

Getting into shape: An empirical comparison of traditional truss-based morphometric methods with a newer geometric method applied to New World cichlids

Kevin J. Parsons^a, Beren W. Robinson^a & Tomas Hrbek^b

^aDepartment of Zoology, University of Guelph, Guelph, Ontario, N1G 2W1, Canada

(e-mail: parsons@uoguelph.ca)

^bCurrent address: Department of Anatomy and Neurobiology, Washington University, St. Louis, MO 63130, U.S.A.

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Synopsis

Body shape is a difficult, but important, trait to quantify. Researchers have traditionally used multivariate analysis of several linear measures ('trusses') across the body form to quantify shape. Newer geometric morphometric methods claim to better estimate shape because they analyze the geometry among the locations of all landmarks simultaneously rather than the linear distances between pairs of landmarks. We tested this claim by comparing the results of several traditional morphometric analyses against a newer geometric analysis involving thin-plate splines (TPS), all applied to a common data set of morphologically variable new world cichlids *Amphilophus citrinellus* and *A. zalius*. The TPS method yielded slightly stronger evidence of morphological differences among forms, although traditional methods also distinguished the two species. Perhaps our most important result was the idiosyncratic interpretation of shape variation among the traditional truss-based methods, whereas the generation of deformation grids using the TPS approach yielded clear and visually interpretable figures. Our results indicate that geometric morphometrics can be a more effective way to analyze and interpret body form, but also that traditional methods can be relied upon to provide statistical evidence of shape differences, although not necessarily accurate information about the nature of variation in shape.

Introduction

Understanding the origins, maintenance and consequences of variation is a fundamental part of biological research, and requires that variation be both precisely and accurately estimated. Complex variation associated with body form is one of the most difficult types of variation to quantify, and the methods used to assess it are collectively referred to as morphometrics. These methods are concerned with quantifying shape variation within and among samples usually to address developmental and evolutionary questions relating to shape change during growth,

across experimental treatments, or among different populations (Rohlf & Marcus 1993). Methods for comparing biological forms range from classic verbal and pictorial representations (Thompson 1917), methods involving the tracing of outlines (Zelditch et al. 1995, Loy et al. 2000), lists of measured linear distances between pairs of identifiable landmarks on an organism (e.g., Robinson et al. 1993), and newer methods involving the geometric locations of landmarks (e.g., Walker & Bell 2000). Our goal is to both illustrate and to compare some of the more commonly used traditional linear-based methods to one of the newer geometric methods. To do this, we analyze the body form of

cichlids sampled from a Nicaraguan lake using a variety of morphometric methods, as opposed to a mathematically formal analysis of different methods involving a set of completely parameterized but abstract and often simpler objects (reviewed in Adams et al. 2003).

The most common approaches, referred to as 'traditional' morphometrics, are only a few decades old (Marcus 1990, Rohlf & Marcus 1993, Adams et al. 2003), and typically apply multivariate statistical methods (e.g., principal components analysis, canonical variate analysis, discriminant function analysis, or multivariate analysis of variance) to a set of variables measured on each individual. Frequently, these variables are linear distances, often called 'trusses' (Strauss & Bookstein 1982), measured between pairs of landmarks on the body, or body parts, of each individual (angles among sets of three landmarks are also sometimes used; Meyer 1987). Most landmark-based approaches involve identifying points that are homologous across the variant forms included in the study.

Increased computing power drove the evolution of traditional morphometrics in the 1960s and 1970s that permitted the simultaneous analysis of multiple traits, and so was an obvious improvement over univariate approaches (e.g., Jolicoeur 1963). We do not review the thousands of traditional morphometric studies in the fish literature except to acknowledge that this work has substantially contributed to our understanding of the causes and consequences of variation in fish form. However, limitations relating to these traditional methods became increasingly obvious and spurred a 'revolution' in morphometrics during the 1980s and 1990s characterized by a shift in focus to the relative geometric positions of landmarks (Rohlf & Marcus 1993, Bookstein 1996, Adams et al. 2003).

Five basic limitations drove the most recent morphometric revolution. First, linear truss lengths are generally strongly positively related to body size. Different methods of removing the effect of size variation from shape variation are possible but these can lead to different estimates of subsequent 'shape' variation (Bookstein et al. 1985), and no standard size adjustment method was ever developed (e.g., Burnaby 1966, Humphries et al. 1981, Reist 1985, Marcus 1990). The size problem is inherent to any data derived from landmarks including the newer geometric approaches, because the geometric coordinates of landmarks are influenced by body size. Second, variation among samples becomes more difficult to assess when homologous landmarks do not define trusses. For example, the trait 'maximum body depth' may not be in a

homologous location on all specimens in a study. One obvious solution to this problem is to only include homologous trusses (defined by homologous landmarks) in the analysis, although this was not always strictly followed. The problem of missing data or absent landmarks is also a persistent problem in morphometric studies. Third, different shapes can yield identical sets of truss lengths because the locations of trusses relative to each other are not quantified. For example, the maximum length and width measurements taken from certain diamond and triangle forms can be identical, indicating that truss-based shape information can potentially ignore important elements of shape information. It was left to the researcher to 'correctly' identify and justify the elements of shape in their analysis. Fourth, the exclusion of potentially important geometric shape information suggests that there can be reduced statistical power to distinguish variation among samples. Lastly, the only quantitative visual representations of shape involved multiple scatter plots or histograms of either numerous univariate measures or statistically derived multivariate measures. There was no way to reconstitute and represent the complete body form(s) assessed using many component trusses. Describing and synthesizing statistical shape parameters is difficult, and our personal experiences suggest that it includes the risk of subjective interpretation. The central problem is that truss-based data does not necessarily provide much information about the relative relationships among all trusses or their landmarks.

