Delineating paleodrainages using morphospace occupation of freshwater mussels

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ABSTRACT

Although Cretaceous fossil freshwater mussels (Unionoidea) frequently possess distinguishing external features upon which identification can be based, most post-K-Pg mussels have less diagnostic features. Discriminating previously unnamed taxa within an assemblage based on morphology is especially difficult when those taxa occupy similar morphospace and do not preserve key characters. Calculating the size of morphospace occupation is identified here as a possible method for estimating the number of taxa present in a given assemblage, assuming the normal range of this value is known for comparable taxa (modern or fossil). Previous neo- and paleontological morphometric analyses performed on freshwater mussels have identified problematic areas in this model: choice of similar proxy taxa to the organisms under study; ontogenetic, sexually dimorphic, and size variation within taxa; taphonomic deformation; and morphological ecological plasticity and evolutionary convergence. The present study attempts to address these last issues.

The shapes of *Anodonta grandis* from two sites in the Red River drainage basin (a North Dakota river and a Minnesota lake, ~190 km apart) were compared using the analysis of 50 pseudolandmarks along the shell outline, anchored on the umbo. Three measures of variation (Foote's disparity, within-group variation, and sum of variance) show that the size of morphospace occupation is not statistically significantly different between the sampled environments, supporting a similarity in the natural amount of variation in each population. Additionally, pseudolandmark analyses show lack of a discernible difference between the shapes of *A. grandis*, suggesting similar habitats and a close phylogenetic relationship. We suggest these techniques provide a potential means to discriminate stable morphological populations (based on morphospace occupation) through space and time. Future studies on additional occurrences within and outside *A. grandis* watersheds should allow baseline statistics on how morphological variation can be applied to mussels of similar form in paleodrainages.

INTRODUCTION

Modern freshwater mussels (Unionoidea) can be used to study fossil assemblages in a number of ways. Important to this study are two uncomfortable ideas: First, that fossil specimens can be misinterpreted into more or fewer taxa than is necessary without the help of soft tissue; and second, that the ecophenotypic variation in these organisms is quite high, resulting in few rules that can be applied to all taxa in terms of variation.

This study attempts to examine the latter question by experimentally testing the morphologic variation in monospecific populations of modern freshwater mussels, in hopes that these methods can be applied to fossil assemblages in order to minimize identification error.

Hypotheses

1. That pseudolandmarks (a new method) are a useful alternative to outline methods on biological shapes that have only one homologous point, particularly freshwater mussels, and allow full use of landmark-based geometric morphometrics.

2. That size of morphospace occupation of freshwater mussels (based on valve morphology) is generally stable within species, regardless of the average morphology of a population.

3. That size of morphospace occupation will increase as populations from larger and larger hydrographic units (e.g., watershed to subbasin to basin) are pooled, and that this change is stable per species.

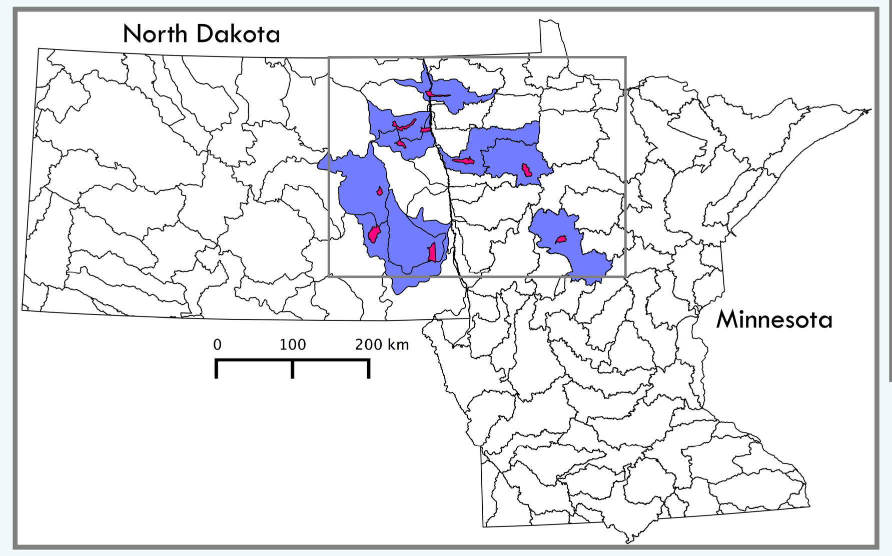
4. That differences in shape are measurable between populations using this method, and that populations representative of smaller hydrographic units will be more similar in shape than those representative of larger units.

