Hystrix, (n.s.) 11 (1) (2000): 49-75

PHYLOGENETIC ANALYSIS OF SKULL SHAPE EVOLUTION IN MARMOTINE SQUIRRELS USING LANDMARKS AND THIN-PLATE SPLINES

DONALD L. SWTDERSKT (*), MIRIAM L. ZELDTTCH (**) AND W. L. FINK (***)

(*) *Museum of Zoology, University of Michignn, Ann Arbor, Michigan, 48109 USA*
(**) Museum of Paleontology, University of Michigan, Ann Arbor, Michigan, 48109 USA
(***) Department of Biology and Museum of Zoology, University of Michigan. Ann Arbor, Michigan, 48109 USA
CorrespondingAuthor: Donald L. Swiderski, Museum of Zoology, Ann Arbor MI 48109 USA
e-mail: dlswider@umich.edu

ABSTRACT. - Several studies have shown that the rcccntly dcvclopcd tcchniqucs of gcomctric morphometrics are extremely powerful descriptive tools. And yet, one potential use of the resulting descriptions, phylogenetic analysis, has generally been neglected. This neglect is understandable because prominent systematists as well as prominent morphometricians have objected to the use of morphometric data in phylogenetic systematics. We agree that some methods of morphometric analysis produce results that cannot be used in phylogenetic systematics. and that some methods of incorporating morphometric results into statements about character transformation are not appropriate. However, we do not agree that these objections to specific techniques support a blanket rejection of the use of morphometric data in systematic studies. In this paper, we review the principles of phylogenetic systematics and show that they are equally applicable to qualitative descriptions of triangles and to quantitative descriptions (shape coordinates of the apex) of those same shapes. Then we show how these principles would he applied to complex shapes like **skulls** of marmotine squirrels, and that the resulting analysis leads to legitimate hypotheses about marmotine phylogeny and the evolution of skull shape in these animals.

Geometric morphometrics has several advantages over traditional methods of analyzing biological shapes (Bookstein, 1990; Bookstein, 1991). One advantage is that the use of landmarks anchors the descriptions of shape differences and the explanations for those shape differences to specific regions of the organism. When landmarks are chosen carefully, the tendency of traditional measurement schemes to overrcprcscnt particular regions or dimensions can be dramatically reduced. Another advantage of this approach is that it provides independent descriptions of size and shape. In addition, it provides a mechanism for decomposing shape differences into a series of components ranging from large-scale features spanning all or most of the form to small-scale features localized to the vicinity of a few closely spaced landmarks. Empirical studies have demonstrated the utility of these methods for the study of allometry (Zelditch and Fink, 1995; Loy et al., 1996; Taylor and Contrafatto, 1996), morphological integration (Zcldditch et al., 1992; Swiderski, 1993), and the relationship between shape and function (Bales, 1996; Courant et al., 1997).

One potential use of geometric morphometrics that has received relatively little atten-

tion is the reconstruction of phylogenetic relationships. For example, Courant et al. (1997) used least squares Procrustes superpositions of cranial landmarks to describe similarities in skull shape among fossorial rodents (Arvicolidae) but did not use the skull shapes in an analysis of arvicolid relationships. Consequently, their results suggest that there could have been convergence, but do not actually document the independent historical transformations of dissimilar ancestors into similar descendants. Rohlf et al. (1996) used UPGMA cluster analysis and minimum spanning trees on canonical variates of partial warps scores to evaluate similarities and differences in skull shape among European moles. Rohlf et al. compared their results to the current taxonomy of the moles, but like Courant et al., did not attempt to incorporate the shape analysis in a phylogenetic analysis. Several other biologists have performed similar studies in which geometric morphometrics were used to describe similarities among taxa, but no phylogenetic analysis was performed to infer the evolutionary relationships of those taxa (,e.g., Bales, 1996; Capanna et al., 1996; Taylor and Contrafatto, 1996). One of the few explicit attempts to reconstruct a history of shape changes was performed by van Dam (1996), who used the fossil record to infer the sequence of tooth shapes in a group of murid rodents, and then used the morphometric analysis to describe the implied shape changes. As far as we know, only Fink and Zelditch (1995) have used cladistic methods of phylogenetic analysis to infer genealogical relationships of taxa from shape differences described by geometric morphometrics.

The lack of cladistic studies using geometric morphometrics is not surprising. Several investigators have argued that cladistic analysis is an inappropriate use of morphometric data (Bookstein, 1994; Adams and Rosenberg, 1998; Rohlf. 1998). Others have argued that cladistic analysis of quantitative data requires manipulations that cannot be justified (Felsenstein, 1988; Garland and Adolph, 1994). In addition, some investigators have argued that morphometric data lack the qualities that are necessary to justify hypotheses of homology, and therefore, are unsuitable for this kind of analysis (Pimentel and Riggins, 1987: Mickevich and Weller, 1990). Taken together, these arguments seem to constitute a daunting obstacle to the cladistic analysis of morphometric data.

We have argued that this obstacle is not as formidable as it appears to be. We agree that many older morphometric methods produce variables that are unsuitable for phylogenetic analysis, but we also find that some of the recently developed landmark-based methods produce variables that are suitable (Zelditch et al., 1995). In addition. we agree that many of the methods used to code morphometric data for phylogenetic analysis employ manipulations that are unjustified, but we have not found these manipulations to be necessary when taxa are well differentiated (Swiderski et al., 1998). Consequently, we argue that some quantitative descriptions of biological shapes can be coded by using the same criteria that are used when those shapes are described qualitatively. Perhaps most important, we have demonstrated that the arguments suggesting that cladistic analysis is an inappropriate use of morphometric data are based on incorrect interpretations of cladistic methodology (Zelditch et al., 1995; Zelditch and Fink, 1998: Zelditch et al., 1998). Thus, even though there is great need for caution in the selection of morphometric variables and in the selection of criteria used for coding, it is possible to produce valid inferences of historical shape change by performing a cladistic analysis of biological shapes that have been described using geometric morphometrics.

The purpose of this paper is to demonstrate how cladistic methods of phylogenetic analysis can be applied to quantitative descriptions of biological shapes. We begin

with a brief review of cladistic methodology, then present two examples to illustrate its application. In the first example, we analyze an artificial data set composed of a series of triangles. In the second example, we analyze differences in skull shape among several species of squirrel-like rodents in the tribe Marmotini.

CLADISTIC METHODOLOGY

The cladistic approach to inferring phylogenetic history is a logical extension of evolutionary theory (Hennig, 1966). Organisms are expected to inherit traits from their ancestors, but they are also expected to acquire modifications of those traits. Subsequent descendants will inherit the modified versions of the traits, and perhaps acquire additional modifications of them. When the lineage branches, the two lines of descendants will accumulate different sets of derived traits. In the absence of convergence, any similarities between representatives of the two lines will be due to the retention of unmodified ancestral traits in both lines. As the lineage continues to branch and other traits are modified (still without convergence) the distribution of derived traits in descendent taxa will exhibit a hierarchical arrangement that retlects the sequence of branching events. Consequently, Hennig argued that the goal of phylogenetic analysis should be to identify nested sets of derived traits and use their distributions to infer the historical sequence of branching events.

Hennig recognized that the principal obstacle to implementing this approach is convergence. Organisms in similar environments may experience similar selection pressures. Consequently, some of their features may be independently modified in ways that make those features more similar in the descendants than they were in the ancestors. These homoplasic similarities could lead to the mistaken inference that the convergent taxa shared a more recent common ancestor with each other than they did with the other members of their respective lineages. However, Hennig reasoned that homologous similarities, those due to common ancestry, would be found throughout the organism, whereas the homoplasic similarities due to a particular convergence would be found in the relatively few traits that were most directly affected by the similar selection pressures. In addition, one species might be convergent with a second species in one set of traits, but convergent with a third species in a different set of traits. Thus, convergent similarities might be misleading, but they would contradict each other. This led Hennig to propose the principle of phylogenetic parsimony. Each judgement of derived similarity supports a hypothesis of homology and monophyly. The judgements that are correct will support hypotheses that corroborate one another, but the judgements that are incorrect due to homoplasy will support contradictory hypotheses (intersecting sets of monophyletic taxa) and ad hoc hypotheses of additional transformations will be necessary to resolve those conflicts. Because homoplasic similarities are not expected to exhibit a coherent pattern, the phylogenetic hypothesis that most accurately retlects the genealogical relationships of the taxa will be the one that requires the fewest ad hoc hypotheses, i.e., the one that is most parsimonious.

Some biologists have claimed that this approach implies an assumption that evolution is parsimonious, that convergent similarities are less common than homologous similarities, and argued that parsimony methods will be mislead if this assumption is incorrect (e.g., Felsenstein, 1978; Saether, 1986). However. Farris (1983, 1986) has shown that parsimony methods do not require that homologous similarities are more common than homoplasic similarities. but only that there is more support for the correct phylogeny than for any one of the alternatives supported by the homoplasies. Thus the real danger is that a particularly large set of functionally or developinentally linked char-

acters has undergone the same series **of** transformations in evolutionarily independent lineages. Accordingly, some systematists have suggested that the characters in these complexes should be assigned lower weights (Hecht and Edwards, 1976; Neff, 1986) or even coded as a single character (Winterbottom, 1990: Mabee, 1993; Fink and Zelditch, 1995) to reduce the influence of correlated homoplasies on the phylogenetic analysis.

The application of Hennig's approach is quite simple. In analyses of qualitatively described traits, the first step is to identify the features that can be used to sort taxa into groups that are different from one another. The next step is to describe each feature and the alternate states found in each group of taxa. Then, integer codes are applied to indicate which taxa have which states. Finally, an analysis is performed to identify which phylogenetic trees imply the fewest number of character state transformations. In analyses of quantitatively described traits, the protocol is slightly different because the measurements are specified a priori and the decision of what to measure is often based on expectations of what should be informative in light of functional models or experience with related taxa. Consequently, the first step is to describe the traits that will be measured, and the second step is to evaluate which traits can be used to sort taxa and describe the alternate states found in each group of taxa. (Much of the debate about coding quantitative data is actually about the validity of alternative criteria proposed for sorting taxa – cf., Farris, 1990; Gift and Stevens, 1997; Swiderski et al., 1998.) Once the states of the potentially informative traits have been described, the subsequent steps are the same as in analyses of qualitatively described traits.