The problem of body size

All landmark-based morphometric methods face the fundamental challenge of removing variation in size from variation in shape. Traditional morphometrics uses one of three general approaches to try to isolate shape from size variation (Humphries et al. 1981, Bookstein et al. 1985): ratios, regression, and multivariate factor or component analysis. The ratio approach takes the value of each truss and divides it by some measure of body size usually in the same scale, and is still a common approach in some fields of biology. It can be adequate for addressing simple questions, such as describing variation within a group on one or a few variables. However, statistical comparisons among groups using ratios (or other simple arithmetic size correction approaches) can be misleading and can result in reduced statistical power (Atchley et al. 1976). Ratios ignore potentially substantial differences among groups in the component variables, and can be

indifferent to allometric differences among populations (ANCOVA using size as a covariate is a better alternative). Bookstein et al. (1985) argued that ratios applied to single traits before multivariate analysis should be avoided because they can result in spurious estimates of shape variation. The problem is that different 'size' parameters (used as the denominator in the ratio) can have large effects on the patterns of variances and covariances among traits. For these reasons, we do not include ratios in our analyses below.

A second common method for removing size effects involves the regression of each truss against a measure of body size and estimating residual truss variation. Residual variation obtained by regression methods again depends on the often-arbitrary choice of a body size variable (such as mass or various possible body length measurements). As a result, residual variation in trusses can still contain size information, depending both on the choice of the body size covariate and the truss under analysis (Bookstein et al. 1985). Performing regressions within populations and using the residual variation in comparisons among populations also assumes that trait allometry is consistent among populations, which should either be explicitly tested or else avoided.

Lastly, a multivariate approach generally using principal components analysis (PCA) has also been used in an attempt to distinguish separate size and shape components. While PCA is traditionally expected to sequester size variation in the first principal component, it can also include significant amounts of shape variation in the first 'size' component and size variation in subsequent components (Marcus 1990). Attempts to modify PCA in order to solve these problems led Humphries et al. (1981) to propose the sheared PCA method. A 'shear' or rotation is applied to PC2 (and other subsequent components) in order to remove variation from that component that is correlated with variation in PC1. The shear is achieved by estimating a mean 'within groups' size vector for all groups standardized to a common centroid, and using this to adjust variation in subsequent components so that it is uncorrelated with respect to the variation on PC1 (see figure 4.3.1 in Bookstein et al. 1985). The original discriminatory information present in the conventional principal components is retained, except that the variation present in the modified components is unrelated to the variation present in PC1. While the shear method removes the mean within-group component of size variation from subsequent components, it ignores possible differences in the form of size variation that may

exist among different groups. The Burnaby (1966) method is an alternative to SPCA that discriminates a principal size component for each group separately, and then estimates shape variation among groups that is uncorrelated with (or orthogonal to) every group's principal size vector. None of the multivariate methods explicitly exclude shape variation from the component that reflects size variation, and so potentially discard unknown amounts of shape variation (Bookstein et al. 1985).

Geometric methods

As the name implies, geometric morphometric methods focus on the geometry of form estimated using the relative locations of landmarks (and sometimes outlines) rather than on linear measurements taken between landmarks. The number of publications using geometric methods has increased exponentially, and now exceeds 100 per year (see figure 1, Adams et al. 2003). This has been assisted by the availability of increasingly user-friendly freeware computer programs and supporting information,¹ reviews of the methods (e.g., Rohlf & Bookstein 1990, Rohlf & Marcus 1993, Adams et al. 2003) and the availability of training workshops. The raw data usually takes the form of two-dimensional Cartesian grid coordinates of landmark points that are homologous among specimens. As in traditional methods, the analysis removes size variation before the analysis of shape variation, which is then described either as differences in the coordinates of corresponding landmarks among specimens, or alternatively estimated using a thin-plate spline (TPS) function to map the deformation in shape of one specimen to another (Bookstein 1991). These approaches generate shape parameters that are generally used in conventional statistical comparisons of shape. The TPS approach can also be used to create deformation grids (or landmark-based vector plots) that depict specimens in close to their original form (depending on the number of landmarks). Deformation grids provide a mathematically rigorous realization of Thompson's (1917) idea of transformation grids, where one object is deformed into another, allowing researchers to more clearly relate form to function over the entire body.

¹ Various free software programs that perform geometric analyses (including TpsDig, TpsRelw, TpsRegr, TpsSmall used here) and much supporting information are available through the morphometrics website at the State University of New York at Stony Brook: <http://life.bio.sunysb.edu/morph/>

It is important to recognize that geometric methods are subject to some of the same problems that plague traditional methods. For example, the selection and number of landmarks used in the analysis is often arbitrary and may influence statistical power. We know of no guidelines for pre-selecting an appropriate number of landmarks on a form and suggest instead that the number of landmarks is a function of the research question and knowledge about the study organism (although when possible 'many' landmarks should be 'evenly spread' over the form; Bookstein et al. 1985). Geometric methods still require the same set of homologous landmarks on all specimens. Unfortunately, specimens can be missing landmarks if they are broken, poorly preserved, or structures are articulated differently, or landmarks found on one taxa are not present on another. Options are limited in these cases. Variant landmarks are either eliminated from the analysis (effectively reducing shape information), or damaged specimens missing landmarks are eliminated from the data set when rare, or missing landmarks are estimated using sample means (Adams et al. 2003). Despite these problems, proponents of the geometric methods have claimed significant progress at solving many of the limitations of traditional morphometric methods (Rohlf & Marcus 1993, Adams et al. 2003).

Our purpose is to test if traditional morphometric methods suffer the limitations of reduced statistical power and reduced accuracy with respect to identifying shape variation compared to a geometric method involving TPS when sensibly applied to a common real world data set. We do this by comparing the effectiveness of traditional truss-based methods and the geometric method at detecting statistically significant morphological differences among groups characterized by body forms that vary in known ways. We also determined if the different analyses yield similarly accurate interpretations of shape variation.

The cichlid data set

Our data set is of a sample of cichlid fishes from Lake Apoyo, Nicaragua. Lake Apoyo has several species of cichlid fish, including *Amphilophus citrinellus*, and *A. zalius*. *A. zalius* occupies the limnetic niche and in appearance is generally more slender, with larger eyes than *A. citrinellus*, which is believed to occupy a benthic niche (Barlow & Munsey 1976). *A. citrinellus* also appears to be more of an ecological generalist (e.g., Meyer 1989, 1990a,b), and can be phenotypically

plastic in response to rearing environment (Barlow & Munsey 1976). Recent population genetic studies by Hrbek et al. (unpublished data) suggest that sympatric speciation may be occurring in Lake Apoyo, with the endemic *A. zalius* possibly derived from *A. citrinellus*. At first glance the two species are morphologically distinct, however, many individuals of intermediate form persist in Lake Apoyo making classification based on phenotype difficult. Here, we classify individuals into one of four groups representing the two species along with two groups of intermediate form, those more like *A. citrinellus* and those more like *A. zalius*.

