SHELL MORPHOMETRICS OF MARINE GASTROPODS: PHYLOGENETICS, VARIATION, AND PLASTICITY

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Abstract. Geometric morphometrics offers a way to quantify the shape of biological structures to be studied in an evolutionary context. Marine gastropod shells are ideal candidates for morphometric study, and are diverse and abundant in Moorea, French Polynesia. In the present study, gastropod specimens from Moorea and surrounding islands were photographed, digitized, and analyzed using computational statistical software. Morphometric traits from multiple species were mapped onto an existing phylogeny and three populations of *Strombus gibberulus* were studied to analyze within and between-group variation. Results show higher phylogenetic shape conservatism at the family level than the species level, suggesting plasticity and more recent adaptations. Variation is significant but minimal between groups, suggesting both high variability and overall conservatism within a species.

Key words: gastropods; Strombus gibberulus; *phylogenetics; variation; trait plasticity; Moorea, French Polynesia*

INTRODUCTION

As a historical science, evolutionary biology applies data and observations available today to infer the greater scope of geologic time and the history of life. To lay the groundwork for ecological study of past and present life, we must consider the evolutionary context of organisms by understanding their relationships. Taxonomists represent the most likely relationships between separate lineages through phylogenetic trees. Traditional phylogenetic analyses rely on morphological or molecular data with discrete states, such as synapomorphies or DNA sequences (Mishler 2005). However, methods that incorporate continuous characters may allow subtle but readily observable differences in morphology to indicate variation within a species or suggest divergence between species (Polly *et al.* 2013).

Morphological analysis, known as morphometrics, is the quantitative description, investigation, and interpretation of size and shape of biological structures (Rohlf 1990). It is an imperative methodological tool in systematics, paleontology, developmental biology, and even forensics (Roth and Mercer 2000). A relatively new method called geometric morphometrics (GM) transforms continuous morphological data in the form of coordinate points into discrete characteristics represented by principal components of shape (Zelditch *et al.* 2012). Requiring only standardized imaging of specimens and freely downloadable software, GM integrates multivariate statistics and geometric principals to convey shape variation between specimens.

This method can expand the possibilities for paleontologists to view and analyze the limited morphological data left by extinct organisms (Bose 2012, Sheets *et al.* 2006). It is also a tool for identifying variation in extant populations to find patterns in developmental or evolution. With further advancement and refining of methods, GM may be used to support phylogenetic relationships between extinct and extant taxa without reliance on genetic material or abundant discrete characteristics, or at least allow mapping of morphology onto phylogenies derived from other data.

The tropical Pacific island of Moorea offers a great diversity of marine gastropods that contribute to the reef ecosystem living within diverse shell forms or adding substrate and homes to other organisms. Their robust shell forms are also major constituents of the marine fossil record and are important for

biostratigraphy and paleoecology (Leighton 2002, Raup and Sepkoski 1982). Despite the relative disparity of shell morphologies, several conserved anatomical features and the rigid nature of their shells make gastropods well suited subjects of morphometric analysis (Smith and Hendricks 2013). Recent studies have visualized morphometric data of gastropods, but are mostly limited to the family Conidae (Cruz *et al.* 2012, Smith and Hendricks 2013). Because of their abundance on Moorea, this study focused on a number of families and species, including *Strombus gibberulus*.

The present study aimed to: (1) assess the phylogenetic conservatism of shape by mapping morphometric characters onto an existing gastropod phylogeny, (2) measure variation among and between *Strombus gibberulus* populations, and (3) identify possible areas of morphological conservatism or plasticity in marine gastropods. I used morphological data obtained through geometric morphometric software to examine relationships between taxa and analyze variation. I hypothesized that, in a phylogenetic context, certain aspects of shell shape are generally conserved among related species, whereas other aspects are more variable between species. If this is accurate, shape trait similarities should be somewhat predictive of phylogenetic relationships. I expected that geographically separated populations would vary significantly in mean shape due to limited gene flow between islands. I also expected to observe the majority of shape change in the spire and aperture of the shell. Information gained from this study may add to our understanding of morphological evolution, biogeography, and trait plasticity.

METHODS

Study site

This study was conducted on Moorea, French Polynesia, from the Richard B. Gump South Pacific Research Station. Most specimens were accessed from the collections of the French research station, le Centre de Recherches Insulaires et Observatoire de l'Environnement (CRIOBE), in Opunohu Bay on Moorea. Others were collected on Motu Tiahura and near the Gump station in Cook's Bay (see Fig. 1).

Sampling

A total of 33 species were selected for the phylogeny to represent a variety of species within caenogastropoda. Each species was represented by one individual specimen due to limited museum samples. Four specimens, Turbo argyrostomus, Terebra maculata, Terebra guttata, and Terebra argus, were collected from the back reef off Point Aroa, near Maharepa on Moorea, during preliminary collection trips made between 5 October and 12 October 2014. The other 29 specimens were accessed from the CRIOBE collections. Species identifications were based on the CRIOBE collection catalogue tags and the reference book by Salvat *et al.* (1984).

