Comparing Size of Morphospace Occupation Among Extant and Cretaceous Fossil Freshwater Mussels Using Elliptical Fourier Analysis

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Abstract

Background. Freshwater mussels of superfamily Unionoidea are a diverse group with an evolutionary history of at least 360 million years. Many fossil and modern species exhibit a generally unsculptured, roughly elliptical shell outline. Such morphology results in difficulties when attempting to identify or classify fossil material, as only hard parts are preserved.

Several latest-Cretaceous fossil localities over a small geographic area in southwestern North Dakota contain poorly preserved edentulous freshwater mussels that effectively lack all but shell outlines. This paper discusses methods that were used to attempt to determine how many fossil taxa were present in one of these assemblages.

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Methods. Elliptical Fourier Analysis was performed on two-dimensional shell outlines of both edentulous fossils and modern taxa to create Fourier scores representing outline shape. Morphospace occupation, or the amount of variation within a sample of shell outlines, was calculated using two methods: within-group dispersion and sum of variance. Morphospace occupation was compared among each of the modern taxa (at both generic and specific levels) and fossil assemblage using confidence intervals, ANOVA, and Tukey's HSD tests.

Results. The amount of outline variation within the fossil assemblage tested is more than some modern genera and species and less than others. Morphospace occupation and confidence intervals are defined for the modern taxa (nine genera and 24 species) that were examined.

Discussion. Although results were inconclusive, discussion points are presented to drive future research. Methodological improvements are suggested including choice of extant (comparative) genera, ontogeny and size, morphological plasticity, phenotypic convergence, taphonomic deformation, and general geometric morphometrics.

1 Introduction

Mussels of the superfamily Unionoidea (order Unionoida) are freshwater, benthic organisms with bivalve shells made of calcium carbonate (Dunca et al., 2005; Bogan, 2008) and an evolutionary history stretching back to at least the Devonian Period (Bogan and Roe, 2008). Freshwater mussels are most notable for utilizing a parasitic larval stage for reproduction, allowing colonization of flowing water with the assistance of fish hosts to move larvae upstream against the current (Cvancara, 1983; Bauer, 2001; Wächtler et al., 2001; Scholz, 2003). Specimens representing eight of the nine extant genera used for this study belong to the family Unionidae (the remaining genus, *Anodontites*, belongs to Mycetopodidae). The family Unionidae is represented by 180 genera worldwide comprising 800–900 species, most of those being members of the subfamily Unioninae (Graf and Cummings, 2006; Bogan, 2008). Fifty-three genera and 302 species of Unionidae exist in the Nearctic (North America and Greenland), making this region the most species-rich in mussels on the planet (Bogan, 2008).

Fossil unionoids from Cretaceous strata of the Western Interior are also quite diverse leading up to near the Cretaceous-Paleogene (K–Pg) extinction horizon at about 65.95 Ma (Kuiper et al., 2008; Hartman and Illies, 2014) and can be biostratigraphically correlated into the North American Land Mammal "Ages" directly below and above the K–Pg boundary horizon (Lancian, Puercan, Torrejonian, and Tiffanian) (Hartman, 1998, 1992; Hartman and Illies, 2014). This fauna underwent a loss of diversity just prior to the K-Pg extinction event, resulting in a reduction of the number of externally sculptured taxa (Hartman and Butler, 1995; Hartman, 1996a,b; Scholz and Hartman, 2007a,b). In southwestern North Dakota and eastern Montana, U.S.A., unionoid taxa with relatively featureless exteriors and a common elliptical shape appear to have survived the extinction event and have represented the primary freshwater mussel in this region ever since (Hartman, 1996a,b). Strongly sculptured forms returned to the fossil record in this area during the Pleistocene Epoch, but never to the ubiquity of Cretaceous taxa.

Over the past fifteen years a small number of localities exhibiting a wholly different type of preservation have been discovered in strata at the base of the Ludlow Member of the Fort Union Formation northwest of Marmarth, Slope County, North Dakota, U.S.A. (Fig. 1). The first of these localities (L6516) was named "Das Goods" for its leaf flora; this name is used herein to refer to the type of preservation exhibited there (Hartman et al., 2001). These fossil localities preserve snails (Class Gastropoda) and unionoids, the former as compressed steinkerns and the latter as molds and casts of the interior and exterior of the valves in mudstone. Pollen analysis has previously shown L6516 to be of latest Cretaceous age (Hartman et al., 2001; Sweet, 2006). Although the Das Goods unionoids are better preserved than other North Dakota freshwater mussels of similar age, the valves are unsculptured, generally elliptical, and retain no morphological characters to aid in identification that can be considered taxonomically useful aside from a lack of hinge teeth (Burton-Kelly, 2008). Clearly, other measures of morphology are required for a meaningful diagnosis.

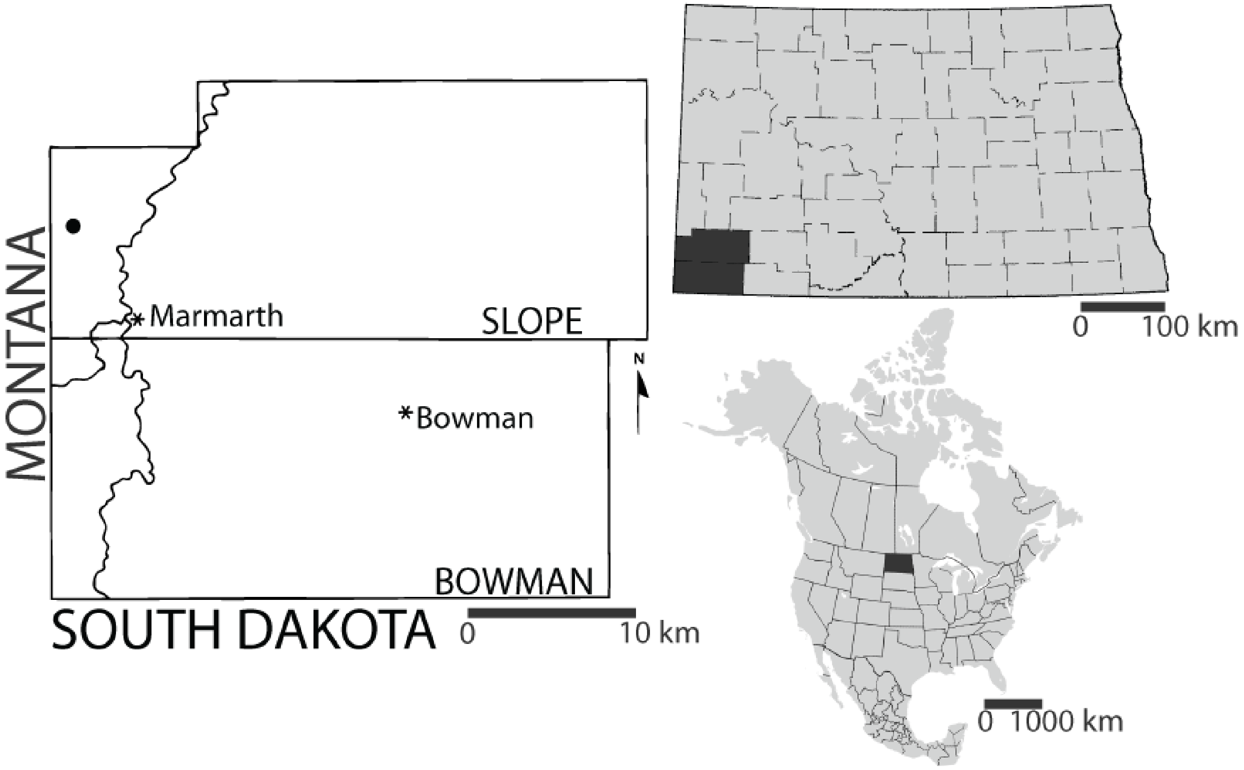

Figure 1: Location of Das Goods locality area (dot) in western Slope County, North Dakota, U.S.A.

"Traditional" morphometrics has long been a process of measuring various discrete distances, manipulating those data, and attempting to determine the taxonomic usefulness of sets of measurements through multivariate statistical methods (e.g., Marcus, 1990). Such approaches have had general success and acceptance in the literature, but do not allow for shape (a description of the organism independent of scaling, rotation, or translation) to be addressed independently of size (e.g., Marcus, 1990; Zelditch et al., 2004). The more recent field of geometric morphometrics seeks to improve mathematical representation of shape by comparing the physical relationship between biologically homologous "landmarks" that have been standardized (Bookstein, 1991; Zelditch et al., 2004). Similarly, outline analysis techniques that standardize data by converting outlines into mathematical functions can be

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used on shapes that have few or no biologically homologous landmarks (Kuhl and Giardina, 1982; Ferson et al., 1985; McLellan and Endler, 1998; Haines and Crampton, 2000; Lestrel et al., 2004; Scholz and Hartman, 2007b; Scholz and Scholz, 2007).

Although computers and statistics will be slow to replace a trained human eye when identifying meaningful biological characters and determining how they relate to an organism within a taxonomic system, geometric morphometric techniques make communication and hypothesis testing of shape data easier and provide a level of confidence. Hypothetically, a computer could analyse all of a specimen's morphological traits and compare it to known taxa or recognize it as unknown (to some degree). Questions concerning morphology need to be carefully constructed in order to utilize the growing mathematical and statistical power at our disposal, yet allow the researcher to have the final word in assessing the accuracy of the results based on his or her own knowledge (Zelditch et al., 2004).

This paper describes one method of geometric morphometrics, elliptical Fourier analysis, to determine the possible number of taxa of freshwater mussel present at fossil locality L6516 by comparing the amount of shape variation in similarly shaped extant freshwater mussels.

2 Abbreviations

2.1 Institutions

DMNS — Denver Museum of Nature and Science, Denver, Colorado, U.S.A.
DU — Drexel University, Philadelphia, Pennsylvania, U.S.A.
GCS — Geological Survey of Canada, Calgary, Alberta, Canada
INHS — Illinois Natural History Survey, Champaign, Illinois, U.S.A.
NCSM — North Carolina Museum of Natural Sciences, Raleigh, North Carolina, U.S.A.
PTRM — Pioneer Trails Regional Museum, Bowman, North Dakota, U.S.A.
SB — Stony Brook University, Stony Brook, New York, U.S.A.
SLU — St. Lawrence University, Canton, New York, U.S.A.

UND — University of North Dakota, Grand Forks, North Dakota, U.S.A.

UND-PC — UND Paleontology Collections

WGA — Wyoming Geological Association, Casper, Wyoming, U.S.A.

2.2 Symbols

AD — Among-groups dispersion

ANOVA — Analysis of Variance

EFA — Elliptical Fourier Analysis

L-number — UND Hartman locality number (Hartman, 1998).