Methods

Sample collection and preparation

Cichlids were sampled by T. Hrbek from Lake Apoyo in November 1999 from four locations around the lake with a 20 m long by 2-m deep gill net (200 mm mesh size) suspended in the water column and set parallel to shore at 3–10 m depths. The net was set for approximately 45 min or until 5 individuals were caught (between 9 and 15 fish per location). Fish were removed, and preserved in 10% buffered formalin.

Digital images of the left side of each of 51 fish were made using a Canon canoscan 7000F scanner. Some individuals had their mouths sewn shut to reduce differences in jaw articulation. K. Parsons sorted individuals into one of four groups: *A. citrinellus* (hereafter referred to as AcI; n = 9), *A. citrinellus*-like intermediates (AcI; n = 14), *A. zalius* (AzI; n = 13), and *A. zalius*-like intermediates (AzI; n = 15) (Figure 1). The principal characteristics used to sort fish into groups were body depth relative to standard length, head shape, and body coloration. *A. zalius* tends to be more fusiform and lightly colored than *A. citrinellus* whose head shows a prominent bulge resulting in a more oblique angle down to the snout. Sorting of the specimens using these criteria allows us to compare how well the different morphometric methods are able to 'discover' these known differences in body form.

Traditional truss-based morphometrics

We measured a total of 20 truss lengths (mm) between 17 homologous landmarks on the left side of each fish, using Scion (NIH) image for Windows (Rasband 2000). Five measurements were made on the head



(a)



(b)



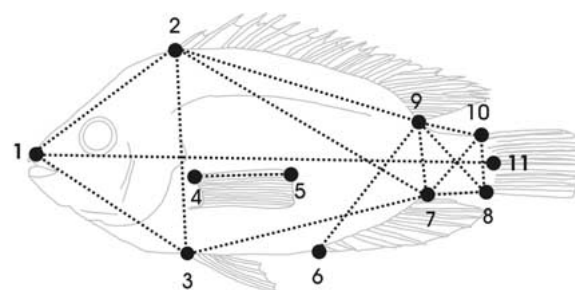
(c)



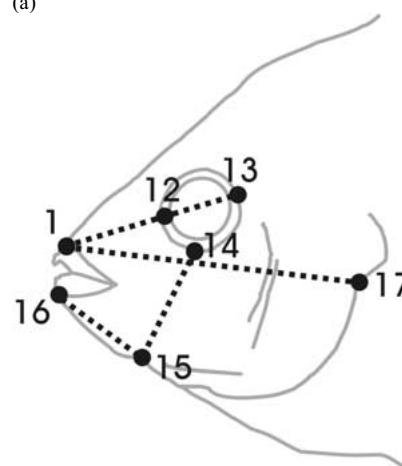
(d)

Figure 1. Representative specimens of the four cichlid groupings used in this study and their abbreviations, (a) *Amphilophus citrinellus* (referred to throughout as Ac), (b) *A. citrinellus*-like intermediate (AcI), (c) *A. zalius*-like intermediate (AzI), and (d) *A. zalius* (Az). Note the gradual compression and lengthening of body form from (a)–(d).

region (Figure 2(b)) following (Barel et al. 1977, Meyer 1987, 1990b), and 15 measurements were made in a truss network distributed over the rest of the body form (Figure 2(a); Strauss & Bookstein 1982).



(a)



(b)

Figure 2. The 17 landmarks used to anchor the 20 truss lengths measured on each specimen for the traditional morphometric analyses: (a) represents body measures, while (b) represents head measures. The Cartesian coordinates of 14 of these landmarks were also used in the geometric TPS analysis. Landmarks numbered 5, 16, and 17 were not included in the TPS analysis because of high variability in their locations (see Methods). Trusses are named in (a) as 1–2: snout to dorsal fin origin (Sndo), 1–3: snout to pelvic fin origin (Snpo), 1–11: standard length (Sl), 2–3: body depth (Bd), 2–9: dorsal base length (Db) 4–5: pectoral fin length (Pl), 3–7: pelvic fin origin to posterior of anal fin (Popaf), 6–9: anal fin origin to posterior of dorsal fin (Afopdf), 2–7: dorsal fin origin to posterior anal fin (Doap), 9–7: anterior caudal peduncle depth (Acd), 8–10: posterior caudal peduncle depth (Pcd), 9–10: dorsal caudal peduncle length (Dcpl), 7–8: ventral caudal peduncle length (Vcpl), 9–8: dorsal anterior to ventral posterior caudal peduncle cross (Dcpx), 7–10: ventral anterior to dorsal posterior caudal peduncle cross (Vcpx), and in (b) as 1–12: snout length (Snl), 12–13: eye diameter (Eyd), 1–17: head length (Hl), 14–15: cheek depth (Chd), 15–16: lower jaw length (Ljl).

We applied four conventional size correction methods to the 20 raw truss lengths in an attempt to statistically remove variation in size from shape. Two methods involved linearly regressing each length against a

measure of body size, either standard length (hereafter referred to as the RSL method), or geometric centroid size (referred to as the RCS method, and using standard length as a truss; e.g., Robinson et al. 1993). Geometric centroid size is a multivariate estimate of size and was calculated as the square root of the sum of squared distances of a set of landmarks (see Figure 2) from their centroid, or equivalently the square root of the sum of the variances of the landmarks about that centroid in x and y directions

We also used two multivariate methods to estimate 'size-free' shape variation: a sheared PCA (Humphries et al. 1981), and the Burnaby (1966) method. The sheared PCA approach (hereafter SPCA) adjusts the variation on PC2 (and subsequent components) with respect to variation on PC1 using a combined within-groups size vector. The Burnaby (1966) size-correction method ('Burnaby' below) quantifies separate within-group components of 'size' variation and then uses a discriminant function to identify shape variation that is constrained to being orthogonal or unrelated to every group's component of size variation.

