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Source: *Paleobiology*, Vol. 36, No. 3 (Summer, 2010), pp. 497-515

Published by: Paleontological Society

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Comparison of morphometric techniques for shapes with few homologous landmarks based on machine-learning approaches to biological discrimination

Bert Van Bocxlaer and Roland Schultheiß

Abstract.—Biometric analyses are useful tools for the study of organisms, their phylogenetic affiliation, and the pattern and rate of their evolution. Various morphometric techniques have been developed to analyze morphological variation, but methodological choices are often made arbitrarily because quantitative comparisons are lacking or inconclusive. Here we address morphometric quantification of taxa with few unambiguously identifiable landmarks (<15), utilizing ornamented and unornamented gastropod shells. Support vector machines were applied to evaluate classification performances of landmark (LMA), elliptic Fourier (EFA), and semi-landmark analysis (SLM). This evaluation is based on the discrimination of between-group differences relative to within-group variation, and thus allows comparing how the techniques treat different types of biological information. The results suggest that EFA performs slightly better than SLM (and certainly LMA) in discerning a priori identified taxa with unornamented shells, but that SLM is significantly superior to other techniques for ornamented shells. Alignment and homology problems may cause the subtle variations in ornamentation to become blurred as noise in EFA, even though EFA is often cited to be able to deal with complex shapes. Performance of LMA depends entirely on how accurately the structure can be covered with landmarks. Guidelines in choosing a morphometric technique in diverse cases are provided.

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Accepted: 5 January 2010

Introduction

Taxonomic classification and our understanding of evolution are historically based on descriptions of the morphology of organisms. Despite recent advances in molecular techniques, the comparison of anatomical and morphological features of organisms remains a central element of (paleo)biology. Differences in morphological complexity among taxa, however, pose a problem if species assignments are based solely on morphology: the fewer the morphological features characterizing a higher taxon, the fewer lower-level taxa we expect to assign to it (e.g., Schopf et al. 1975; Schopf 1982). The resulting classification will reflect differences in perceived morphological complexity, not necessarily underlying biological relationships. This problem affects extant organisms to a much lesser extent than fossils because numerous characters, regularly included in the description of living forms, are unavailable in the fossil record.

Differences in morphological complexity influence taxonomy and, therewith, the reconstruction of evolutionary patterns, so their effect can be far reaching, certainly in paleontology. Therefore, morphological identification of biological species has received reasonable attention from paleontologists (e.g., Jackson and Cheetham 1990; Jablonski 1999). Because the projection of phylogeny into a multivariate morphospace has been shown to be an effective tool in evolutionary studies (e.g., Clabaut et al. 2007; Sidlauskas 2008), evaluation of the performance of diverse analyses in documenting various aspects of morphology is urgently needed.

In the twentieth century, biology and the analysis of morphology underwent a metamorphosis from a mainly descriptive to a quantitative science (Thompson 1942; Bookstein 1998; Adams et al. 2004). Quantification considerably increases objectivity in evolutionary research and is regularly required for

statistical applications to the analysis of biological data (biometry). Morphometrics, the quantitative description, analysis, and interpretation of shape and shape variation, has become a fundamental part of biological research, especially in paleontology, where many other biometric techniques are not applicable (Rohlf 1990b; Gould 1991). As such, a plethora of techniques exists at present. The first morphometric techniques developed were traditional morphometrics (TM) (Marcus 1990; Reymert 1991), in which variables usually correspond to caliper-measured distances, angles, and derived ratios (Rohlf and Marcus 1993; Zelditch et al. 2004). Although TM may, depending on the underlying hypothesis, prove informative, a second series of techniques, unified under the name of geometric landmark morphometrics (LM), is from a theoretical viewpoint more powerful. Whereas the information in TM is inherent to the measured distances between landmarks, LM techniques additionally take the geometrical relationships among the variables into account (Rohlf and Marcus 1993; Zelditch et al. 2004; Sheets et al. 2006). The latter techniques thus attempt to capture the shape (geometry) of the studied structure two- or three-dimensionally. This theoretical superiority of LM has some empirical support (Parsons et al. 2003), but LM techniques have their own limitations (see, e.g., Zelditch et al. 2004). Because of these limitations, using unambiguously assignable, homologous landmarks to extract morphological information from taxa with a low degree of morphological complexity or from isolated mineralized elements of more complex taxa may pose severe problems for the application of LM. Although many morphological characters may be available for analyses at the generic and specific levels for Gastropoda (e.g., Vermeij and Carlson 2000; Wagner 2002; Papadopoulos et al. 2004), Bivalvia (e.g., Anderson and Roopnarine 2003), and Bryozoa (e.g., Cheetham et al. 2007), these taxa have a low degree of morphological complexity relative to Vertebrata, Cephalopoda, Echinodermata, Anthozoa, and Arthropoda (Schopf et al. 1975). Difficulties in using LM to document morphological variation in taxa

with a relatively low morphological complexity remain a potential obstacle to examining underlying paleobiological and evolutionary signals.

Proposals to counter this problem are morphometric approaches that attempt to incorporate information of curves. Outline methods such as eigenshape analysis and Fourier shape analysis have been explored to a much more limited extent than landmark-based methods (Zelditch et al. 2004), although they have been suggested to be useful tools for the analysis of biological shapes in the absence of sufficient homologous landmarks (Rohlf 1990b; Bookstein 1997; Haines and Crampton 2000; Sheets et al. 2006). Nonetheless, many workers still consider it advantageous to base morphometric analyses on the positions of homologous landmarks, even if there are few (e.g., Bookstein et al. 1982; Hammer and Harper 2006). Criticism to outline analyses in general and elliptic Fourier analysis (EFA) in particular includes a variety of issues, the most fundamental being that the markers are not homologous and that the lack of biological significance of the Fourier coefficients hampers interpretation of the results (Bookstein et al. 1982). The homology issue has been countered on various bases (Ehrlich et al. 1983; Read and Lestrel 1986; MacLeod 1999), and may be addressed by a reorientation of the outlines using at least two well-defined markers (Rohlf 1990a). Admittedly, difficulties may exist in interpreting Fourier coefficients (Lestrel 1997), but perhaps these have been overestimated (Monti et al. 2001). Moreover, the early finding that EFA is a very powerful technique (Rohlf and Archie 1984) has not been countered by later studies (Navarro et al. 2004; Sheets et al. 2006). Other, more recent developments to include curve information are semi-landmark approaches (SLM), which attempt to incorporate information on curves into the landmark-based formalism (Bookstein 1996, 1997; Green 1996; Sheets et al. 2004, 2006). This is performed by expanding the set of traditional landmarks with a set of non-discrete anatomical loci (semi-landmarks) that have arbitrary spacing along homologous, open or closed curves. Various

ways of incorporating semi-landmark data into geometric landmark analyses are discussed by Sheets et al. (2006), and SLM has been applied successfully in various case studies (e.g., Wood et al. 2007; Zelditch et al. 2008).

Because the diverse methods involve very different mathematical procedures, and thus utilize different classes of data, they may interpret types of biological information differently, potentially resulting in significantly different outcomes depending on method rather than sampled variation. Although efforts to compare the mathematical procedures underpinning morphometric techniques have been undertaken (e.g., Rohlf 1999, 2000; Sheets et al. 2006), comparisons within a biological framework remain rare. Several outline methods have been compared in performance for objects with very few landmarks (Sheets et al. 2006), but in terms of preference of a particular methodological technique, such comparisons remained largely inconclusive. Moreover, although outline methods are commonly applied to shapes that lack even a few homologous landmarks, their application to shapes with a restricted number of landmarks (~15) and the associated explanatory power in such cases remain poorly explored, and explorations are qualitatively based only (Loy et al. 2000). The result is that decisions to adopt a particular technique for shapes with a restricted number of landmarks are subjective, given that direct quantitative comparison of landmark and outline methods has not been performed. This may be due to the lack of a standardized procedure. Optimization of morphological analyses requires documentation of the abilities of various techniques to discriminate between-group differences from within-group variation.

This study proposes a statistical framework to investigate how three morphometric techniques (LMA, SLM, and EFA) assemble biological information from biological shapes with a limited number of landmarks. Our framework provides a powerful statistical solution to the quantitative comparison of morphometric data sets in the absence of multivariate normality and dimensionality

reduction. Using fossil and recent material of ornamented and non-ornamented gastropod shells with different degrees of intermediate shapes, we evaluate how these methods perform in biological discrimination under a variety of conditions. By quantifying performance, we hope to facilitate methodological decisions in morphometric studies of objects whose homologous landmarks are limited and/or dubious.

Material

We analyzed two sets of specimens from the Malawi basin (East African Rift System). The first consists of 94 modern and middle Holocene *Lanistes* (Ampullariidae) shells. The shells belong to the nominal species *L. ellipticus* Martens, 1866; *L. nasutus* Mandahl-Barth, 1972; *L. nyassanus* Dohrn, 1865; *L. ovum* Troschel, 1845; and *L. solidus* Smith, 1877 (Fig. 1) (Mandahl-Barth 1972, but see Schultheiß et al. 2009 for taxonomic discussion). We consider this data set to be representative of non-ornamented gastropods. Discerning several non-ornamented species requires accurate quantification of general shell shape parameters, such as shell globosity, spire height, and spire angle. Specimens in the second set are from a taxon including both unornamented and strongly sculptured shells. This data set of 64 shells comprises several very closely related Malawian endemics of the genus *Melanoides* (Thiaridae), i.e., the nominal species *M. polymorpha* (Smith, 1877), *M. pupiformis* (Smith, 1877) and *M. turritispira* (Smith, 1877) (Fig. 2) (Mandahl-Barth 1972, but see Genner et al. 2007 for taxonomic discussion). This data set allows us to study the effect of shell ornamentation on the analyses, and thus to test the effectiveness of the morphometric techniques in discerning more complex shell morphologies (or structures in general). Again, modern and middle Holocene material was included. For both *Lanistes* and *Melanoides* we grouped fossil and modern material of the same species. Although this ignores more subtle, intraspecific differences between modern and fossil populations, we consider it appropriate for the present study