LSD — Least significant difference [test]

MANOVA — Multivariate Analysis of Variance

S-number — UND Hartman specimen number

ΣV — Sum of variance

T-number — Burton-Kelly temporary specimen number

WD — Within-group dispersion

3 Materials & Methods

Quantitative study of fossil material has long been an important part of paleontology (e.g. and within Sepkoski, 2012; Burma, 1948, 1949; Shaw, 1956; Sepkoski, 2005; Thompson, 1945). The type and number of direct measurements that can be made necessarily vary according to the taxa being studied and the quality of their preservation. The measurements that can be made on unionoid mussels similar to the Das Goods fossils are limited by their relatively featureless exterior; many studies of better-preserved specimens have chosen to investigate only length, height, thickness (inflation), umbonal position, and various derived ratios (Eager, 1948, 1974, 1977, 1978; Aldridge, 1999; Scholz and Scholz, 2007). Although such traditional morphometric measurements lead to a better understanding of the relation of shell shape to habitat and life habits, such as burrowing depth and rate (Eager, 1948, 1974, 1978; Innes and Bates, 1999), they can be used for identification and classification only in the most general way. Recently, popular geometric morphometric methods such as elliptical Fourier analysis (EFA) and landmark methods (LM) are an attempt to mathematically capture as much quantitative shape information as possible for use in multivariate statistical tests (Kuhl and Giardina, 1982; Rohlf and Archie, 1984; Ferson et al., 1985; Foote, 1989; Crampton and Haines, 1996; Haines and Crampton, 2000; Schmittbuhl et al., 2003; Bookstein, 1991; Zelditch et al., 2004).

Confounding the use of any quantitative shape measure for these purposes is the noted morphological plasticity of unionoids with regard to habitat, leading to repeated convergence in shape of either closely or distantly related unionoid phylogenies (Balla and Walker, 1991; Eager, 1948, 1974, 1977; Hinch and Bailey, 1988; Watters, 1993; Burton-Kelly, 2013). Unfortunately, geometric morphometric methods cannot—on their own—distinguish convergent forms or resolve homologies. Instead, they can be used as another way to visualize an organism (or part of an organism) in addition to qualitative presence/absence characters so that specific questions about shape types or features can be addressed. Such shape data can then be used to support an argument for or against homology or convergence with other related taxa.

The majority of the fossil unionoid specimens used in this study preserve little more than an outline of the valve or valves and incomplete growth line traces, leaving almost nothing that can be treated as an identifiable character (Fig. 2). In order to differentiate between subjectively apparent morphological groups (akin to fossil leaf morphotypes after Johnson, 2002) in these assemblages and to try to identify possible affinities of these morphotypes to extant genus or possible genera, EFA was chosen as a method in order to capture unionoid valve outline data rather than point (landmark) data. The EFA method used herein was created by Ferson et al. (1985) and improved upon in FORTRAN/MS-DOS$^{\text{TM}}$ program format by Crampton and Haines (1996). EFA produces a series of scores (often termed

"Fourier Coefficients") that define the shape of a closed curve; these scores can then be used in multivariate analyses to examine similarity or difference between individuals or groups of specimen outlines. A thorough explanation of the theory behind EFA is given by Crampton and Haines (1996), Haines and Crampton (2000), and Scholz (2003).

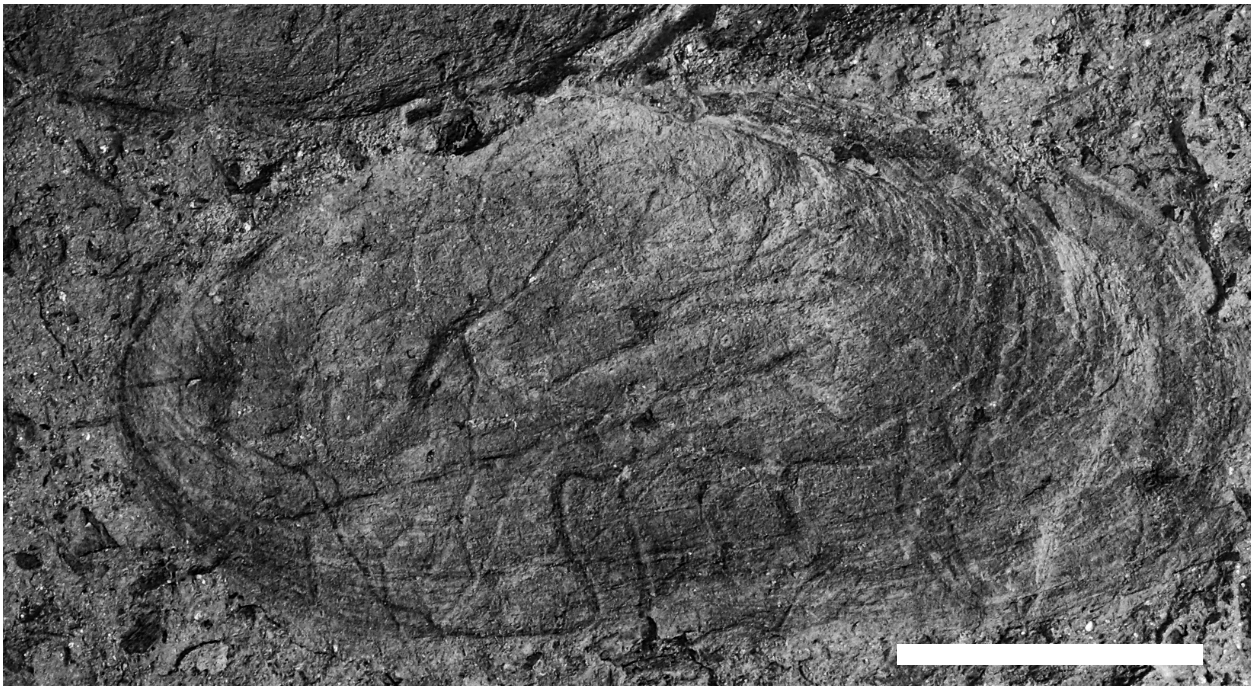

Figure 2: Example of a unionoid specimen from Das Goods (L6516). Scale bar is 2 cm.

An important objective of this study is to improve EFA of shell outline for the analysis of the size of morphospace occupation of the fossils from L6516. Optimization of these methods is based on the metrics of within-group dispersion (WD) and sum of variance (ΣV). Within-group dispersion was calculated as the mean of all pairwise [Euclidean, multidimensional] distances between samples within a group (Foote, 1989) and sum of variance as the trace (the sum of the diagonal elements) of the covariance matrix, calculated as a bootstrapped value (Rodgers, 1999; Zelditch et al., 2004; Hesterberg et al., 2005). The variables of smoothing, number of Fourier harmonics, and normalization to a certain Fourier harmonic can all be tested for with a synthetic group to determine the combination that results in the highest discreteness (AD/WD), the ratio of among-group dispersion (AD) to within-group dispersion (Foote, 1989). A model system made of specimens that fall into "easily identifiable" morphological groups would have a low within-group dispersion (variation) and a high morphological disparity. Optimization is limited to the current data set, and other data sets would most likely be optimized with different input values during EFA. Optimized methods for specimens of extant genera can be applied to the mussel specimens from the Das Good assemblage, as long as the extant taxa possess similar morphologies (see section 3.2).

3.1 Material: Fossil Specimens

Specimens specific to this project were collected over a period of two summers, composed of two incomplete field days in August 2006 and three complete days in August and September 2007 by the author with field assistance from Joseph Hartman (UND), Arthur Sweet (GCS), Matthew Borths (SB), Marron Bingle (WGA), Tanya Justham (Gza Geoenvironmental), Kristyn Voegele (DU), and the UND Introduction to Paleontology class of fall 2007. Material was previously collected in August 2000 by Joseph Hartman, S. Bowman, and David Lamb, and in June 1999 by Kirk Johnson (DMNS), R. Barclay (DMNS), Bowman, and G. Knauss. The locality was first recorded in July 1998 by Johnson and Tim Farnham. Extraction methods were similar to those outlined by Johnson (2002) for the fossil leaves found at this locality. Fossils were removed by quarrying large blocks with hoe picks and then splitting these blocks parallel to bedding planes with chisels and brick hammers. Due to the thinness of the bed interval producing fossils of interest at these sites, care was taken to minimize the amount of overburden removed and to focus on this single producing horizon (Burton-Kelly, 2008). Several dozen individual specimens have been recovered from this locality, many described in more detail by Burton-Kelly (2008).

3.2 Material: Extant Specimens

Specimens of extant freshwater mussels of confirmed identification were needed for optimizing the quantitative methods used below and to compare modern shapes to fossils from

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L6516. Extant specimens were chosen for comparison based on 1) an edentulous or nearly edentulous hinge; 2) lack of surface sculpture; 3) lack of extraneous dorso-posterior "wings" (cf. *Cristaria*); and 4) preference for silty or muddy substrates. Extant specimens were identified to the species level according to collection labels (Appendix A). As morphology was of greatest concern, no significant attempt was made to confirm identifications or resolve possible synonymies.

3.3 Specimen Imaging

Most fossil specimens were photographed with a digital FujiFilm FinePix S1 Pro camera, which produced images of 5 megapixel resolution. Some fossil and extant specimens were scanned with an HP Scanjet 4070 Photosmart scanner at 400 DPI resolution. Extant specimens were photographed with a variety of camera models depending on home institution and photographer.

Specimens (both fossil and extant) were oriented so that the commissural plane was parallel to the plane of focus. Scanned specimens were laid flat (interior down) on the scanning bed. In most cases the specimen label was included in the photograph or scan. All fossil specimens from the Das Goods locality (L6516) were given a Hartman (UND) specimen (S) number. Extant specimens were numbered internally to this study with a prefix (T) (Appendix A). Data management is described in Burton-Kelly (2008).

3.4 Specimen Outline Digitization

Before digitization, specimen images were oriented in Adobe® Photoshop® with the longest axis of the specimen generally horizontal (any deviation from this was adjusted for by rotation of the outline during EFA). Outlines of 27 unionoid valves from L6516 (of unknown affinity) and 384 valves of eight extant mussel genera from family Unionidae (*Anodonta, Anodontoides, Gonidea, Pilsbryoconcha, Pyganodon, Simpsonaias, Strophitus*, and *Utterbackia*) and one genus from family Mycetopodidae (*Anodontites*) were manually digitized using tpsDig

2.05 (Rohlf, 2008) (Appendix A). Outlines were manually digitized using the pencil tool in a clockwise direction, beginning and ending at the umbo or the nearest approximation that could be determined. Manual rather than automated outline tool digitization was chosen because of the lack of a well-defined edge on most fossil specimens. Although interpreted outlines that were traced manually over photographs (CorelDraw® or Adobe® Illustrator®) could be subsequently digitized automatically, this would still result in a digitized outline based on a manually defined edge.

Digitization of specimens was performed with accuracy to the valve outline in mind. Small irregularities in outlines were included where possible in order to capture as much "natural" variation as possible, under the assumption that small-scale variations in shell shape (because of variations in the life history of the individual) are phenotypically representative. Most specimens were digitized at an arbitrary screen size dependent on the resolution of the original image and the size of the monitor (in this case, 38.1 cm diagonal, resolution 1280 by 1024 pixels). Preliminary study suggested that the amount of error in manually digitized outlines was reduced as the size of the specimen image during digitization was increased; increasing the amount of smoothing during EFA also reduces WD and ΣV for repeated digitizations of the same specimen, however increased smoothing seems to progressively remove detail from the outline (Burton-Kelly, 2008).