Geometric morphometrics

Fourteen of the seventeen landmarks used to anchor the truss lengths for the traditional methods described above (Figure 2) were also used in our geometric analysis of body form. We excluded landmarks 5, 16, and 17 because they were considerably more variable or were articulated landmarks, making them less suitable for a geometric approach (Adams 1999 describes how articulated landmarks can be included in geometric approaches). The reduced number of landmarks in our geometric analyses may reduce the power to distinguish groups using the geometric data compared to the traditional methods (where power is influenced by the number of landmarks and the number of trusses, making strict comparisons among different methods difficult).

The x and y coordinates of each landmark were collected from every specimen using the software TpsDig (see Appendix 1). Landmark coordinates were then adjusted using a generalized procrustes analysis (GPA; previously referred to in the literature as generalized least squares or GLS analysis; Rohlf & Slice 1990, Rohlf & Marcus 1993). This procedure centers (translates) each specimen onto a common centroid reflecting the centroid of each specimen, then scales all specimens to a common unit size by dividing each total

configuration by centroid size (Bookstein 1986), and lastly rotates each specimen to a common orientation that minimizes the squared differences between corresponding landmarks ('optimal rotation,' Rohlf & Slice 1990, Adams et al. 2003).

We estimated a consensus (also sometimes referred to as a 'mean') form using all 51 cichlid specimens composed of the mean coordinates for each landmark averaged across all specimens. The GPA-adjusted landmark coordinates were then modeled against the consensus form using the software TPS (Bookstein 1991). TPS functions can quantify shape differences among groups by estimating the minimal shape parameters (referred to as partial warps) needed to deform or 'warp' the form of the consensus configuration to each specimen. We estimated 22 partial warps, using the software TpsRelw (Rohlf 1998; see Appendix 1 for further details of how TPS was applied here). Partial warp scores contain shape information that can be analyzed using conventional multivariate statistical methods (e.g. Caldecutt & Adams 1998, Bookstein et al. 1999, Rüber & Adams 2001, Klingenberg & Leamy 2001, Adams et al. 2003). However, partial warps are a more dynamic measure of shape information than linear trusses because they include detailed information about the relative geometric locations of landmarks. Partial warps are distinct from 'principal warps' in that the former reflect an individual's shape attributes in a space defined by the latter, which represents shape variation in the entire sample. 'Relative warps' also sometimes appear in the literature and represent the canonical axes estimated from a PCA of partial warp scores (Adams et al. 2003).

Statistical analysis of group differences

We used multivariate discriminant function analysis (DFA) on the sets of shape measures derived from each of the four traditional size-removal methods (RSL, RCS, SPCA, and Burnaby) and the TPS method in an attempt to discriminate the four cichlid groups (Marcus 1990, Manly 1994). The RSL and RCS methods yielded residual truss lengths, the SPCA and Burnaby methods yielded size-adjusted factor scores, and the geometric TPS method yielded partial warp scores, all of which were used as the independent parameters in the DFA.

Discriminant function analysis estimates a set of functions, each of which is a linear combination of weighted variables that best differentiates defined

groups by maximizing the ratio of the between-groups sums of squares and cross products to within-groups SSCP (Manly 1994). Morphological 'scores' can be estimated for individual specimens on each of these axes (also referred to as 'roots') that summarize shape variation associated with that axis. The PCA is one alternative to DFA, and is sometimes considered a more conservative and appropriate method for assessing morphological variation among groups. PCA is a data projection technique for summarizing variability in complex correlated data sets using a simple algorithm that finds major axes of variation in the data (Marcus 1990, Manly 1994). One potential drawback with respect to discrimination is that it assumes differences among groups will primarily involve the traits with the greatest variance. If not, then PCA potentially confounds components of variation within and among groups. There is no *a priori* reason to make such an assumption among groups that have only recently diverged, as we believe is the case here (Barlow & Munsey 1976, Hrbek et al. unpublished data).

DFA permits a statistical test of the null hypothesis of no morphological differences among at least two groups. We use the magnitude of the multivariate *p*-values estimated from the DFA to test this hypothesis. We also compare the five morphometric approaches using the percentages of correct classification (e.g., the classification of each specimen by a DFA compared to our *a priori* classification into the four groups). Lastly, we use a squared Mahalanobis distance matrix to estimate the divergence between pairs of group centroids (F-test of distances between pairs of group centroids using the within-group Mahalanobis distances to estimate error variation; Manly 1994). We apply a sequential Bonferroni adjustment to individual *p*-values resulting from these six pair-wise comparisons so as to maintain a method-wide alpha-value of 0.05 (Rice 1989).

Interpreting shape variation

In addition to determining if there is significant evidence of shape differences among groups, most researchers are interested in having a clear and correct description of the morphological variation in order to assist future classification or to link form with function. The aspects of morphological variation reflected in each discriminant function (root) can be interpreted on the basis of correlations between each original trait (e.g., the size-adjusted truss lengths) and the discriminant root scores. These correlations are referred to

as the canonical loadings of the original traits onto each root, and if greater than 0.3 or less than -0.3 are often interpreted as contributing to shape variation on that root (Elazar 1982). We use this standard approach to interpret variation in shape among the four cichlid groups.

To interpret shape differences among groups using the TPS method, we regressed geometric-based shape information (partial warps) against the first canonical root scores from the DFA using the software TpsRegr (Rohlf 1998). This procedure creates deformation grid plots that reflect how one form can be stretched, pulled, and shrunk in order to deform it into another related form. The deformation grids shown here reflect the degree and type of shape change required to deform the population's consensus form into the most extreme form of each group.

Results

Tests of group differences

The four traditional methods yielded no overlap in the morphological variation between the Az and Ac species groups in scatter plots of scores on the first two discriminant axes (Figure 3(a–d)), whereas these two clusters touch in the TPS analysis (Figure 3(e)). Az and Ac groups were consistently differentiated along the first axis (root 1) in all five methods, with the intermediate forms generally falling between the two species in the traditional methods. This was not the case in the TPS method where the two intermediate groups (AcI and AzI) were as differentiated from each other on the first root as the Ac and Az groups. Intermediate groups were differentiated from their respective species group on the second root (Figure 3(e)). Morphological variation between intermediate and species groups on the second root was also apparent in the RCS results. All five methods found statistically significant evidence of multivariate differences in morphology between at least two groups (Table 1). The rank ordering of the methods from most to least significant was the TPS method, followed by the Burnaby, RCS, SPCA, and RSL methods.