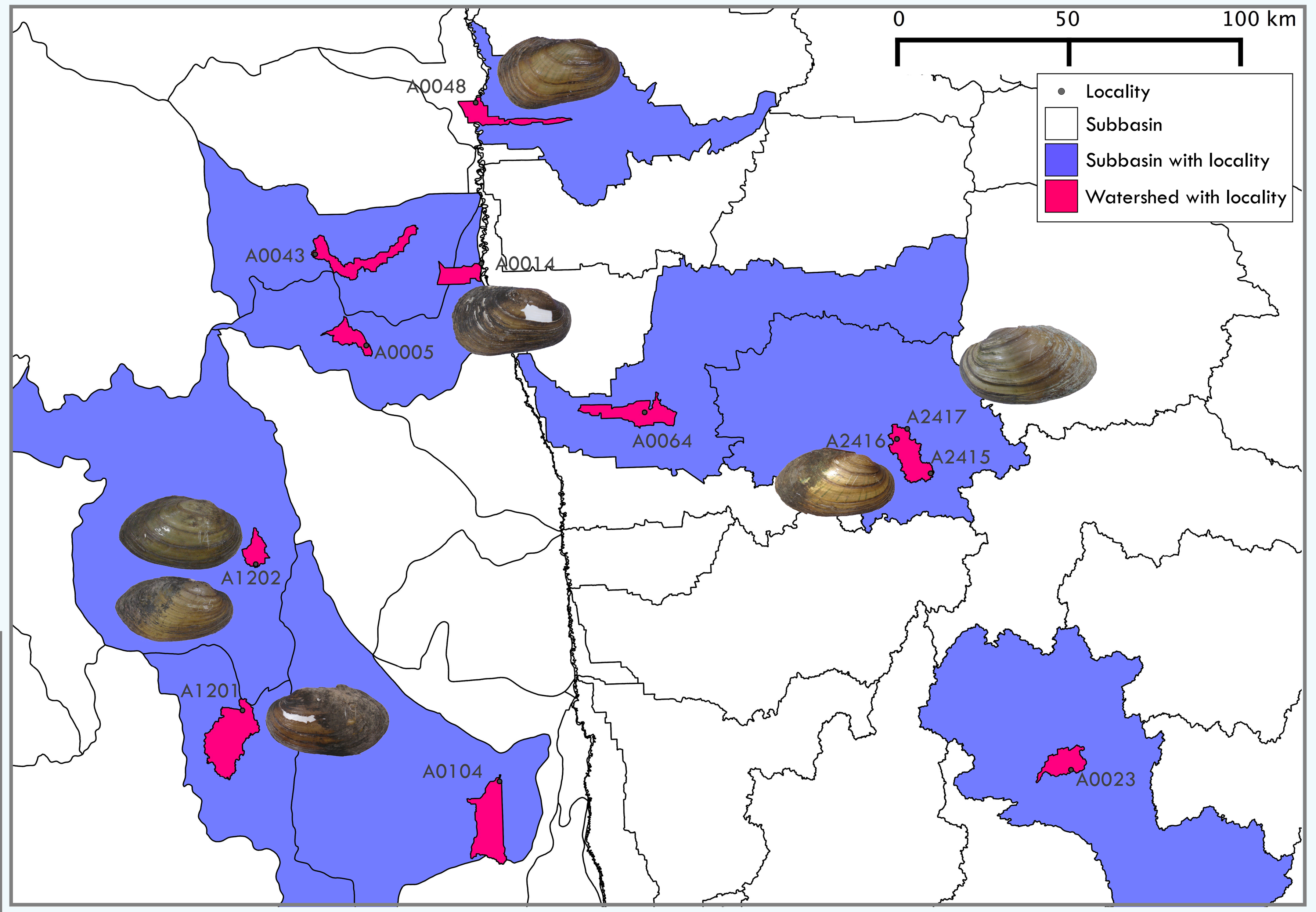
LOCALITIES

Localities were selected based on the availability of material in the UND Geology and Geological Engineering Paleontological Collections. Several thousand modern freshwater mussel (Unionoidea) specimens represent the effort of many people over more than two decades to catalog the molluscan fauna of North Dakota and eastern Minnesota.