Elliptical Fourier analysis was performed using the program HAngle (Crampton and Haines, 1996) with a smoothing of two, 12 Fourier harmonics, and normalized to the second harmonic (an ellipse).

4 Results

The statistical tests performed assume that a) extant mussel genera occupy the same or greater amount of morphospace based on shell shape than fossil mussel genera, b) each extant specimen used is representative of its assigned genus and species, c) fossil specimens represent the complete ecological assemblage. Each statistical test has additional underlying assumptions (detailed in Burton-Kelly (2008)) that contribute to the power of that test.

The size of morphospace occupation of extant edentulous freshwater mussel genera was defined by calculating the WD and ΣV based on the Fourier scores produced by elliptical Fourier analysis of individual valves from each genus. The resulting morphospace occupation envelope for each genus or group of genera of known size that can be compared with theoretical fossil genus groups to determine whether they are probable. Multivariate tests were also used to determine whether extant genera could be identified as different based on Fourier scores; if so, one may assume that these same methods can be used to test theoretical fossil genus groups. All statistical analyses were performed in PAST (Hammer et al., 2008).

4.1 Extant Genera and L6516

4.1.1 Within-group dispersion; ANOVA and confidence intervals

The average within-group dispersion of selected extant genera with L6516 specimens is 0.0790 with a range of 0.0629 (0.0427 to 0.1057) (Fig. 3) and extant species with L6516 specimens is 0.0704 with a range of 0.0615 (0.0427 to 0.1042) (Fig. 4). Calculated WD for L6516 specimens is 0.0890, within the range of extant genera and species, statistically significantly higher than 66% (6 out of 9) of the extant genera and 88% (21 out of 24) of the extant species based on 95% confidence intervals (Fig. 3). A one-way ANOVA found a statistically significant difference among WD values for extant genera and L6516 specimens ($F(9, 9750) = 520.2$, $p < 0.01$). All but five post hoc pairwise Tukey's HSD tests (L6516-*Pyganodon*, L6516-*Gonidea*, *Anodontoides*-*Gonidea*, *Gonidea*-*Strophitus* and *Anodontoides*-*Strophitus*) are statistically significant ($p < 0.05$) (Fig. 5). A one-way ANOVA found a statistically significant difference among WD values for extant species ($F(24, 4717) = 113.5$, $p < 0.05$). Sixty-four out of 300 post hoc pairwise Tukey's HSD tests were statistically significant (Fig. 6). 95% confidence intervals based on the t distribution agree with these tests (Burton-Kelly, 2008).

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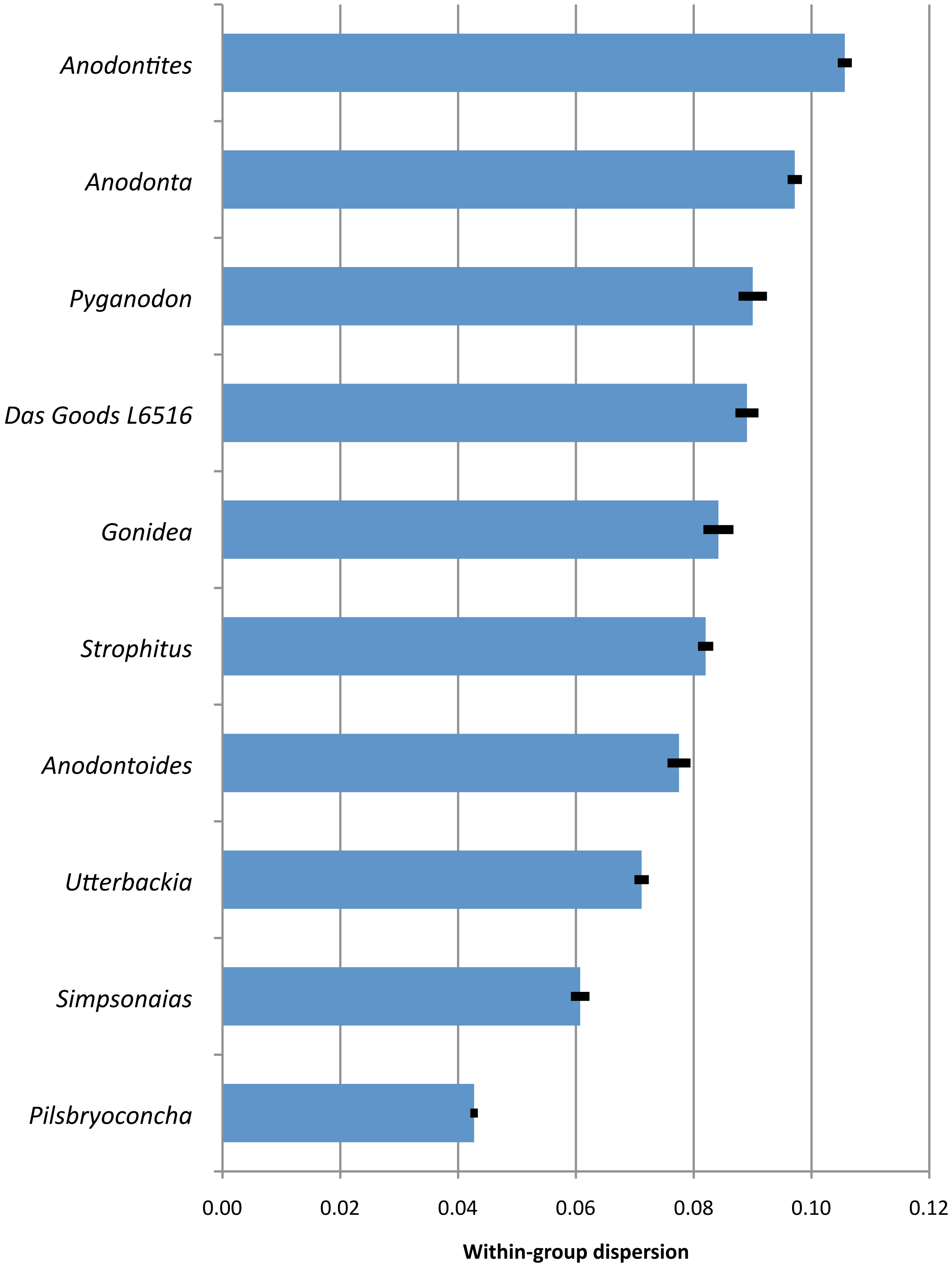

Figure 3: Comparison of within-group dispersion of some edentulous freshwater mussel genera based on outline shape. Height of bar represents WD value (= mean pairwise Euclidean distance among specimens in the same genus). Error bars represent 95% confidence interval based on the t distribution.

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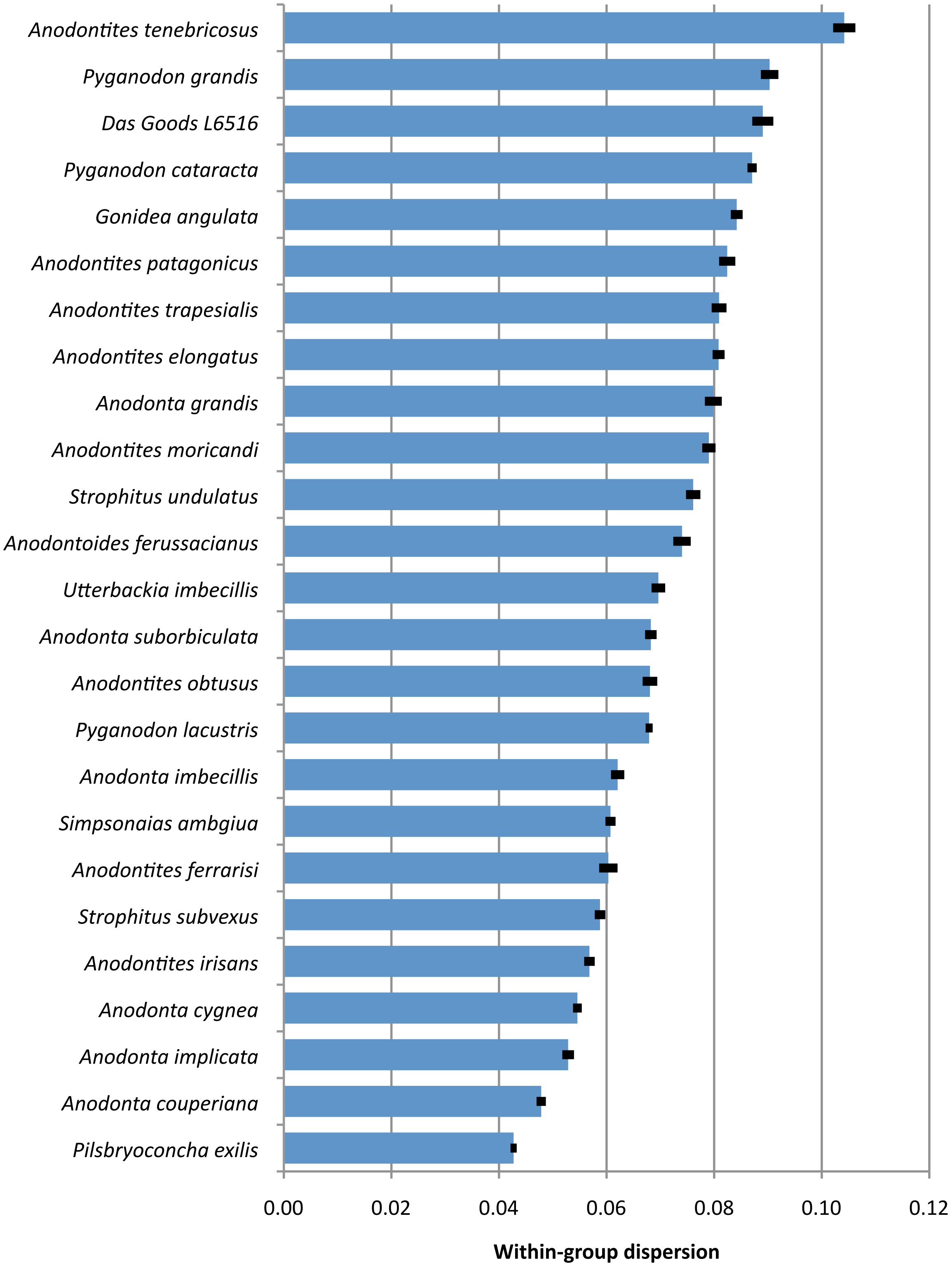
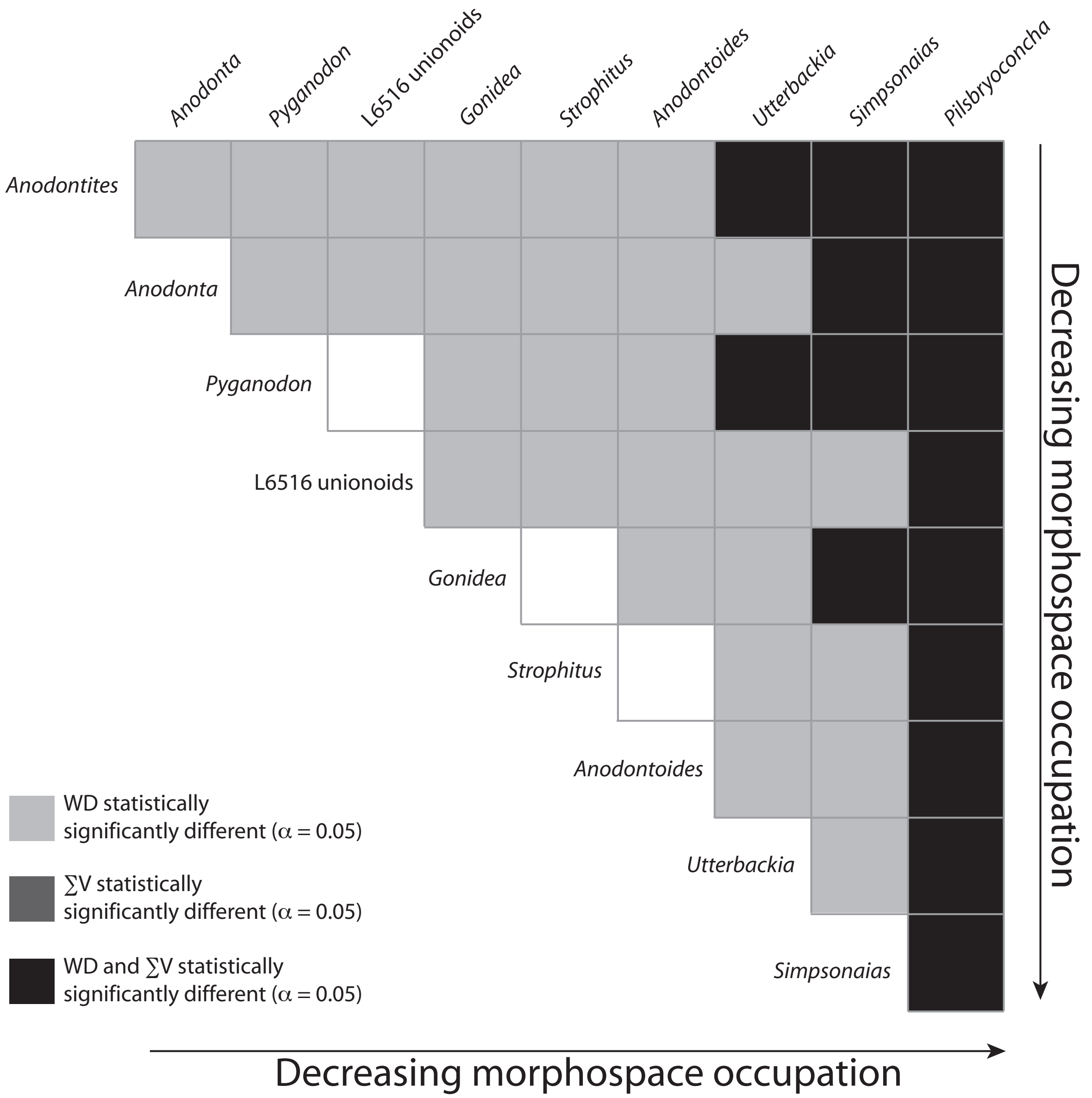