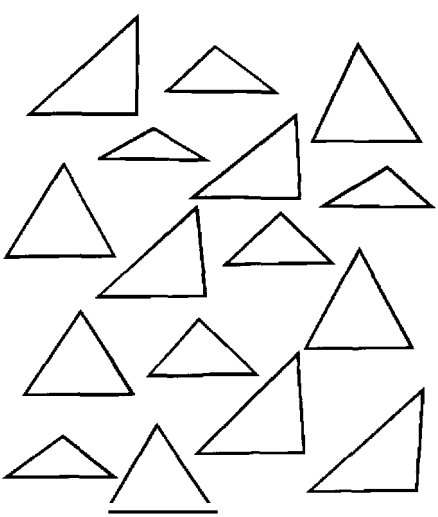

Figure 1. An artificial data set composed of a set of triangles

TRIANGLES

As discussed above. phylogenetic inference is based on hypotheses of homology and monophyly, and these hypotheses are based on judgements of the similarity of the traits observed in the taxa. In morphometric analyses, the traits are sizes and shapes. Sizes are one-dimensional scalars. There might be disagreement concerning which measurement of size is more appropriate (e.g., surface area or volume), but not about how to judge the similarity of volumes or areas. In contrast, shapes are multi-dimensional, so the evaluation of similarities of shapes is more complex. In this section, we illustrate the problem of comparing shapes, and our solution of the problem, with a set of triangles. Later in this paper, we show how this solution can be applied to analyses of more complex shapes like those of mammalian skulls.

Qualitative Analysis. Figure 1 shows several triangles, each representing an individual specimen of one of the species being analyzed. For any inferences of homologous shapes to be valid, the triangles must be defined by the same three points in all taxa, and oriented in the same way for all comparisons. For example, the two lower points might be the anterior ends **of** the zygomatic

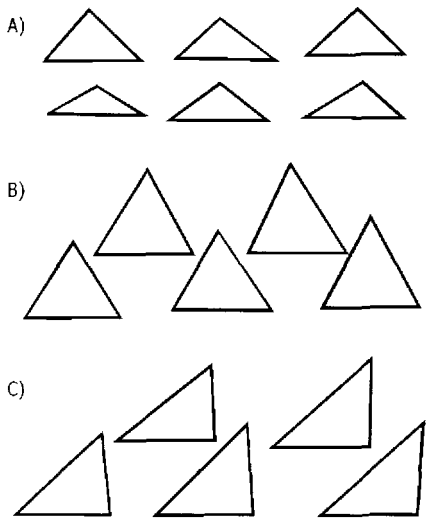

Figure 2. The triangles from Figure 1 sorted by shape. A) short and relatively symmetrical, B) tall and relatively symmetrical, C) tall and asymmetrical.

arches on the left and right sides, and the apex might be the distal end of the mid-line suture between the two nasal bones. Because this is a constructed example. we will assume that all of the triangles have been oriented appropriately.

There are several sets of attributes that could be used to describe the shape of a triangle. Two commonly used features are the aspect ratio, which describes the height of the apex relative to the length of the base, and skewness, which indicates whether the apex is centered over the baseline or displaced toward one end. Together, these two features describe the shape of a triangle completely and without redundancy. In Figure 2, the triangles have been sorted into three groups: **A)** relatively short and approximately symmetrical, B) relatively tall and approximately symmetrical. and C) relatively tall and skewed to the right.

Now that the three sets of shapes have been recognized, the next step is to code those shapes for the phylogenetic analysis. If there were only two sets of shapes, perhaps those in groups **A** and B, this would be simple. The shape shared by the ingroup (the taxa of interest) and the outgroup (selected close relatives) would be assigned state 0 to reflect the hypothesis that this shape was inherited by both groups from their common ancestor and therefore primitive. The other shape would be assigned state 1 to reflect the hypothesis that this state is derived and the taxa that share this shape are a monophyletic subgroup of the ingroup. If the two sets of shapes are those in **A** and C, we still have evidence of only a single shape change, even though it is necessary to describe the changes in terms of both the aspect ratio and skewness. Only when all three shapes are present is there evidence of shape changes in two different directions. Unfortunately, there also are nine possible histories of transformations connecting these three states (Figure 3). The shapes of these triangles provide no information that can be used to choose among these nine character state trees; information from other characters is needed. It is possible to reduce the set of possible character state trees, but only if there is only one state that is shared by the ingroup and the outgroup.

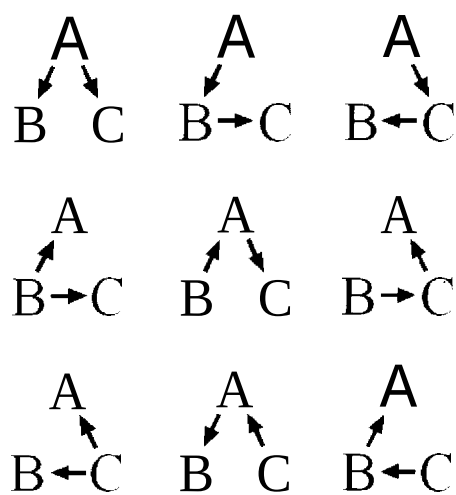

Figure 3. Nine possible transformation series for three character states.

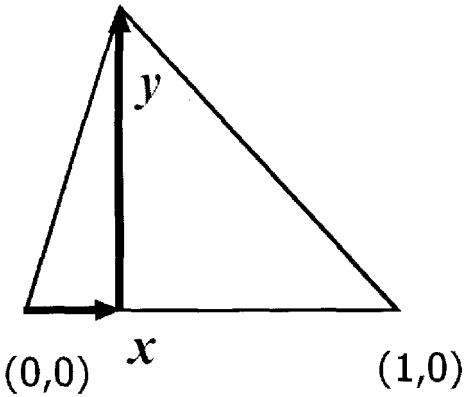

Figure 4. Graphical representation of shape coordinates.

Under those circumstances, it would be reasonable to hypothesize that the state shared by the ingroup and the outgroup is primitive. Even so, there would still be three equally plausible transformation histories, and no way to choose among them. Because there is no way to choose among the alternative hypotheses, multistate characters are analyzed as unordered, which means that no hypothesis is specified.

Quantitative analysis. **As** in the qualitative description, there are several combinations of variables that could be used to quantitatively describe the shapes of triangles. One particularly convenient set of variables is the pair of shape coordinates of the apex (Bookstein et al., 1985). The first step of computing shape coordinates is to rescale each triangle so that its baseline has unit length. The subsequent steps compute the vectors that describe the orthogonal projection of the apex onto the baseline (Figure 4). In essence, the shape coordinates of the apex are linear, quantitative versions of skewness (x) and aspect ratio (y) of the triangle.

The correspondence between shape coordinates and familiar qualitative descriptors is useful, but the real utility of shape coordinates is that they completely describe the two-dimensional shape of the triangle in two linear and independent variables. Consequently, the diversity of shapes can be displayed as a scatter-plot of the shape coordinates. In Figure 5A, the triangles from Figures **2A** and 2B are aligned by their baselines, which have been rescaled to the same length. Figure 5B shows only the locations of the apical points, with ellipses to outline each group. The two clusters of points do not overlap, so the clusters can be coded as separate character states. The transformation can be described as a shift along the y-axis (i.e., a change in the relative height of the apex), but the direction of that transformation cannot be determined from the information given so far. However, if the shape of the outgroup is known, then the direction can be specified as an increase or decrease relative to the shape shared by both the outgroup and some members of the ingroup. In other words, the hypothesis of transformation can be polarized to indicate

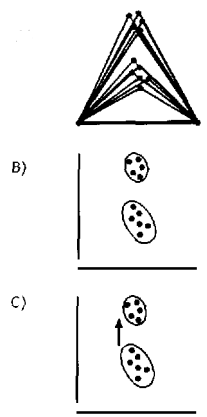

Figure 5. Graphical representation of coding two sets of triangles, 1. transformation parallel to one morphometric axis. **A)** short symmetrical triangles and tall symmetrical triangles superimposed at their baselines, B) scatter-plot of shape coordinates of the apical points, C) scatter-plot with arrow to indicate the inferred direction of transformation.

which shape is inferred to be derived and which group is inferred to be monophyletic. In Figure 5C, an arrow is included to indicate the polarized hypothesis that the shape change is an increase in the relative height of the apex.

In Figure 5, the values of the shape coordinates are not shown. They were used to generate the scatter-plot, but they are irrelevant to the subsequent analysis. Only two pieces of information are used to formulate the hypothesis of transformation: the presence of two distinct groups of shapes, and the shape of the outgroup. The integers that are assigned as character state codes are nothing more than labels that reflect the hypothesis of transformation. These labels are not intended to represent the magnitude of that transformation on any scale.

Figure 6 shows the case in which the transformation is not parallel to the axes of the quantitative description, using the triangles from Figures **2A** and 2C. As in the qualitative analysis, the description of this change is more complex because it requires two variables (x and y), but that does not mean that the change occurred in two steps. **A** different method of quantification may produce a description that requires only one variable. As in the previous example, the hypothesis of a transformation is based on the recognition that there are two distinct groups of shapes, not on the description of the difference between those groups.

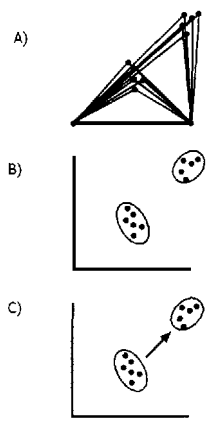

Figure 6. Graphical representation of coding two sets of triangles, 2, transformation that is not parallel to either morphometric axis. **A)** short symmetrical triangles and tall asymmetrical triangles superimposed at their baselines, B) scatter-plot of shape coordinates of the apical points, C) scatter-plot with arrow to indicate the inferred direction of transformation.

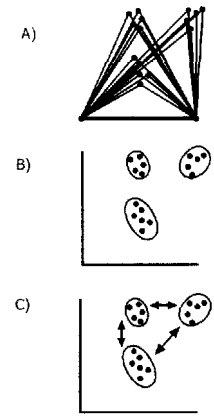

Figure 7. Graphical representation of coding three sets of triangles. **A)** all three sets superimposed at their baselines, B) scatter-plot **of** shape coordinates of the apical points, C) scatter-plot with two-headed arrows to indicate uncertainty about the directions of transformation.

Figure 7 shows the quantitative analysis of **all** three groups of triangles from Figure 2. **As** in the qualitative analysis, the available information supports the hypothesis that there are two derived states. Also as before, that information does not indicate whether those states are steps in a historical sequence, nor what that sequence was. Other characters must be used to infer the phylogenetic relationships of the taxa. Then, the historical sequences of these shapes can be interpreted in the light of those relationships. In the absence of any evidence about the historical sequence, all possible sequences must be considered equally plausible, as indicated by the two-headed arrows.