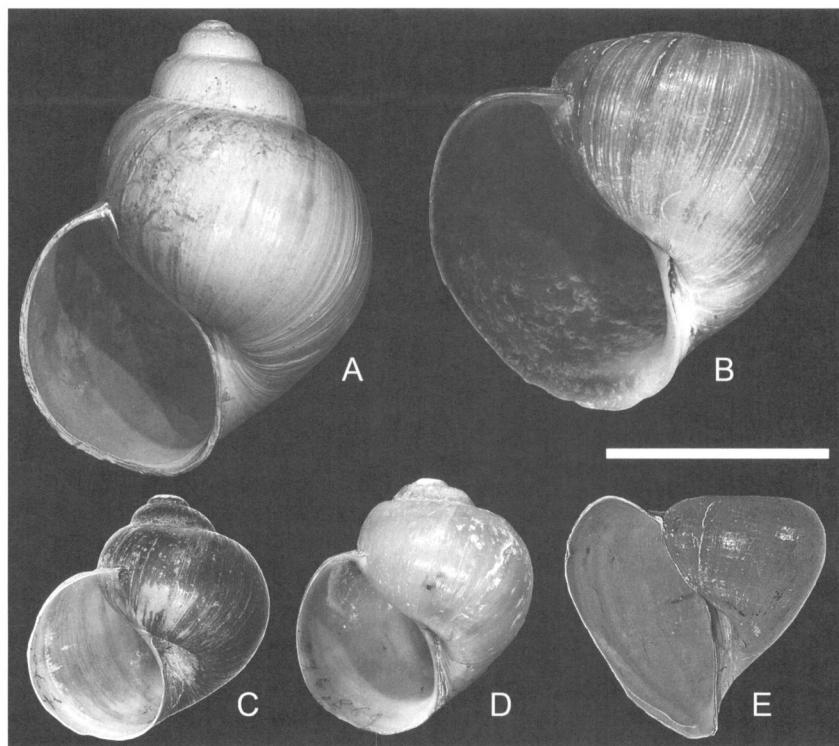


FIGURE 1. Holocene *Lanistes* species of the Malawi basin: A, *L. ovum* (SRI SH02-08-92.4.52). B, *L. nyassanus* (RUP-DVD LAM-02). C, *L. ellipticus* (MRAC MB 796-867). D, *L. solidus* (MRAC MB 796-869 III). E, *L. nasutus* (BMNH; Eccles collection). Spiral height, resulting from the displacement along the spiral axis during ontogeny, determines the main morphological differences between these species. Scale bar, 30 mm.

because our interest lies in general patterns. Collection numbers identify the institutions that house the shells included in this study: BMNH, the National History Museum

(London, U.K.); RUP, the Research Unit Palaeontology of Ghent University (Ghent, Belgium); MRAC, the Royal Museum of Central Africa (Tervuren, Belgium); SRI, the Senckenberg Research Institute (Frankfurt am Main, Germany); and UGSB, the Department of Animal Ecology and Systematics of the Justus Liebig University (Giessen, Germany). Tentative identifications of the species were based on the commonly adopted qualitative identification system of *Lanistes* and *Melanoides* in Lake Malawi, including anatomical and conchological characters (Crowley et al. 1964; Mandahl-Barth 1972; Brown 1994). In addition, we used habitat information and ecological preferences for *Lanistes* wherever possible (Louda and McKaye 1982; Louda et al. 1983; Louda et al. 1984). Numerous specimens identified by earlier authorities (Crowley et al. 1964; Mandahl-Barth 1972; Brown 1994) have been included to ensure consistent interpretation of diagnostic features and therewith accurate identification.

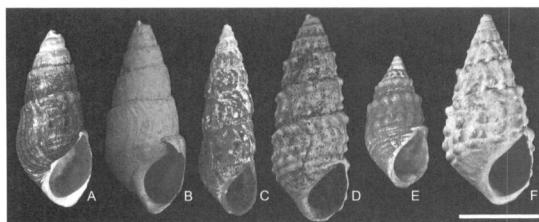


FIGURE 2. Endemic, Holocene *Melanoides* species of the Malawi basin included in this study: A, B, *M. polymorpha* (MRAC MB 796-885 I; SRI SH02-08-92.4.69). C, D, *M. pupiformis* (MRAC MB 796-882; SRI SH02-08-92.4.75). E, F, *M. turritispira* (RUP Pain; SRI SH02-08-92.8.183). *M. polymorpha* and *M. turritispira* are highly variable in general shell morphology (mainly in spiral height and the spiral angle). *M. turritispira* shells often are strongly nodular, with a marked spiral ridge below the suture, giving the shell a steplike appearance, whereas *M. polymorpha* has a less marked spiral ridge and no nodules. *M. pupiformis* has in general a higher spire than the other two species and a variable ornamentation. Scale bar, 5 mm.

Methods

Morphometric Methods.—Shells were digitized in apertural view, with the spiral axis horizontal and the outer lip so that a line tangent to the apertural margin, perpendicular to the horizontal plane could be constructed. Because shell shape in gastropods accrues from aperture shape, coiling, ontogenetic change in aperture shape, and ontogenetic change in coiling, animals with slightly different ages will likely have shells with a different number of whorls. Moreover, ecological conditions may speed up or slow down shell accretion. As a result, biologically homologous points can be difficult to identify along the coiling axis. We addressed this problem by selecting specimens with a similar number of whorls, and by counting the whorls beginning with the body whorl in our landmark-based approaches, rather than following the ontogenetic growth process. This buffers the analysis against minor differences in the number of whorls, because anchoring on the body whorl shifts differences in the number of whorls to the tiny apical whorls. Digitization was performed using a digital SLR camera for *Lanistes* and an Olympus DC 10 camera mounted on an Olympus SZX 12 stereomicroscope for *Melanoides*. Images were modified using Adobe CS2 products. Slightly damaged shells were reconstructed in order to reduce non-biological, postmortem, or taphonomic noise. If unambiguous reconstruction was impossible, the material was omitted from the analyses. All morphometric analyses were based on the same set of digital images.

In complex shapes, like *Melanoides* shells, morphospace occupation will be determined by the combined effect of general shape (similar to the case of *Lanistes*) and ornamentation parameters. An accurate morphological study of such ornamented shells requires the ability to discern the variation induced by the respective sets of parameters. We used TM to separate general shell shape and ornamentation signals. To isolate the ornamental signal, we compared the distance along the outline between landmarks L4 and L9 with the straight distance between both (Fig. 3E).

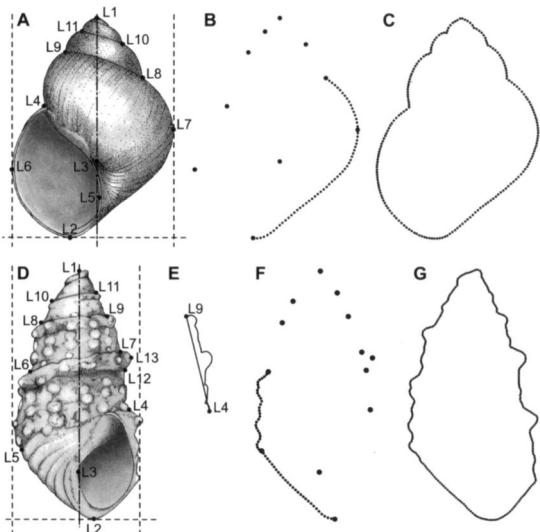


FIGURE 3. Markers utilized to document the shell morphometry for the various analyses performed. Drawings of the shells of *Lanistes ovum* (A) and *Melanoides turritispira* (D), indicating the axis of the spire (dash-dotted line) and the framework of parallel and perpendicular lines (dashed lines) to position landmarks at the extremes of body whorl curvatures. Landmarks utilized for the landmark analysis (LMA) are indicated in A (L1–L11; *Lanistes*) and D (L1–L13; *Melanoides*). B, F, Plots of the landmarks and the 60 semi-landmarks along the open curve utilized during semi-landmark analysis (SLM) for *Lanistes* and *Melanoides*, respectively. C, G, Plots of the outline of the *Lanistes* shell (C) and the *Melanoides* shell (G) as reconstructed from 200 and 300 equidistantly located points around the outline, respectively. These points were the input for the elliptic Fourier analysis (EFA). E, The two line segments measured to document shell ornamentation for *Melanoides* via traditional morphometrics.

Because differences in inflatedness of whorls between groups are negligible, we consider this ratio to be a good predictor of the degree of shell ornamentation in *Melanoides* independent of general shell shape. We then compared the ornamentation signal obtained by TM with the results of other morphometric techniques, in order to examine the abilities of the various techniques to document shell ornamentation. Measurements were obtained using ImageTool 3.0 (Wilcox et al. 2002).

Prior to landmark analysis (LMA), elliptic Fourier analysis (EFA), and semi-landmark analysis (SLM), the digital images were compiled in tps format using TpsUtil, version 1.38 (Rohlf 2006). Landmark analysis was performed for both *Lanistes* and *Melanoides* with 11 and 13 landmarks respectively

(Fig. 3A,D). Landmark digitization was performed utilizing TpsDig 2, version 2.12 (Rohlf 2008). Of these, respectively seven and nine landmarks are of type 2, and in each genus four landmarks are probably of type 3 (Bookstein 1991). When landmarks were concealed on the images, the position of landmarks was reconstructed by utilizing needles and photo overlay. We included type 3 landmarks because of the restricted coverage by type 2 landmarks, therewith relaxing the homology criterion. This has been done to a comparable or larger degree in previous landmark studies on gastropod shells (Carvajal-Rodríguez et al. 2005; Conde-Padín et al. 2007a,b, 2009; Genner et al. 2007). Replication of landmark assignment indicated that general differences (i.e., error) associated with the relocation of homologous points on each shell were minimal (*Lanistes*: $0.29 \pm 0.21\%$ centroid size; *Melanoides*: $0.18 \pm 0.13\%$ centroid size; see Appendix 1 of the supplemental material, online at <http://dx.doi.org/10.1666/08068.s1>). In order to reduce the effect of the relocation error, landmark coordinates of each shell were averaged and these average coordinates were utilized for subsequent geometric analysis. The landmark coordinates were made invariant to size, orientation, and position by a least-squares Procrustes fitting in PAST, version 1.80 (Hammer et al. 2001) prior to the analysis. After transforming landmark coordinates to 2-D Procrustes coordinates the mean was subtracted, yielding Procrustes residuals (Dryden and Mardia 1998; Hammer and Harper 2006). These Procrustes residuals were projected in tangent space utilizing TpsSmall, version 1.20 (Rohlf 2003) to assess the correlation between Procrustes and Kendall tangent space distances. This ensured that the amount of shape variation in the data set was small enough to allow statistical analysis in linear tangent space as an approximation of nonlinear Kendall shape space (Rohlf 1999). This has been performed with similar results for SLM. The Procrustes residuals were used for ordination, classification, and cross-validation.