Figure 4: Comparison of within-group dispersion of some edentulous freshwater mussel species based on outline shape. Height of bar represents WD value (= mean pairwise Euclidean distance among specimens in the same species). Error bars represent 95% confidence interval based on the t distribution.



	Anodonta	Pyganodon	L6516 unionoids	Gonidea	Strophitus	Anodontoides	Utterbackia	Simpsonaias	Pilsbryoconcha
Anodontites	▒	▒	▒	▒	▒	▒	■	■	■
Anodonta		▒	▒	▒	▒	▒	▒	■	■
Pyganodon				▒	▒	▒	■	■	■
L6516 unionoids				▒	▒	▒	▒	▒	■
Gonidea						▒	▒	■	■
Strophitus							▒	▒	■
Anodontoides							▒	▒	■
Utterbackia								▒	■
Simpsonaias									■

▒ WD statistically significantly different (α = 0.05)
▓ ∑V statistically significantly different (α = 0.05)
■ WD and ∑V statistically significantly different (α = 0.05)

Figure 5: Summary of statistically significant differences among WD and ΣV for modern genera and L6516 specimens. Size of morphospace occupation decreases to the bottom right.

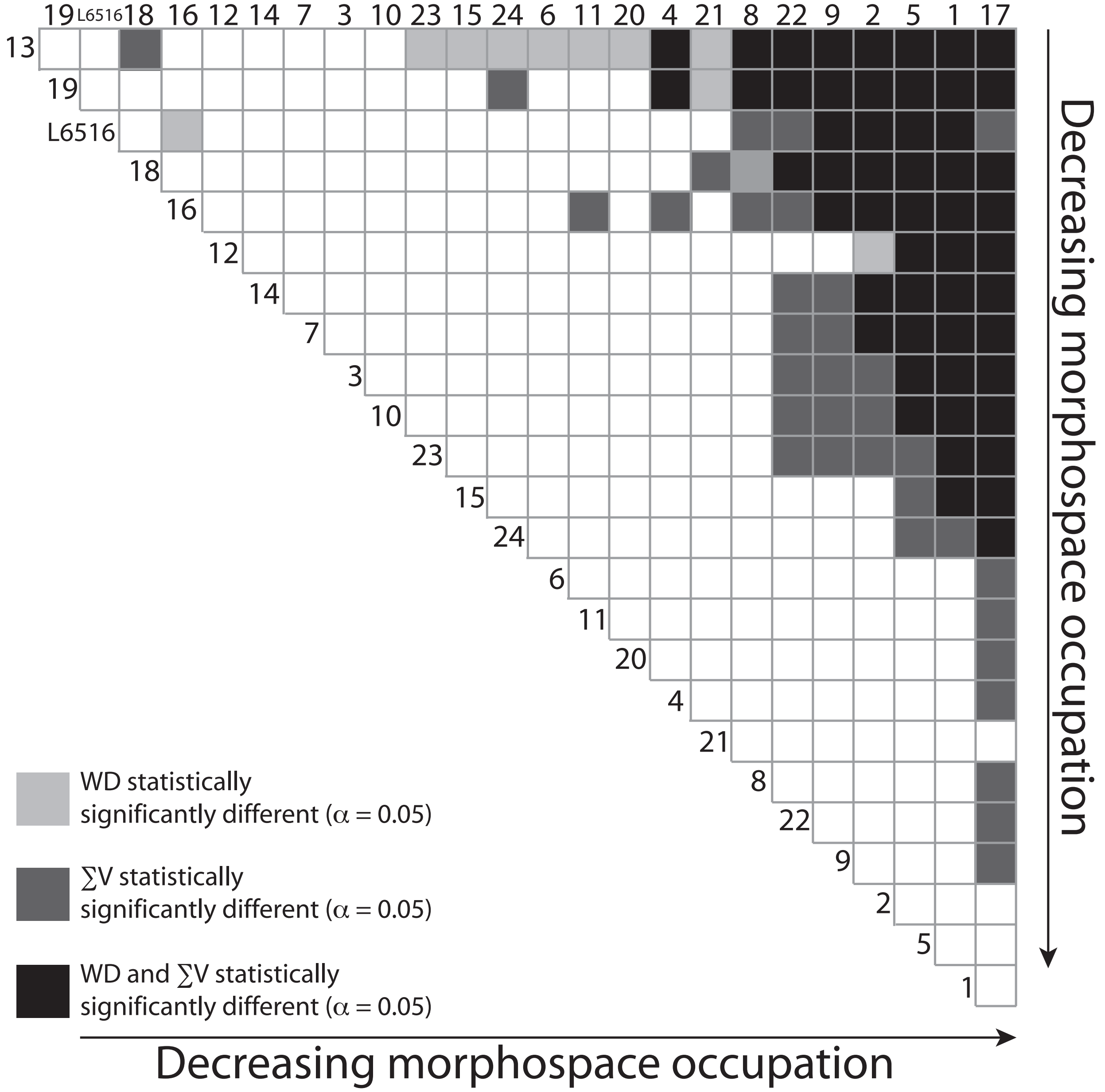


Figure 6: Summary of statistically significant differences among WD and ΣV for modern species and L6516 specimens. Size of morphospace occupation decreases to the bottom right. Key to species (alphabetical): 1–*Anodonta couperiana*, 2–*Anodonta cygnea*, 3–*Anodonta grandis*, 4–*Anodonta imbecillis*, 5–*Anodonta implicata*, 6–*Anodonta suborbiculata*, 7–*Anodontites elongatus*, 8–*Anodontites ferrarisi*, 9–*Anodontites irisans*, 10–*Anodontites moricandi*, 11-*Anodontites obtusus*, 11–*Anodontites obtusus*, 12–*Anodontites patagonicus*, 13–*Anodontites tenebricosus*, 14–*Anodontites trapesialis*, 15–*Anodontoides ferussacianus*, 16–*Gonidea angulata*, 17–*Pilsbryoconcha exilis*, 18–*Pyganodon cataracta*, 19–*Pyganodon grandis*, 20–*Pyganodon lacustris*, 21–*Simpsonaias ambigua*, 22–*Strophitus subvexus*, 23–*Strophitus undulatus*, 24–*Utterbackia imbecillis*.

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4.1.2 Sum of variance; confidence intervals

The average sum of variance of selected extant genera and L6516 specimens is approximately 0.0035 with a range of 0.0052 (0.0009 to 0.0061) (Fig. 7) and extant species with L6516 specimens is 0.0028 with a range of 0.0101 (0.0008 to 0.0108) (Fig. 8). Fifteen pairs of groups (genera or L6516) were found to possess statistically significant ΣV values based on non-overlapping confidence intervals (Fig. 5), disagreeing with the WD results because of larger confidence intervals, however, the rank order of genera based on ΣV is the same. Eighty-seven pairs of groups (modern species or L6516 specimens) were found to possess statistically significant ΣV values based on non-overlapping confidence intervals (Fig. 6). ΣV of L6516 specimens was only statistically significantly different than one of the selected genera (*Pilsbryoconcha*), making it statistically significantly greater than 11% of those genera based on 95% bootstrapped confidence intervals (Figs. 7 and 5).

4.1.3 Multivariate analyses; MANOVA, PCA, CVA

A MANOVA revealed statistically significant differences among outline shapes of extant genera and L6516 specimens based on the multivariate means of Fourier scores ($p = 0$, $\alpha = 0.05$; Wilk's $\lambda = 0.0149$, F(198, 3198) = 10.56; Pillai trace = 2.965, F(198, 3492) = 8.666). All post hoc pairwise Hotelling's T^2 tests show statistically significant differences among genera (Burton-Kelly, 2008). These results are supported by the permutation test for two multivariate groups (Burton-Kelly, 2008).