The geometric TPS method yielded the highest percentage of individuals correctly classified to the four original groupings (92%, Table 1). The TPS method also resulted in the highest percentages of correct group classification for both the Az and Ac species groups (respectively 100% and 89%). Comparing the methods

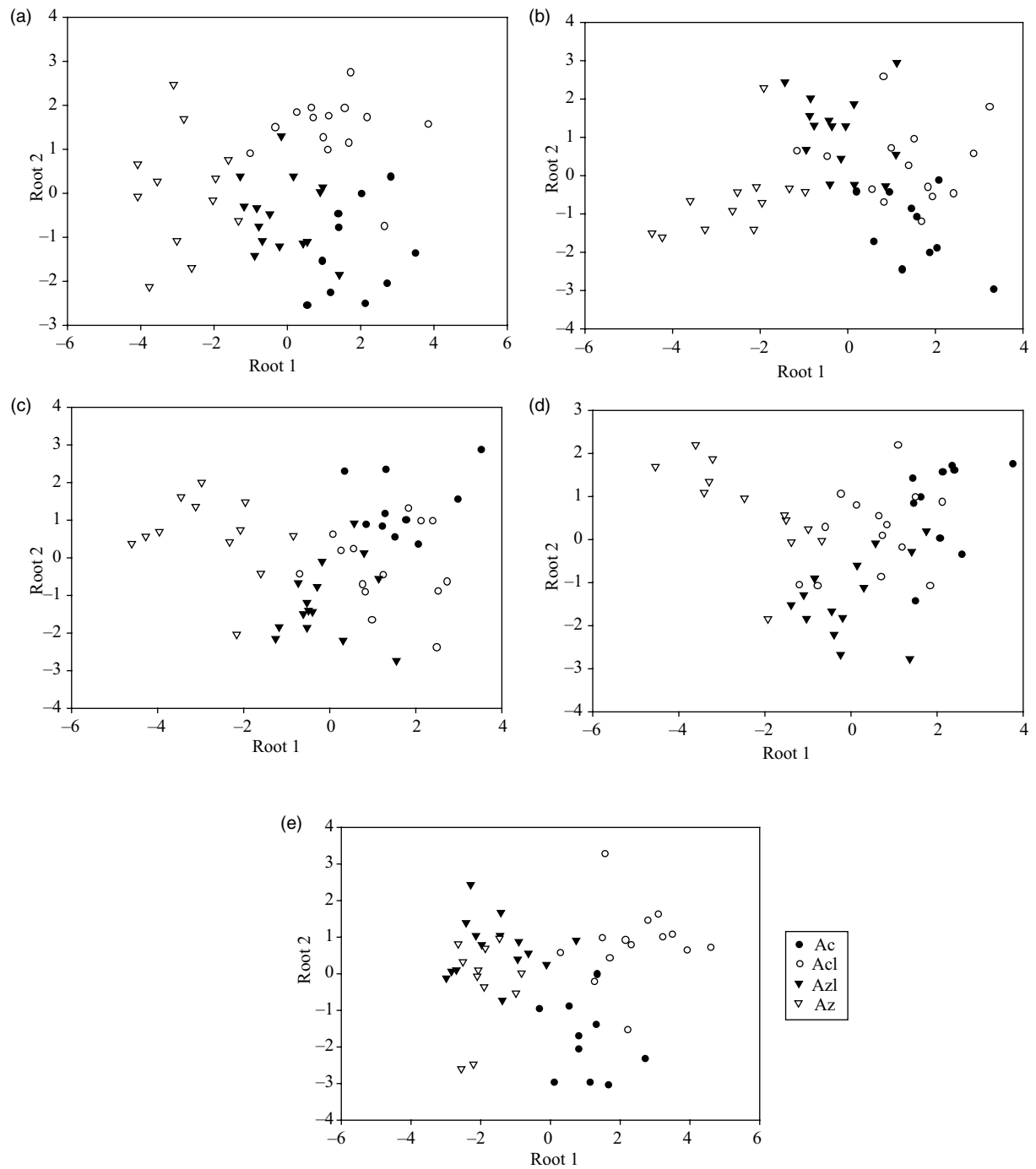


Figure 3. Scatter plots representing variation in shape within and among the four cichlid groups: *A. citrinellus* (Ac), *A. citrinellus*-like intermediates (Acl), *A. zalius*-like intermediates (Azl), and *A. zalius* (Az). Shape variation estimated for the first two discriminant functions (roots 1 and 2) are plotted for each method: (a) RSL (this method uses residual variation as the shape data after each truss is linearly regressed against standard length), (b) RCS (also uses residuals although estimated from a regression against multivariate centroid size), (c) Burnaby (uses size-adjusted multivariate shape information), (d) SPCA (a shared PCA), (e) TPS methods (geometric TPS).

by percentages of least successful group classification also suggests that the TPS method was superior with its poorest classification rate at 86% (AcI group) compared to the traditional methods where poorest classification rates were less than or equal to 78%.

The geometric TPS method also appeared best at establishing significant morphological differences among pair-wise group comparisons (Table 2). All of the methods found evidence of differences between the most divergent Az and Ac species groups (Figures 1 and 3). The TPS, RCS, and Burnaby methods also found evidence of differences between the Az and AcI

groups. Only the TPS method found evidence of an additional difference between the Ac and AzI groups. Evidence of significant differences between pairs of groups was always greatest (e.g., smallest p-value) using the TPS method. No method found evidence of significant differences between body forms of the AcI and AzI intermediate groups.

Interpreting shape variation

Both the number of trusses and how trusses contributed to shape differences among groups depended on the traditional method used (Table 3). The RCS method identified the lowest number of trusses related to shape differences (4 trusses loading on root 1), the SPCA method revealed more (10 trusses loading on roots 1–3), while the Burnaby and RSL methods had the greatest number of loadings (14 on roots 1–3). There was little consistency between these last two methods because only one of the 14 trusses (pectoral length) loaded in the same direction onto the same root (R1). Somewhat more consistent results were found for the SPCA and Burnaby results because eight trusses loaded in a similar fashion on the same root (traits: Snl, Hl, Bd, Acd, Popaf, Dcpl, Sndo, Snp; see Figure 2 for truss descriptions). It is surprising that only two trusses (Pl and Acd) consistently loaded onto root 1 for any two traditional methods given that variation between the two cichlid species occurred along this root. On balance, there is little evidence that the traditional methods consistently identified traits that contributed to the morphological differentiation between cichlid groups.