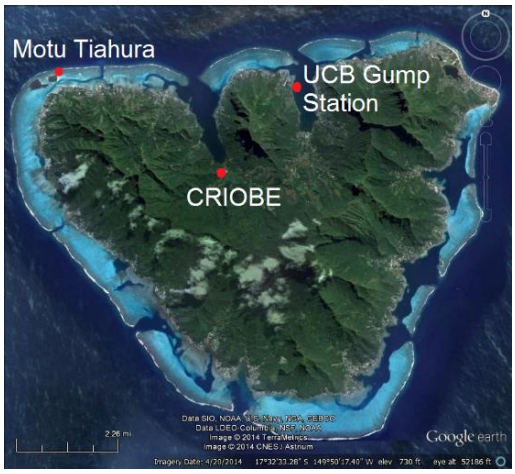

FIG. 1. Map of Moorea, French Polynesia. Points of note: Motu Tiahura, UCB Gump Station, and CRIOBE.

The species *Strombus gibberulus* was chosen for the study of variation between populations for its abundance on Moorea and sample size from CRIOBE. A total of 58 *S. gibberulus* shells were obtained, belonging to three different locations in French Polynesia: 16 from Tubuai, Austral Islands (23°21'24"S, 149°26'56"W); 25 from Maupiti, Society Islands (16°26'38"S, 152°15'35"W); and 17 from Moorea, Society Islands (17°29'11"S, 149°54'37"W). All 17 specimens from Moorea were collected in the

sheltered sand flats of Motu Tiahura on 26 October 2014 (Wilson 2009). All other *S. gibberulus* specimens were previously collected and accessed from the CRIOBE collections. Specimens with broken parts were omitted. Figure 2 shows a map of the three island populations.

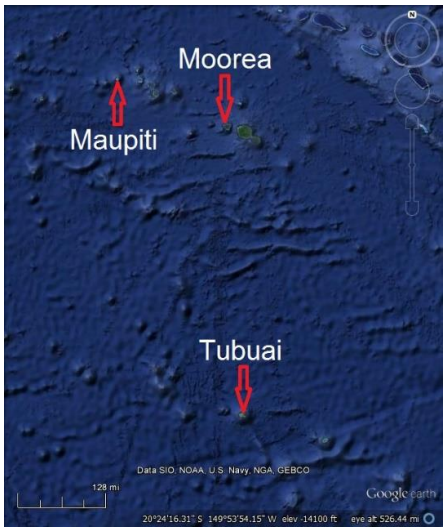

FIG. 2. Map of islands home to the study populations: Moorea, Maupiti, and Tubuai (image from Google Earth).

Imaging and Digitizing

All of the aforementioned specimens were photographed with a Canon EOS digital camera on 17, 23, and 30 October 2014 in the CRIOBE optics room. Specimens were placed on a grid of 1 cm squares and stabilized by Styrofoam to correct imbalances. Each specimen was carefully oriented with the aperture parallel to the grid and directly facing the camera. The camera was mounted 50cm above the grid. Two lights were pointed at a 45 degree angle downward on both sides of the specimen.

Photographs were digitized in tpsDig v. 1.40 to capture the x,y coordinates of landmark points (Rohlf 2004). 10 homologous landmark points were captured for each specimen for both the phylogenetic and shape variation study. Landmarks (LM) 1, 2, 6, and 7 are Type I landmarks, which are based on histological evidence and are highly preferred for representing direct juxtapositions of tissue types or probable homologies (Bookenstein 1991). LM1 is the apex of the shell. LM2 is the lower suture of the penultimate whorl on the aperture side (right profile). LM6 is the point opposite LM2 on the body whorl side (left profile). LM7 is the junction between the end of the suture and the apertural lip. Landmarks 3, 4, and 9 are Type II landmarks, which are based on geometric evidence and not entirely histological. LM3 is the intersection of the inner and outer apertural lip at the posterior canal. LM4 is the anterior-most point of the columella. LM9 is the anterior apertural suture, or the intersection of the siphonal notch and the columella. Landmarks 5, 8, and 10 are Type III landmarks, which are more arbitrary points, usually defined by distance from other points. LM5 is midway along the outer-most curve of the body whorl (left profile). LM8 is the outer-most point of the outer apertural lip. LM10 is the midpoint of the columella on the inner apertural lip. Figure 3 shows the 10 selected landmark points marked on a *Strombus gibberulus* specimen.