A plot of the first two principal components (variance-covariance matrix with singular value decomposition in PAST) of the EFA output for the extant specimens does not show large differentiation between all groups of specimens representing the different genera (Fig. 9). However, some genera are notably distinct from others when 95% confidence limits are used. The *Gonidea* morphospace envelope does not overlap with that of *Anodonta*, *Pilsbryoconcha*, *Strophitus* or *Utterbackia*. *Pilsbryoconcha* is distinct from *Simpsonaias* and *Strophitus* in the same manner. The first three principal components account for 25.8%,

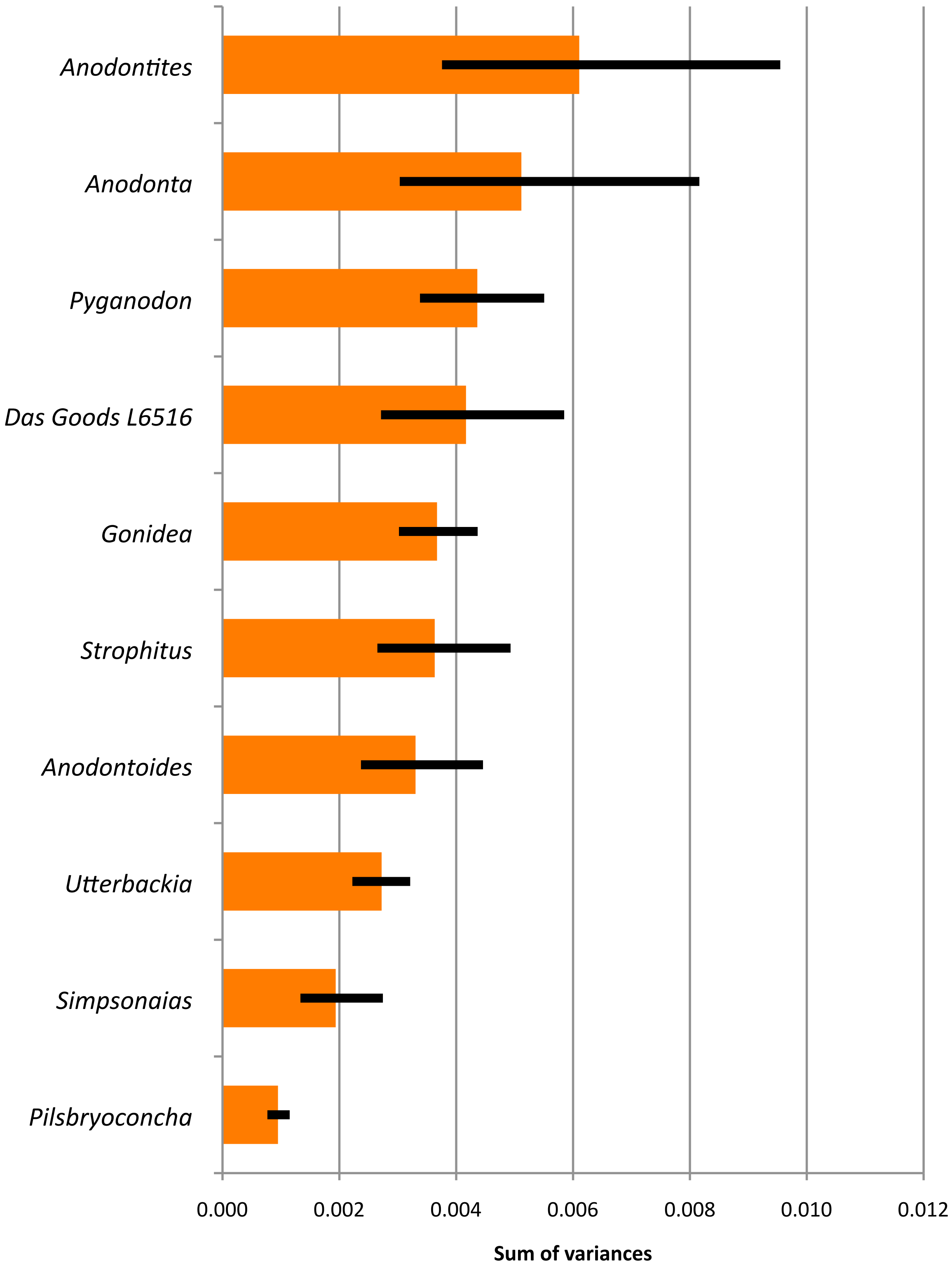

Figure 7: Comparison of sum of variance of some edentulous freshwater mussel genera based on outline shape. Height of bar represents ΣV value based on bootstrapped ($N = 1000$) sum of variances, error bars represent bootstrapped ($N = 1000$) 95% confidence intervals.

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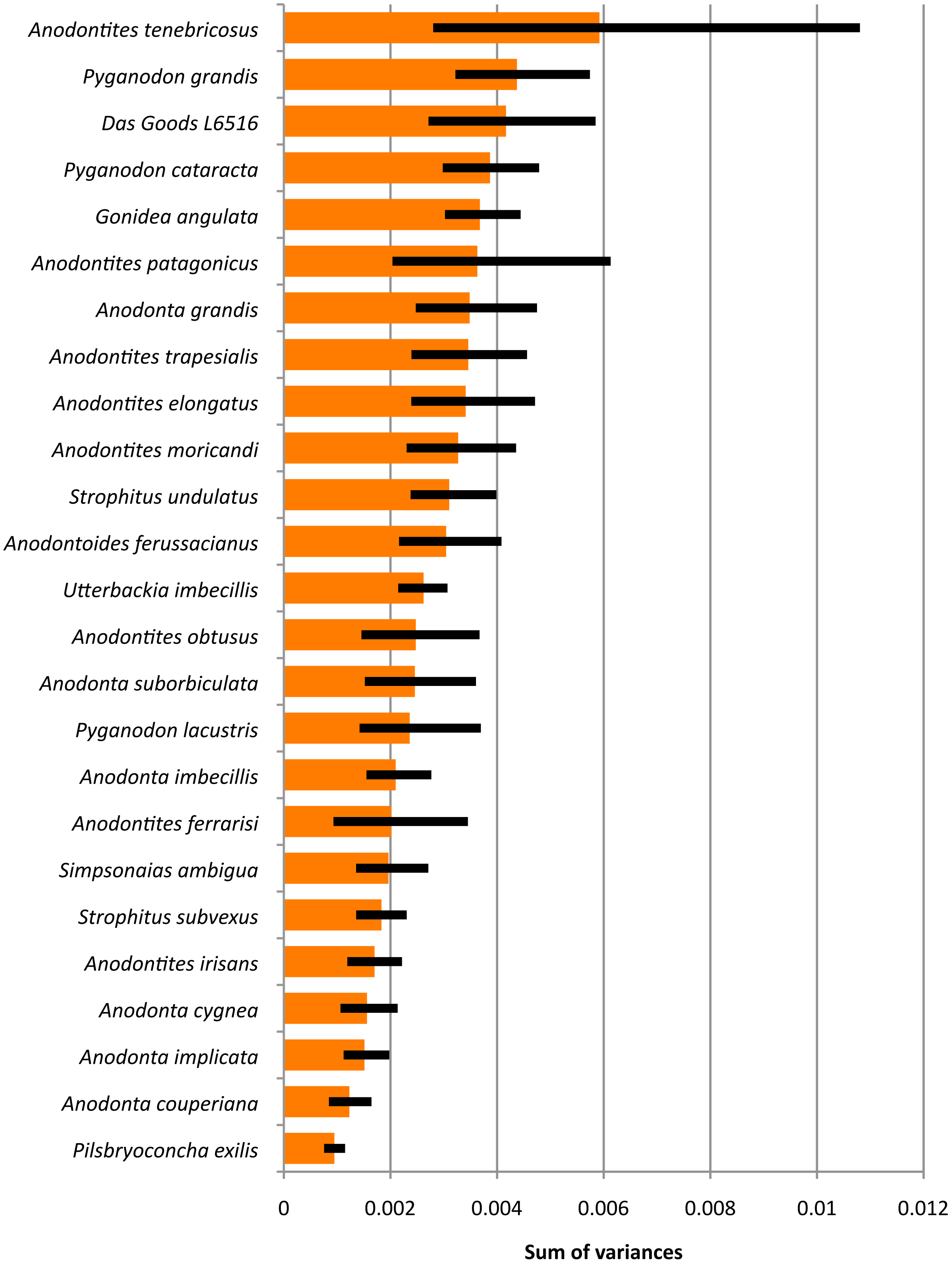

Figure 8: Comparison of morphological variation of some edentulous freshwater mussel species based on outline shape. Height of bar represents ΣV value based on bootstrapped ($N = 1000$) sum of variances, error bars represent bootstrapped ($N = 1000$) 95% confidence intervals.

24.9% and 8.6% of the variance, respectively. 95% of the variance is explained in the first 14 principal components. Principal component loadings show a positive relationship between the first principal component and EFA harmonics B5, B7, and A6 (in descending order), the second principal component and harmonics B2, B5, B7, A3, and B6; a negative relationship of the first principal component with harmonics B2, A3, B4, B8, B6, and A5; and the second principal component and harmonics A3, A5, A7, and B4. A plot of the first two canonical variates designed to maximize among-group differences shows similar results (Fig. 10).

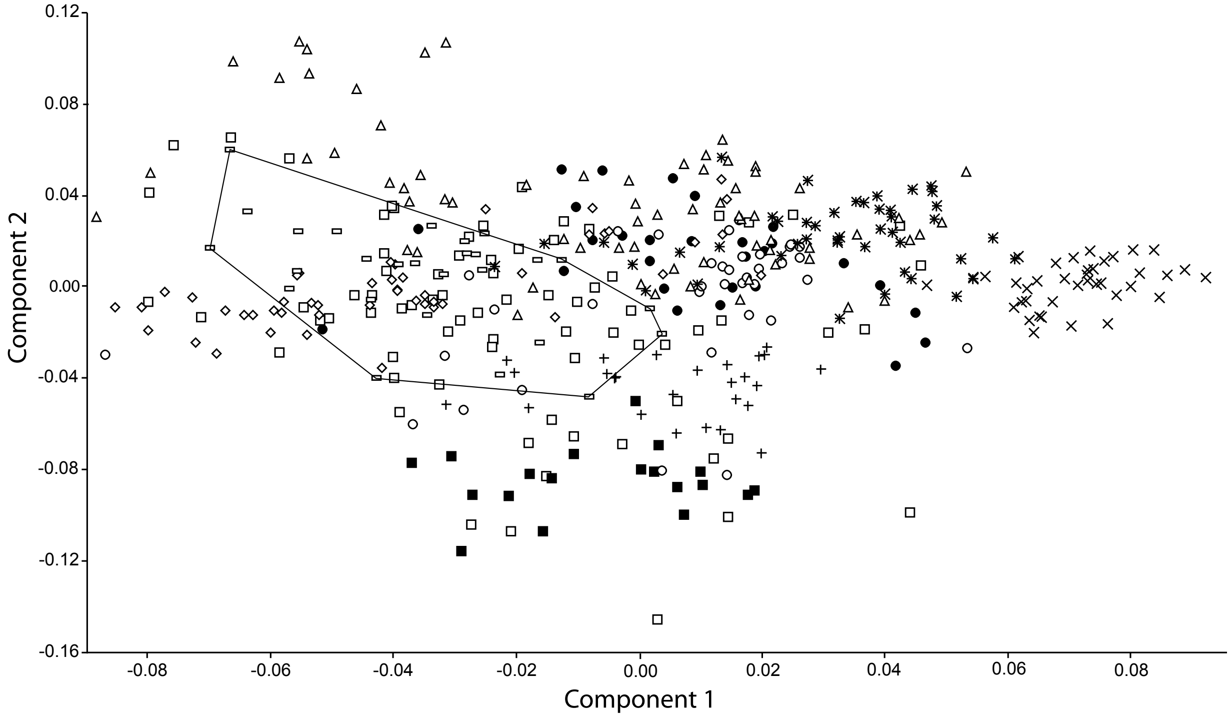

Figure 9: Principal component plot of elliptical Fourier coefficients of selected modern edentulous freshwater mussels and fossil unionoids from L6516 (as outlined). Key to symbols: open triangle–*Anodonta*, open square–*Anodontites*, open circle–*Anodontoides*, filled square–*Gonidea*, x–*Pilsbryoconcha*, filled circle–*Pyganodon*, cross–*Simpsonaias*, open diamond–*Strophitus*, star–*Utterbackia*, open rectangle–L6516 unionoids.