Elliptic Fourier analysis (Kuhl and Giardina 1982) is, within the class of conventional

Fourier procedures, the best suited to handle complicated shapes, and is thus the most powerful Fourier technique (Rohlf and Archie 1984; Lestrel 1989; Hammer and Harper 2006). In two-dimensional EFA, the increments in the *x* coordinate from point to point around the outline define a periodic function along the outline, which can be subjected to Fourier decomposition into sinusoidal components (Hammer and Harper 2006). The increments in the *y* direction are subjected to a similar, independent decomposition. This results in a set of four coefficients for each harmonic, namely the sine and cosine amplitudes of the *x* and the *y* increments. The more harmonics used, the better the reconstructed outline contour fits the original object. For complex shapes, more harmonics are thus needed to describe the contour accurately, but the number of points along the outline should at least be twice the number of harmonics used (Hammer et al. 2001; Hammer and Harper 2006). Outline digitization was performed with TpsDig 1, version 1.14 (Rohlf 2004). As a starting point for digitization of each *Lanistes* contour, the position of landmark L7 was used, and each shell outline was characterized by 200 equidistant markers (Fig. 3C). The contour data were normalized to be invariant to size, rotation, and starting point (Lestrel 1989), because the alignment was reliable. For *Melanoides*, landmark L4 was used as the digitization starting point and, owing to the complexity of the shell shape, 300 equidistant markers were used to characterize the shell accurately (Fig. 3G). Contour data were normalized to be invariant to size, but not to rotation and starting point, because these latter normalizations reduced the accuracy of alignment in the *Melanoides* data set significantly: several shells were aligned with a rotation error of 5–20°, and some even ~180°. Therefore, manual alignment was preferred for *Melanoides*. As the outline was obtained automatically, practically no error was associated with tracing contours. Elliptic Fourier analysis was performed with PAST, version 1.80 (Hammer et al. 2001), utilizing all 20 harmonics available in order to reproduce the original shape adequately. Independent analyses with SHAPE (Iwata and Ukai 2002),

where more harmonics can be utilized, were performed to evaluate whether 20 harmonics suffice to characterize the complex outline of some strongly ornamented *Melanoides* shells. Because increasing the number of harmonics up to 30 did not result in a visible difference in shell representation, nor in morphospace occupation, 20 harmonics were considered to be sufficient. The resulting Fourier coefficients were used subsequently for ordination, classification, and cross-validation.

For semi-landmark analysis, the landmarks utilized for the LMA were combined with an open curve along the body whorl (Fig. 3B,F). Along this curve, 60 equidistant points (semi-landmarks) were sampled via the resample-curve-by-length option in TpsDig 2, version 2.12 (Rohlf 2008), and are thus not entirely homologous. The curve was anchored on LM8 and LM2 for *Lanistes* and LM6 and LM2 for *Melanoides*. Variation in scale, orientation, and position was removed via Procrustes superimposition using the program CoordGen6h (Sheets 2008), after which points were slid along the curve with the program SemiLand6, utilizing the minimum Procrustes distance criterion (Sheets 2008). This alignment criterion, also called perpendicular projection, removes differences in semi-landmark positions along the curve between the reference form and each specimen by estimating the direction tangential to the curve and removing the component of the difference that lies along this tangent (Sheets et al. 2004). The resulting partial Procrustes superimposition coordinates were then utilized for ordination, classification, and cross-validation.

Ordination.—We used nonmetric multidimensional scaling (nmMDS) (Cox and Cox 2000) with the Procrustes residuals (LMA), the Fourier coefficients (EFA), and the partial Procrustes superimposed coordinates (SLM) to visualize the data in the diverse treatments. Nonmetric MDS is promoted as a highly reliable ordination method because of its limited assumptions (McCune and Grace 2002; Brehm and Fiedler 2004). This rank-based multivariate analysis utilizes an iterative search aiming to find the positions of n entities in a k -dimensional space that

minimizes the stress of the k -dimensional configuration (Venables and Ripley 2002). We constructed a Euclidean distance matrix of each data set (and each genus) that was iterated for ordination in two dimensions ($k = 2$). Preliminary runs showed convergence to be reached mostly before 50 iterations, so we set the maximum number of iterations to 300. To avoid being trapped in local minima we performed the analyses with 1000 random starting configurations for each data set, following the suggestions of Minchin (1987). The stress values (i.e., goodness-of-fit) obtained for the nmMDS solutions (Fig. 4) were multiplied by 100, and evaluated using Kruskal's and Clarke's rules of thumb (Kruskal 1964; Clarke 1993). The values indicated ordination to be good (values ≤ 10), with limited risk of drawing false inferences. Nonmetric MDS was performed in R (R Development Core Team 2007) using the packages MASS (Venables and Ripley 2002) and vegan (Oksanen et al. 2008). The results of nmMDS are very similar to those of the more commonly adopted ordination technique of principal components analysis (Hotelling 1933) (see Appendix 2 in the online supplemental information (<http://dx.doi.org/10.1666/08068.s1>)).

Correlation of Pairwise Distances.—In order to quantify the similarity between the LMA, EFA, and SLM data sets for each taxon, we analyzed the correlation of pairwise distances. This assesses the degree of overall similarity in how the morphometric techniques, with their underlying theoretical differences, sample the same shell shapes. For these pairwise tests, Euclidean distance matrices were constructed utilizing, respectively, the Procrustes residuals (LMA), the Fourier coefficients (EFA), and the partial Procrustes superimposition coordinates (SLM). The analysis was conducted via Mantel tests (Mantel 1967), which randomly permute rows and columns, yielding distribution matrix correlations for unrelated matrices (Dietz 1983). We used the package "ade4" (Chessel et al. 2004) in R (R Development Core Team 2007) with 1000 permutations.

Classification and Cross-Validation.—Although the above tests allow statistical comparison of

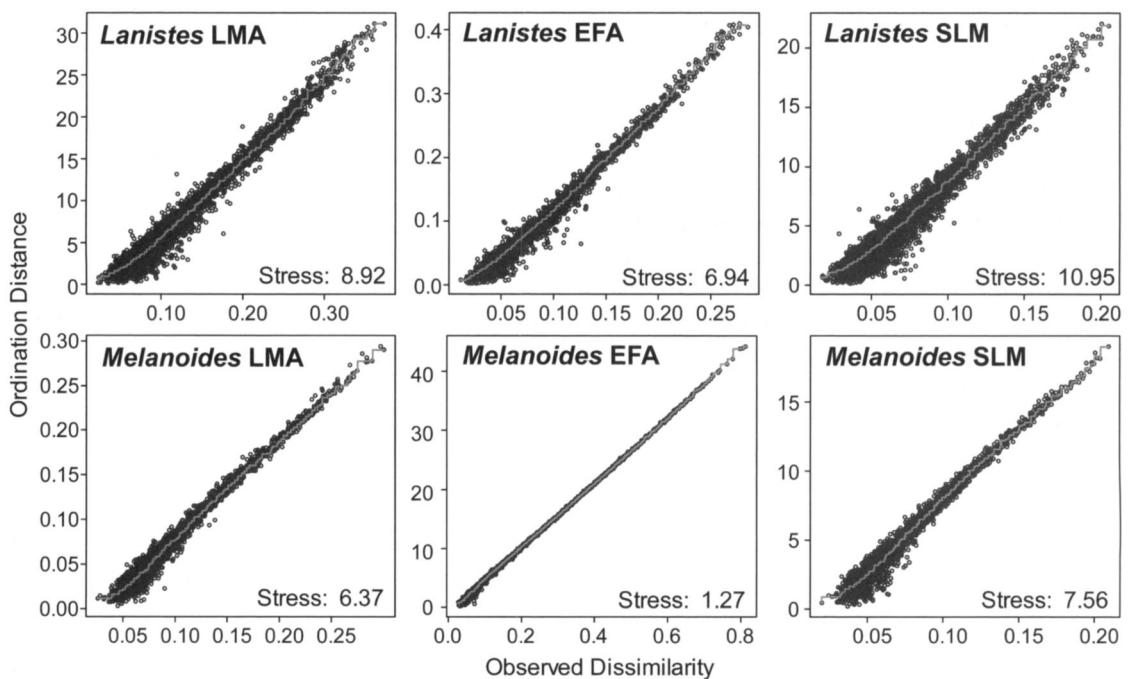


FIGURE 4. Plots of observed dissimilarities in the original distances versus the reproduced distances by nonmetric multidimensional scaling (Shepard plots) provide an indication of the stress in the nonmetric multidimensional scaling. The step-function of $d\text{-hat}$ values (central line) results from monotone transformation of the input data. The better the reproduced distances fit to the step-line, the better the rank-ordering of distances is performed by the solution; deviations from the step-line indicate a lack of fit. All six plots show a strong linear relationship between the two distance matrices, fitting the step-line well. The resulting, comparatively low stress values (≤ 10) indicate ordination to be good and robust.

how morphological variation was recorded within data sets, they yield no information on biological patterns. Such biological interpretation of differences and similarities requires discrimination of within-group variation relative to between-group differences. A difficulty of this approach is that patterns of morphospace occupation do not necessarily reflect true biological patterns, and/or that biological affinities of the biota analyzed are often unknown. For several reasons, we used classifying support vector machines (SVMs) as an alternative to classical statistical analyses that discriminate between two or more groups specified a priori, e.g., canonical variate analysis (CVA). First, multivariate normality is an assumption of linear CVA, but for morphometric data sets it often cannot be obtained. Second, CVA requires that there be more specimens than variables, because matrix inversion of the pooled within-group variance-covariance matrix requires the matrix to be fully ranked. This poses problems for

procedures that involve large numbers of variables, as, e.g., EFA and SLM. Consequently, the dimensionality of the data has to be reduced prior to discriminant analysis. This is potentially problematic—earlier work (Sheets et al. 2006) has suggested that dimensionality reduction can affect the classification performance. This may be because techniques for reducing dimensionality, e.g., PCA, perform a rigid rotation of space to represent the largest amount of variance possible on each axis regardless of the source of variation. The signals of between-group differences and within-group variation may thus become blurred, potentially affecting subsequent discrimination. Finally, more sophisticated classification techniques may be more effective in discriminating performances of various morphometric techniques, where CVA remains inconclusive (Sheets et al. 2006). Indeed, preliminary experiments comparing CVA and SVM classification do not deny superiority of SVMs (see Appendix 3 in the online

supplemental material at <http://dx.doi.org/10.1666/08068.s1>.