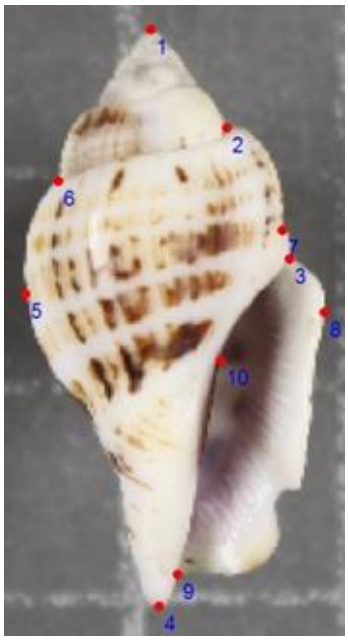

FIG. 3. *Strombus gibberulus* with landmark points 1-10.

Geometric Morphometrics

Landmark coordinates for all specimens were analyzed in MorphoJ v. 1.06b (Klingenberg 2011). Specimens for the phylogenetic study (33 individuals) and the population study (58 individuals) were divided into separate datasets. They were superimposed by Procrustes Fit, which maps the landmark configuration of each specimen in a dataset onto each other so that corresponding points are as close as possible. This process scales specimens to comparable sizes based on centroid size and minimizes the sum of squared distances between corresponding points (Rohlf 1999).

A covariance matrix was generated, then a Principal Component Analysis was run on each dataset in MorphoJ. PCA is an ordination method which manipulates multidimensional data by rotating the frame of reference such that the maximum variability is visible (Campbell and Atchley 1981). This frame of reference becomes Principal Component Axis 1 (PC1). The next highest axis of variability is PC2, and so on until variability is negligible. In a practical sense, this allows us to identify the modes of change that account for the most variation and provides linear numerical values for further analysis.

For both datasets, the eigenvalues and percentages of total variance were calculated for principal components 1-4.

All geometric morphometric techniques used in this study are explained in detail in "A Practical Companion to Geometric Morphometrics for Biologists: Running analyses in freely-available software" (Zelditch *et al.* 2012).

Phylogenetic tree mapping

To represent the evolutionary relationships between the studied taxa, an informal phylogenetic supertree was constructed. A formal supertree compiles the character matrices of multiple source trees to recreate an optimized comprehensive tree (Bininda-Emonds 2004). If matrices are not available, an informal supertree can be formed by grafting together hierarchically nested source trees. In this study, a tree taken from "Phylogeny and Evolution of the Mollusca" (Ponder *et al.* 2008), obtained from a Bayesian analysis using a combined molecular and morphological dataset, was used as the base of the supertree up to the family level. This tree was based heavily on the molecular data of Colgan *et al.* (2007) and the morphological data of Ponder and Lindberg (1997). The Strombidae tree tip was obtained from Latiolais *et al.* (2006). The branch of other families were obtained from the World Register of Marine Species' taxon tree database (Boxshall *et al.* 2014). Due to several trees missing from Treebase, the trees used in this study were carefully reconstructed by hand in Mesquite (Maddison and Maddison 2014). It is important to note that the out-group, Turbinidae, belongs to Vetigastropoda, whereas all other taxa belong to Caenogastropoda.

Principal component 1 and 2 values from the initial PCA were rescaled in Excel to ensure all values were above zero. PC1 and PC2 values were each mapped onto the informal supertree separately to compare the degree of phylogenetic shape conservatism. These are titled Tree 1 and Tree 2, respectively. Branches were color-coded by PC score category for easier interpretation. An image of a specimen representative of each family was also added to show the basic form of the taxa.

The two morphometric characters were combined in Mesquite and mapped onto a single tree, titled Tree 3. The percentage of sister clades that match the same PC score category was calculated. A discussion of the overall trends and possible implications was also included.

Variation of S. gibberulus *populations*

In addition to the transformation grids shown for PC1 and PC2 of both datasets, a graphic was created which depicts the mean shape and standard deviations to further represent the range of shape change.

A method used to measure the geographic home ranges of different populations was applied to the distribution of morphometric variation (Fieberg and Kochanny 2005). From the scatterplot of principal component axes 1 and 2, home ranges encompassing 95 percent of the distribution of each population were

plotted in R (R Core Team 2014). The relative home range sizes were calculated. Overlap indices between the ranges were also calculated with the adehabitatHR package (Calenge 2006) as a proxy for measuring morphometric variation overlap.

Because PCA lacks an underlying model and does not recognize a specified classifier variable, a Discriminant Function Analysis (DFA) was run on the *S. gibberulus* dataset in MorphoJ with respect to locality. This method is used to determine whether or not a set of variables differ significantly between groups. However, DFA tends to over-estimate the separation between groups, so a leave-one-out cross-validation is necessary to assess the reliability (Lachenbruch 1967).

RESULTS

Phylogenetic tree mapping

Principal components 1 and 2 accounted for a cumulative 84.086% of total variance among the 33 species (see Fig. 4). Because these accounted for the most variance, PC1 and PC2 were used to map morphometric characters onto the phylogenetic supertree.