5 Discussion

Locality L6516 unionoids cannot be definitively placed within the context of the size of morphospace occupation of extant edentulous taxa (i.e., the L6516 specimens did not occupy

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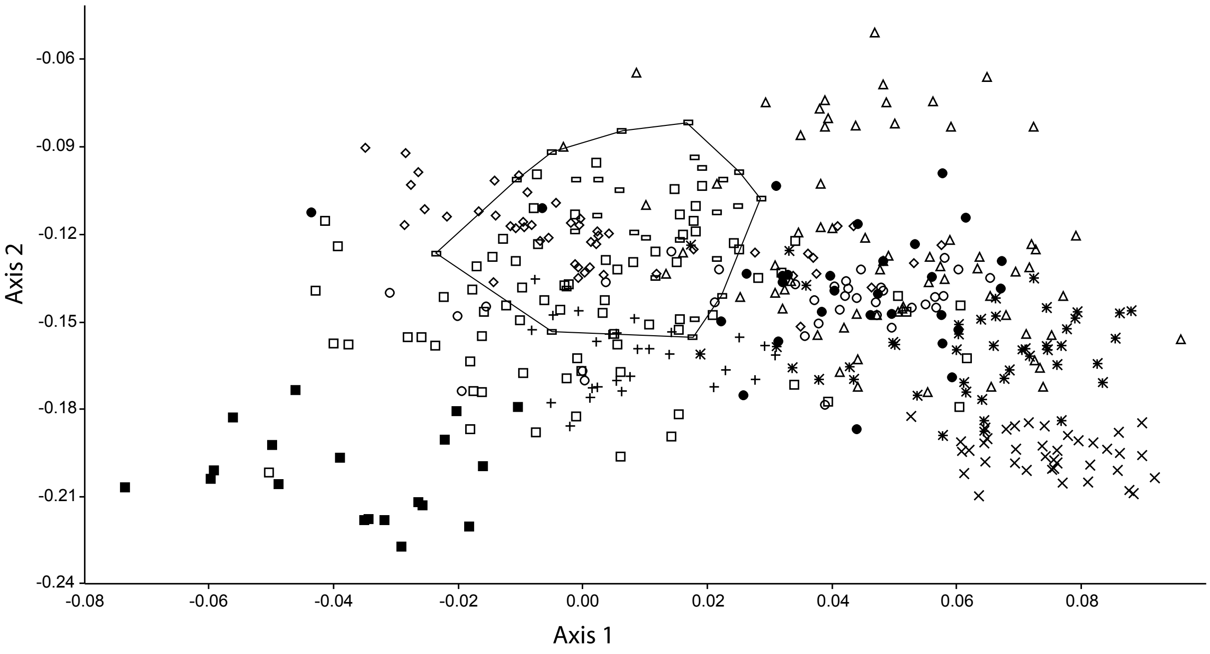

Figure 10: Canonical variate plot of elliptical Fourier coefficients of selected modern edentulous freshwater mussels and fossil unionoids from L6516 (as outlined). Key to symbols: open triangle–*Anodonta*, open square–*Anodontites*, open circle–*Anodontoides*, filled square–*Gonidea*, x–*Pilsbryoconcha*, filled circle–*Pyganodon*, cross–*Simpsonaias*, open diamond–*Strophitus*, star–*Utterbackia*, open rectangle–L6516 unionoids.

significantly more or significantly less morphospace than all other genera and species tested). Qualitatively, however, the possibility of more than one morphotype at L6516 is clear to the naked eye, but how can these methods be improved to identify and quantify these differences?

Some questions that underly these results are 1) whether a quantitative or qualitative method is more useful in determining what defines a taxon and 2) whether quantitative methods are worth the trouble. Identification to the species level of any organism should be based on discrete characters, which can be described qualitatively as well as mathematically, but each method has flaws in the way they can be interpreted: both qualitative and quantitative characters are variable according to preservation or individual variation or pathology, and quantitative characters can be additionally manipulated with different methods of significance testing or variation in alpha levels.

The absence of definitive results from present analyses to use size of morphospace occupation to calculate the number of unionoid genera or species present at L6516 is not necessarily because of poor methods (although improvements are described below). Perhaps the L6516 unionoids occupy less morphospace than some extant edentulous genera and species and still represent multiple morphotypes that, when the size of morphospace occupation for each is added together, it is still less than those extant genera with high within-group variation. To solve this problem, subgroups of the L6516 unionoid faunule can be selected manually (based on qualitative assessment of morphotypes) or automatically (based on all possible combinations of specimens) and tested against extant taxa. A growing number of studies have attempted to correlate amounts of genotypic and phenotypic variation (Davis et al. (2003) (bacteria), Alvarez-Molina (2004) (freshwater mussels), Mock et al. (2004) (freshwater mussels), Relethford (2004) (humans), Wong et al. (2004) (freshwater fish), Sommer (2007) (freshwater mussels), Burton-Kelly and Hartman (2009) (freshwater mussels), Smith (2009) (humans)). Future studies of multiple groups with a larger geometric morphometric variation component have the chance to become meaningful across the fields of biology and paleontology.

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5.1 Methodological Issues and Suggested Solutions

A number of issues exist with the methods used above that need to be addressed. Although it is not the opinion of the authors that the results specified above are inaccurate, criticism can be made of specific aspects of the methodology that can be improved and extraneous variation removed from the calculations of size of morphospace occupation.

5.1.1 Choice of Extant Genera

The primary concern when interpreting these data is whether the extrapolation from the extant forms selected can be applied to the fossil unionoids from Das Goods. This refers specifically to the choice of the extant genera and species used to set the baseline of size of morphospace occupation. The extant genera initially selected were edentulous forms that were relatively simple to obtain, which is far from a systematic approach. Only nine out of over two dozen extant genera lacking hinge teeth were analyzed.

This concern is an important one when utilizing extant forms to determine the taxonomic identity of fossil assemblages. Without selecting a specific set of extant genera identified by a quantitative shell character and analyzing a large number of each of those genera, the actual position of the fossil assemblage within the range of size of morphospace occupation can only be a rough estimate. Put another way, had the three genera (*Anodontites*, *Anodonta*, and *Pyganodon*) or two species (*Anodontites tenebricosus* and *Pyganodon grandis*) occupying more morphospace than the L6516 unionoids been left out by accident or design, there would have been more support for the possibility that more than one genus-sized group of unionoids occurs at the Das Goods locality (Figs. 3, 7, 4 and 8).

If all extant edentulous unionoid mussels been included in the analysis, however, the argument would clearly be against the possibility of multiple genera at Das Goods. Unfortunately, by this definition (and working within the sample group) all genera except for *Anodontites* and all species except for *Anodontites tenebricosus* would be suspect, when clearly each genus has different valve shapes and soft-part morphology. This is not to discount the work described above, but to recognize that these methods can only be used to find extreme groups at the generic and specific levels among what is hopefully a meaningful comparative group of taxa.

5.1.2 Ontogeny and Size

Capturing variation in unionoid mussels is difficult because of ontogenetic variation within designated genera and species. Additionally, because of the environmental plasticity of the unionoids, they are subject to variation in growth rate among habitats even along their ontogenetic trajectories. Optimally, morphospace occupation would be calculated with specimens of the same age from the same site, which would theoretically be the same size because of their common habitat. This would control for ontogeny and size, however, an adequate sample size would be difficult to obtain for every genus and species used in this project. Whether size or ontogeny of freshwater mussels has a more stable relationship to shape is unclear. If a size/shape or age/shape relationship were calculated, either age (based on growth lines, and able to be estimated even in the L6516 specimens) or size (based on length if using outlines or centroid size if using landmarks) could be utilized as a means of standardization, potentially allowing specimens from multiple localities to be used in calculating generic or specific morphospace occupation.

5.1.3 Morphological Plasticity and Phenotypic Convergence

Specimens of the same genera were obtained from multiple museums (Appendix A) and publications. The species measured in this study represent a variety of habitats in watersheds throughout the world. Understanding the plasticity of the unionoids according to habitat (leading to convergence through space and time) is key to improving on the result of this and studies, necessarily extrapolating from the present to the past.

Optimally, all extant specimens (of all applicable extant genera and species, discussed above) would be collected from a similar environment as the paleoenvironment represented at Das Goods—the muddy bottom of a long-lived pond or lake. This would help to reduce the amount of calculated morphospace occupation resulting from specimens from different environments possessing differing morphologies. Additionally, specimens of a single genus or species would be most likely to be similar if collected from the same habitat in the same watershed, although locating hundreds of specimens collected in this manner would be difficult, if not impossible, without a designated collecting expedition (Burton-Kelly and Hartman, 2009). Such a project would create possibilities of comparing the morphospace occupation of taxa from multiple habitats and watersheds with fossil localities, and with each other, to determine the interaction of morphospace occupation with habitat, population dynamics and geography in an attempt to document some of the gaps in the fossil record (Burton-Kelly, 2013). Investigations of this type have recently been accomplished by Costa et al. (2008), on marine clams, showing that morphological distances between species can be less than the morphological distance between different populations of the same species.

5.1.4 Taphonomic Deformation

An original goal of Burton-Kelly (2008) was to determine the potential amount of deformation undergone by the L6516 fossils due to lithostatic loading and unloading. The gastropod steinkerns in particular have been compressed to some degree parallel to the bedding plane, which suggesting that unionoids have as well. Physical tests to determine the possible amount of outline deformation due to compaction have not yet been accomplished; outlines were used as-is.