Support vector machines are a set of related learning methods for classification and regression. The use of SVMs has been growing exponentially since the mid-1990s (Cortes and Vapnik 1995) although the theoretical basis was formulated earlier (Vapnik 1979). SVMs are now recognized as the most successful techniques for pattern recognition, with generalization performances (i.e., error rates on test sets) either matching or outperforming competing methods (Burges 1998; Brown et al. 2000; DeCoste and Schölkopf 2002). They are based on the statistical learning theory, which is traditionally concerned with balancing empirical risk and the capacity of a learning machine in order to obtain a small actual risk (i.e., a good generalization performance) (Vapnik 1998; Zhang et al. 2004). In the case of linearly separable binary classification, the input data are considered as two sets (two-class) of p -dimensional vectors (data point with p features), and classification decisions are based on linear combinations of the features. An SVM classifier will attempt to construct a separating $p-1$ dimensional hyperplane in that space, which maximizes the separation between the two sets of data. The larger the distance of the hyperplane to the neighboring data points of both classes, the better the generalization is, and, therefore, the smaller the empirical classification error obtained by the classifier will be. In some cases, the classes are not linearly separable or the separation of more than two sets of data is required. The first problem can be solved by the introduction of kernels, which project the input data to a high-dimensional or even infinitely dimensional feature space (Schölkopf and Smola 2001). Translating the training sets into a higher-dimensional space exposes the learning system to the risk of finding trivial solutions that overfit the data, but SVMs elegantly sidestep this difficulty by choosing the maximum margin separating hyperplane from among the many that allow separation in the feature space (Brown et al. 2000). Expansion of SVMs from two-class (binary) to multiclass problems occurs by decomposing a single multiclass problem into

multiple binary problems. The SVM analyses have been performed with Weka 3.5.7 (Witten and Frank 2005), utilizing the sequential minimal optimization algorithm (SMO) (Platt 1998). This algorithm was modified (Keerthi et al. 2001) and expanded to account for multiclass problems via pairwise classification (Hastie and Tibshirani 1998). For our analyses a polynomial kernel was utilized. Analyses were performed with the Procrustes residuals of the LMA, the sinusoidal components of the EFA, and the partial Procrustes superimposition coordinates of the SLM. Training of the SVM classifier was performed utilizing K-fold cross-validation (kCV), a technique that takes a set of n examples and partitions them into k sets (folds) of n^*k^{-1} examples. For every iteration over the folds, a classifier is trained on $k-1$ folds and then tested on the remaining fold. Machine learning algorithms were utilized to train and test learning schemes with 2, 5, and 10 folds, partitioning the data into 50% training and 50% validation, 80% training and 20% validation, and finally, 90% training and 10% validation, respectively. The larger the training fraction, the better each class (in our case = (morpho)species) will be characterized, and thus the more reliable the validation ought to be. Cross-validation requires each class to be represented well enough to make training and testing possible. Therefore, these analyses were performed only with well-represented species (in essence $\sim 10\%$ of the total number or more). The *Lanistes* data set comprises *L. ovum* ($n = 26$), *L. solidus* ($n = 38$), and *L. nyassanus* ($n = 22$), and the *Melanoides* data set consists of *M. polymorpha* ($n = 19$), *M. pupiformis* ($n = 6$), and *M. turritispira* ($n = 39$). Because tests from 50 up to 1000 iterations showed differences to be not significant ($p_{(same)} \geq 0.226$), we performed 100 iterations per experiment. We calculated one F -measure per fold, and averaged all F -measures per iteration (e.g., in 2CV, two F -measures were obtained per iteration, and these were averaged per iteration). A pool of 100 F -measures (number of iterations) was thus obtained for each experiment (2CV, 5CV, 10CV), and the final performance was documented by comparing these pools. Precision

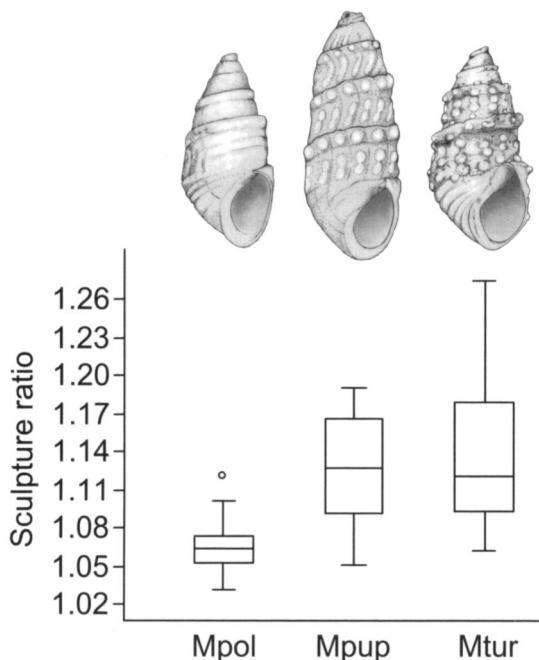


FIGURE 5. Box plots indicate the strength of ornamentation as measured by traditional morphometrics in *M. polymorpha* (Mpol), *M. pupiformis* (Mpup), and *M. turritispira* (Mtur). The sculpture ratio was calculated from the measurements of the straight distance and the distance along the outline between two landmarks on the outline (Fig. 3, E). Outliers ($>1.5 \times$ the interquartile range away from the box ends) are indicated with circles. The difference between *M. polymorpha* and *M. turritispira* is highly significant (Table 1).

and recall were evenly weighted ($\beta = 1$) and the *F*-measure (van Rijsbergen 1979) is defined as

$$F_1 = \frac{2 * \text{Precision} * \text{Recall}}{\text{Precision} + \text{Recall}}$$

with

$$\text{Precision} = \frac{TP}{TP + FP}$$

$$\text{Recall} = \frac{TP}{TP + FN}$$

TP denotes the number of true positives, FP the number of false positives, and FN the number of false negatives. Precision is thus the proportion of examples that truly have class x among all those that were classified as class x . Recall, equivalent to the true positive rate, is the proportion of examples that were classified as class x among all examples that truly have class x . The false positive rate is the

TABLE 1. Traditional morphometrics documenting shell ornamentation in *Melanoides*. Mann-Whitney pairwise comparison of the equality of the medians (Bonferroni corrected\uncorrected) of shell sculpture ratios in *M. polymorpha* (Mpol), *M. pupiformis* (Mpup), and *M. turritispira* (Mtur) indicates a significant difference in shell sculpture between Mpol and Mtur; significant values are indicated in bold.

	Mpol	Mpup	Mtur
Mpol			<0.001
Mpup		0.051	0.777
Mtur	<0.001		1.000

proportion of examples which were classified as class x , but belong to another class.

Results

Traditional Morphometrics.—This type of morphometrics was only applied to *Melanoides* to document differences in shell sculpture independently from general shell parameters. The Shapiro-Wilk normality test indicated that the shell sculpture ratios for *M. polymorpha* ($p = 0.268$) and *M. pupiformis* ($p = 0.987$) are likely normally distributed, since the null hypothesis (normality) cannot be rejected. Those of *M. turritispira* are not ($p = 0.025$), because lumping modern and fossil shells created a bimodal pattern. Figure 5 shows the sculpture ratios for the three species. The nonparametric Kruskal-Wallis test showed significant differences ($p < 0.001$) in these ratios between the three species. Mann-Whitney pairwise comparisons indicate that at least *M. polymorpha* and *M. turritispira* have a highly significant difference in shell sculpture (Table 1). The difference in ornamentation between *M. polymorpha* and *M. pupiformis* is much less pronounced.

Landmark Analysis.—The stress associated with nmMDS was fairly low (*Lanistes*: 8.92; *Melanoides*: 6.37; Fig. 4), suggesting that the LMA plots in Figures 6 and 7 are reliable two-dimensional representations of the data. For both genera studied, considerable overlap of at least two species is observed.

Elliptic Fourier Analysis.—The stress associated with plotting the data in two dimensions was low compared to those values obtained for the other analyses (*Lanistes*: 6.94; *Melanoides*: 1.27; Fig. 4). The EFA and LMA plots