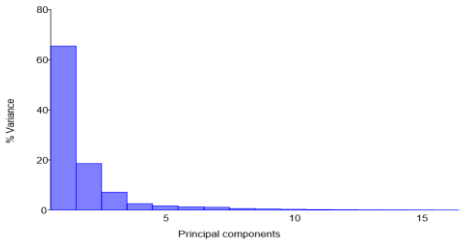

FIG. 4. Eigenvalues, percentage of total variance, for PCs 1-4.

A scatter plot of PC1 and PC2 scores was created to illustrate the disparity between species and represent changes in shape space between different lineages (see Fig. 5).

Tree 1 mapped morphometric character PC1. Tree 2 mapped PC2. Tree 3 mapped both characters together. See Appendix A for phylogenetic Tree 1 (Fig. 6), Tree 2 (Fig. 7), and Tree 3 (Fig. 8).

There are a total of 35 identifiable paired sister clades, characterized by groups separated by a single node. Ancestral forms are

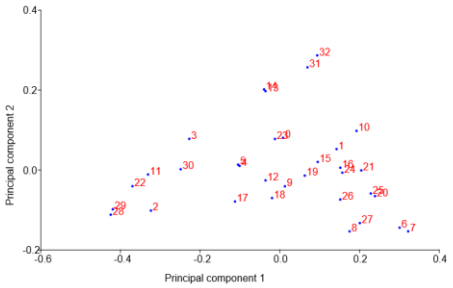

FIG. 5. PC scores for each species studied.

implied by the averaging of preceding clades, whose branches are also shown color coded by Mesquite. If any of these sister groups are represented in the same PC score category, they are counted as matching.

In Tree 1, 10/35 (28.6%) of sister clades match. In Tree 2, 7/35 (20%) match. In Tree 3, 11/35 (31.4%) match. This measurement includes all ancestral clades represented. However, if we only look at the tips where actual study taxa are mapped, with only 26 pairings, Tree 1 has 7/26 (26.9%), Tree 2 has 5/26 (19.2%), and Tree 3 has 9/26 (34.6%).

Variation of S. gibberulus *populations*

Transformation grids and eigenvector diagrams show the shape changes and variation associated with PC1 and PC2 for *Strombus gibberulus* (see Fig. 9).

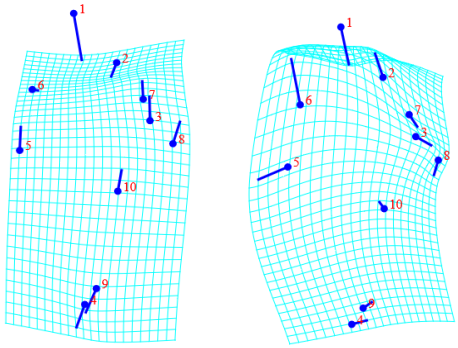

FIG. 9. *S. gibberulus* PC1 and PC2 lollipop graphs and transformation grids.

A diagram of mean shape and standard deviations of PC1 and PC2 represents the range of shape change by juxtaposing the extremes (see Fig. 10).

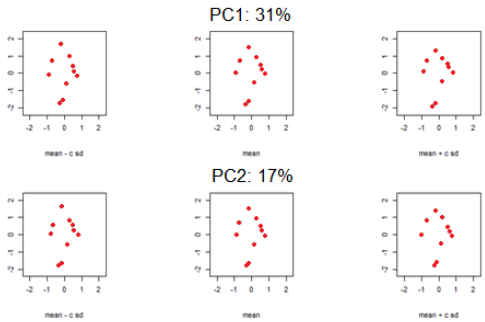

FIG. 10. *S. gibberulus* PC1 and PC2. Shape range with mean and standard deviations.

As seen best in the plot of standard deviations, both PCs represented an elongation of the spire. Specifically, PC1 shows that as the spire (Landmark 1) and penultimate sutures (LM2 and 6) elongate, the columella (LM4 and 10) is shorter relative to total size. Relative to overall shape, the aperture does not show significant change. PC2 also shows spire stretching, but mainly between the apex and the sutures. As seen best in the transformation grid (Fig. 9), PC2 represents an apparent

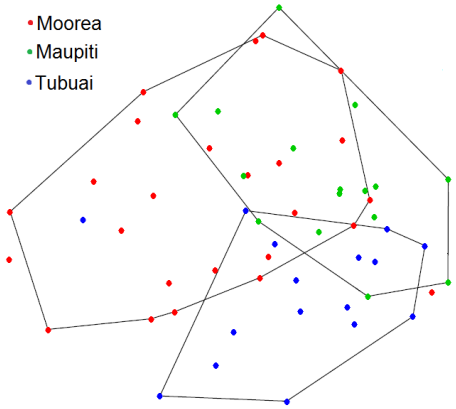

FIG. 12. *S. gibberulus* morphometric home ranges. Moorea=red, Maupiti=green, Tubuai=blue.

bending of the shell around the aperture and a widening of the central body whorl.