Table 1. Results of separate discriminant function analyses (DFA) using data generated by each of the five morphometric methods.

| Method | Multivariate DFA | | Percent correct classification by group | | | | |
|---------|------------------|------------------|---|-----|-----|-----|----|
| | p-value | Wilk's λ | All groups | Az | AzI | AcI | Ac |
| TPS | 0.0026 | 0.050 | 92 | 100 | 92 | 86 | 89 |
| RSL | 0.0081 | 0.101 | 86 | 92 | 80 | 93 | 78 |
| RCS | 0.0045 | 0.081 | 86 | 85 | 87 | 93 | 78 |
| SPCA | 0.0075 | 0.087 | 80 | 83 | 73 | 86 | 80 |
| Burnaby | 0.0028 | 0.076 | 84 | 85 | 87 | 86 | 78 |

The geometric morphometric TPS (thin-plate spline method) and the four traditional methods: RSL and RCS (respectively residuals of each truss estimated by linearly regressing each truss length against either standard length or centroid size), the Burnaby, and SPCA methods (sheared PCA). The five columns at the right reflect the correct classification rate of specimens by each DFA in the following order: for all four cichlid groups together, and for each group separately: Az (*Amphilophus zaliosus*), AzI (*A. zaliosus*-like intermediate), AcI (*A. citrinellus*-like intermediate), and Ac (*A. citrinellus*).

Table 2. Results of separate F-test of Mahalanobis distances between pairs of group centroids estimated by a discriminant function analysis of each type of morphometric data.

| Method | p-values of comparisons between pairs of cichlid groups | | | | | |
|---------|---|---------------|--------|--------|---------------|---------|
| | Az–Ac | Az–AcI | Az–AzI | Ac–AcI | Ac–AzI | AcI–AzI |
| TPS | 0.00008 | 0.0065 | 0.070 | 0.41 | 0.0032 | 0.16 |
| RSL | 0.00405 | 0.0266 | 0.060 | 0.21 | 0.0511 | 0.47 |
| RCS | 0.00292 | 0.0092 | 0.071 | 0.21 | 0.0571 | 0.31 |
| SPCA | 0.00179 | 0.0230 | 0.062 | 0.21 | 0.0935 | 0.38 |
| Burnaby | 0.00403 | 0.0092 | 0.049 | 0.41 | 0.1455 | 0.32 |

The statistical significance (in bold) of pair-wise comparisons for each method was assessed using a sequential Bonferroni approach that maintained an alpha level of 5% over all six tests in each row (Rice 1989). Descriptions of the morphometric methods and the cichlid groups are found in Table 1.

Table 3. Results from the traditional morphometric methods (described in Table 1) used to interpret shape variation among the cichlid groups.

| Truss | Traditional truss-based morphometric method | | | |
|---|---|-----------|-----------------------|-----------------------|
| | RSL | RCS | SPCA | Burnaby |
| Snout length | | | R2 (0.44) | R1 (-0.61), R2 (0.44) |
| Lower jaw length | | | R1 (0.59) | R1 (-0.54), R2 (0.38) |
| Eye diameter | R2 (-0.49) | | R1 (0.54) | R1 (-0.59) |
| Cheek depth | R1 (0.34) | | | |
| Head length | | R1 (0.30) | R3 (0.39) | R3 (0.34) |
| Dorsal fin origin to anal posterior | R1 (0.78), R2 (-0.35) | | | |
| Pectoral length | R1 (0.37), R2 (-0.37) | | | R1 (0.30) |
| Dorsal fin base | R1 (0.64) | | | R3 (-0.34) |
| Body depth | R1 (0.73) | | R2 (0.31), R3 (-0.31) | R3 (-0.40) |
| Dorsal caudal peduncle cross | R2 (-0.40) | R1 (0.35) | | |
| Ventral caudal peduncle cross | | | | |
| Anterior caudal peduncle depth | R1 (0.34) | | R1 (-0.37) | R1 (-0.33) |
| Posterior caudal peduncle depth | R1 (0.60) | | | |
| Pelvic origin to posterior anal fin | R1 (0.33), R3 (-0.49) | | R2 (0.37) | R2 (0.38) |
| Ventral caudal peduncle length | R1 (0.54) | | | |
| Dorsal caudal peduncle length | R1 (0.35) | | R2 (0.45) | R2 (0.43) |
| Standard length | R1 (-0.39), R3 (-0.36) | R1 (0.48) | | R2 (-0.32) |
| Snout to dorsal fin origin | | R1 (0.32) | R2 (-0.36) | R2 (-0.41) |
| Snout to pelvic fin origin | R1 (-0.76) | | R3 (0.51) | R3 (0.54) |
| Anal fin origin to posterior dorsal fin | | | | R3 (-0.31) |
| Total trusses | 14 | 4 | 10 | 14 |

Important canonical loadings (correlations greater than an absolute value of 0.3 shown in parentheses) between the original trusses and the discriminant axes (roots: R1–R3) are given for each method. Trusses are linear measures between pairs of landmarks as illustrated in Figure 2. At the bottom of each column is the total number of trusses that contribute important shape information in each analysis.

These inconsistencies raise questions of the reliability of any interpretations of shape variation. We expected on functional grounds (Webb 1984, Walker 1997) that the more benthivorous *A. citrinellus* would have smaller eyes, blunter heads, a deeper and more robust body shape, and a shorter deeper caudal peduncle compared to the more pelagic *A. zalius*, which would exhibit a longer and more slender or compressed body form. Because all five methods discriminated between the two species primarily along the first principal axis, we focus on interpreting this component of shape variation.