MARMOTINES

We constructed the examples above so that the groups of shapes would be clearly distinct. The natural world is seldom so neat. Below, we present a more realistic example in which we produce quantitative descriptions of skull shape in marmotine squirrels. then use those descriptions to infer what transformations of skull shape occurred during the evolution of marmotines.

Background. The tribe Marmotini is a monophyletic group that includes marmots (*Marmota*), antelope squirrels (*Ammospermophilus*), ground squirrels (*Spermophilus*), and prairie dogs (*Cynomys*) (Hafner. 1984). Several lines of evidence suggest that marmotines diverged from primitive tree squirrels in the late Oligocene (Bryant, 1945; Hight et al., 1974; Ellis and Maxson, 1980; Emry and Thorington, 1982; Hafner, 1984). Since their origin, marmotines have undergone considerable diversification in adult body size, diet and foraging habits (Howell, 1938; Bryant, 1945; Black; 1963; Hafner, 1984). Comparable changes in size and behavior are associated with the evolution of skull shape in many mammalian lineages (cf., Radinsky, 1982; Janis and Ehrhardt, 1988: MacFadden, 1992). It would not be surprising to find similar associations in the marmotines.

Six marmotine species and three outgroups are included in the analysis below (Appendix 1). All four genera and most of the commonly recognized subgenera of marmotines are included. In addition. these six marmotines span most of the range of adult body size in the tribe and exemplify most of the different diet and foraging habits found in the tribe. The three outgroup species (two tree squirrels, *Sciurus niger* and *Tami-*

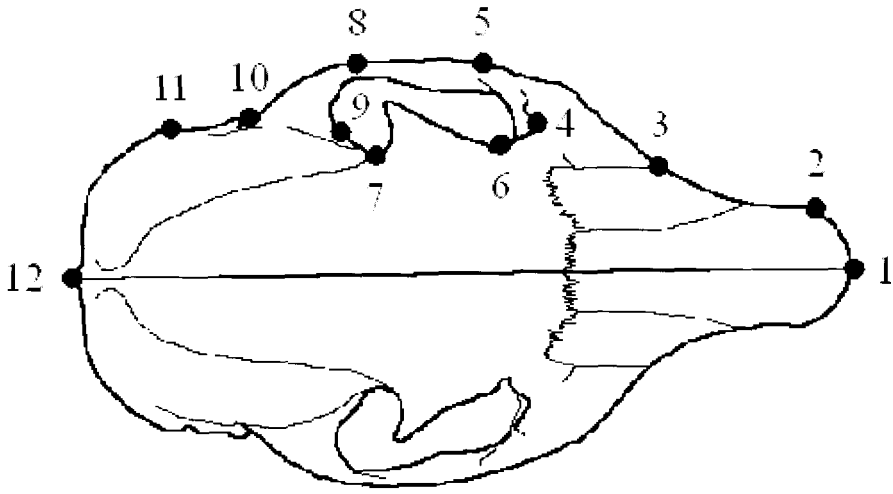

Figure 8. Line drawing of a representative specimen **of** *Sciurus niger*, showing locations of the land**marks.** Ij tip of the rostrum at the midline suture. 2) lateral limit of the dorsal margin of the nares. 3) antero-dorsal end of the zygomatic arch and plate, 4) notch above the lacrimal process. 5) anterior end of the masseter lateralis fossa, 6) margin of the orbit at the supraorbital notch or foramen, 7) notch behind the postorbital process, 8) posterior end of the masseter lateralis fossa, 9) anterior end of the glenoid fossa, 10) posterior end of the glenoid fossa, 11) mastoid process, 12) posterior edge at the midline.

asciurus hudsonicus, and a chipmunk, *Tamias striatus)* are included to represent some of the size and dietary diversity found among the closely related outgroups.

The six marmotine species included in this example are only about 1/10 of the extant species recognized by most marmotine taxonomists. Because this analysis includes only a small fraction of the marmotine species. it is unlikely that the results will be an accurate reflection of the marmotine phylogeny. Therefore, the purpose of this demonstration is not to produce a definitive answer to the question of marmotine relationships, but to illustrate the methods that would be used in a more complete analysis. The question to be addressed in the analysis of each shape feature is whether the diversity of shapes is distributed in a way that justifies a specific hypothesis of homology and monophyly.

Shape Analysis. We began the analysis of skull shape by digitizing 12 landmarks on each skull (Figure 8). These landmarks were chosen because they mark prominent aspects of shape that could be compared among taxa. For example, landmark 11, the mastoid process, marks the widest point on the braincase, and with landmark 12 marks the edge of the occipital region. Another important consideration in the selection of landmarks was to use points that are easily recognizable, but not prone to breakage. We did not use the tip of the post-orbital process because this structure is often broken, and we did not use the anterior end of the base of the process because it is smoothly continuous with the margins of the orbit. We did use the notch behind the process, which also represents the antero-medial corner of the temporal fossa. We used the supraorbital and lacrimal notches because both are easy to locate, and because both have consistent positions relative to the orbit. In contrast, we did not use landmarks on the sutures on the snout because the locations of these sutures are quite variable within species and often differ between individuals of the same species with similar snout shapes. Thus, landmarks on these sutures might be useful for describing the shapes of these bones, but would not be very useful for describing the shape of the snout. For similar reasons, we used landmarks on the zygomatic arch that are associated with muscle attachments or the jaw joint and in stable locations around the arch, and did not use sutures of the bones forming the arch. Several of these features are easier to see in lateral view than in dorsal view, so markers were placed in the field of view adjacent to their locations.

To eliminate the effects of asymmetry. landmarks 2-11 were digitized on both sides and shape coordinates were computed for all 22 landmarks using points 1 and 12 to define the baseline (midline). The signs of the y coordinates of landmarks on the right side were reversed, effectively reflecting the right side onto the left. Then, the x and y coordinates of each pair of corresponding landmarks were averaged for each specimen. These 12 pairs of symmetrized shape coordinates and the shape coordinates of the baseline were the input for the thin-plate spline analysis.

For the spline analysis, one specimen of *S. niger* was used as the reference form (or starting form). The symmetrized shape coordinates of each landmark were compared by rank order to identify a specimen that does not have an unusual arrangement of landmarks. Our goal was to find a specimen that has a normal shape for that species, so that the other specimens in the study would be described in terms that referred to the shape of that species. One of the principal advantages of using landmarks is that they attach descriptions of shape to specific locations on the form. Using a reference that is a representative form of one species enhances this advantage by ensuring that the descriptions refer to features of a biological form. This advantage can be enhanced further if the reference has a primitive or juvenile form, which makes it possible to describe the other shapes as modifications of the reference, not simply as different from

the reference. Using a reference that is a mean of several dissimilar species dilutes the advantages of landmarks by allowing shape description to refer to features of an artificial construct that may not represent any biological form. **In** addition, using the mean shape as the reference form means that the starting configuration will change with the addition of each new specimen, whereas using a specific shape as the reference means that specimens can be added to the study and described in the same terms. In our view, changing inferences about patterns of shape evolution should reflect changing hypotheses of what is primitive, not changing sample sizes.

We compared shapes using partial warps scores (Bookstein, 1991), and scores for the uniform component (Bookstein, 1996). Our reasons for using these scores rather than relative warps are related to our reasons for using a specific reference rather than a sample mean. Partial warps describe differences from the reference in terms of features of the reference. Relative warps are principal components of partial warp scores for all the specimens in the study. Like the mean, principal components can change every time

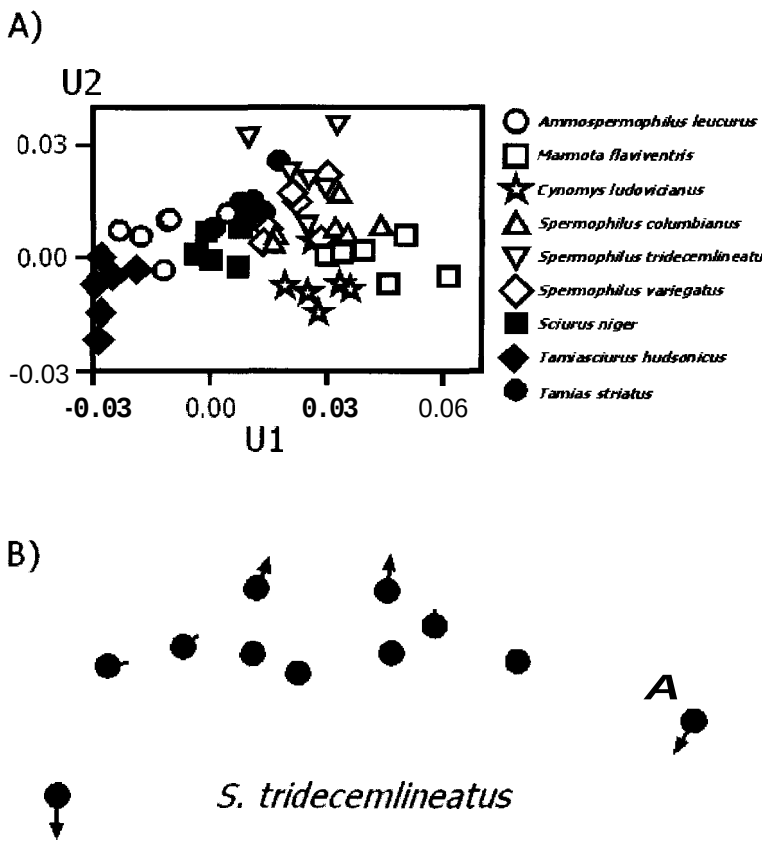

Figure 9. Variation in the component of marmotine skull shape described by the uniform analysis. **A)** scatter-plot of uniform component scores. U1 **is** shear, **U2** is dilation and compression. B) vector diagram of the uniform component of the deformation of the reference into the configuration of a representative specimen of *S. tridecemlineatus.*

specimens are added and deleted. More important, principal components are determined by the patterns of variation and covariation in the sample, so that relative warps are a function of dissimilarity over all the landmarks, over all the specimens. In our view, these features of principal components make relative warps analysis unsuitable for phylogenetic studies because they defeat the purpose of using landmark-based morphometrics. (For more discussion of the issues related to reference choice and the use of partial warps rather than relative warps, the reader is referred to the following papers: Swiderski, 1993; Fink and Zelditch, 1995; Zelditch and Fink, 1995, 1998; Zelditch et al; 1995, 1998; Swiderski et al., 1998)

The programs TPSSPLIN (Rohlf, 1997) and TPSRELW (Rohlf, 1998) can both be used to generate partial warp scores. The reference form used by TPSRELW is a consensus form (a mean form constructed by Procrustes analysis), but the reference used by TPSSPLIN can be any form the user specifies. Because we were using a particular specimen as the reference, we used TPSSPLIN to generate partial warp scores. TPSSPLIN does not compute scores for the uniform component according to Bookstein's (1996) new protocol, so we wrote a program in QBASIC to implement Bookstein's protocol and compute the uniform components of our selected reference, and the scores on those components for each specimen. To illustrate the uniform deformations of a particular specimen, we used the scores to compute the landmark displacements that can be attributed to this component, and we used VECTOR SPECTOR (Humphries, 1994) to draw those displacements. We also used VECTOR SPECTOR to produce vector diagrams of selected non-uniform deformations. Following Bookstein (1991), we have numbered the warps in order of increasing localization, which reflects the order of their computation. Scatter-plots of scores (both uniform and non-uniform components) were produced in SYSTAT. The reference is plotted at the origin of each graph.