5.1.5 Technical Cooperation

Outline analysis and geometric morphometrics have a great deal of potential for use in the natural sciences, however, there needs to be more cohesiveness within the field regarding standardization and communication. Transformation of data for use between different software packages was extremely arduous. Standardization (or documentation of standards) of data formats will allow workers to exchange information, freely and without loss of fidelity to be used in different programs. The release of different standardized datasets would allow newcomers to the field to learn how to use the methods involved, and experienced workers to attempt new and better methodologies. Simple, clear communication will be key for the newer morphometric procedures to be used by those who did not create them. Detailed, step-by-step procedural methods need to be recorded and published, not so that newcomers can produce data without understanding morphometric theory, but so mistakes can be avoided, problems identified, and solutions created for difficult tasks (this was attempted in Burton-Kelly (2008)). Software (for data capture and analysis) needs to be documented, including reference to the theory behind the point and click interface (for an excellent example, see Hammer et al., 2008).

Many custom scripts and small programs had to be written to streamline the data capture and manipulation for this project; for someone less able or willing to produce such custom software, use of such a large data set may be overwhelming. As newer morphometric procedures are utilized by more workers, more support for the existing software will drive improvement of data manipulation capabilities (for interoperability of different software packages that were not originally designed to work together), while hopefully allowing for detailed control of data when experimenting with new methods.

New and promising statistical and modeling techniques have recently been released, including geodesic distance shape analysis (GDA) (Klassen et al., 2004; Prieto-Marquez et al., 2007), various methods of shape classification (Joshi and Srivastava, 2003; McNeill and Vijayakumar, 2005), soft independent modeling of class analogy (SIMCA), and partial least square discriminant analysis (PLSDA) (Costa et al., 2008).

6 Conclusions

This project fell short of its intended goal of determining the number of unionoid taxa present at Das Goods locality (L6516), but did produce useful results for comparing fossil assemblages and extant taxa. Methodological problems that were encountered over the course of the project were addressed with the intent that future studies will produce more taxonomically useful results. Specific conclusions are listed below.

1. The size of morphospace occupation of extant edentulous freshwater mussels can be calculated and ranked according to the within-group dispersion and sum of variance measures based on elliptical Fourier scores of the outlines of the valves.
2. The unionoid mussels preserved at the Das Goods locality (L6516) do not possess statistically significantly different morphological variation (using the within-group dispersion and sum of variance measures) than the selection of extant genera and species used, based on elliptical Fourier scores of mussel valve marginal outlines.
3. Methodological problems, including choice of extant genera and species, ontogeny and size of taxa, morphological plasticity and convergence, and taphonomic deformation of the fossil specimens, contribute to exaggerated size of morphospace occupation.
4. Standardization of morphometric techniques, datasets, and procedures will greatly simplify classification based on computer-intensive methods.

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671 # A Specimens of Extant Freshwater Mussels

Table 1: Specimens used for this project. Species identifications were identified by the source. T numbers were used for this project only. Valves are identified as left (l) or right (r), and whether the interior or exterior was photographed. The last five columns mark whether each specimen (and which valve) was used in that analysis: EFA genus–elliptical Fourier analysis to compare extant genera with L6516 unionoids, EFA species–elliptical Fourier analysis to compare extant species with L6516 unionoids, Dig. length–determining the effect of digitization length on variation, Smoothing–determining the effect of smoothing values on variation.

Species	**No.**	**Valves**	**Source**
Anodonta anatina	T0009	r ext	Menker (2005)
Anodonta beringiana	T0010	r ext	Menker (2005)
Anodonta californiensis	T0011	r ext	Menker (2005)
Anodonta coarctata	T0012	r ext	Menker (2005)
Anodonta couperiana	T0013	r ext	Menker (2005)
Anodonta couperiana	T0127	ext	A. Bogan (NCSM)
Anodonta couperiana	T0128	ext	A. Bogan (NCSM)
Anodonta couperiana	T0129	ext	A. Bogan (NCSM)
Anodonta cygnea	T0014	l ext	Menker (2005)
Anodonta cygnea	T0130	ext	A. Bogan (NCSM)
Anodonta cygnea	T0131	ext	A. Bogan (NCSM)
Anodonta globusa	T0015	r ext	Menker (2005)
Anodonta grandis	T0085	l int, r ext	Howells et al. (1996)
Anodonta grandis	T0086	l ext, r int	Howells et al. (1996)
Anodonta grandis	T0087	r ext	Howells et al. (1996)
Anodonta grandis	T0088	l ext	Howells et al. (1996)
Anodonta grandis	T0089	r ext	Howells et al. (1996)

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Table 1 – *continued from previous page*

Species	**No.**	**Valves**	**Source**
Anodonta grandis	T0090	l ext	Howells et al. (1996)
Anodonta grandis	T0132	l int, r ext	Howells et al. (1996)
Anodonta grandis	T0133	l ext, r int	Howells et al. (1996)
Anodonta grandis	T0134	int	UND-PC
Anodonta grandis	T0135	l int	UND-PC
Anodonta grandis	T0136	int	UND-PC
Anodonta grandis	T0137	r int	UND-PC
Anodonta grandis	T0138	int	UND-PC
Anodonta grandis	T0139	r int	UND-PC
Anodonta grandis	T0140	r int	UND-PC
Anodonta grandis	T0141	int	UND-PC
Anodonta grandis	T0142	int	UND-PC
Anodonta grandis	T0143	int	UND-PC
Anodonta imbecillis	T0091	l ext, r int	Howells et al. (1996)
Anodonta imbecillis	T0092	l int, r ext	Howells et al. (1996)
Anodonta imbecillis	T0093	l ext, r int	Howells et al. (1996)
Anodonta imbecillis	T0144	l ext	Howells et al. (1996)
Anodonta imbecillis	T0145	r ext	Howells et al. (1996)
Anodonta imbecillis	T0146	r ext	Howells et al. (1996)
Anodonta implicata	T0016	r ext	Menker (2005)
Anodonta implicata	T0094	l int, r ext	Strayer and Jirka (1997)
Anodonta implicata	T0095	l ext	Strayer and Jirka (1997)
Anodonta kennerlyi	T0017	r ext	Menker (2005)
Anodonta nuttalliana	T0018	r ext	Menker (2005)
Anodonta sp.	T0096	l int, r ext	Howells et al. (1996)
Anodonta suborbiculata	T0019	r ext	Menker (2005)
Anodonta suborbiculata	T0076	l ext	Parmalee and Bogan (1999)
Anodonta suborbiculata	T0097	l ext	Howells et al. (1996)
Anodonta suborbiculata	T0147	r ext	Howells et al. (1996)
Anodonta suborbiculata	T0148	l ext	Howells et al. (1996)

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Table 1 – *continued from previous page*

Species	**No.**	**Valves**	**Source**
Anodonta suborbiculata	T0386	r ext	Cicerello and Schuster (2003)
Anodontites crispatus	T0414	r ext	Simone (2006)
Anodontites elongatus	T0415		Simone (2006)
Anodontites elongatus	T0416		Simone (2006)
Anodontites elongatus	T0417		Simone (2006)
Anodontites elongatus	T0418		Simone (2006)
Anodontites elongatus	T0419		Simone (2006)
Anodontites elongatus	T0420		Simone (2006)
Anodontites elongatus	T0421		Simone (2006)
Anodontites elongatus	T0422		Simone (2006)
Anodontites ferrarisi	T0423		Simone (2006)
Anodontites ferrarisi	T0424		Simone (2006)
Anodontites irisans	T0425		Simone (2006)
Anodontites irisans	T0426		Simone (2006)
Anodontites moricandi	T0427		Simone (2006)
Anodontites moricandi	T0428		Simone (2006)
Anodontites moricandi	T0429		Simone (2006)
Anodontites moricandi	T0430		Simone (2006)
Anodontites obtusus	T0431		Simone (2006)
Anodontites obtusus	T0432		Simone (2006)
Anodontites patagonicus	T0433		Simone (2006)
Anodontites patagonicus	T0434		Simone (2006)
Anodontites patagonicus	T0435		Simone (2006)
Anodontites patagonicus	T0436		Simone (2006)
Anodontites tenebricosus	T0437		Simone (2006)
Anodontites tenebricosus	T0438		Simone (2006)
Anodontites tenebricosus	T0439		Simone (2006)
Anodontites tenebricosus	T0440		Simone (2006)
Anodontites tenebricosus	T0441		Simone (2006)
Anodontites tenebricosus	T0442		Simone (2006)

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Table 1 – *continued from previous page*

Species	**No.**	**Valves**	**Source**
Anodontites tenebricosus	T0443		Simone (2006)
Anodontites tenebricosus	T0444		Simone (2006)
Anodontites tenebricosus	T0445		Simone (2006)
Anodontites tenebricosus	T0446		Simone (2006)
Anodontites tenebricosus	T0447		Simone (2006)
Anodontites tortilis	T0448		Simone (2006)
Anodontites trapesialis	T0449		Simone (2006)
Anodontites trapesialis	T0450		Simone (2006)
Anodontites trapesialis	T0451		Simone (2006)
Anodontites trapesialis	T0452		Simone (2006)
Anodontites trapesialis	T0453		Simone (2006)
Anodontites trapesialis	T0454		Simone (2006)
Anodontites trapesialis	T0455		Simone (2006)
Anodontites trapesialis	T0456		Simone (2006)
Anodontites trapesialis	T0457		Simone (2006)
Anodontites trapesialis	T0458		Simone (2006)
Anodontites trapesialis	T0459		Simone (2006)
Anodontites trapesialis	T0460		Simone (2006)
Anodontites trapesialis	T0461		Simone (2006)
Anodontites trapesialis	T0462		Simone (2006)
Anodontites trapesialis	T0463		Simone (2006)
Anodontites trapesialis	T0464		Simone (2006)
Anodontoides connasaugaensis	T0030	r ext	Menker (2005)
Anodontoides denigrata	T0031	r ext	Menker (2005)
Anodontoides denigrata	T0387	r ext	Cicerello and Schuster (2003)
Anodontoides ferussacianus	T0032	r ext	Menker (2005)
Anodontoides ferussacianus	T0077	l ext	Parmalee and Bogan (1999)
Anodontoides ferussacianus	T0098	l int, r ext	Strayer and Jirka (1997)
Anodontoides ferussacianus	T0099	r ext	Strayer and Jirka (1997)
Anodontoides ferussacianus	T0100	r ext	Strayer and Jirka (1997)

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Table 1 – *continued from previous page*

