025–9029, 13684, 13686, 14030–14033, 14823, 14961, 14962; Costa Rica, FMNH 43977, 123130–123132; El Salvador, MVZ 130866–130868, 130870, 130873–130879; Grenada, FMNH 34167; Guatemala, FMNH 41932–41934, 64474, 64479, 64636, 64948, 64949, 64953, 64955; Honduras, FMNH 47635, 49853, 49854; Mexico, IPN 6725–6729, 6731, 13020–13025, 23169, 23171, 24573–24576, 24578, 34942, 34947–34949, 34959, 34963, 34972, 34982, 36586, 36587, 36591–36595, 37280, 37282, 37285, 37639–37645; Panama, FMNH 19207, 128111, 128112, 128113, 128114, 128115; MVZ 135977, 164782, 164785; Trinidad, FMNH 61923, MVZ 167449, 183524; Venezuela, CVULA 173, 175, 280, 353, 354, 500, 501, 504, 505, 514, 518, 519, 521, 522, 534, 751, 753, 1478, 1814, 1888, 2310, 2311, 2460, 2542, 2762, 2789, 3248, 3381, 3382, 3482, 3488.

A. intermedius ($N = 50$), El Salvador, MVZ 130909, 130910; Mexico & IPN 1786, 1787, 4651, 13035–13037, 13864, 15138, 15139, 15155, 15512, 15513, 15515, 15521, 15885, 15887, 16216, 16217, 16226, 21056, 21080, 21081, 21082, 24606, 24609, 24610, 24611, 27971, 27972, 28509, 28512, 28514, 28515, 28522, 28523, 35488, 35490, 35491, 35493, 35495, 35496, 35502, 35588, 35589, 35700, 41111; Panama, MVZ 118796, 118797.

A. lituratus ($N = 77$): Belize, FMNH 58198, 58199, 58201, 58202, 106808, 106809, 120856, 120859, 120860; Bolivia, FMNH 21483, 115032–115035; Brazil, FMNH 18224, 52355, 92070, 94662, 94663, 99664; Colombia, ICN 71, 8518, 10877, 10941, 13685, 13805, 14034, 14167, 14436, 14908, 14909, 15116–15118; Ecuador, FMNH 124906, 124915, 124930, 124936; Mexico, IPN 13856, 13855, 41112, 41113; Paraguay, MVZ 144429, 144430, 144436, 144452, 144453, 144459, 144460, 144463; Peru, FMNH 129112, 129113, 129117, 129118, 157744, 157751, 157759, 157760, 166558, 166559; Venezuela, CVULA 2803, 2807, 2809, 2810, 2813, 3240–3242, 3246, 3448, 5810, 5822, 5844, 5983, 6064, 6202, 6203.

A. obscurus ($N = 47$): Bolivia, FMNH 104663, 104664, 104665, 104666, 104668; Brazil, 94691, 94692, 136862, 136863, 136864, 136865, 136866, 141708, 141709, 141710, 141711, Colombia, ICN 10562, 10563, 10566, 10572, 10573, 10581, 10584, 12693, 14904, 14910; Ecuador, FMNH 124858, 124861–124865; Peru, FMNH 55393, 55397, 55398, 89070–89073; Surinam, FMNH 93197; Trinidad, FMNH 54033; Venezuela, CVULA 828, 1728, 1733, 1734, 3416, 3417.

A. planirostris ($N = 46$): Bolivia, FMNH 104669, 115029, 115031; Brazil, 92067, 92069, 92073; British Guiana, FMNH 22474; Colombia, ICN 10637, 10641, 10644, 10645, 10646, 10647, 11387, 14112, 14361–14363, 14365–14367; Ecuador, FMNH 41436–41438, 124873, 124875, 124876, 124890, 124892, 124894, 124903–124905; French Guiana, FMNH 14886; Surinam, FMNH 93198–93202; Venezuela, CVULA 1690, 1713, 1714, 1717, 1719, 1763; West Indies, FMNH 47738.