Uniform.— This feature describes shearing (U1), in which medial and lateral landmarks are displaced in opposite directions, and dilation-elongation (U2), in which the skull becomes wider and shorter, or longer and narrower (Figure 9). In the scatter-plot of scores for this component, there is a noticeable gap in the distribution of ***A. leucurus*** specimens. Five specimens are on the left with the *T. hudsonicus* cluster, and one ***A. leucurus*** specimen is on the right with the other taxa. If the gap separated all ***A. leucurus*** and *T. hudsonicus* from the others, then we would consider it reasonable to interpret this gap as evidence of evolutionary divergence separating these two groups. We also might interpret this gap as evidence of divergence despite the one unusual specimen of **A.** *leucurus*, if we had reason to dismiss that individual as an outlier. However, there are similar gaps in the distributions of several other species, supporting the inference that sample sizes are too small to judge which specimens are outliers. Because none of the gaps anywhere in this scatter-plot support an unambiguous grouping of species (two or more species on each side of the gap with none spanning the gap), our judgement is that no informative characters can be inferred from this plot.

Warp 1.—As is common for elongate forms, the largest scale warp describes a pattern of landmark displacement in which the landmarks near the center of the form move in one direction and the landmarks near the ends move in the opposite direction (Figure 10). When the landmarks are displaced parallel to the long axis of the form, they produce a gradient of relative elongation in one direction. Thus, negative scores on the *x*-axis (as in *T. hudsonicus)* indicate a longer braincase and shorter snout than in the reference (*S. niger*). Positive scores, which are not found in these taxa except for very low scores in some specimens of *S. niger*, would indicate a shorter braincase and longer snout than in the reference specimen. In *T. stria-*

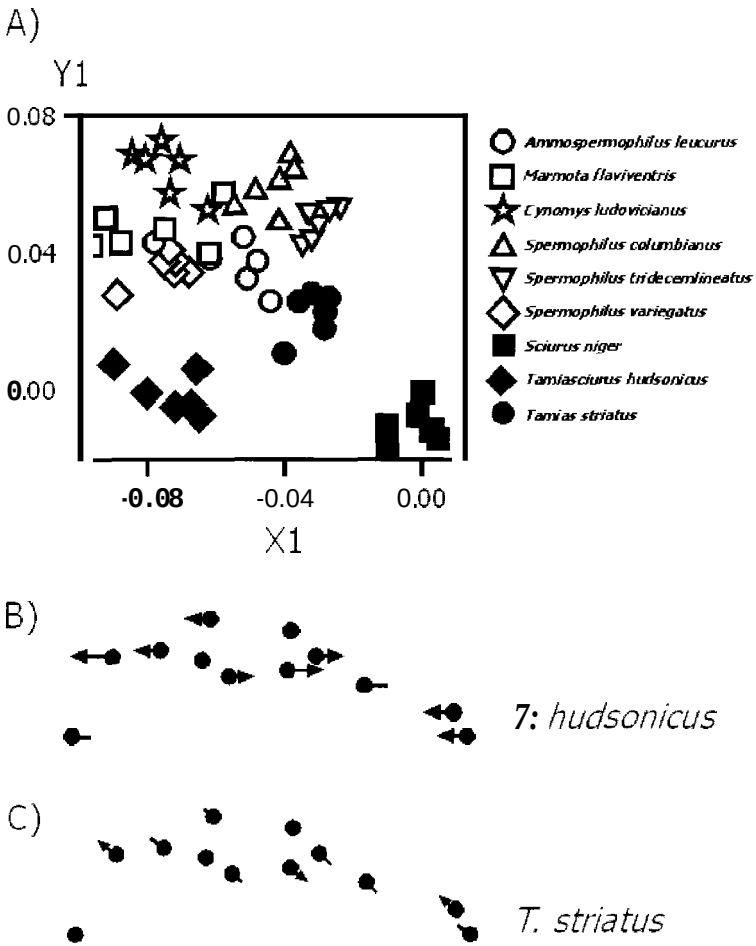

Figure 10. Variation in the component **of** skull ahape described by **warp** 1. A) scatter-plot of partial warp scores. B) vector diagram of the deformation described by partial warp 1 for a representative specimen of *T. hudsonicus*. C) vector diagram of the deformation described by partial warp 1 for a representative specimen of *T. striatus*.

tits and the marmotines, negative scores on the x-axis are combined with positive scores on the y-axis. reflecting the fact that their braincases are wider as well as longer, and their snouts are narrower, as well as shorter. The scatter-plot for this feature shows several species with ranges that do not overlap any other. An especially large gap separates *S. niger* from everything else, smaller gaps separate *T. hudsonicus*, *T. striatus*, *S. tridecemlineatus* and *S. columbianus*. To code this feature, it is necessary to consider whether each of these species is truly distinct from the four species with overlapping ranges. It is also necessary to consider whether any of the species with separate ranges can be grouped together (i.e., can a hypothesis of shared transformation be justified despite their differences).

Of the five non-overlapping species, *S.*

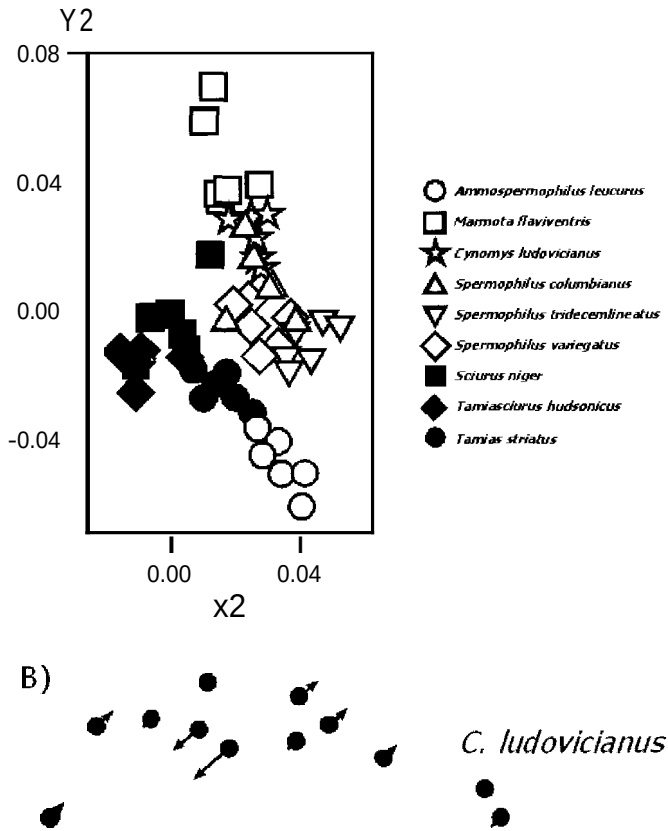

Figure 11. Variation in the component of skull shape described by warp 2. **A)** scatter-plot of partial warp scores with an ellipse enclosing the scores of *S. niger* specimens. B) vector diagram of the deformation described by partial warp 2 for a representative specimen of *C. ludovicianus*.

columbianus and *S. tridecemlineatus* are closest to each other. These two are also the closest to the four overlapping species. In fact, a boundary drawn between *S. columbianus* and the overlapping species would have some rather sharp bends in it, suggesting that *S. columbianus* is not really differentiated from the others. If *S. columbianus* is recognized as divergent, then both *S. tridecemlineatus* and *C. ludovicianus* should be recognized as sharing the same transformation and all three species should be assigned the same character state code. However, one reason for not doing this is the overlap of *M. fluviventris* and *C. ludovicianus*, suggesting these species may not be differentiated. Another obstacle is the fact that a different direction of transformation (+*x*) provides an equally valid justification for assigning a shared character state to *S. tridecemlineatus*, *T. striatus* and *S. niger*. In fact, there are at least two other equally valid, equally narrow dividing lines that could be drawn on this scatter-plot to demarcate groups.

Given the number of conflicting groupings that can be based on this plot, there is good

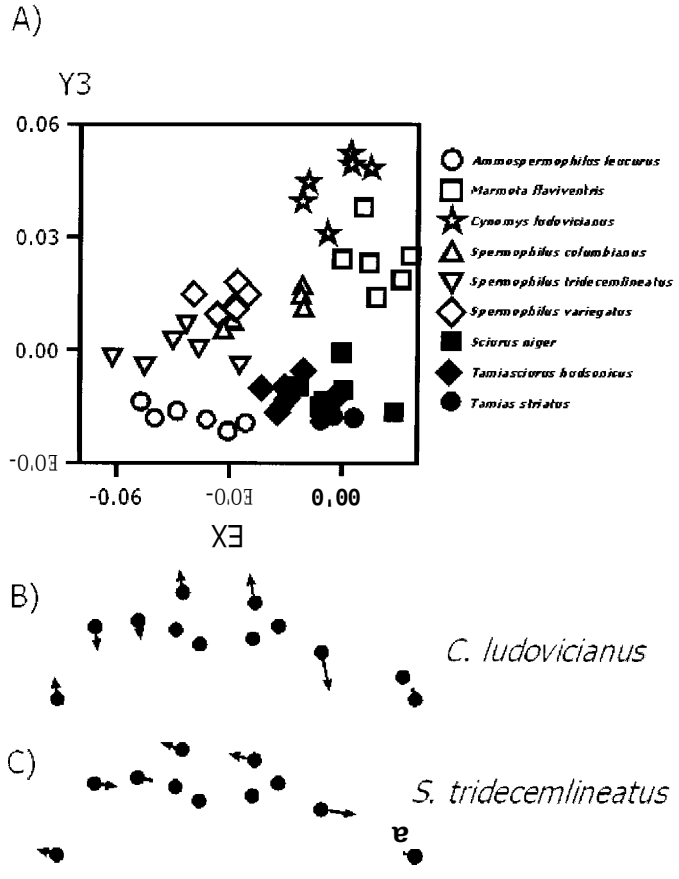

Figure 12. Variation in the component of skull shape described by warp 3. A) scatter-plot of partial warp scores. B) vector diagram of the deformation described by partial warp 3 for a representative specimen of *C. ludovicianus*. C) vector diagram of diagram of the deformation described by partial warp 3 for a representative specimen of *S. tridecemlineatus*.

reason to doubt whether any of them are valid. An additional indication that none of these narrow divisions represents real evolutionary divergence is the fact that there are two or three larger gaps within the distributions of most of the species. Accordingly, our interpretation of the plot is that there are only three character states separated by substantial gaps. One state diagnoses *T. striatus* and all of the marmotines; each of the other two states diagnoses one of the tree squirrels (Table 1). Because there are three states, this trait will be analyzed as unordered.