These collections comprise specimens from all of the 13 species represented in North Dakota, but only a few sites yielded a large enough sample size for geometric morphometric analysis. Three species in particular were well-sampled at more than one locality and were chosen for this study: *Anodonta grandis*, *Lampsilis radiata*, and *Lampsilis siliquoidea*.

Specimens from six sites (A0014, A0048, A1201, A1202, A2416, and A2417; see map) representing eight monospecific populations were located in North Dakota and Minnesota in the Hudson Bay drainage. One site from Lake Erie (C0168; not mapped) representing one monospecific population was located in the St. Lawrence River drainage. This locality was chosen as an outgroup for *Lampsilis siliquoidea*.





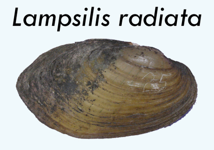
HYDROGRAPHIC RELATIONSHIPS

	Hudson Bay, Atlantic Ocean (Canada)					Atlantic Ocean (Canada)
Region	Souris-Red-Rainy (U.S.A., Canada)					St. Lawrence River (U.S.A., Canada)
Subregion	Red River (U.S.A., Canada)					St. Lawrence River (U.S.A., Canada)
Basin	Lower Red River (U.S.A., Canada)					Great Lakes (U.S.A., Canada)
Subbasin	Lower Sheyenne River (ND)	Clearwater (MN)	Middle Sheyenne River (ND)	Grand Marais Creek (ND)	Lower Red River (ND)	Lake Erie (U.S.A., Canada)
Watershed	Valley City (ND)	Lost River (MN)	Cooperstown (ND)	County Ditch No. 175 (ND)	City of Drayton (ND)	
Subwatershed	Hobart Lake (ND)	Pine Lake (MN)	Ueland Dam (ND)	Judicial Ditch No. 68 (ND)	City of Drayton (ND)	
Site	A1201 *Lampsilis radiata*	A2416 *Lampsilis radiata*; A2417 *Anodonta grandis*, *Lampsilis radiata*	A1202 *Anodonta grandis*; *Lampsilis radiata*	A0014 *Lampsilis siliquoidea*	A0048 *Lampsilis siliquoidea*	C0168 *Lampsilis siliquoidea*







Analyses were based upon the position of each site within the hierarchy of hydrographic units. The within-group variation (size of morphospace occupation) was measured for species at sites within the same hydrologic unit: *Lampsilis radiata* within the Pine Lake subwatershed, *Anodonta grandis*, *Lampsilis radiata*, and *Lampsilis siliquoidea* within the Lower Red River basin, and *Lampsilis siliquoidea* within rivers eventually reaching the Atlantic Ocean.

METHODS

A new geometric morphometrics method is introduced here as "pseudolandmark method" in order to use landmark analyses on outlines. This method differs from sliding semilandmarks, which create perimeter landmarks based on the position of the centroid. Since it is the only biologically homologous point readily available on many species of freshwater mussel, the umbo was chosen as the starting and ending point for a series of landmarks spaced evenly and measured clockwise around a valve oriented with the umbo to the upper left (shown below).

Manual digitization of specimens and analysis of populations would have been labor-intensive, so the following steps were automated with Photoshop CS2 actions and ImageJ macros.

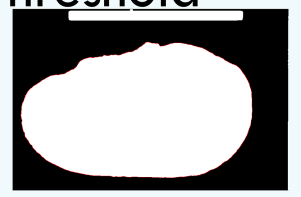
Scan

Specimens were scanned with labels and scales in color at 400 dpi. Specimens were split into separate files.

Blur

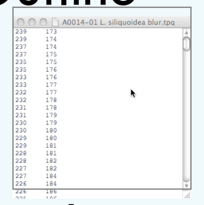
Images were blurred in Photoshop CS2 (Box Blur, 4px radius) to smooth edges and improve contrast.

Threshold



ImageJ was used to automatically threshold images (Hysteresis plugin) and automatically trace outlines. Outline traces were started at the umbo.

Outline



Outlines were saved as a series of XY coordinate pairs.