Some of the canonical loadings arising from the RSL method were consistent with these expectations (Table 3). *A. citrinellus* had on average longer pectoral fins, and deeper and shorter bodies compared to *A. zalius* (traits: Pl, Chd, Doap, Bd, Acd, Pcd, Sl, Snp). However, other results suggested that *A. citrinellus* also had somewhat longer body length elements than *A. zalius* (traits: Db, Popaf, Vcpl, Dcpl). The results of the SPCA analysis indicated that *A. zalius* had on average smaller jaws as expected,

but also smaller eyes, and a deeper caudal peduncle compared to *A. citrinellus*. (traits: Eyd, Ljl, Acd). The Burnaby results indicated that *A. citrinellus* had on average smaller eyes, longer pectoral fins, shorter heads, but unexpectedly smaller mouths, and narrower caudal peduncles (traits: Eyd, Pl, Ljl, Snl, Acd). Lastly, the RCS method indicated that *A. citrinellus* had larger heads and caudal peduncles as expected but also longer bodies compared to *A. zalius* (Traits: Hl, Sndo, Dcpx, Sl). These results demonstrate that our attempt to reconstruct variation in body form on the basis of truss measures depends idiosyncratically on the morphometric method used.

Shape differences identified by the geometric TPS analysis were both more consistent with our expectations, and were also easier to interpret because variation can be represented through deformation grid diagrams of the entire body form (Figure 4). This removes the need to interpret many separate traits through loadings. Subtle variation can also be accentuated by applying a magnification factor to the entire grid system. The body form of *A. zalius* appeared generally more

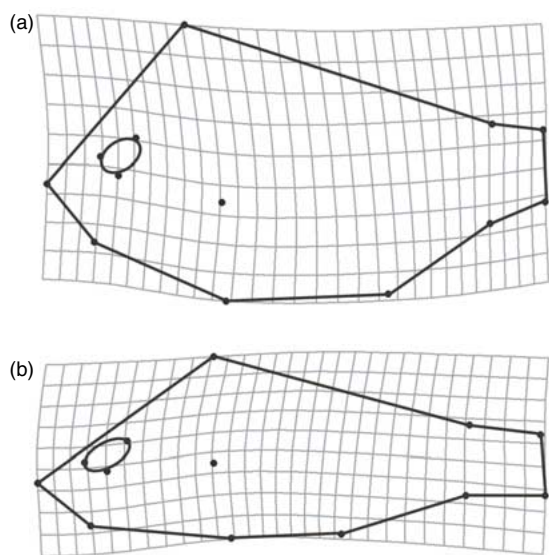


Figure 4. Deformation grids depicting shape differences (at $3\times$ magnification) between (a) *A. citrinellus*, and (b) *A. zaliusus*. Note the longer more compressed shape in (b) and the shorter, more robust shape of (a) that reflects the body forms shown in Figure 1. Refer to Figure 2 for the locations of landmarks on the body. Deformation grids were estimated by regressing geometric shape information (the partial warps generated by the TPS analysis) against the scores on the first root of the DFA using the software TpsRegr (© Rohlf 1998).

compressed and elongate than that of *A. citrinellus* (Figure 4(a) and (b)). More subtly, *A. zaliusus* had an elongated caudal peduncle, a pectoral fin insertion closer to the horizontal midline, and an elongated head compared to *A. citrinellus*.

Discussion

The geometric-based TPS and the four traditional truss-based morphometric methods gave similar statistical results with respect to identifying the two cichlid species as morphologically divergent. However, the methods differed in the strength of the evidence for differences with respect to intermediate forms, and also yielded different interpretations of variation in body form among groups. All methods found that *A. zaliusus* and *A. citrinellus* were the most morphologically distinct groups, but only the geometric method provided some evidence of a distinct intermediate group. These findings are similar to other comparisons of traditional and geometric morphometric methods applied to a common data set, and suggest greater discriminating

power is available using geometric-based methods (e.g., Adams & Rohlf 2000).

The roughly similar statistical power to discriminate *A. zaliusus* and *A. citrinellus* by the various methods is reassuring, and suggests to us that prior morphometric studies using truss-based measures can be reliable. The similarity of the evidence for differences at the scale of species differences probably reflects our use of an almost identical set of landmarks in all analyses, although here, the traditional methods incorporated three additional landmarks not included in our geometric TPS analysis. In other words, the somewhat increased discriminating power of the geometric methods may be even greater than shown here. This interpretation is supported by the increased sensitivity of the TPS method that alone detected differences between the Ac and AzI intermediate forms. This suggests that the TPS method used additional shape information, most likely present in the relative locations of landmarks. In traditional morphometrics, the relative locations of trusses can only be incorporated into the analysis by the researcher after statistical analysis.

We also found variation in the statistical power among the traditional methods. For example, greater power was associated with the RCS compared to RSL methods. This may be because standard length itself contained important variation among groups, although we do not know whether this represented variation in size, shape or both (Table 3). This leads us to believe that standard length was not effective as a size parameter here, perhaps because it includes shape information that is effectively lost when used as a 'size' covariate. This is surprising because standard length and centroid size were positively correlated in this data set (Pearson's $r = 0.72$, $p < 0.01$). This indicates that correlations among possible size parameters does not guarantee similar results, and so cannot be used to justify any one particular size parameter in a given morphometric analyses (Bookstein et al. 1985).

The traditional methods also resulted in inconsistent interpretations of shape variation among groups. Discrepancies existed among traditional methods with respect to which traits contributed to shape variation based on our interpretation of canonical loadings. For example, a trait such as body depth made a significant contribution to shape differences between the two species in the RSL method but not in other methods. Such idiosyncrasies should not exist if each method is correctly assessing variation in shape. One possible explanation for this effect is that only the variable with

the highest individual F-statistic from a set of correlated variables will be weighted heavily by the optimization criteria used by discriminant function analysis. For that set of variables, other correlated variables that do not provide additional discriminatory power will be assigned low coefficients even though they may contain almost the same amount of information as the first variable (Humphries et al. 1981). The different size-adjustment strategies used by the traditional truss-based methods likely influences the rank ordering of the F-statistics of a set of correlated measures, sufficient to result in some spurious ordering of even the most discriminatory traits. This could have generated the differences in the trait loadings among the traditional methods shown in Table 3, and so could contribute to the inconsistencies in our interpretations of shape variation. This is not always the case, as shown by fewer discrepancies found by Wimberger (1991) between two traditional morphometric methods used to analyze diet-induced shape variation in *Geophagine* cichlids.

The TPS generated deformation grids were an effective visual representation of shape change among the cichlid groupings here (compare Figures 1 and 4), and so facilitated our analysis of body form. For example, differences between Az and Ac forms matched our expectations based on patterns of morphological divergence between benthic and pelagic forms of lake fishes elsewhere (Webb 1984, Walker 1997, Robinson & Schluter 2000), and matched the characteristics used to sort the specimens into groups in the first place.