Warp 2.—This warp is a pattern of landmark displacements which is similar to warp 1, but covers a smaller region of the skull (Figure 11). Thus, the region around the eye and the anterior end of the zygomatic arch is expanded in *C. ludovicianus*, and the region behind the post-orbital process is contracted. In *M. flaviventris*, a larger positive *y*-component reflects its relatively greater width around the anterior end of the zygomatic arch, and also its more triangular braincase. Similarly, large negative scores for the *x*-component in *A. leucurus* reflect the narrowness of its skull near the anterior

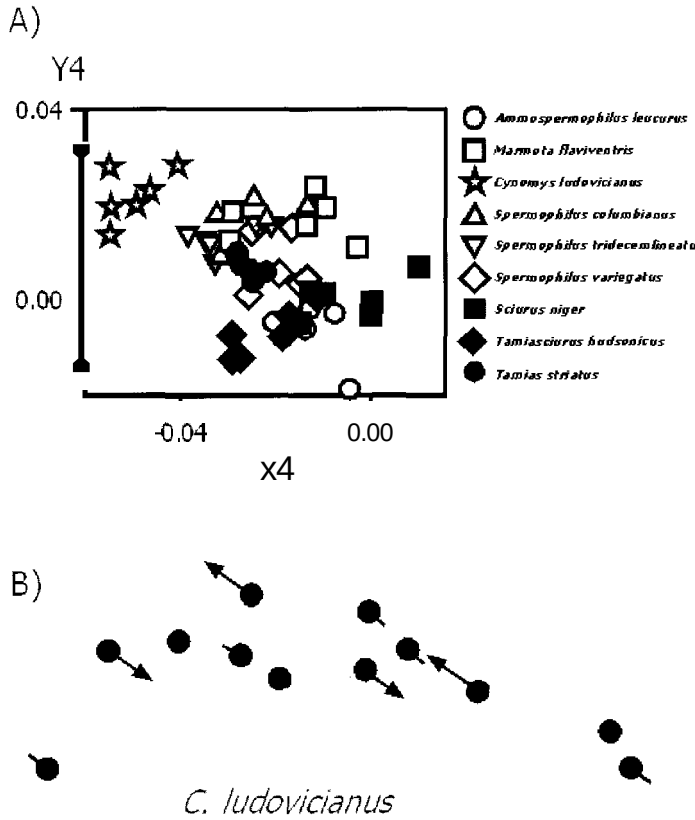

Figure 13. Variation in the component of skull shape described by warp 4. **A)** scatter-plot of partial warp scores. B) vector diagram of the deformation described by partial warp 4 for a representative specimen of C. *ludovicianus*.

end of the zygomatic arch, and also its relatively broad and square braincase.

The scatter-plot for this feature, like that for the uniform, appears to have two distinct clusters of specimens which might reflect evolutionary divergence except for the fact that one species has members in both clusters. Here the gap suggests divergence from the outgroup by all marmotines except ***A.*** *leucurus*. The species that spans the gap is one of the outgroups, *S. niger*. As before, we cannot be certain that one particular individual is an outlier, so we cannot ignore the one specimen of *S. niger* on the right side of the gap. Therefore. our judgement is that no informative characters can be inferred from this plot. either.

Warp 3.—In this feature, the outer (lateral) portion of the zygomatic arch is displaced relative to its ends (Figure 12). In addition, the posterior end of the skull is displaced in the same direction as the outer portion of the zygomatic arch. Transformations of this feature retlect a relatively triangular zygomatic arch and tapered braincase (-*x*), especially in representatives of ***A.*** *leucurus* and *S. tridecemlineatus*, or a relatively square zygomatic arch and narrow braincase (+*y*), as in C. *ludovicianus* and ***M.*** *flaviventris*.

At least three groups can be recognized in

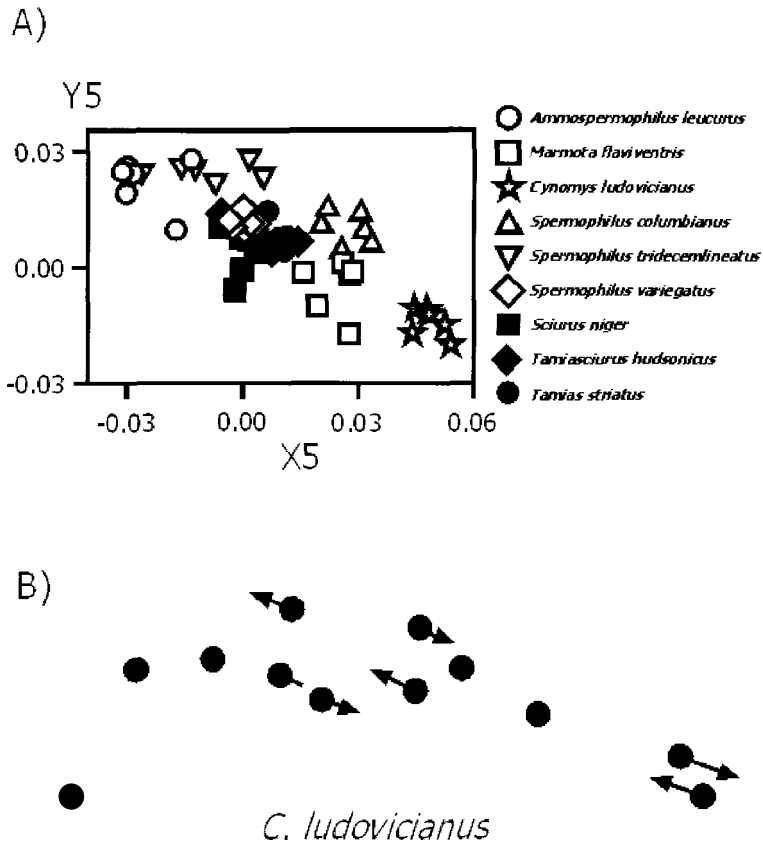

Figure 14. Variation in the component of skull shape described by warp 5. **A)** scatter-plot of partial warp scores. B) vector diagram of the deformation described by partial warp 5 for a representative specimen of C. *ludovicianus*.

this scatter-plot. There is unambiguous separation of ***M. flaviventris*** and C. *ludovicianus* from all other species. Another large gap separates *S. tridecemlineatus*, *S. variegatus* and *S. columbianus* from A. *leucurus* and the outgroups. There is one *S. tridecemlineatus* specimen in this gap, but it is still possible to draw a line between the two groups. **A** third gap separates A. *leucurus* from the outgroups. *S. tridecemlineatus* appears to diverge from *S. variegatus* and *S. columbianus* in the same direction that A. *leucurus* diverges from the outgroups (-*x*), but *S. tridecemlineatus* still overlaps both *S. variegatus* and *S. columbianus*. Without this overlap, *S. tridecemlineatus* might be assigned the same character state as **A.** *leucurus*, or even assigned a unique character state. Because there is overlap here, we have coded this feature as an unordered multistate character with 4 states (Table 1).

Warp 4.—**In** this feature, the largest displacements are at landmarks 3, **6,** 8 and 11 (Figure 13). The large negative *x*-scores in *C. ludovicianus* again reflect the relatively greater angularity of its zygomatic arch. The distribution of scores for this feature has one obvious gap separating C. *ludovi-*

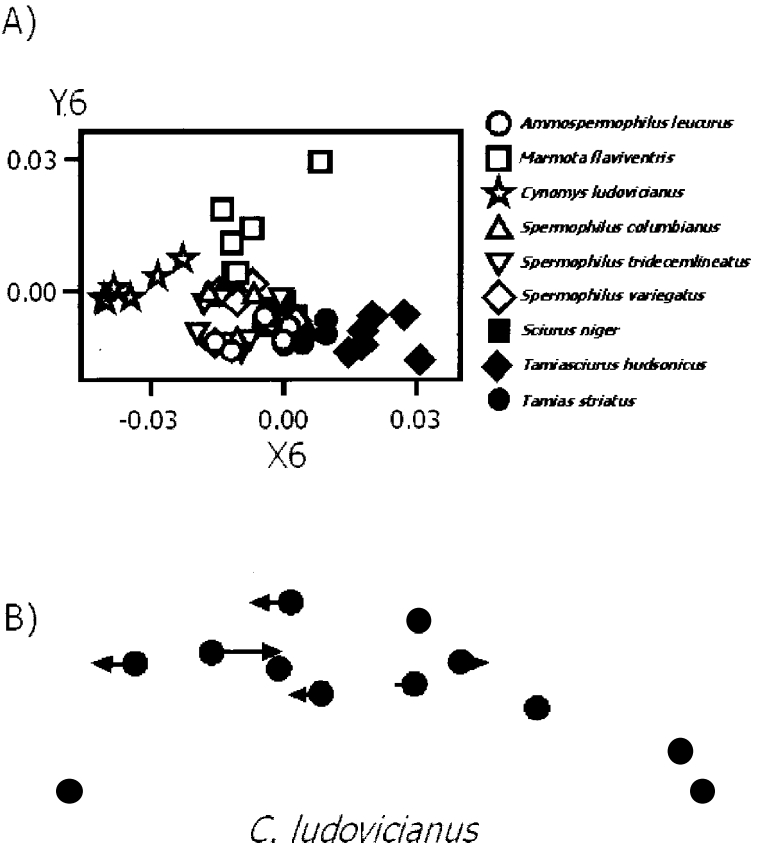

Figure 15. Variation in the component of **skull** shape described by warp 6. A) scatter-plot of partial warp scores. B) vector diagram of the deformation described by partial warp 6 for a representative specimen of *C. ludovucianus*.

cianus from all other species. It is also possible to draw a line separating **A.** *leucurus* and the outgroups from the other taxa. There is no overlap, but there is also more than one specimen responsible for the narrowness of this gap. In addition, the gap is smaller than almost all distances between individuals within species. Consequently, we only recognize the gap separating C. *ludovicianus* as clear evidence of an evolutionary transformation. Because the divergence ***of*** a single species is not phylogenetically informative, we have not included this character in Table 1.