Eigenvalues and percentages of total variance were calculated for principal components 1-4 (see Figure 11). The corresponding eigenvalue for each PC is a percentage of the total variance. Principal components 1 and 2 accounted for a cumulative 49.006% of total variance among

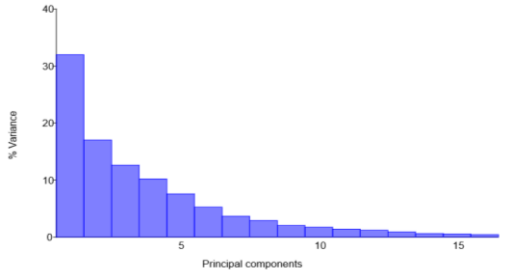

FIG. 11. *S. gibberulus* Eigenvalues, percentage of total variance, for PCs 1-4.

the 58 specimens. Despite this low percentage, PC1 and PC2 represented the most variance of any two PCs and contained the best data for PC score comparisons.

A graph of morphometric home ranges based on PC axes 1 and 2 was created to illustrate shape-space distributions among the populations (see Figure 12). The distributions are fairly wide and considerable overlap can be observed. The following tests were done to

TABLE 1. Sample size and home range size for each locality.

Location	Sample size	Range size
Maupiti	25	5.95
Moorea	17	3.83
Tubuai	16	2.90

verify the reliability of the data

Home range size may appear to be affected by sample size (see Table 1), but a simple linear regression failed to identify any functional relationship (F ratio below critical value).

The unit of the overlap index is a ratio between 0 and 1, 0 meaning completely separated and 1 meaning they share the exact same range. Home range overlap may appear related to geographical separation, but the overlap values are too similar and too small in sample to draw conclusive relationships (see Table 2). However, the largest overlap is observed between the closest locations, Moorea and Maupiti, which may hint at a trend.

The discriminant function analysis of the populations gave parametric p-values well

TABLE 2. Range overlap, total distance, and latitudinal distance between compared locations.

Compared locations	Range overlap	Total distance (km)	Latitudinal distance
Maupiti-Moorea	0.560	275.4	115.9
Moorea-Tubuai	0.440	654.5	652.7
Maupiti-Tubuai	*0.454*	822.8	768.7

below 0.05, but the cross-validation showed considerable mismatching (see Table 3 in Appendix B). Cross-validation involves running a second DFA that groups the specimens from two localities together rather than apart. The results in Table 3 show how well specimens in each pairing are matched correctly. The values in Table 3 represent the number of specimens that were matched to a given locality. Out of 116 total, 88 were matched correctly (75.9%) and 28 were mismatched (24.1%).

DISCUSSION

Phylogenetic tree mapping

The mapping of morphometric characters onto a combined supertree of accepted morphological and molecular data showed a moderate amount of conservatism at the family level, but showed a considerable amount of divergence even between related gastropod families.

On Tree 1, most clades are fairly neutral, including the out-group, Turbinidae. Though distantly related, Terebridae and Cerithiidae show close similarities on the lower extreme of PC scores. This is likely because these families have convergently evolved an elongated spire and shorter columella. On the other end of the spectrum is Conidae, which are uniquely shaped. The families Strombidae and Mitridae each show high differentiation in score category between species. This may be due to the larger sample collected relative to other families and their great diversity in French Polynesia.

On Tree 2, most clades are closer to the low end of scores, with the out-group far to the upper extreme as one would expect. The wide-shelled Littorinidae show the most similarity to the out-group Turbinidae, which may suggest an ancestral tendency to form wider, rounded shells. On Tree 2, Terebridae are mapped more similarly to their relatives the Conidae than on Tree 1. At a glance, these two observations may suggest that Character 2 is more indicative of phylogenetic relationship than Character 1. However, matching sister clades on both trees reveals a much higher rate of matching on Tree 1. To the contrary, this higher rate of matching on Tree 1 may be explained by the high number of clades occupying the middle range of PC score categories.

The combined mapping on Tree 3 shows clear trends among family level clades and balances the trends of Tree 1 and Tree 2. At the middle range, Mitridae and Buccinoidae show differentiation at the species level, but stability at the family level. At the lower extreme, Terebridae and Cerithiidae are most similar, with taxa in the true *Terebra* genus showing the most divergence. Conidae occupy the upper extreme and show a similarity to their cousin Olividae. However, two observations are particularly interesting. One, the out-group Tubinidae occupies the very middle of the overall range. Although this out-group is basal to the other clades, we cannot necessarily expect it to represent the ancestral form, yet this limited data may weakly suggest that. The other peculiarity is that the two groups representing the outer extremes, Terebridae and Conidae, are shown as closely related. Although this relationship is currently supported by molecular evidence (Ponder *et al.* 2008), this observation poses a morphological conundrum that requires further study.