Species	**No.**	**Valves**	**Source**
Anodontoides ferussacianus	T0149	ext	A. Bogan (NCSM)
Anodontoides ferussacianus	T0150	ext	A. Bogan (NCSM)
Anodontoides ferussacianus	T0151	int	UND-PC
Anodontoides ferussacianus	T0152	int	UND-PC
Anodontoides ferussacianus	T0153	int	UND-PC
Anodontoides ferussacianus	T0154	int	UND-PC
Anodontoides ferussacianus	T0192	int	UND-PC
Anodontoides ferussacianus	T0193	int	UND-PC
Anodontoides ferussacianus	T0194	int	UND-PC
Anodontoides ferussacianus	T0195	int	UND-PC
Anodontoides ferussacianus	T0196	int	UND-PC
Anodontoides ferussacianus	T0197	int	UND-PC
Anodontoides ferussacianus	T0388	r ext	Cicerello and Schuster (2003)
Anodontoides radiatus	T0033	r ext	Menker (2005)
Gonidea angulata	T0041	r ext	Menker (2005)
Gonidea angulata	T0347	r int	K. Cummings (INHS)
Gonidea angulata	T0348	int	K. Cummings (INHS)
Gonidea angulata	T0349	int	K. Cummings (INHS)
Gonidea angulata	T0350	int	K. Cummings (INHS)
Gonidea angulata	T0351	int	K. Cummings (INHS)
Gonidea angulata	T0352	l int	K. Cummings (INHS)
Gonidea angulata	T0353	int	K. Cummings (INHS)
Gonidea angulata	T0354	l int	K. Cummings (INHS)
Gonidea angulata	T0355	r int	K. Cummings (INHS)
Gonidea angulata	T0356	r int	K. Cummings (INHS)
Gonidea angulata	T0357	l int	K. Cummings (INHS)
Gonidea angulata	T0358	r int	K. Cummings (INHS)
Gonidea angulata	T0359	l int	K. Cummings (INHS)
Pilsbryoconcha exilis	T0051	l ext	Menker (2005)
Pilsbryoconcha exilis	T0175	ext	A. Bogan (NCSM)

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Table 1 – *continued from previous page*

Species	**No.**	**Valves**	**Source**
Pilsbryoconcha exilis compressa	T0469	l int, r ext	A. Bogan (NCSM)
Pilsbryoconcha exilis compressa	T0470	l int, r ext	A. Bogan (NCSM)
Pilsbryoconcha exilis compressa	T0471	l int, r ext	A. Bogan (NCSM)
Pilsbryoconcha exilis compressa	T0472	l int, r ext	A. Bogan (NCSM)
Pilsbryoconcha exilis compressa	T0473	l int, r ext	A. Bogan (NCSM)
Pilsbryoconcha exilis compressa	T0474	l int, r ext	A. Bogan (NCSM)
Pilsbryoconcha exilis compressa	T0475	l int, r ext	A. Bogan (NCSM)
Pilsbryoconcha exilis compressa	T0476	l int, r ext	A. Bogan (NCSM)
Pilsbryoconcha exilis compressa	T0477	l int, r ext	A. Bogan (NCSM)
Pilsbryoconcha exilis compressa	T0478	l int, r ext	A. Bogan (NCSM)
Pilsbryoconcha exilis compressa	T0479	l int, r ext	A. Bogan (NCSM)
Pilsbryoconcha exilis compressa	T0480	l int, r ext	A. Bogan (NCSM)
Pilsbryoconcha exilis compressa	T0481	l int, r ext	A. Bogan (NCSM)
Pilsbryoconcha exilis compressa	T0482	l int, r ext	A. Bogan (NCSM)
Pilsbryoconcha exilis compressa	T0483	l int, r ext	A. Bogan (NCSM)
Pilsbryoconcha exilis compressa	T0484	l int, r ext	A. Bogan (NCSM)
Pilsbryoconcha exilis compressa	T0485	l int, r ext	A. Bogan (NCSM)
Pilsbryoconcha exilis compressa	T0486	int	A. Bogan (NCSM)
Pyganodon cataracta	T0106	l ext	Strayer and Jirka (1997)
Pyganodon cataracta	T0107	l ext	Strayer and Jirka (1997)
Pyganodon cataracta	T0108	r ext	Strayer and Jirka (1997)
Pyganodon cataracta cataracta	T0054	r ext	Menker (2005)
Pyganodon cataracta marginata	T0055	r ext	Menker (2005)
Pyganodon doliaris	T0056	l ext	Menker (2005)
Pyganodon gibbosa	T0057	l ext	Menker (2005)
Pyganodon grandis	T0078	l ext	Parmalee and Bogan (1999)
Pyganodon grandis	T0109	r ext	Strayer and Jirka (1997)
Pyganodon grandis	T0110	r ext	Strayer and Jirka (1997)
Pyganodon grandis	T0111	r ext	Strayer and Jirka (1997)
Pyganodon grandis	T0112	r ext	Strayer and Jirka (1997)

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Table 1 – *continued from previous page*

Species	**No.**	**Valves**	**Source**
Pyganodon grandis	T0113	r ext	Strayer and Jirka (1997)
Pyganodon grandis	T0177	ext	A. Bogan (NCSM)
Pyganodon grandis	T0178	r ext	A. Bogan (NCSM)
Pyganodon grandis	T0396	r ext	Cicerello and Schuster (2003)
Pyganodon grandis corpulenta	T0058	r ext	Menker (2005)
Pyganodon grandis grandis	T0059	r ext	Menker (2005)
Pyganodon grandis simpsoniana	T0060	r ext	Menker (2005)
Pyganodon grandis stewartiana	T0061	r ext	Menker (2005)
Pyganodon hallenbecki	T0062	r ext	Menker (2005)
Pyganodon lacustris	T0114	r ext	Strayer and Jirka (1997)
Pyganodon lacustris	T0115	r ext	Strayer and Jirka (1997)
Pyganodon lacustris	T0116	r ext	Strayer and Jirka (1997)
Pyganodon lacustris	T0117	r ext	Strayer and Jirka (1997)
Pyganodon lacustris	T0118	l ext	Strayer and Jirka (1997)
Pyganodon lacustris	T0119	r ext	Strayer and Jirka (1997)
Pyganodon teres	T0063	r ext	Menker (2005)
Simpsonaias ambigua	T0064	r ext	Menker (2005)
Simpsonaias ambigua	T0079	l ext	Parmalee and Bogan (1999)
Simpsonaias ambigua	T0182	ext	A. Bogan (NCSM)
Simpsonaias ambigua	T0183	ext	A. Bogan (NCSM)
Simpsonaias ambigua	T0273	ext	A. Bogan (NCSM)
Simpsonaias ambigua	T0274	l ext	A. Bogan (NCSM)
Simpsonaias ambigua	T0275	ext	A. Bogan (NCSM)
Simpsonaias ambigua	T0276	ext	A. Bogan (NCSM)
Simpsonaias ambigua	T0277	ext	A. Bogan (NCSM)
Simpsonaias ambigua	T0278	ext	A. Bogan (NCSM)
Simpsonaias ambigua	T0279	ext	A. Bogan (NCSM)
Simpsonaias ambigua	T0280	ext	A. Bogan (NCSM)
Simpsonaias ambigua	T0281	ext	A. Bogan (NCSM)
Simpsonaias ambigua	T0282	ext	A. Bogan (NCSM)

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Species	**No.**	**Valves**	**Source**
Simpsonaias ambigua	T0397	r ext	Cicerello and Schuster (2003)
Strophitus subvexus	T0070	r ext	Menker (2005)
Strophitus subvexus	T0283	ext	A. Bogan (NCSM)
Strophitus subvexus	T0284	ext	A. Bogan (NCSM)
Strophitus subvexus	T0285	ext	A. Bogan (NCSM)
Strophitus subvexus	T0286	ext	A. Bogan (NCSM)
Strophitus undulatus	T0081	l ext	Parmalee and Bogan (1999)
Strophitus undulatus	T0120	l int, r ext	Strayer and Jirka (1997)
Strophitus undulatus	T0121	l int, r ext	Strayer and Jirka (1997)
Strophitus undulatus	T0122	l ext, r int	Howells et al. (1996)
Strophitus undulatus	T0189	ext	A. Bogan (NCSM)
Strophitus undulatus	T0287	ext	A. Bogan (NCSM)
Strophitus undulatus	T0288	ext	A. Bogan (NCSM)
Strophitus undulatus	T0289	ext	A. Bogan (NCSM)
Strophitus undulatus	T0290	ext	A. Bogan (NCSM)
Strophitus undulatus	T0291	ext	A. Bogan (NCSM)
Strophitus undulatus	T0292	ext	A. Bogan (NCSM)
Strophitus undulatus	T0293	ext	A. Bogan (NCSM)
Strophitus undulatus	T0294	ext	A. Bogan (NCSM)
Strophitus undulatus	T0295	ext	A. Bogan (NCSM)
Strophitus undulatus	T0296	l ext	A. Bogan (NCSM)
Strophitus undulatus	T0297	ext	A. Bogan (NCSM)
Strophitus undulatus	T0298	ext	A. Bogan (NCSM)
Strophitus undulatus	T0299	r ext	A. Bogan (NCSM)
Strophitus undulatus	T0300	r ext	A. Bogan (NCSM)
Strophitus undulatus	T0398	r ext	Cicerello and Schuster (2003)
Strophitus undulatus pavonia	T0123	r ext	Strayer and Jirka (1997)
Strophitus undulatus tennessensis	T0071	r ext	Menker (2005)
Strophitus undulatus undulatus	T0072	r ext	Menker (2005)

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Table 1 – *continued from previous page*

Species	**No.**	**Valves**	**Source**
Strophitus connasaugaensis	T0080	l ext	Parmalee and Bogan (1999)
Utterbackia imbecillis	T0073	r ext	Menker (2005)
Utterbackia imbecillis	T0082	l ext	Parmalee and Bogan (1999)
Utterbackia imbecillis	T0124	r ext	Strayer and Jirka (1997)
Utterbackia imbecillis	T0190	ext	A. Bogan (NCSM)
Utterbackia imbecillis	T0191	ext	A. Bogan (NCSM)
Utterbackia imbecillis	T0301	ext	A. Bogan (NCSM)
Utterbackia imbecillis	T0302	ext	A. Bogan (NCSM)
Utterbackia imbecillis	T0303	l ext	A. Bogan (NCSM)
Utterbackia imbecillis	T0304	ext	A. Bogan (NCSM)
Utterbackia imbecillis	T0305	ext	A. Bogan (NCSM)
Utterbackia imbecillis	T0306	ext	A. Bogan (NCSM)
Utterbackia imbecillis	T0307	ext	A. Bogan (NCSM)
Utterbackia imbecillis	T0308	ext	A. Bogan (NCSM)
Utterbackia imbecillis	T0309	ext	A. Bogan (NCSM)
Utterbackia imbecillis	T0310	ext	A. Bogan (NCSM)
Utterbackia imbecillis	T0311	ext	A. Bogan (NCSM)
Utterbackia imbecillis	T0312	ext	A. Bogan (NCSM)
Utterbackia imbecillis	T0313	ext	A. Bogan (NCSM)
Utterbackia imbecillis	T0314	ext	A. Bogan (NCSM)
Utterbackia imbecillis	T0315	ext	A. Bogan (NCSM)
Utterbackia imbecillis	T0316	ext	A. Bogan (NCSM)
Utterbackia imbecillis	T0317	ext	A. Bogan (NCSM)
Utterbackia imbecillis	T0318	ext	A. Bogan (NCSM)
Utterbackia imbecillis	T0400	r ext	Cicerello and Schuster (2003)
Utterbackia peggyae	T0074	r ext	Menker (2005)
Utterbackia peninsularis	T0075	r ext	Menker (2005)

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