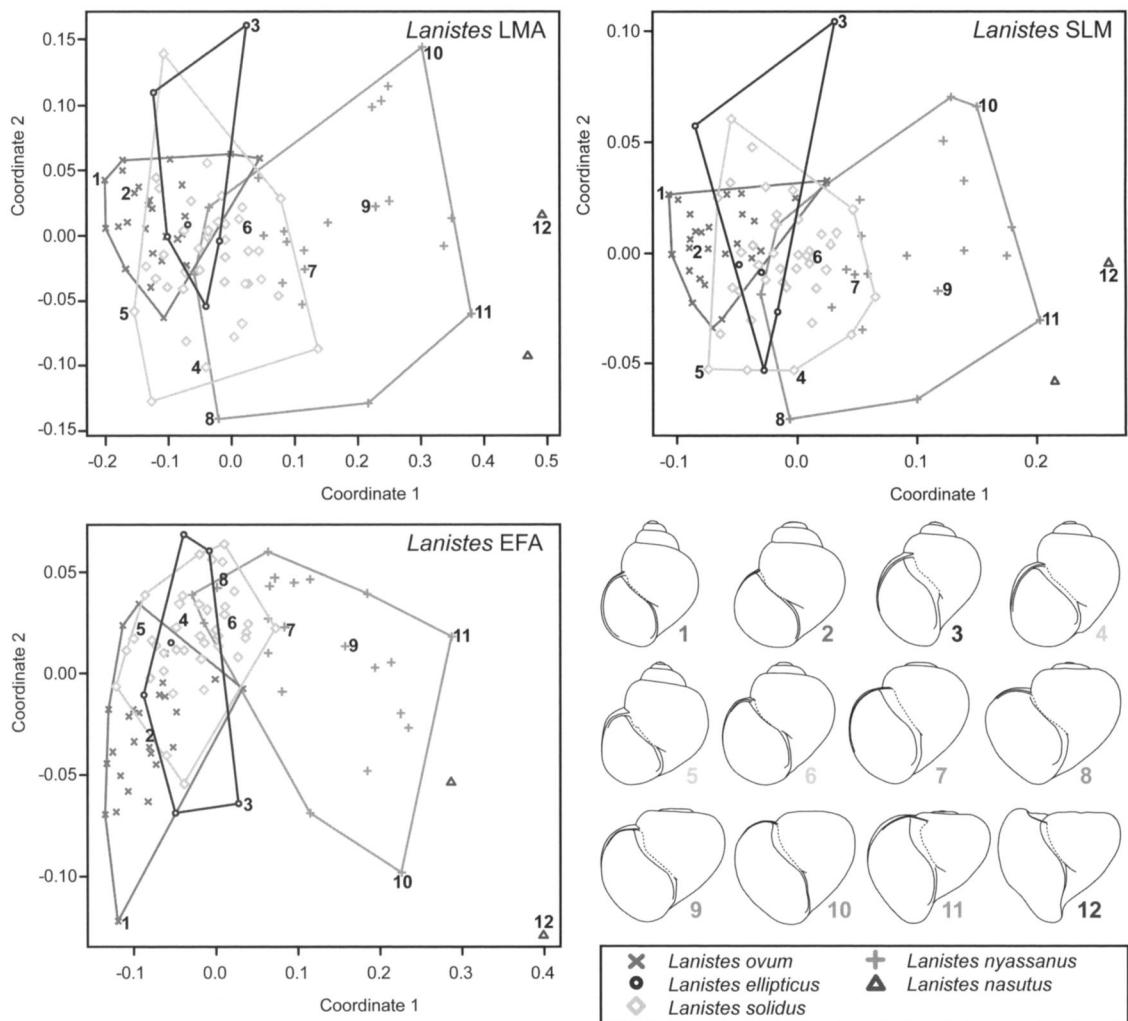


FIGURE 6. Nonmetric multidimensional scaling plots for *Lanistes*. The plots visualizing the landmark (LMA), elliptic Fourier (EFA), and semi-landmark (SLM) analyses indicate differences to be minor, although coordinate 2 in the EFA plot is mirrored compared to LMA and SLM. Schematic shell shape has been indicated for several specimens (not to scale), allowing comparison of morphospace occupation.

for *Lanistes* are somewhat different (Fig. 6), with the positions mirrored along coordinate 2 (e.g., compare positions of specimens 10 and 11). Although some specimens have shifted positions somewhat compared to the surrounding specimens, this does not result in group displacements. For *Melanoides*, differences between the LMA and EFA plots are more limited than for *Lanistes* (Fig. 7).

Semi-Landmark Analysis.—The stress-values associated with nMDS of the partial Procrustes superimposition coordinates were higher than those for the above-mentioned analyses (*Lanistes*: 10.95; *Melanoides*: 7.56; Fig. 4). Although comparatively high, the

stress associated with plotting the multivariate data sets in two dimensions remains acceptable according to the general criteria (Kruskal 1964; Clarke 1993). This is corroborated by the very high similarity between the LMA and SLM plots for each genus (Figs. 6, 7). The differences between the EFA and SLM plots are similar to those for EFA versus LMA.

Correlation of Pairwise Distances.—Nonmetric MDS plots indicate that the three methods of data acquisition (LMA, EFA and SLM) capture morphological diversity rather similarly, which could, from visual inspection of the plots, lead to similar or almost identical

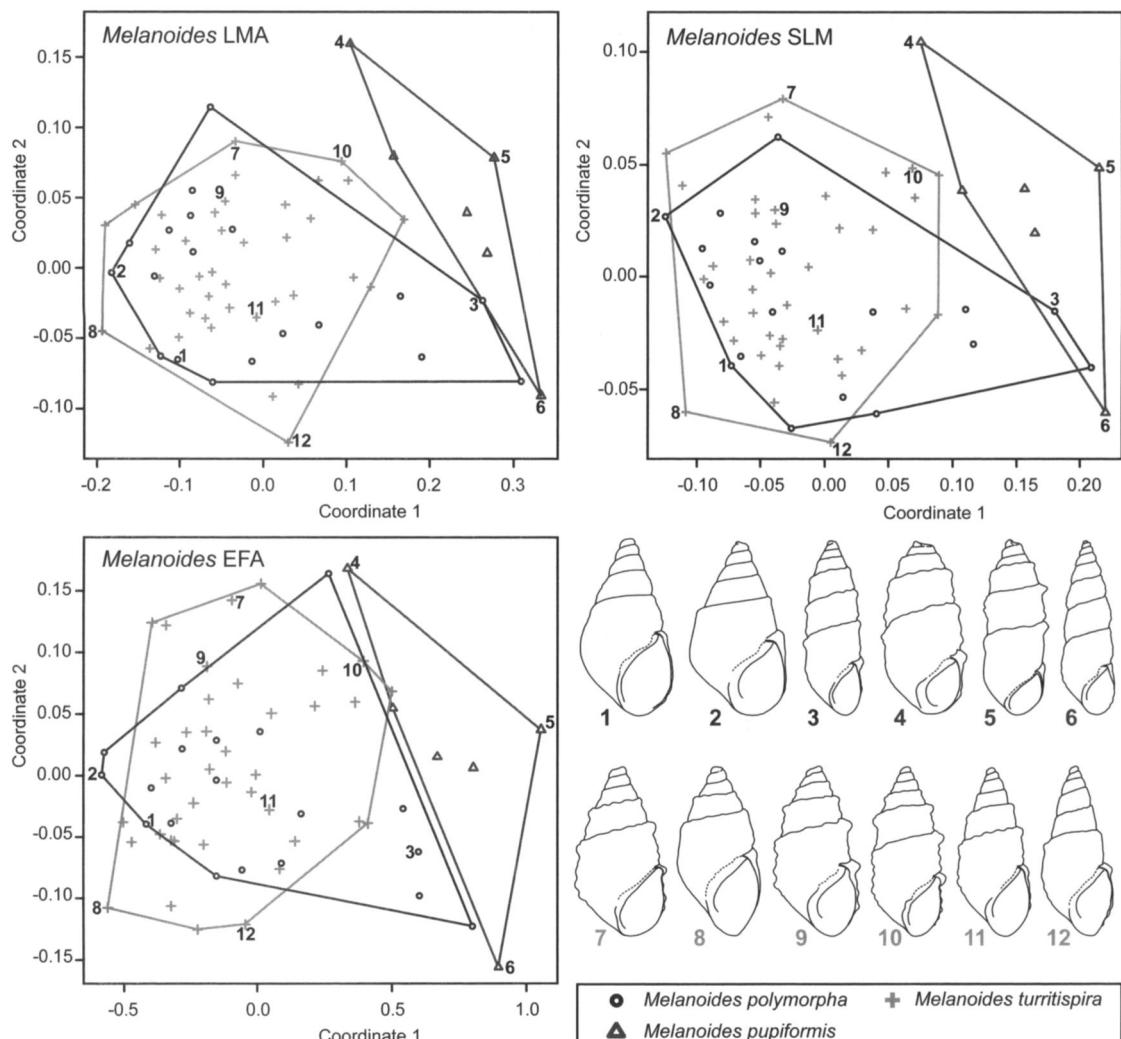


FIGURE 7. Nonmetric multidimensional scaling plots for *Melanoides*. As for *Lanistes* (Fig. 6), the plots visualizing the landmark (LMA), elliptic Fourier (EFA) and semi-landmark (SLM) analyses indicate that differences are minor. Again, schematic shell shape has been indicated for several specimens (not to scale), allowing comparison of morphospace occupation.

biological conclusions. Mantel tests were utilized to quantify similarities in the morphological information recorded by the LMA, EFA and SLM data sets, and comparison of derived distance matrices reveals a strong positive correlation ($z = 0.867\text{--}0.979$; Table 2), corroborating the similarities visualized by nmMDS. This suggests that although the various techniques utilize different theoretical frameworks, there are no inconsistencies in the morphological diversity they record.

Classification and Cross-Validation.—Although all methods sample morphological diversity consistently, differences may affect

biological discrimination. Figure 8A depicts the abilities (evaluated by *F*-measure values) of different morphometric techniques in assigning specimens from the *Lanistes* data set to the species they have been identified with a priori (*L. ovum*, *L. solidus*, and *L. nyassanus*). This suggests that EFA performs somewhat better than SLM and LMA in discriminating between-group differences from within-group variation. Because the values for the *F*-measures are not normally distributed for each treatment (2CV, 5CV, or 10CV) (Shapiro-Wilk: LMA 2CV $p = 0.174$; LMA 5CV $p = 0.026$; LMA 10CV $p = 0.642$;

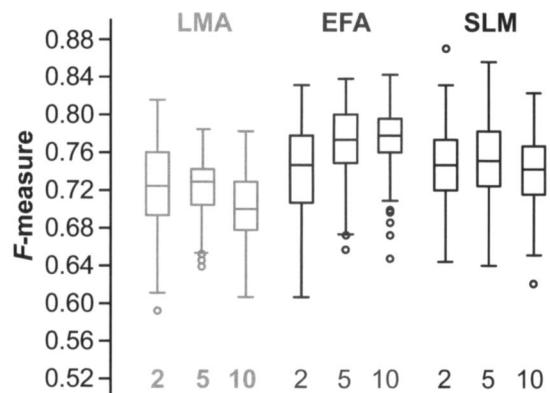
TABLE 2. Comparison of pairwise distances for all data sets. Correlations between target matrices as obtained by an analysis of the correlation of pairwise distances indicate a strong positive correlation, and thus high similarity, in all *Lanistes* and *Melanoides* data sets, respectively. The z -values are provided in the upper triangles, and the associated p -values in bold in the lower triangles. Mantel tests indicated each correlation to be highly significant, but note that the morphological shapes sampled by the morphometric techniques are the same and thus not independent. Hence, the p -values may not be reliable.

<i>Lanistes</i>			<i>Melanoides</i>		
LMA	EFA	SLM	LMA	EFA	SLM
0.935	0.979		0.884	0.968	
<0.001			<0.001		0.867
<0.001	<0.001		<0.001	<0.001	

EFA 2CV $p = 0.068$; EFA 5CV $p = 0.013$; EFA 10CV $p < 0.001$; SLM 2CV $p = 0.500$; SLM 5CV $p = 0.927$; SLM 10CV $p = 0.715$), the assumptions for an Analysis of Variance (ANOVA) are violated. A Kruskal-Wallis test was therefore preferred as a nonparametric alternative. It reports a highly significant difference ($p < 0.001$) in F -measures between the treatments. The Bonferroni-corrected pairwise comparison (Table 3) does not reject that EFA 5CV and 10CV, the two most accurate cases representing EFA, are drawn from the same population, but it does suggest that this population is significantly different from those of all the other treatments, except for SLM 5CV. The F -measures of SLM 5CV may be derived from the same population as that of EFA 5CV ($p = 0.196$), but not as that of EFA 10CV ($p = 0.009$). We therefore conclude that EFA seems to perform slightly but, at least for 10CV, significantly better than SLM, and definitely better than LMA in biological discrimination.