Variation of S. gibberulus *populations*

The study of populations was limited by sample size. The CRIOBE collection contained

additional *S. gibberulus* shells from the island of Anaa, but only 4 individuals were available. Specimen collection from different islands and larger sample sizes could lead to a more accurate relationship of geographic separation to morphometric variation overlap.

Although a simple linear regression showed that the home range overlap was not correlated with geographic distance, it may be better explained by a more precise model of geographic range overlap or morphometric variation overlap. This method of applying geographical models to morphometrics is uncommon, but worth further investigation in order to visualize theoretical shape space as easily as physical spaces.

As suggested by the Kernel overlap ratios in Table 2, each population overlaps each other by about 0.5, meaning the populations are roughly halfway between what one would expect from identical populations and fully divergent populations. This makes sense from an evolutionary perspective. Populations separated by a considerable geographic distance are subject to genetic drift and/or differential selection factors, leading to phenotypic variation. However according to the results and implications of Madeira *et al*. (2012), morphometric characters show no significant differentiation among geographic regions of the same habitat, whereas genetic characters do show significant differentiation. This may be due to similar environmental conditions and selection pressures in similar habitats. Another explanation is that marine gastropods have a planktonic larval stage, allowing offspring to disperse with ocean currents across long distances to connect populations and increase gene flow (Kyle and Boulding 2000).

The between group variation is not completely different from the variation observed within each population. The results of the DFA cross-validation test show that when specimens were grouped together, they were mismatched about 24% of the time. Therefore individuals are sometimes more similar to others of a different population than to others of the same population. This trend of close similarity between populations is also true for humans. Witherspoon *et al*. (2007) demonstrates that many genetic profiles are more similar between people of different ethnic backgrounds. In a social sense, this suggests that racial distinctions are insignificant. In an evolutionary sense, it shows that traits are highly variable even among related individuals, yet some traits are so conserved that they remain commonly shared among a lineage.

Shell shape plasticity

The shape changes observed between different *S. gibberulus* shells, though subtle, partially match the initial expectations. The observations would suggest that spire height is quite plastic, even within the same species. Aperture width did not show much change, but this may be due to landmark placement. Because the lip of the aperture has few distinguishable features common to all specimens, a less reliable Type III landmark was used (see LM8 in Fig. 3).

It is important to note that more information may be available from semilandmark curve tracing, however this study was limited to landmark points. The bending observed from PC2 may be an effect of slight orientation error when photographing specimens.

Although only the shape range and plasticity of *S. gibberulus* was thoroughly studied, future studies could compare different taxa. Broad shape ranges of the phylogeny taxa were not represented graphically because small directional changes are lost amid the enormous variation. Taxa were also arbitrarily selected, so certain shape trends would have been exaggerated due to sampling.

These observations suggest that gastropod shells are quite plastic, especially the spire region. Gastropods shells are likely very adaptable, but probably require isolation from dispersing planktonic larvae to speciate.

Future study

Geometric morphometrics can be used on any group of specimens with rigid structures and homologous features, lending itself to the study of invertebrate hard parts and vertebrate bones. Morphometric data can also be compared with ecological, molecular, or

environmental data to identify correlations or support a hypothesis. Free to download programs supporting this growing method of study, such as R and MorphoJ, offer a wide range of tools to answer biological questions. Authors of these programs and packages are often receptive to questions from users.

Although the paleontological applications of GM were emphasized in the Introduction, it also has uses in ecology and conservation. For example, the endangered *Partula* species on Moorea may only exist in secluded populations. Because specimen collection is prohibited, molecular data is unavailable for genetic diversity studies. This could be substituted by morphometric data collected by careful photography for the purpose of measuring population diversity after this unfortunate bottleneck.

Another important application would be to collect gastropod specimens from different environments or substrates to compare shape difference between these groupings and explore possible environmental factors influencing shell development or trait selection.