Analysis

Resample and custom DOS batch files (Burton-Kelly, 2008) were used to create 50 pseudolandmarks (spaced evenly around the specimen perimeter) from XY coordinates in TPS format.

Pseudolandmark analysis was performed with the IMP Suite: Foote's Disparity was calculated using DisparityBox6, distance between group means was calculated using TwoGroup6. Pseudolandmark analysis was performed twice, with 50 pseudolandmarks, an arbitrary value which needs to be examined in the future.

RESULTS

Size of Morphospace Occupation

Anodonta grandis: Analysis of pseudolandmarks failed to produce statistically significantly higher amounts of morphospace occupation as hydrographic unit scale increased. The Foote's disparity of combined sites in the same hydrographic unit falls between the lowest and highest values of the sites that were combined. This suggests that the populations at both sites are similar enough in shape that within-population variation outweighs cross-population variation; when populations are combined, enough overlap occurs to lower the mean distance between individuals.

Lampsilis radiata: Specimens at all four sites produce non-different values of Foote's disparity based on 95% confidence intervals. Foote's disparity is statistically significantly different between grouped specimens from the same watershed (A2416-A2417) and one same-basin group (A1201-A1202-A2416).

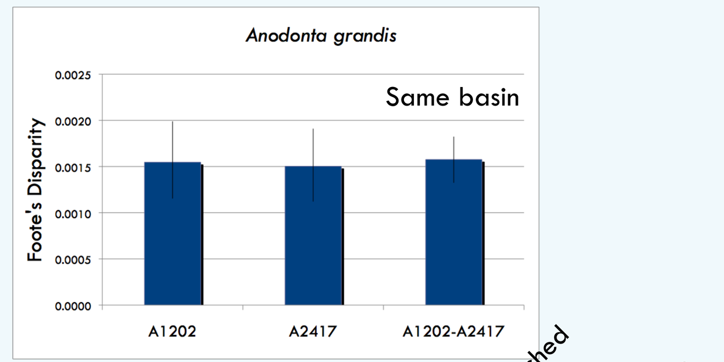
Lampsilis siliquoidea: Specimens from all three sites produce non-different values of Foote's disparity. Foote's disparity is statistically significantly different between grouped specimens from the same basin (A0014-A0048) and one same-ocean group (A0048-C0168).

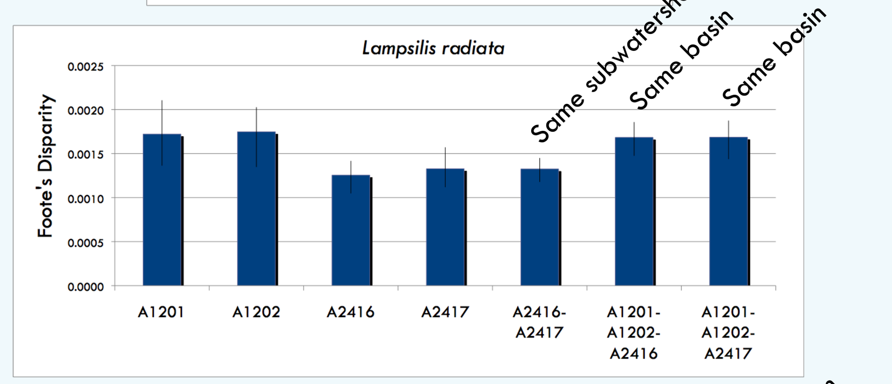
Mean Shape Distance

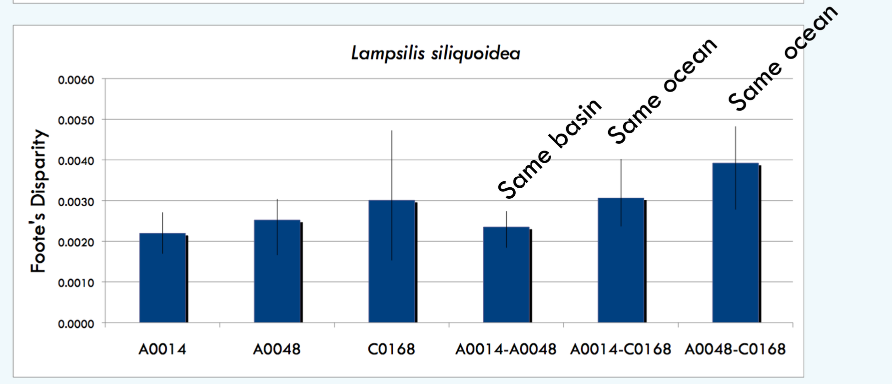
Anodonta grandis: The bootstrapped full Procrustes distance between the two *A. grandis* populations (from the same basin) was 0.0167 (CI 0.0122 to 0.0247). With only two sites, no comparisons could be made.