Warp 5.—In this feature, large displacements at landmarks 5, 6. 7 and 8 are combined with contrasting displacements of the landmarks at the tips of the snout (Figure 14). Thus this warp describes changes in which the elongation of the outer portion of the zygomatic arch (further contributing to its relatively greater angularity) are combined with blunting of the snout. Near the center of the scatter plot for this feature is a dense cluster with several species broadly overlapping. Two groups of species appear to diverge from this cluster in two directions. One group includes C. *ludovicianus*,

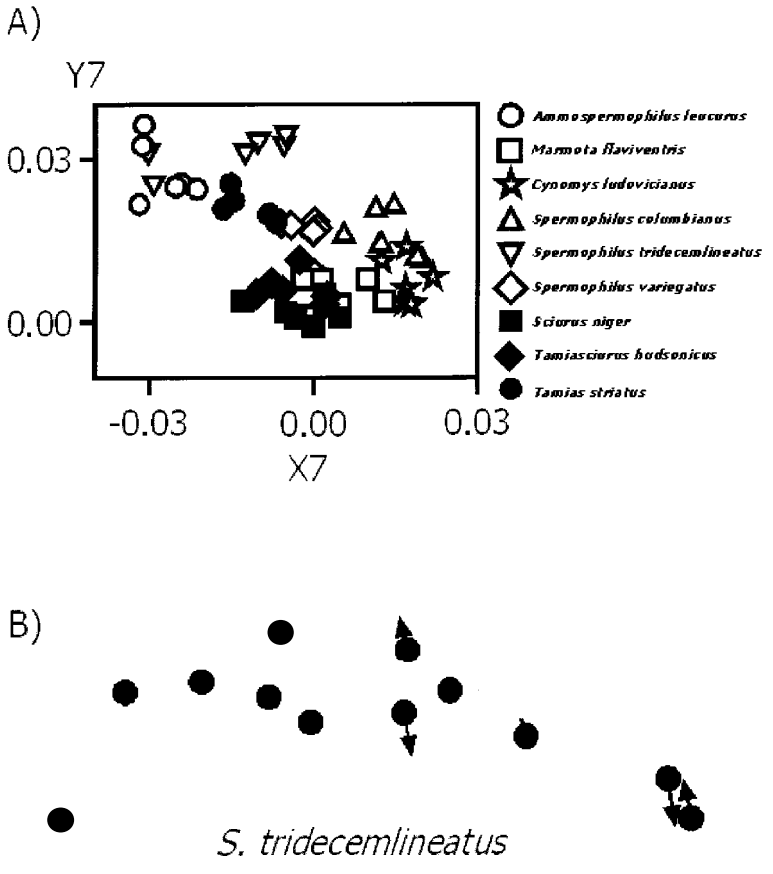

Figure 16. Variation in the component of skull shape described by warp 7. **A)** scatter-plot of partial warp scores. B) vector diagram of the deformation described by partial warp 7 for a representative specimen of *S. tridecemlineatus*.

M. flaviventris and *S. columbianus* (+*x*); the other group includes **A.** *leucurus* and *S. tridecemlineatus* (+*y*). As in the previous feature, there is no overlap between groups, but the difference between groups is less than most differences within species. Again, the only large unambiguous difference is the one separating C. *ludovicianus*, so this phylogenetically uninformative character also is not included in Table 1.

Warp 6.—In this feature, the largest displacements are at landmarks 8, 10 and 11 (Figure 15). The large negative *x* scores for C. *ludovicianus* reflect posterior extension of the zygomatic arch, reduction of the posterior root of the arch, and posterior displacement of the mastoid producing a more squared outline for the braincase. The somewhat smaller positive *x* scores for *T. hudsonicus* primarily reflect a relatively broader posterior root of the zygomatic arch. The scatter-plot for this feature also shows a dense cluster near the center, from which both C. *ludovicianus* and ***T. hudsonicus*** are unambiguously differentiated. Some specimens of *M. flaviventris* have relatively large +*y* scores, but this species is not completely differentiated from the

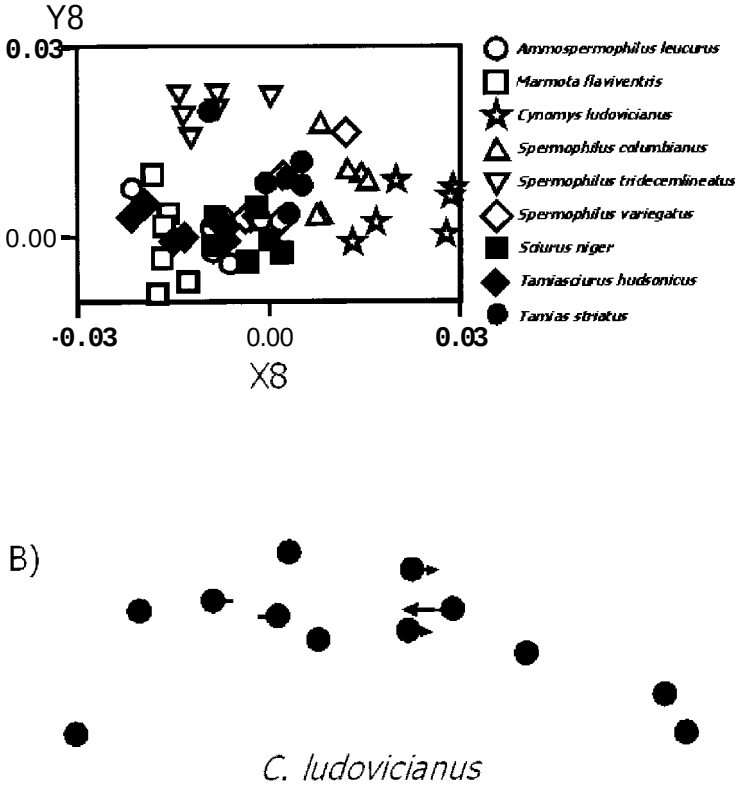

Figure 17. Variation in the component of skull shape described by warp 8. **A)** scatter-plot of partial warp scores. B) vector diagram of the deformation described by partial warp 8 for a representative specimen of C. *ludovicianus*.

central cluster. Thus we have coded this feature as a three-state character in which two states are unique to single species (Table 1).

Warp 7.—In this feature, the largest displacements are at the tip of the snout and near the eye (Figure 16). The positive *y* scores for *S. tridecemlineatus*. *A. leucurus* and *T. striatus* reflect their relatively large eyes and more tapered snouts. The positive *x* scores for C. *ludovicianus* reflect a sharper point at the tip of the snout (but not a general tapering) and a relatively small contraction of the base of the post-orbital process.

At first glance the scatter-plot for this feature appears to have three or four distinct clusters of specimens. Closer examination reveals that each gap runs through the range of at least one species. Thus, the gaps appear to be artifacts of small sample size, not evidence of evolutionary change. In other words, no character state transformations can be inferred from this plot.

Warp 8.—In this feature, there is a large displacement of landmark 4 on the anterior of the orbit, and contrasting displacements of landmarks 5 and 6 on the lateral and medial sides of the orbit (Figure 17). Positive *x* scores for C. *ludovicianus* again reflect greater angularity at the anterior end of the

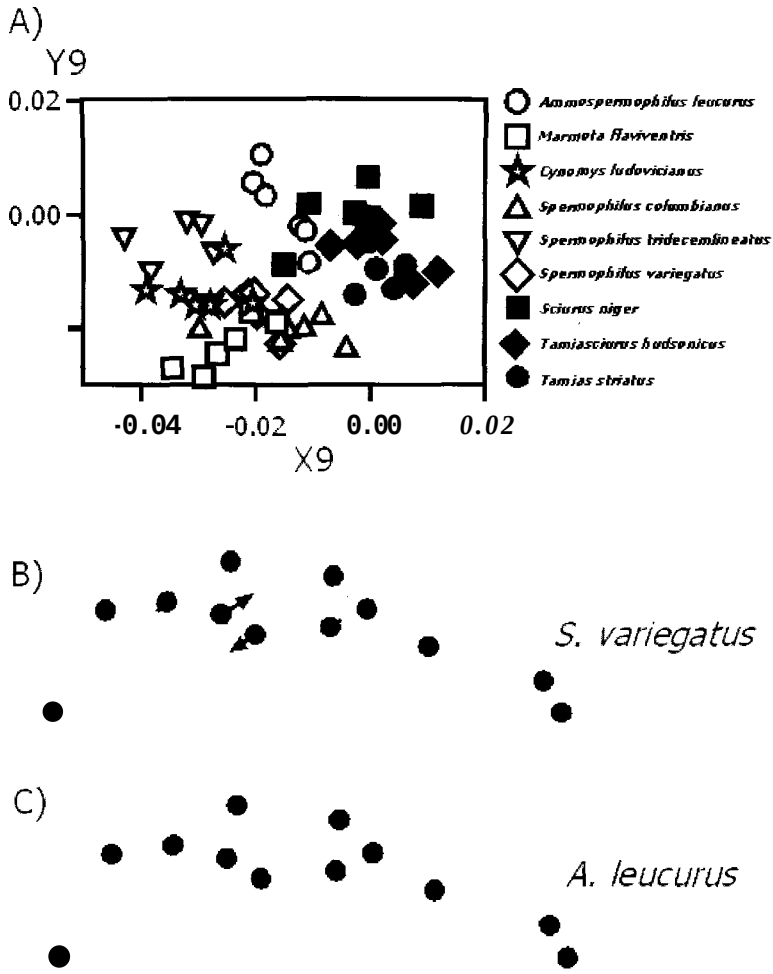

Figure 18. Variation in the component of **skull** shape described by warp 9. **A)** scatter-plot of partial warp scores. B) vector diagram of the deformation described by partial warp 9 for a representative specimen of *S. variegatus*. C) vector diagram of the deformation described by partial warp 9 for a representative specimen of *A. leucurus*.

zygomatic arch (in conjunction with slight reduction of the posterior root of the zygomatic arch). Positive *y* scores for *S. tridecemlineatus* reflect a somewhat square zygomatic arch in these animals as well, but in this case it is due to medio-lateral expansion **of** the anterior end rather than an anterior displacement of the antero-lateral corner.