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APPENDIX A

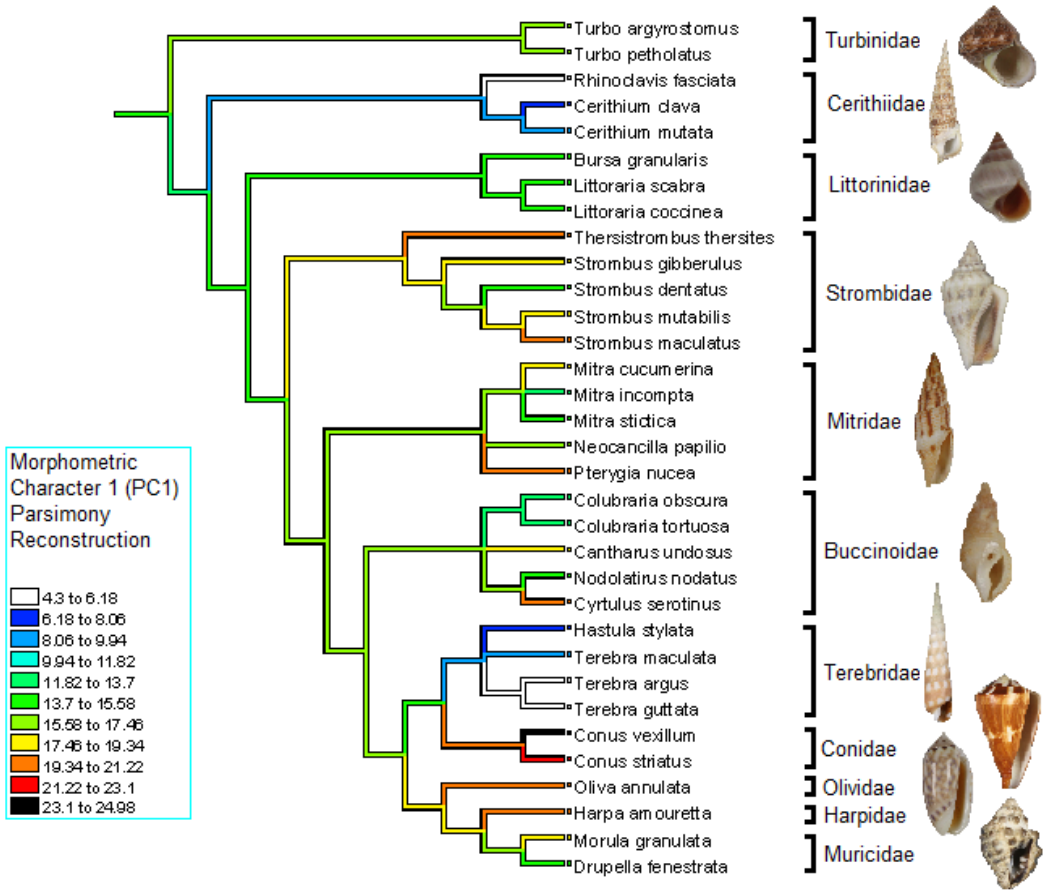

FIG. 6. Tree 1. Phylogenetic tree of the 33 gastropod species, mapped with Morphometric Character 1 (PC1). Colors correspond to PC score categories.