Mantel tests indicate that similarities in the *Melanoides* data sets, compiled with diverse morphometric approaches, are not as strong as in the *Lanistes* data sets. Likewise, Figure 8B shows much more pronounced differences in performance during cross-validation for *Melanoides* than for *Lanistes*. This relates to large differences in the abilities of the utilized approaches to assign specimens to the species they have been identified with a priori. Again, the values for the F -measures are not normally distributed for each treatment

A *Lanistes*



B *Melanoides*

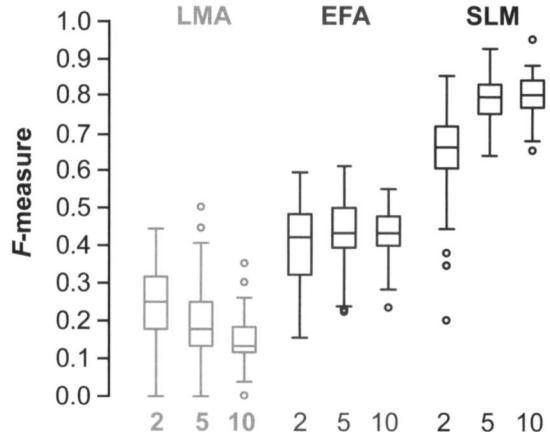


FIGURE 8. Box plots of the F -measure values for the diverse experiments (with 100 iterations each) allow comparison of cross-validation performance for *Lanistes* (A) and *Melanoides* (B). For *Lanistes*, elliptic Fourier analysis performs best, whereas SLM performed best for *Melanoides*. Differences in the power of discernment are far more pronounced for the *Melanoides* data sets than for *Lanistes*. LMA, landmark analysis; EFA, elliptic Fourier analysis; SLM, semi-landmark analysis; numbers below the box plots indicate the number of folds in the cross-validation. Outliers ($>1.5 \times$ the interquartile range away from the box ends) are indicated with circles.

(Shapiro-Wilk: LMA 2CV $p = 0.272$; LMA 5CV $p = 0.038$; LMA 10CV $p = 0.051$; EFA 2CV $p = 0.016$; EFA 5CV $p = 0.023$; EFA 10CV $p = 0.100$; SLM 2CV $p < 0.001$; SLM 5CV $p = 0.881$; SLM 10CV $p = 0.136$). The Kruskal-Wallis test reports a highly significant difference ($p < 0.001$) in F -measures between the treatments. Table 4 gives Bonferroni-corrected Mann-Whitney pairwise comparison values. Comparison of SLM 5CV and SLM 10CV did not yield a significant difference, but

TABLE 3. Comparison of *F*-measure values for *Lanistes*; standard parameters for the *F*-measures obtained after 100 iterations are provided in the upper part and the results of Mann-Whitney pairwise comparisons (Bonferroni-corrected) below. The table indicates the performance of EFA 5CV and 10CV to be significantly better than that of other treatments (except for SLM 5CV in comparison to EFA 5CV). *k*CV indicates the number of folds in the cross-validation; significant values are indicated in bold. LMA, landmark analysis; EFA, elliptic Fourier analysis; SLM, semi-landmark analysis.

Treatment	LMA			EFA			SLM		
	2CV	5CV	10CV	2CV	5CV	10CV	2CV	5CV	10CV
Mean	0.723	0.723	0.700	0.742	0.770	0.774	0.745	0.754	0.740
SD	0.047	0.030	0.038	0.049	0.038	0.035	0.042	0.042	0.040
Var	0.002	0.001	0.001	0.002	0.001	0.001	0.002	0.002	0.002
Median	0.723	0.729	0.700	0.747	0.774	0.777	0.746	0.751	0.742
LMA 2CV									
LMA 5CV		1.000							
LMA 10CV	0.006		<0.001						
EFA 2CV	0.225	0.018		<0.001					
EFA 5CV	<0.001	<0.001		<0.001		0.002			
EFA 10CV	<0.001	<0.001		<0.001		<0.001		1.000	
SLM 2CV	0.100	0.001	<0.001		1.000		<0.001		<0.001
SLM 5CV	<0.001	<0.001	<0.001		1.000		0.196	0.009	1.000
SLM 10CV	0.664	0.047	<0.001		1.000		<0.001	<0.001	1.000
									0.505

these two treatments outperformed all other treatments in the classification. In fact, SLM 2CV also outperformed all LMA and EFA treatments.

In order to infer the causes of these differences in biological discrimination, we examined two additional experimental treatments: (1) the combination of LMA and EFA data into one data set, and (2) the utilization of the EFA coordinates as input to the semi-landmark analysis by applying the above-

mentioned SLM protocol and setting the centroid size to a default of 1.0. *Lanistes* was chosen as test case, and results are documented in Table 5. The table indicates that all classifications based on the use of EFA coordinates as semi-landmarks (SLA) perform significantly worse than standard EFA classifications, although the utilized coordinates are identical. Secondly, when the LMA and EFA data were combined, performance was worse than for EFA 5CV and 10CV, but

TABLE 4. Comparison of *F*-measure values for *Melanoides*; standard parameters for the *F*-measures obtained after 100 iterations are provided in the upper part and the results of the Mann-Whitney pairwise comparisons (Bonferroni-corrected) below. The table indicates that SLM 5CV and SLM 10CV outperform all other treatments. *k*CV indicates the number of folds in the cross-validation; significant values are indicated in bold. LMA, landmark analysis; EFA, elliptic Fourier analysis; SLM, semi-landmark analysis.

Treatment	LMA			EFA			SLM		
	2CV	5CV	10CV	2CV	5CV	10CV	2CV	5CV	10CV
Mean	0.246	0.187	0.147	0.405	0.436	0.436	0.647	0.787	0.800
SD	0.096	0.091	0.061	0.105	0.087	0.060	0.103	0.057	0.050
Var	0.009	0.008	0.004	0.011	0.008	0.004	0.011	0.003	0.002
Median	0.247	0.180	0.133	0.421	0.436	0.432	0.660	0.793	0.802
LMA 2CV									
LMA 5CV		<0.001							
LMA 10CV	<0.001		0.022						
EFA 2CV	<0.001	<0.001		<0.001					
EFA 5CV	<0.001	<0.001		<0.001		1.000			
EFA 10CV	<0.001	<0.001		<0.001		1.000			
SLM 2CV	<0.001	<0.001		<0.001	<0.001		<0.001		<0.001
SLM 5CV	<0.001	<0.001		<0.001	<0.001		<0.001		<0.001
SLM 10CV	<0.001	<0.001		<0.001	<0.001		<0.001		1.000

TABLE 5. Comparison of *F*-measure values for additional tests; standard parameters for the *F*-measures obtained after 100 iterations are provided in the upper part and the results of Mann-Whitney pairwise comparisons (Bonferroni-corrected) below. The table compares the performance of elliptic Fourier analysis (EFA) for *Lanistes* with the alternative approaches of combining EFA and landmark analysis data sets (COM) and utilizing EFA coordinates as semi-landmarks (SLA). At least EFA 5CV and EFA 10CV perform significantly better than all non-EFA treatments. Remarkable is that SLA 5CV and SLA 10CV perform significantly worse than all other techniques. *k*CV indicates the number of folds in the cross-validation; significant values are indicated in bold.

Treatment	EFA			COM			SLA		
	2CV	5CV	10CV	2CV	5CV	10CV	2CV	5CV	10CV
Mean	0.742	0.770	0.774	0.742	0.739	0.716	0.716	0.687	0.656
SD	0.049	0.038	0.035	0.050	0.033	0.030	0.051	0.042	0.039
Var	0.002	0.001	0.001	0.002	0.001	0.001	0.003	0.002	0.001
Median	0.747	0.774	0.777	0.743	0.740	0.715	0.718	0.692	0.659
EFA 2CV									
EFA 5CV	0.002								
EFA 10CV	<0.001	1.000							
COM 2CV	1.000	0.001	<0.001						
COM 5CV	1.000	<0.001	<0.001	1.000					
COM 10CV	<0.001	<0.001	<0.001	<0.001	<0.001				
SLA 2CV	0.019	<0.001	<0.001	0.018	0.061	1.000			
SLA 5CV	<0.001								
SLA 10CV	<0.001								

intermediate between that for the LMA and EFA data sets alone.

Discussion

Visual inspection and quantitative comparison of the two-dimensional nmMDS plots of each genus suggest a high similarity in the variance recorded by the respective data sets. Biological interpretation based on the total variation recorded within these plots would lead to highly similar, if not identical, conclusions. These findings are consistent with those of Loy et al. (2000). However, classification performances for the *a priori* identified taxa by each data set (LMA, EFA, SLM) for each genus (*Lanistes*, *Melanoides*) suggest that the methods differ significantly in their ability to perceive between-group differences and within-group variation. Although EFA performed slightly better than SLM for *Lanistes*, the limited differences between EFA and SLM suggest that both methods capture general shape parameters of relatively simple objects well. It cannot be ruled out that the small difference in classification performance relates to dissimilarities in our way of sampling objects (Fig. 3): while EFA sampled the complete shell outline, SLM

sampling focused on the body whorl. Additional variance in the penultimate and earlier whorls may therefore have helped EFA in the classification. Our results also confirm the problems of documenting shapes with few unambiguously assignable, homologous landmarks via LMA. The cross-validation performance for the relatively simple objects utilized here is suboptimal at best. The intermediate biological discrimination performance obtained by combining the EFA and LMA data sets seems to result from averaging out differences in the biological patterns obtained by two theoretically different methods. Overfitting might also contribute to this result (D. H. Sheets personal communication 2008). The larger differences in the Mantel test results for *Melanoides* compared to *Lanistes* may be due to different ways or different abilities in capturing shell ornamentation. Each *Melanoides* species included in our analyses shows a high diversity in general shell shape. General shell shape by itself will thus not allow high rates of correct assignment. However, the combination of general shell shape and significant differences in ornamentation allows successful classification, as indicated by the cross-validation

performance of SLM. The method of EFA has more problems than SLM in combining these signals into consistent pictures for each species. This is likely the result of the difficulties EFA experiences in discerning sculpture signals from noise. As it is, EFA analyses the complete shape of the structure and compares shapes for proportional differences (spiral height, spiral angle, aperture size, etc.), rather than examining minor differences in, for example, the periphery of a whorl. A possible source of this problem is alignment. This was corroborated by the results of our analysis utilizing EFA coordinates as semi-landmarks (SLA, Table 5). The EFA coordinates were subjected to the whole procedure, resulting in partial Procrustes superimposition values, but, as indicated by the inferior performance, the procedure was not able to deal well with the way EFA assembled information in the absence of homology. After all, a point n could be located on one whorl in one outline and on a different whorl in the outline of another specimen. Whereas EFA seems to be able to deal well with non-homologous partitions of the curve, SLM assumes homology and appears heavily affected upon violation of the assumption. The fact that EFA is robust to this issue almost necessarily implies a downside: the structure is aligned generally, but not accurately. Subtle differences in outline, which would require accurate alignment based on homology of every part of the curve in order to provide a consistent signal in the data set, are partly (or completely) lost in the noise of aligning the structure only generally.