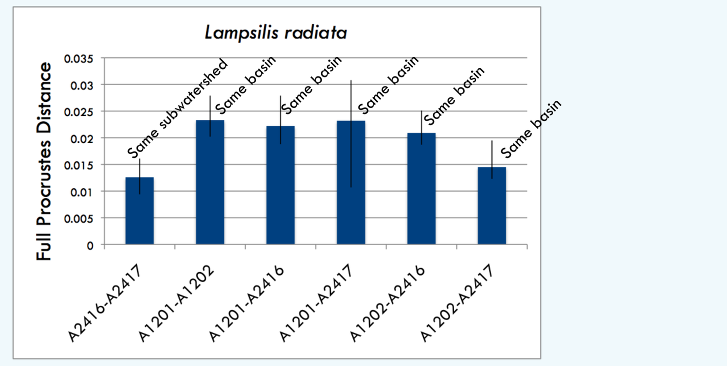
Lampsilis radiata: With the exception of the A1201-A2417 and A1202-A2417 pairings, full Procrustes distances between populations limited to the same basin was statistically significantly larger than distances between populations limited to the same subwatershed (A2416-A2417). Physical stream distance between localities may have an effect that is not shown when comparing hydrologic unit scale.

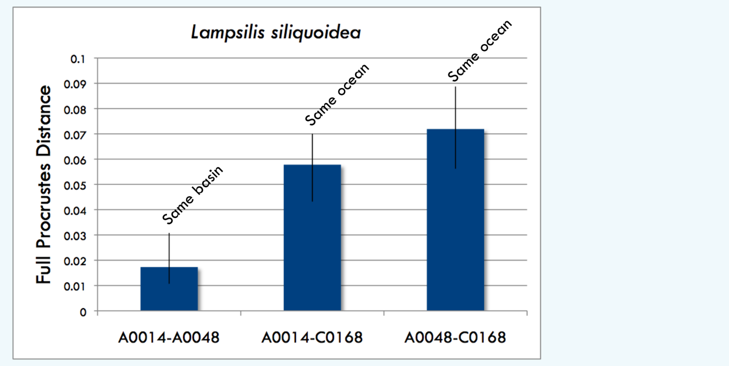
Lampsilis siliquoidea: The distance between populations limited to the same basin was statistically significantly lower than the distance between populations limited to the same ocean (which may be an arbitrary hydrologic unit scale). This result suggests that other anlyses may be attempting to compare populations at too fine a scale for these methods to allow.











CONCLUSIONS

1. Pseudolandmarks show promise for describing the shape of organisms with a single homologous point on an outline; however specific problems need to be solved. Accuracy in placement of the umbo during digitization may not be consistent between specimens, which would lead to increased variation within the population being studied. The number of pseudolandmarks should be, to follow general practice, no more than half the number of specimens used, which in this case was not followed. The difference in results between this method and outline methods (specifically elliptical Fourier analysis) was not tested in this study but should be to determine the efficacy of pseudolandmarks (see Scholz's [2003] comparison of EFA with sliding semilandmarks).

2. For the few populations examined, size of morphospace occupation does not differ to a statistically significant degree within species. It has yet to be determined whether this holds true for populations over a wider geographic area.

3. Statistical significance in morphospace occupation of grouped populations was never a general rule, although improvement of the pseudolandmark method to minimize digitization error may improve the comparison of within-group and without-group size of morphospace occupation. More populations would add to this understanding.

4. In a very general sense, paired populations selected from larger hydrologic units were more disparate in shape than those from smaller hydrologic units. Departure from this possible trend may stem from failing to take stream distance into account; populations from different basins may in fact be geographically closer to one another when measured along the stream network than populations within the same basin.

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