One general criticism leveled at geometric approaches is that they cannot be easily used to interpret functional morphology because localized shape change cannot necessarily be quantified to a single measurable trait as in truss-based methods (e.g., Schlichting & Pigliucci 1998, p. 211). In our minds, this argument boils down to the lengths and widths of specific objects being more functionally informative than the relative positions of the landmarks that define those very measurements. There may be some truth to this because morphometric studies span a continuum from highly focused studies of a few very specific traits such as particular elements of a pharyngeal jaw apparatus (e.g., Wainwright et al. 1991) to exploratory studies simultaneously focused on many aspects of morphology as performed here. Quantitative tests of the relationships between form and function are often reductionist and focus on a few traits, such as the length or width of one element from a single bone. This focus on a particular trait can provide clarity in a complex system, variation is easier to quantify, and an understanding of functional

links may be more attainable using such a reductionist approach.

This view should be tempered by a number of additional considerations. First, is the realization that assessing body form is basically a geometric problem and so geometric methods may have much to offer us as we learn how to use them to redefine traits based on geometry rather than linear dimensions (e.g., Albertson & Kocher 2001, Rüber & Adams 2001). Second, if discrepancies occur among traditional methods in correctly identifying and quantifying morphological variation, then any conclusions reached on the basis of such studies may be suspect. Third, functional studies rely on the inclusion of *appropriate* trusses that must be known *a priori* (Adams 1999). A strength of geometric approaches, particularly during exploratory studies, is that many aspects of shape variation are captured among a set of landmarks so that specific anatomical variation can be detected without having to be known or explicitly included in the analysis ahead of time. Subsequent more detailed analyses can focus on specific traits identified by the geometric analysis either by reverting to the use of lengths of specific traits, or by the geometric analysis of landmarks at a finer scale in a particular region of interest (e.g., Albertson & Kocher 2001).

Traditional morphometric methods are also perhaps thought of as being more flexible than geometric methods because truss lengths, lengths of gill rakers and sizes of internal organs can more easily be combined in a single analysis. Such a synthesis is also possible using geometric-based shape information. For example, a geometric analysis of external body form can be used to contribute partial warp scores of body form to a multivariate analysis that also includes parameters quantifying variation in gill raker length, organ dimensions, and other traits. In this case these additional traits would have to be isometrically scaled to body size by dividing each by centroid size (it is ironic that the generalized procrustes analysis used in geometric-based analyses performs a size adjustment involving ratios, given the problems with ratios noted above, although it does so at the multivariate scale). The creation of deformation grids from such an analysis will not include information about these internal features. One last situation where traditional methods may be better suited is when the goal is to compare the results of a contemporary study with an earlier study (although geometric analysis may again contribute valuable new insights even in this situation).

Geometric morphometrics is increasingly being viewed as a powerful tool in the analysis of form

(Rohlf & Marcus 1993, Bookstein 1996, Adams et al. 2003). In our study, the TPS approach was an effective method from a statistical, and especially from a descriptive standpoint. Its applicability to the study of fish shape is reflected in a rapidly expanding citation base that allows many entries into the field that are less technical than the original papers explaining geometric methods (e.g., Fink & Zelditch 1995, Corti & Crosetti 1996, Corti et al. 1996, Fink & Zelditch 1996, Caldecutt & Adams 1998, Cavalcanti & Lopes 1999, Loy et al. 1996, 1998, 2000, Walker & Bell 2000). No doubt this expanding literature has come about because of the availability of free, increasingly user-friendly software, and better resources that explain its components and utility (e.g., Rohlf & Bookstein 1990, Rohlf & Marcus 1993, Marcus et al. 1996, Dryden & Mardia 1998, Costa & Cesar 2000, Da Fontoura & Cesar 2000, Adams et al. 2003). Technical advances are continuing in the use of geometric methods for the analysis of three-dimensional data, contour type data, and articulated structures. Researchers are also applying geometric methods to study other questions relating to body form, such as fluctuating asymmetry, quantitative genetics, selection, and phylogenetic analyses (reviewed in Adams et al. 2003). The promise of broadly accessible new morphometric methods has only become apparent relatively recently despite the proclamation of a revolution in the field a decade ago (Rohlf & Marcus 1993, Adams et al. 2003). At the risk of ignoring these recent problems, we are optimistic that it has never been easier to get into shape.

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Appendix 1. We performed six steps in our analysis of shape using the TPS geometric morphometric procedure. Each step is given a process or purpose, comments, the software used, and additional possible options available with that software. All of this TPS software (and more) is available from the State University of New York (SUNY) at Stony Brook morphometrics website (<http://life.bio.sunysb.edu/morph/index.html>).

| Process or purpose | Comments | Software | Additional options |
|---|--|-------------------------------|---|
| 1 Put multiple pictures into one metafile/building a TPS file from pictures | Allowed for easy digitization of landmarks as one could scan through multiple pictures | TpsUtil © Rohlf 2001 | Specimens can be randomized |
| 2 Digitize landmarks from pictures, collection of x, y coordinates | Sequence in which landmarks were placed was consistent | TpsDig © Rohlf 1998 | Size scale can be added, specimens can be outlined, linear measures can be made |
| 3 Test if sample forms are reasonably comparable | Rejects extremely different sample forms for TPS analysis | TpsSmall © Rohlf 2000 | GLS processed x, y coordinates can be collected |
| 4 Performs generalized procrustes analysis (GPA), historically referred to as general least squares (GLS) analysis; estimates partial warp scores | x, y coordinates used to create a consensus configuration and shape information (partial warp scores) for each specimen, collectively referred to as the weight matrix | TpsRelw © Rohlf 2001 | Relative warps analysis (PCA of partial warp scores), visualization of relative warps in a deformation grid |
| 5 Statistical analysis of shape data (partial warps) | Partial warp scores entered into a DFA, 3 canonical roots scores produced for each specimen | Statistica (Statsoft 1998) | Standard univariate and multivariate tests |
| 6 Estimation of deformation grids | Regressed DFA root 1 against GPA-corrected x, y coordinates | TpsRegr © Rohlf 2000 | Shape change expressed in grids can be magnified, centroid size data can be collected |