The performance of LMA, although acceptable for *Lanistes*, is very poor for *Melanoides*. More than anything else, this illustrates that homologous landmarks may not allow capturing the differences in curvature and sculpture of objects. The inferior classification performance of LMA in complex shells illustrates the notorious difficulty in accounting for shell sculpture in morphometric analyses of gastropod shells. The 11 and 13 landmarks utilized in our analyses, similar to the average number utilized in most gastropod studies (Carvajal-Rodríguez et al. 2005; Conde-Padín et al. 2007a,b, 2009; Genner et al.

2007), allow but a partial documentation of between-group differences and within-group variance. Relaxing the homology criterion further may increase the resolution, but even if appropriate, it would strongly undermine the very criteria on which LMA is based. To deal with information along curves is the reason why landmark morphometrics were extended to semi-landmark techniques (Bookstein 1996, 1997; Green 1996; Sheets et al. 2004).

Conclusions

Here we presented the first comparison of LMA, EFA, and SLM for objects with few discernable homologous landmarks in a biological framework. Although research on additional taxa is desired to elaborate insights into the biological patterns that diverse morphometric analyses yield, and although the preferred morphometric technique depends strongly on the objectives of a study, we can provide the following general guidelines:

If documentation of a specific parameter, such as shell ornamentation, is desired, isolation of the specific morphological signal from that of other morphological parameters is recommended. Our study indicates that traditional morphometrics may be very effective in isolating such specific signals. If a variety of morphological parameters are to be documented, geometric morphometrics provide a useful set of tools. For simple objects, such as the *Lanistes* shells included in the present study, where morphological disparity is dominated by differences in general shape parameters, EFA seems to be a most valuable strategy. It is expected to be especially fruitful when most unambiguously assignable homologous landmarks are located along the outline of the structure, but are restricted in number because of the homology criterion. Although SLM may also perform well in some of these cases, a careful assessment of homology is required, whereas EFA remains relatively unaffected by homology problems. However, if general shape parameters and more subtle signals, like those of ornamentation, are to be integrated, EFA seems to be inappropriate and the performance of SLM

surpasses that of all other approaches. In EFA, general shape parameters mask the subtle differences, possibly because of inadequate alignment by lack of homology. The performance of LMA depends entirely on how accurately the structure can be covered with landmarks and how much information on curves between landmarks is desired. For simple objects with few unambiguously assignable, homologous landmarks, the performance of LMA is suboptimal at best.

Acknowledgments

Access to material was provided by F. Schrenk (Senckenberg Research Institute), G. Gruber, O. Sandrock (Hessisches Landesmuseum Darmstadt), J. Ablett (Natural History Museum) and D. Van den Spiegel (Royal Museum of Central Africa). H. D. Sheets is wholeheartedly acknowledged for his interest, thoughtful discussion, and generous help with the analyses, in particular semi-landmark approaches. The manuscript also benefited from constructive advice by Ø. Hammer, A. Cardini, P. J. Wagner, J. A. Todd, A. Gautier, T. Wilke, D. Van Damme, K. N. Mertens, and two anonymous referees. R. Roelant, M. Van Bel, and S. Van Landeghem provided expertise concerning classification, support vector machines, and learning algorithms; E. Coppejans and T. Schils provided access to the binocular microscope and camera. B.V.B. is Aspirant of the Flanders Research Foundation (FWO-Vlaanderen). The research received funding from the Flanders Research Foundation and the Ghent University Research Council (BOF) to B.V.B. and from DFG-Project SCHR352/9-1 and SYNTHESYS grant GB-TAF-3455 to R.S.

Literature Cited

- Adams, D. C., F. J. Rohlf, and D. E. Slice. 2004. Geometric morphometrics: ten years of progress following the 'revolution.' *Italian Journal of Zoology* 71:5–16.
- Anderson, L. C., and P. D. Roopnarine. 2003. Evolution and phylogenetic relationships of Neogene Corbulidae (Bivalvia; Myoidea) of tropical America. *Journal of Paleontology* 77:1086–1102.
- Bookstein, F. L. 1991. *Morphometric tools for landmark data: geometry and biology*. Cambridge University Press, Cambridge.
- . 1996. Applying landmark methods to biological outline data. Pp. 59–70 in K. V. Mardia, C. A. Gill, and I. L. Dryden, eds. *Image fusion and shape variability*. University of Leeds, Leeds, U.K.
- . 1997. Landmark methods for forms without landmarks: morphometrics of group differences in outline shape. *Medical Image Analysis* 1:225–243.
- . 1998. A hundred years of morphometrics. *Acta Zoologica* 44:7–59.
- Bookstein, F. L., R. E. Strauss, J. M. Humphries, B. Chernoff, R. L. Elder, and G. R. Smith. 1982. A comment upon the uses of Fourier methods in systematics. *Systematic Zoology* 31:85–92.
- Brehm, G., and K. Fiedler. 2004. Ordinating tropical moth ensembles from an elevational gradient: a comparison of common methods. *Journal of Tropical Ecology* 20:165–172.
- Brown, D. S. 1994. *Freshwater snails of Africa and their medical importance*. Taylor and Francis, London.
- Brown, M. P. S., W. N. Grundy, D. Lin, N. Cristianini, C. W. Sugnet, T. S. Furey, M. Ares, and D. Haussler. 2000. Knowledge-based analysis of microarray gene expression data by using support vector machines. *Proceedings of the National Academy of Sciences USA* 97:262–267.
- Burges, C. 1998. A tutorial on support vector machines for pattern recognition. *Data Mining and Knowledge Discovery* 2:121–167.
- Carvajal-Rodríguez, A., P. Conde-Padín, and E. Rolán-Alvarez. 2005. Decomposing shell form into size and shape by geometric morphometric methods in two sympatric ecotypes of *Littorina saxatilis*. *Journal of Molluscan Studies* 71:313–318.
- Cheetham, A. H., J. Sanner, and J. B. C. Jackson. 2007. *Metrarabdotos* and related genera (Bryozoa: Cheilostomata) in the Late Paleogene and Neogene of tropical America. *Paleontological Society Memoir* 67. *Journal of Paleontology* 81(Suppl. to No. 1).
- Chessel, D., A.-B. Dufour, and J. Thioulouse. 2004. The ade4 package – I: one-table methods. *R News* 4:5–10.
- Clabaut, C., P. M. Bunje, W. Salzburger, and A. Meyer. 2007. Geometric morphometric analyses provide evidence for the adaptive character of the Tanganyikan cichlid fish radiations. *Evolution* 61:560–578.
- Clarke, K. R. 1993. Non-parametric multivariate analyses of changes in community structure. *Austral Ecology* 18:117–143.
- Conde-Padín, P., A. Carvajal-Rodríguez, M. Carballo, A. Caballero, and E. Rolán-Alvarez. 2007a. Genetic variation for shell traits in a direct-developing marine snail involved in a putative sympatric ecological speciation process. *Evolutionary Ecology* 21:635–650.
- Conde-Padín, P., J. W. Grahame, and E. Rolán-Alvarez. 2007b. Detecting shape differences in species of the *Littorina saxatilis* complex by morphometric analysis. *Journal of Molluscan Studies* 73:147–154.
- Conde-Padín, P., A. Caballero, and E. Rolán-Alvarez. 2009. Relative role of genetic determination and plastic response during ontogeny for shell-shape traits subjected to diversifying selection. *Evolution* 63:1356–1363.
- Cortes, C., and V. N. Vapnik. 1995. Support vector networks. *Machine Learning* 20:273–297.
- Cox, T. F., and M. A. A. Cox. 2000. *Multidimensional scaling*. Chapman and Hall, New York.
- Crowley, T. E., T. Pain, and F. R. Woodward. 1964. A monographic review of the Mollusca of Lake Nyasa. *Annales du Musée Royal de l'Afrique Centrale, Sciences Zoologiques* 131:1–58.
- DeCoste, D., and B. Schölkopf. 2002. Training invariant support vector machines. *Machine Learning* 46:161–190.
- Dietz, E. J. 1983. Permutation tests for association between two distance matrices. *Systematic Zoology* 32:21–26.
- Dryden, I. L., and K. V. Mardia. 1998. *Statistical shape analysis*. Wiley, New York.

