

**QUANTIFYING VARIABILITY AND UNDERSTANDING SPECIES DELIMITATION: A CASE STUDY  
INTEGRATING MORPHOLOGICAL AND GENETIC DATASETS IN TEREBRATULIDE  
BRACHIOPODS**

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Species conceptualization and delimitation have always been controversial and highly debated topics in biology. From an operational standpoint, setting species boundaries requires multiple sources of information (e.g. morphology, genetics, ecology, biogeography, etc.). Paleontologists, however, are limited to fewer lines of evidence when delimiting species and rely heavily on morphology. Considering the fundamental role morphology plays in species recognition in the fossil record, analyzing morphology in a quantitative manner and examining phenotypic variability in both fossils and extant close relatives is fundamental. Furthermore, we can take advantage of living specimens to incorporate additional sources of data, such as genetics and biogeography, in order to characterize species in a more accurate manner. Therefore, the objective of our research is to understand how extant terebratulide brachiopod species are delimited, both morphologically and genetically, and compare observed ranges of phenotypical variability to the fossil record to make better informed decisions when recognizing extinct species.

In order to quantify variability in extant terebratulide brachiopods, we chose to analyze long loops—the calcareous structures that support the lophophore. We CT scanned a total of 59 individuals (41 specimens of *Laqueus*, 13 of *Terebratalia*, and 5 of *Dallinella*). The 3D isosurface models generated were then analyzed using 3D geometric morphometrics, which uses landmark coordinates to examine changes in the geometry of morphologic structures using statistical analyses. To simplify patterns of variation, exploratory methods, such as Principal Component Analysis (PCA) and Canonical Variate Analysis (CVA), were implemented. To test if shape is dependent on species designation and size, Procrustes ANOVA analyses testing landmark coordinates against species identification and against size, respectively, were performed. Based only on loop morphology, our results show that all of the species analyzed were statistically different from one another, including those with a contentious taxonomic history. Our results suggest that even when external characters are not considered (e.g. shell ornamentation and folding), the loop offers sufficient resolution to discriminate among named species.

To translate these results to the fossil record, we integrated our 3D dataset with 2D images of the outlines of their respective dorsal valves to test for correspondence between loops and overall shell shape. This is particularly important since long loops are rarely preserved in the fossil record. If we can find a correlation between long loop shape and outline shape, we can then make assumptions of species delimitation using only outline data. We digitized the outlines and analyzed them using Elliptical Fourier Analysis (EFA) to quantify variation of the shell shape. In this method, shape is described by harmonics, each representing a closed contour. Once Fourier coefficients are obtained, they were used as input for PCAs, as well as statistical analyses to assess if there is significant difference between the outline shapes from different named species. Based on preliminary results, shell outlines do not appear to offer enough resolution to discriminate among different named species.

Finally, to add another source of information that will help us test our morphological predictions on species boundaries, we propose analyzing genetic data of living brachiopods. The species *Terebratalia transversa*, *T. coreanica*, *Laqueus erythraeus*, *L. vancouveriensis*, and *L. rubellus* will be examined using a RADSeq protocol. The resulting DNA sequences will be analyzed under the multispecies coalescent model to estimate species delimitation and population genetic parameters, such as heterozygosity and population differentiation [*FST*]. Comparing genetic and morphologic species delimitation results will help us determine if species can be

successfully discriminated using only morphology, which is the main source of information in the fossil record. Studying variability in extinct and living specimens will help clarify how extant species are delimited based on genetic, and morphological characteristics, as well as test and improve our understanding of morphologically defined fossil species as evolutionary biological entities. This comparison of fossil and extant species delimitation in brachiopods is essential to evaluate the common assumption in paleontology that morphology alone proxies for biological species.