There is one unambiguous gap separating C. *ludovicianus* from the other taxa. Some individuals of *S. columbianus* have similar scores, but there is considerable overlap between *S. columbianus* and *S. variegatus*. Consequently, *S. columbianus* and *S. variegurus* cannot be differentiated. Similarly, all specimens of *S. tridecemlineatus* have large +*y* scores, but a specimen of *T. striatus* has an equivalent score, so these species also cannot be differentiated. Again, the only large indisputable difference is the one sep-

Table 1 – Data matrix

	W1	w3	W6	w9
S. niger	1	*0*	*0*	0
***T.** hudsonicus*	*2*	*0*	1	0
T. striatus	0	*0*	*0*	0
A. *leucurus*	*0*	1	*0*	0
M. flaviventris	*0*	*3*	*0*	1
S. variegatus	*0*	***2***	*0*	**1**
S. tridecemlineatus	0	*2*	*0*	I
S. columbiunus	***0***	*2*	*0*	**1**
C. ludovicianus	***0***	*3*	*2*	I

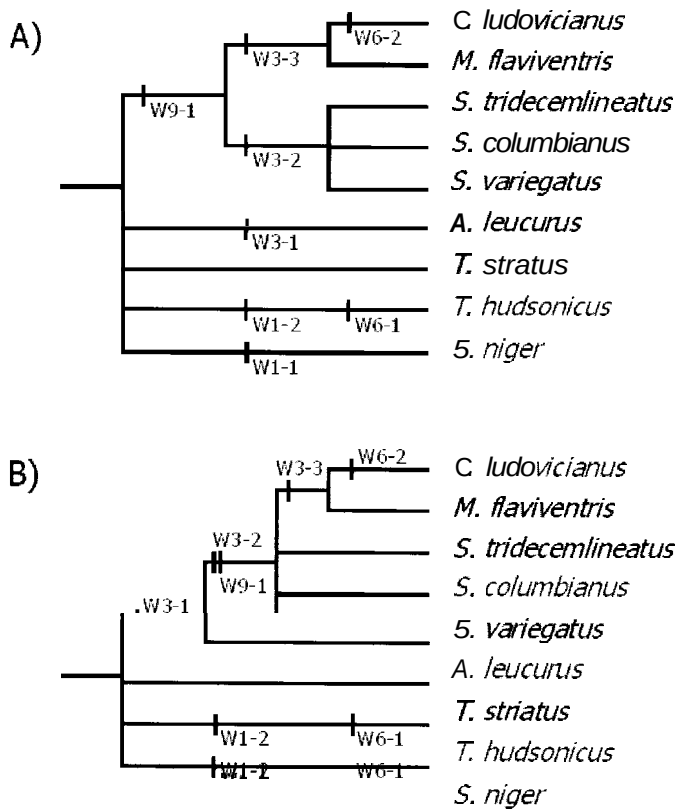

Figure 19. Cladograms showing the phylogenetic relationships that can be inferred from this analysis of marinotine skull shapes. **A)** Character W3 interpreted as diagnosing three evolutionarily independent groups. B) Character W3 interpreted as diagnosing three sequentially nested groups.

arating *C. ludovicianus*, so this phylogenetically uninformative character also is not included in Table 1.

Warp 9.—This feature describes contrasting displacements of landmarks 7 and 9 (Figure 18). In *S. variegatus* and most other marmotines, negative scores on both *x*- and *y*-axes reflect their relatively narrower and deeper notch behind the post-orbital process. In some **A.** *leucurus* and *S. tridecemlineatus*, the *y* scores are nearly zero, indicating that the notches of these specimens are simply narrower.

The scatter-plot for this feature shows a gap separating most of the marmotines from ***A.*** *leucurus* and the outgroups. In most places this is a rather broad gap, relative to the distances between individuals within species. Only one specimen of *S. niger* intrudes into this gap, but does not cross it. Accordingly, we have coded this feature as a two-state character with **A.** *leucurus* and the outgroups sharing state 0 and all other taxa sharing state 1 (Table 1).

Phylogeneiic analysis. Table 1 lists the character state codes for all 9 taxa for the four features that could be coded. Because there are so few characters, the relationships of these six taxa cannot be completely resolved. However, it is possible to extract some information by rooting the tree among the outgroups, as suggested by previous studies of marmotine phylogeny (Bryant, 1945; Black, 1963; Hight et al., 1974: Ellis and Maxson, 1980; Hafner, 1984). Based on this rooting. warp 9 can be interpreted as supporting a monophyletic group that includes all marmotines except ***A.*** *leucurus*. Within this group, two subgroups with different states for warp 3 can be recognized. Using only the evidence at hand, it is not possible to determine whether one or both groups are monophyletic; different trees would be inferred from different interpretations of the relationships of the warp's character states. Figure 19A shows the relationships that would be inferred if state 0 is considered primitive and states 1, 2 and 3 each diagnose a separate lineage. Figure 19B shows the phylogenetic relationships that would be inferred if the character states are ordered from 0 to 3, with each derived state diagnosing a progressively smaller group. Several other trees are equally plausible. Because this analysis is based on only a small portion of the species in the Marmotini, and because each species is represented by only six specimens, we do not view Figure 19 as a meaningful statement of marmotine relationships. Considerably more work will be needed before we have a clear picture of marmotine relationships and the evolutionary history **of** skull shape in this group.

DISCUSSION

On the surface, phylogenetic analysis of qualitatively scored traits simply analyzes the distribution of coded character states and identifies the tree that implies the fewest changes between states. However, if this analysis is performed within the Hennigian paradigm, the states and the tree have deeper meanings. In this conceptual framework, the states represent initial hypotheses of homology and monophyly proposed to explain the diversity of traits in the taxa under investigation. and the tree represents the branching pattern that requires the fewest ad hoc hypotheses to resolve conflicts among the initial hypotheses (i.e., the most parsimonious tree). Because the character states encode hypotheses that explain diversity, the analysis of their distributions to identify the most parsimonious tree is logically separate and distinct from the analysis that describes the diversity. It is this disjunction between the phylogenetic analysis and the morphological analysis that allows systematists to score morphological features as categorical variables and compare them as logically equivalent. Coding is not a statement that two differences are equivalent evolutionary changes (e.g., addi-

tion of a fold on a tooth and fusion of two wrist bones); rather, it is a statement of a hypothesis that they are equivalent indicators of phylogenetic relationships. The same logic means that quantitatively described traits can be coded to reflect hypotheses about their evolution, and that doing so requires more than simply rescaling the original measures.

To apply the logic of the Hennigian approach, the descriptions of the traits must meet certain requirements. One important requirement is that the traits must be described in enough detail that it is possible to judge whether they refer to comparable features in different organisms (Pimentel and Riggins, 1987; Zelditch et al., 1995). Only if the features are comparable does it make sense to attribute differences to evolutionary transformations, and to attribute similarities to a single transformation in a common ancestor. In other words, coding can only be a rational hypothesis of transformation when there are grounds for interpreting similarities and differences in terms of descent with modification.

Partial warps decomposition of the thin-plate spline and the new formula for describing the uniform component both provide the necessary grounds for coding (Zelditch et al., 1995; Swiderski et al., 1998; Zelditch et al., 1998). This is because these components describe specific patterns of landmark displacement. Consequently, the scores of any particular component reflect the variability of a particular region of the reference form. If that reference is a single individual or an average of individuals from a single species (preferably representing a single age class), then the region is a feature of an organism, and the diversity in shape can be interpreted in terms of descent with modification. Thus, partial warp analysis and the uniform analysis of an appropriate reference form provide descriptions of shape differences that can legitimately be used in a cladistic analysis of phylogenetic relationships.

In our analysis of skull shape in marmotines, we used one individual from one of the outgroups as a starting form. The shapes of all the other individuals were described in terms of differences from the reference form (i.e., non-zero scores on the partial warps). Then we proposed hypotheses interpreting these scores as evidence of a change in the underlying morphology, but only if we judged that the scores could be sorted into two or more distinct groups. For example, partial warp 3 describes a pattern of landmark displacement involving large movements at six landmarks on the zygomatic arch and posterior of the braincase. The scores for this feature indicated considerable diversity in the ways in which individual specimens differ from the reference with respect to the relative positions of these landmarks. We then moved from the morphometric analysis to the first steps of the phylogenetic analysis. Based on the scores, we inferred that there was an evolutionary transformation of the underlying anatomical structures (the zygomatic arch and braincase) in which the lineages leading to *C. ludovicianus* and *M. flaviventris* diverged from the lineages leading to the other species. In addition, we inferred from the similarity of their scores that this transformation occurred in the common ancestor of *C. ludovicianus* and *M. flaviventris*, and that none of the other species in this study are derived from that ancestor. Because none of the other data at hand contradicts that interpretation, our phylogenetic tree (based only on these data) suggests that *C. ludovicianus* and *M. flaviventris* represent a monophyletic group.

Thus, the methods of geometric morphometrics are powerful tools for recognizing differences among biological shapes. This does not mean that the shape differences described using these methods can be equated automatically with descriptions of the historical evolutionary transformation. A phylogenetic analysis of the observed differ-

ences is needed to infer the history of shape change. This caveat is not unique to the methods of geometric morphometrics. Rather, the unique feature of some of these methods is that their descriptions of shape differences can be used in a subsequent analysis, which proposes and evaluates hypotheses of evolutionary change. When used in this way, geometric morphornetric analyses can play an important role in studies of morphological evolution and phylogenetic relationships.

References

Adams, D. C. and Rosenberg, M. S., 1998. Partial warps, phylogeny, and ontogeny: A comment on Fink and Zelditch (1995). Syst. Biol.. 47: 168-173.

Bales, C. S., 1996. Skull evolution in the Rhinocerotidae (Mammalia, Perissodactyla): Cartesian transformations and functional interpretations. J. Mamm. Evol., 3: 261-279.

Black, C. C., 1963. A review of the North American Tertiary Sciuridae. Bull. Mus. Comp. Zool., 130: 109-248.

Bookstein, F. L., 1990. Introduction to methods for landmark data. In: Rohlf, F. J. and Bookstein, E L. (eds.), Proceedings of the Michigan Morphometrics Workshop, Special Publication Number 2. The University of Michigan Museum of Zoology, Ann Arbor: 215-225

Bookstein, F. L., 1991. Morphometric Tools for Landmark Data: Geometry and Biology. Cambridge University Press, New York.