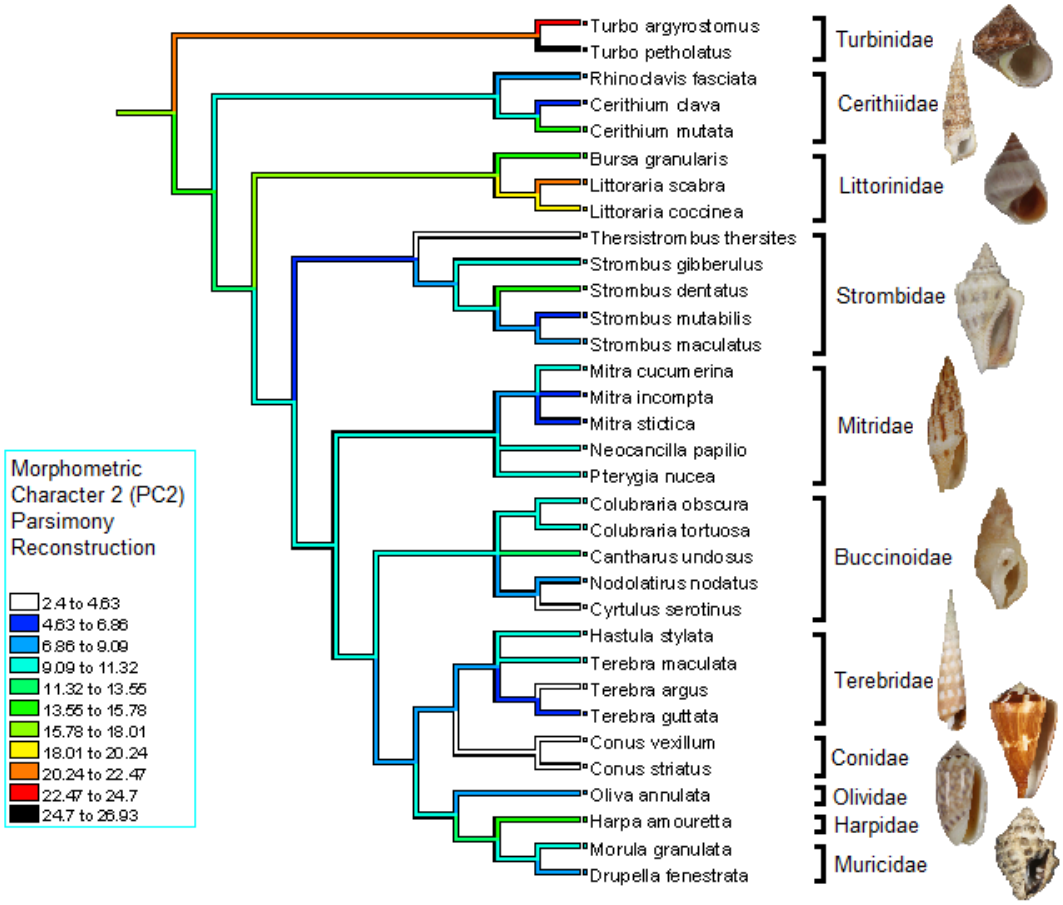

FIG. 7. Tree 2. Phylogenetic tree, mapped with morphometric character 2 (PC2).

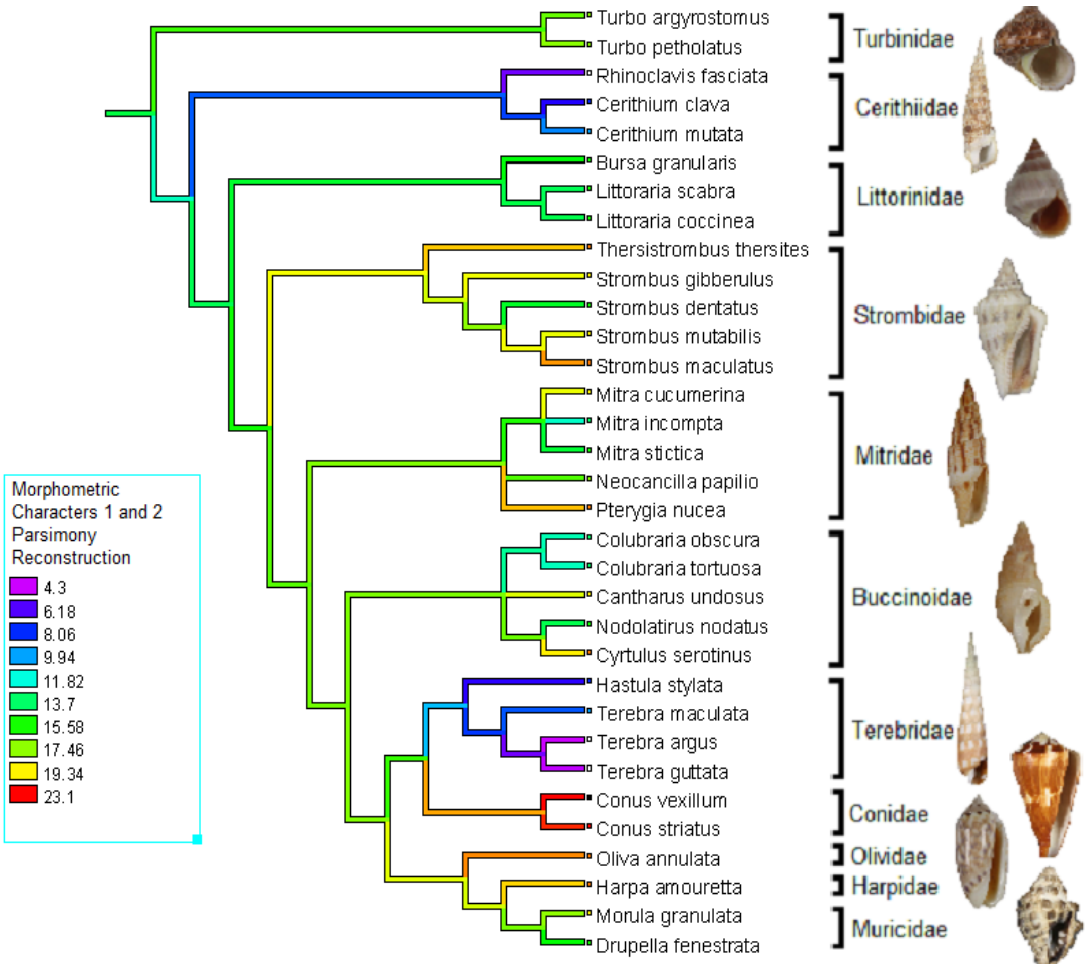

FIG. 8. Tree 3. Phylogenetic tree, mapped with the PC1 and PC2 combined.

APPENDIX B

TABLE 3. Cross-validation of Discriminant Function Analysis on grouped populations.

Group	Maupiti	Moorea	Total
Maupiti	19	6	25
Moorea	6	11	17
Group	Moorea	Tubuai	Total
Moorea	13	4	17
Tubuai	2	14	16
Group	Maupiti	Tubuai	Total
Maupiti	20	5	25
Tubuai	5	11	16