- Ehrlich, R., R. B. Pharr, and N. Healy-Williams. 1983. Comments on the validity of Fourier descriptors in systematics: a reply to Bookstein et al. *Systematic Biology* 32:202–206.
- Gemner, M. J., J. A. Todd, E. Michel, D. Erpenbeck, A. Jimoh, D. A. Joyce, A. Piechocki, and J.-P. Pointier. 2007. Amassing diversity in an ancient lake: evolution of a morphologically diverse parthenogenetic gastropod assemblage in Lake Malawi. *Molecular Ecology* 16:517–530.
- Gould, S. J. 1991. The disparity of the Burgess Shale arthropod fauna and the limits of cladistic analysis: why we must strive to quantify morphospace. *Paleobiology* 17:411–423.
- Green, W. D. K. 1996. The thin-plate spline and images with curving features. Pp. 79–87 in K. V. Mardia, C. A. Gill, and I. L. Dryden, eds. *Image fusion and shape variability*. University of Leeds, Leeds, U.K.
- Haines, A. J., and J. S. Crampton. 2000. Improvements to the method of Fourier shape analysis as applied in morphometric studies. *Palaeontology* 43:765–783.
- Hammer, Ø., and D. A. T. Harper. 2006. Paleontological data analysis. Blackwell, Oxford.
- Hammer, Ø., D. A. T. Harper, and P. D. Ryan. 2001. PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4:1–9.
- Hastie, T., and R. Tibshirani. 1998. Classification by pairwise coupling. *Annals of Statistics* 26(1):451–471.
- Hotelling, H. 1933. Analysis of a complex of statistical variables into principal components. *Journal of Educational Psychology* 24:417–441.
- Iwata, H., and Y. Ukai. 2002. SHAPE: a computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors. *Journal of Heredity* 93:384–385.
- Jablonski, D. 1999. The future of the fossil record. *Science* 284:2114–2116.
- Jackson, J. B. C., and A. H. Cheetham. 1990. Evolutionary significance of morphospecies: a test with cheilostome Bryozoa. *Science* 248:579–582.
- Keerthi, S. S., S. K. Shevade, C. Bhattacharyya, and K. R. K. Murthy. 2001. Improvements to Platt's SMO algorithm for SVM classifier design. *Neural Computation* 13:637–649.
- Kruskal, J. 1964. Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. *Psychometrika* 29:1–27.
- Kuhl, F. P., and C. R. Giardina. 1982. Elliptical Fourier features of a closed contour. *Computer Graphics and Image Processing* 18:236–258.
- Lestrel, P. E. 1989. Method for analyzing complex two-dimensional forms: elliptical Fourier functions. *American Journal of Human Biology* 1:149–164.
- . 1997. Fourier descriptors and their applications in biology. Cambridge University Press, Cambridge.
- Louda, S. M., and K. R. McKaye. 1982. Diurnal movements in populations of the prosobranch *Lanistes nyassanus* at Cape Maclear, Lake Malawi, Africa. *Malacologia* 23:13–21.
- Louda, S. M., W. N. Gray, K. R. McKaye, and O. J. Mhone. 1983. Distribution of gastropod genera over a vertical depth gradient at Cape Maclear, Lake Malawi. *Veliger* 25:387–392.
- Louda, S. M., K. R. McKaye, T. D. Kocher, and C. J. Stackhouse. 1984. Activity, dispersion, and size of *Lanistes nyassanus* and *Lanistes solidus* (Gastropoda, Ampullariidae) over the depth gradient at Cape Maclear, Lake Malawi, Africa. *Veliger* 26:145–152.
- Loy, A., S. Busilacchi, C. Costa, L. Ferlin, and S. Cataudella. 2000. Comparing geometric morphometrics and outline fitting methods to monitor fish shape variability of *Diplodus puntazzo* (Teleostea: Sparidae). *Aquacultural Engineering* 21:271–283.
- MacLeod, N. 1999. Generalizing and extending the eigenshape method of shape space visualization and analysis. *Paleobiology* 25:107–138.
- Mandahl-Barth, G. 1972. The freshwater Mollusca of Lake Malawi. *Revue de Zoologie et de Botanique Africaines* 86:257–289.
- Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* 27:209–220.
- Marcus, L. F. 1990. Traditional morphometrics. In F. J. Rohlf and F. L. Bookstein, eds. *Proceedings of the morphometrics workshop*. University of Michigan Museum of Zoology Special Publication 2:77–122.
- McCune, B., and J. B. Grace. 2002. Analysis of ecological communities. MJM Software Design, Gleneden Beach, Ore.
- Minchin, P. R. 1987. An evaluation of the relative robustness of techniques for ecological ordination. *Plant Ecology* 69:89–107.
- Monti, L., M. Baylac, and B. Lalanne-Cassou. 2001. Elliptic Fourier analysis of the form of genitalia in two *Spodoptera* species and their hybrids (Lepidoptera: Noctuidae). *Biological Journal of the Linnean Society* 72:391–400.
- Navarro, N., X. Zatarain, and S. Montuire. 2004. Effects of morphometric descriptor changes on statistical classification and morphospaces. *Biological Journal of the Linnean Society* 83:243–260.
- Oksanen, J., R. Kindt, P. Legendre, B. O'Hara, G. L. Simpson, M. H. H. Stevens, and H. Wagner. 2008. Vegan: community ecology package, Version R, package version 1.13–1.
- Papadopoulos, L. N., J. A. Todd, and E. Michel. 2004. Adulthood and phylogenetic analysis in gastropods: character recognition and coding in shells of *Lavigeria* (Cerithioidea, Thiaridae) from Lake Tanganyika. *Zoological Journal of the Linnean Society* 140:223–240.
- Parsons, K. J., B. W. Robinson, and T. Hrbek. 2003. Getting into shape: an empirical comparison of traditional truss-based morphometric methods with a newer geometric method applied to New World cichlids. *Environmental Biology of Fishes* 67:417–431.
- Platt, J. 1998. Fast training of support vector machines using sequential minimal optimization. Pp. 41–65 in B. Schölkopf, C. Burges, and A. Smola, eds. *Advances in Kernel methods: support vector learning*. MIT Press, Cambridge.
- R Development Core Team 2007. R: a language and environment for statistical computing, Version 2.6. Foundation for Statistical Computing, Vienna.
- Read, D. W., and P. E. Lestrel. 1986. Comment on uses of homologous-point measures in systematics: a reply to Bookstein et al. *Systematic Zoology* 35:241–253.
- Reyment, R. A. 1991. *Multidimensional palaeobiology*. Pergamon, Oxford.
- Rohlf, F. J. 1990a. Fitting curves to outlines. In F. J. Rohlf and F. L. Bookstein, eds. *Proceedings of the morphometrics workshop*. University of Michigan Museum of Zoology Special Publication 2:167–177.
- . 1990b. Morphometrics. *Annual Review of Ecology and Systematics* 21:299–316.
- . 1999. Shape statistics: Procrustes superimpositions and tangent spaces. *Journal of Classification* 16:197–223.
- . 2000. Statistical power comparisons among alternative morphometric methods. *American Journal of Physical Anthropology* 111:468–478.
- . 2003. tpsSmall, Version 1.20. Department of Ecology and Evolution, State University of New York at Stony Brook.
- . 2004. tpsDig 1. Department of Ecology and Evolution, State University of New York at Stony Brook.
- . 2006. tpsUtil, file utility program, Version 1.38. Department of Ecology and Evolution, State University of New York at Stony Brook.
- . 2008. tpsDig 2, Version 2.12. Department of Ecology and Evolution, State University of New York at Stony Brook.

- Rohlf, F. J., and J. W. Archie. 1984. A comparison of Fourier methods for the description of wing shape in mosquitoes (Diptera: Culicidae). *Systematic Zoology* 33:302–317.
- Rohlf, F. J., and L. F. Marcus. 1993. A revolution in morphometrics. *Trends in Ecology and Evolution* 8:129–132.
- Schölkopf, B., and A. J. Smola. 2001. Learning with kernels: support vector machines, regularization, optimization, and beyond. MIT Press, Cambridge.
- Schopf, T. J. M. 1982. A critical assessment of punctuated equilibria. 1. Duration of taxa. *Evolution* 36:1144–1157.
- Schopf, T. J. M., D. M. Raup, S. J. Gould, and D. S. Simberloff. 1975. Genomic versus morphologic rates of evolution: influence of morphologic complexity. *Paleobiology* 1:63–70.
- Schultheiß, R., B. Van Boekelaer, T. Wilke, and C. Albrecht. 2009. Old fossils—young species: evolutionary history of an endemic gastropod assemblage in Lake Malawi. *Proceedings of the Royal Society of London B* 276:2837–2846.
- Sheets, H. D. 2008. IMP: integrated morphometrics package. Department of Physics, Canisius College, Buffalo, N.Y.
- Sheets, H. D., K. Keonho, and C. E. Mitchell. 2004. A combined landmark and outline-based approach to ontogenetic shape change in the Ordovician Trilobite *Triarthrus becki*. Pp. 67–81 in A. Elewa, ed. *Applications of morphometrics in paleontology and biology*. Springer, New York.
- Sheets, H. D., K. M. Corvino, J. M. Panasiewicz, and S. R. Morris. 2006. Comparison of geometric morphometric outline methods in the discrimination of age-related differences in feather shape. *Frontiers in Zoology* 3:15.
- Sidlauskas, B. 2008. Continuous and arrested morphological diversification in sister clades of characiform fishes: a phylo-morphospace approach. *Evolution* 62:3135–3156.
- Thompson, D. A. W. 1942. *On growth and form*. A new edition. University Press and Macmillan, Cambridge, U.K.
- van Rijsbergen, C. J. 1979. *Information retrieval*. Butterworths, London.
- Vapnik, V. N. 1979. Estimation of dependences based on empirical data. Springer, New York.
- _____. 1998. *Statistical learning theory*. Wiley, New York.
- Venables, W. N., and B. D. Ripley. 2002. *Modern applied statistics with S*. Springer, New York.
- Vermeij, G. J., and S. J. Carlson. 2000. The muricid gastropod subfamily Rapaninae: phylogeny and ecological history. *Paleobiology* 26:19–46.
- Wagner, P. J. 2002. Phylogenetic relationships of the earliest anisostrophically coiled gastropods. *Smithsonian Contributions to Paleobiology* 88:1–152.
- Wilcox, C. D., S. B. Dove, W. D. McDavid, and D. B. Greer. 2002. *ImageTool 3.0 for Windows*. University of Texas Health Science Center, San Antonio.
- Witten, I. H., and E. Frank. 2005. *Data mining: practical machine learning tools and techniques*. Morgan Kaufmann, San Francisco.
- Wood, A. R., M. L. Zelditch, A. N. Rountrey, T. P. Eiting, H. D. Sheets, and P. D. Gingerich. 2007. Multivariate stasis in the dental morphology of the Paleocene–Eocene condylarth *Ectocion*. *Paleobiology* 33:248–260.
- Zelditch, M. L., D. L. Swiderski, H. D. Sheets, and W. L. Fink. 2004. *Geometric morphometrics for biologists: a primer*. Elsevier, London.
- Zelditch, M. L., A. R. Wood, R. M. Bonett, and D. L. Swiderski. 2008. Modularity of the rodent mandible: integrating bones, muscles and teeth. *Evolution and Development* 10:756–768.
- Zhang, L., W. Zhou, and L. Jiao. 2004. Hidden space support vector machines. *IEEE Transactions on Neural Networks* 15:1424–1434.