Bookstein, F. L., 1994. Can biometrical shape be a homologous character? In: Hall, B. K. (ed.), Homology: The Hierarchical Basis of Comparative Biology. Academic Press, New York: 197-227

Bookstein, F. L., 1996. Standard formula for the uniform shape component in landmark data. In: Marcus. L. F., Corti, M., Loy, A., Naylor, G. J. P. and Slice, D. E., (eds.), Advances in Morphometrics, NATO ASI Series. Series A, Volume 284. Plenum, New York: 153-168

Bookstein, F. L., Chernoff, B., Elder, R., Humphries, J., Smith, G. and Strauss, R., 1985. Morphometrics in Evolutionary Biology. Academy of Natural Sciences, Philadelphia.

Bryant, M. D., 1945. Phylogeny of the Nearctic Sciuridae. Amer. Mid. Natur., 33: 257-390.

Capanna, E.. Bekele, A., Capula, M.. Castiglia, R., Civitelli, M. V., Codjia, J. T. C., Corti, M. and Fadda, C., 1996. A multidisciplinary approach to the systematics of the genus *Arvicanthis* Lesson, 1842 (Rodentia, Murinae). Mammalia, 60: 677-696.

Courant, F., David, B., Laurin, B. and Chaline. J., 1997. Quantification of cranial convergences in arvicolids (Rodentia). Biol. J. Linn. Soc., 62: 505-517.

Ellis, L. S. and Maxson, L. R., 1980. Albumin evolution within New World squirrels (Sciuridae). Amer. Mid. Natur., 104: 57-62.

Emry, R. J. and Thorington, Jr., R. W., 1982. Descriptive and comparative osteology of the oldest fossil squirrel, *Protosciurus* (Rodentia: Sciuridae). Smithsonian Contributions to Paleobiology, 47: 1-35.

Farris, J. S., 1983. The logical basis of phylogenetic analysis. In: Platnick, N. I. and Funk, V. A. (eds.), Advances in Cladistics, volume 2: Proceedings of the second meeting of the Willi Hennig Society. Columbia University Press, New York: 7-36

Farris, J. S., 1986. On the boundaries of phylogenetic systematics. Cladistics, 2: 14-27.

Farris, J. S., 1990. Phenetics in camouflage. Cladistics, 6: 91-100.

Felsenstein. J., 1978. Cases in which parsimony and compatibility methods will be positively misleading. Syst. Zool.. 27: 401-410.

Felsenstein, J., 1988. Phylogenies and quantitative characters. Ann. Rev. Ecol. Syst., 19: 445-471.

Fink, W. L. and Zelditch, M. L., 1995. Phylogenetic analysis of ontogenetic shape transformations: a reassessment of the piranha genus *Pygocentrus* (Teleostei). Syst. Biol., 44: 343-360.

Garland, Jr., T., and Adolph, S. C., 1994. Why not to do two species comparative studies: Limitations on inferring adaptation. Physiol. Zool., 67: 797-828.

Gift, N. and Stevens, P. F., 1997. Vagaries in the delimitation of character states in quantitative variation—an experimental study. Syst. Biol., 46: 112-125.

Hafner, D. J., 1984. Evolutionary relationships of the Nearctic Sciuridae. In: Murie, J. O. and Michener, G. R. (eds.), The Biology of Ground-Dwelling Squirrels. University of Nebraska Press, Lincoln, Nebraska: 3-23

Hecht. M. K. and Edwards, J. L., 1976. The determination of parallel or monophyletic relationships: the proteid salamanders—a test case. Amer. Natur., 110: 653-677.

Hennig. W., 1966. Phylogenetic Systematics. University of Illinois Press, Urbana.

Hight, M. E.. Goodman, M. and Prychodko. W., 1974. Immunological studies of the Sciuridae. Syst. Zool., 23: 12-25.

Howell, A. H., 1938. Revision of the North American ground squirrels. with a classification of the North American Sciuridae. North American Fauna, 56: 1-256.

Humphries, J., 1994. VECTOR SPECTOR, beta version, 1.00a. Cornell University, Ithaca, New York.

Janis, C. M. and Ehrhardt, D., 1988. Correlation of relative muzzle width and relative incisor width with dietary preference in ungulates. Zool. J. Linn. Soc., 92: 267-284.

Loy, A., Cataudella, S. and Corti, M., 1996. Shape changes during the growth of the sea bass, *Dicentrarchus labrax* (Teleostea: Perciformesj, in relation to different rearing conditions. An application of thin-plate spline regression analysis. In: Marcus. L. F., Corti, M., Loy, A., Naylor, G. J. P. and Slice. D. E., (eds.), Advances in Morphometrics, NATO ASI Series. Series A, Volume 284. Plenum, New York: 399-405

Mabee. P. M., 1993. Phylogenetic interpretation of ontogenetic change: sorting out the actual and artefactual in an empirical case study of centrarchid fishes. Zool. J. Linn. Soc., 107: 175-291.

MacFadden. B. J., 1992. Fossil Horses: Systematics, Paleobiology and Evolution of the Family Equidae. Cambridge University Press, Cambridge, U.K.

Mickevich, M. F. and Weller, S. J., 1990. Evolutionary character analysis: tracing character change on a cladogram. Cladistics, 6: 137-170.

Neff, N. A., 1986. A rational basis for a priori character weighting. Syst. Zool.. 35: 110-123.

Pimentel, R. A. and Riggins, R., 1987. The nature of cladistic data. Cladistics, 3: 201-209.

Radinsky, L. B., 1982. Evolution of skull shape in carnivores. 3. The origin and early radiation of the modern carnivore families. Paleobiology, 8: 177-195.

Rohlf, F. J., Loy, A. and Corti, M., 1996. Morphometric analysis of Old World Talpidae (Mammalia. Insectivora) using partial warp scores. Syst. Biol., 45: 344-362.

Rohlf, F. J., 1997. TPSSPLIN, version 1.15. SUNY at Stony Brook, Stony Brook, New York.

Rohlf, F. J., 1998. On applications of geometric morphometrics to studies of ontogeny and phylogeny. Syst. Biol., 47: 147-158.

Rohlf, F. J., 1998. TPSRELW, version 1.17. SUNY at Stony Brook. Stony Brook, New York.

Sæther, O. A., 1986. The myth of objectivity—Post-Hennigian deviations. Cladistics, 2: 1-13.

Swiderski, D. L., 1993. Morphological evolution of the scapula in tree squirrels, chipmunks and ground squirrels (Sciuridae): an analysis using thin-plate splines. Evolution, 47: 1854-1873.

Swiderski, D. L., Zelditch, M. L. and Fink, W. L., 1998. Why morphometrics is not special: coding quantitative data for phylogenetic analysis. Syst. Biol., 47: 508-519.

Taylor, P. J. and Contrafatto, G., 1996. Mandible shape and size in three species of musk shrews (*Crocidura* Wagler, 1832) from southern Africa. Mammalia, 60: 753-765.

van Dam, J., 1996. Stephanodonty in fossil murids. A landmark-based approach. In: Marcus, L. F., Corti, M., Loy, A., Naylor, G. J. P. and Slice, D. E., (eds.), Advances in Morphometrics, NATO ASI Series. Series A, Volume 284. Plenum, New York 449-478.

Winterbottom, R., 1990. The *Trimmatonz nannus* species complex (Actinopterygii, Gobiidae): phylogeny and progenetic heterochrony. Syst. Zool., 39: 253-265.

Zelditch, M. L., Bookstein, F. L. and Lundrigan, B. L., 1992. Ontogeny of integrated skull growth in the cotton rat *Sigmodon fulviventer*. Evolution, 46: 1164-1180.

Zelditch. M. L. and Fink, W. L., 1995. Allometry and developmental integration of body growth in a piranha, *Pygocentrus nattereri* (Teleostei: Ostariophysi) J. Morphol., 223: 341-355.

Zelditch. M. L. and Fink, W. L., 1998. Partial warps, phylogeny and ontogeny: a reply to Adams and Rosenberg. Syst. Biol., 47: 344-348.

Zelditch, M. L., Fink, W. L. and Swiderski, D. L., 1995. Morphometrics, homology and phylogenetics: Quantified characters as synapomorphies. Syst. Biol., 44: 179-189.

Zelditch, M. L., Fink. W. L., Swiderski, D. L. and Lundrigan. B. L.. 1998. On applications of geometric morphometrics to studies on ontogeny and phylogeny: a reply to Rohlf. Syst. Biol., 47: 158-166.

APPENDIX 1 – LIST OF SPECIMENS.

All specimens are from the University of Michigan Museum of Zoology, Mammal Division. m = male, f = female, ? = unknown.

OUTGROUPS

Sciurus niger: USA, N. Carolina, Anson Co.: 123729, f. USA, N. Carolina, Craven *Co.:* 123565, m, 123731, m. USA, N. Carolina, Duplin Co.: 123566, f. USA, N. Carolina, Hoke Co.: 123733, f. USA, S. Carolina, Georgetown *Co.,* 125705, m.

Tamiasciurus hudsonicus: USA, Michigan, Clare Co.: 85195, m. USA, Michigan, Iosco Co.: 85202, f. USA, Michigan, Presque Isle Co.: 86232, f. USA, Michigan. Van Buren Co.: 82640, f. USA, Michigan, Washtenaw Co.: 79823, m, 79824, m.

Tamias striatus: USA, Michigan, Gogebic Co.: 53592, f. USA, Michigan, Chippewa Co.: 126668, m. USA, Michigan, Mackinac Co.: 162429, m; 162432, m; 162433, m; 162434, f.

MARMOTINES

Ammospermophilus leucurus: USA, California, Inyo Co.: 108235. m; 108236, m; 108237, f; 108243, m; 108245, m; 108246, f.

Cynomys ludovicianus: USA, Kansas, Ness Co.: 67352, m; 67354, ?. USA, Nebraska, Sheridan Co.: 75513, m. USA, New Mexico. Quay Co.: 108049 f. USA, S. Dakota. Custer Co.: 96071, m; 97078. f.

Marmota flaviventris: USA, Idaho, Butte Co.: 78814, f; 78816, m; 78817, f. USA, Idaho, Fremont Co.: 162546 f. **USA,** Montana, Ravalli Co.: 57974, f. USA, Montana, Sweet Grass Co.: 87343, f.

Spermophilus columbianus: Canada. Alberta, Rio Alto Ranch (50°34' N, 114°20' W): 158291, 158294, 158295, 158302, f; 158303, f. Canada, Alberta, Hailstone Butte (50°12'N, 114°27'W): 158460 f.

Spermophilus tridecemlineatus: USA, Iowa, Crawford Co.: 162866, f; 162872, f; 162873, m; 162875, f; 162878. f; 162879, m.

Spermophilus variegatus: USA, Arizona, Cochise Co.: 66337, m; 66338, f; 66340. m; 77493, f; 77494, m; 77495, m.