Reference: *Biol. Bull.* **220:** 224–237. (June 2011)
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Quantifying Complex Shapes: Elliptical Fourier Analysis of Octocoral Sclerites

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Abstract. Species descriptions of most alcyonacean octocorals rely heavily on the morphology of sclerites, the calcium carbonate spicules embedded in the soft tissue. Sclerites provide taxonomic characters for species delineation but require qualitative descriptions, which introduce ambiguities in recognizing morphological features. Elliptical Fourier analysis of the outline of sclerites was used to quantify the morphology of eight species of gorgoniid octocoral in the genus *Pseudopterogorgia*. Sclerites from one to seven colonies of each species were compared. Scaphoids and spindles were examined separately; rods and octoradiates were excluded from the analyses because of their morphologic similarity across all species. Discriminant analysis of elliptical Fourier descriptors (EFDs) was used to determine whether the elliptical Fourier analysis could be used to identify the specimens. Sclerites were highly variable even within a single colony. Correct species assignments of individual sclerites were greater than 50% for both scaphoids and spindles. Species assignments based on averages of the EFDs for each colony approached 90%. Elliptical Fourier analysis quantifies morphological differences between species and measures colony variance in sclerite size and shape among colonies and species. Phylogenetic analysis based on EFDs did not capture monophyletic groups. The quantification of complex shapes such as sclerites provides an important tool in alpha taxonomy but may be less useful in phylogenetic analyses.

Introduction

The theoretical issue of what is a species and the practical issues of delineating species have beleaguered biologists for centuries (Wilkins, 2009). Darwin (1859) noted the difficulties of defining species, and the discussion of the issue continued through the 20th century and into the 21st (see Mayr, 1970; De Queiroz, 2007). De Queiroz (2007) suggests that much of the difficulty comes from definitions that place "speciation" at different points on the temporal sequence from a single population to distinct reciprocally monophyletic clades. The debate over the appropriateness of the varying "species concepts" will undoubtedly continue. However, sorting individuals into species categories is central to research ranging from physiology to conservation biology. Inaccurate species descriptions or inaccurate classification affects estimations of species' habitat ranges, physiologic tolerance, and population size; and as Knowlton and Jackson (1994) noted, these errors have consequences for our understanding of ecologic and evolutionary theory and management and effects of global climate change. Developing methodologies that accurately characterize species and minimize ambiguity in assigning individuals to species groups is important. Also important, however, is recognizing that traits used in discerning the evolutionary status of groups are not necessarily practical tools for identification, and traits that can be used to identify specimens do not necessarily provide information about the evolutionary relatedness of species.

In this paper we employ a mathematical characterization of form (elliptical Fourier analysis) in concert with multivariate and phylogenetic analysis in an attempt to provide objective criteria to classify a group of Caribbean octocorals on the basis of calcium carbonate skeletal elements called sclerites. Caribbean octocorals are emblematic of a large array of taxa in which species identification is often based on qualitative comparisons of complex morphologic structures. The taxonomy and systematics of the group have been particularly difficult because species descriptions and higher

Received 9 March 2010; accepted 2 May 2011.

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Abbreviations: EFD, elliptical Fourier descriptor.

level phylogenetics have been based on phenotypically plastic traits such as colony form and sclerite shape. A clear taxonomy for the group is important because octocorals are common, in some cases dominating many Caribbean and Indo-Pacific coral reefs, and are important members of deep-sea communities. As is often the case for marine invertebrates, ecological research has been hampered by both the difficulty of identifying individuals and the small (and declining) number of experts capable of such identification.

The focus of this study is Caribbean members of the genus *Pseudopterogorgia* (Alcyonacea:Gorgoniidae). Like all of the gorgoniids, individuals in this genus form plume-like colonies consisting of central branches and smaller side branches, branchlets, all of which are supported by an axis composed of gorgonin (solidified collagen) (Goldberg, 1976). The polyps, which populate all of the branches, are embedded in mesoglea, a gelatinous, highly hydrated layer (Lewis and Wallis, 1991) that surrounds the axis. The sclerites are found on the surface of the branches, embedded throughout the mesoglea, and around the individual polyps of the colony. Sclerite formation occurs within vacuoles of scleroblasts (Dunkelberger and Watabe, 1974; Kingsley and Watabe, 1982) where the sclerites develop as an aggregate of calcite fibers, which are laid in concentric layers. The finer structures of sclerites form *via* nanoparticle aggregation and predisposed crystal orientation (Sethman *et al.,* 2007). The sclerites incorporate an organic matrix, which presumably controls form.

Pseudopterogorgia spp. are members of the order Alcyonacea, a group that has been divided into the soft corals and the gorgonians on the basis of colony form and the presence of an inner axis. The categories are purely descriptive, and recent treatments of the order had divided the order into five subordinal groups. However, a phylogenetic treatment of the order based on two mitochondrial genes did not support monophyly of the subordinal classifications and found that 9 of 14 tested families, including the Gorgoniidae, were polyphyletic (McFadden *et al*., 2006).

The alpha taxonomy of the *Pseudopterogorgia* spp., as is the case for most of the alcyonaceans, is based on a mix of colony and sclerite morphologic traits. Bayer (1961) in his treatment of the shallow-water West Indian fauna recognized 12 *Pseudopterogorgia* species, and distinctions between species are often based on qualitatively described differences in the morphology of sclerites. Although there have been efforts to systematize terminology (*i.e.,* Bayer *et al*., 1983), characterization of the shape and ornamentation of sclerites requires considerable experience, and intra-colony, intercolony, and population-level variation are poorly understood, which makes both species delineation and subsequent identification of specimens difficult.

Sclerites have been divided into a variety of types on the basis of form. Some types are found in multiple genera and families; others are unique to a single genus. Although the sclerite types are presumed to be homologous within families, homologies across taxa and between different sclerite types are unknown. *Pseudopterogorgia* spp. contain scaphoids that are predominantly on the branch surface; spindles and octoradiates found in the mesoglea; and rods that are found as an "armor" on the polyp surface. Species descriptions tend to highlight qualitative differences in the shape of scaphoids and quantitative differences in the lengths of scaphoids, spindles, and rods (Bayer, 1961). The highly complex form of the sclerites does not lend itself to quantification, and most quantitative analyses of sclerite form have been based on simple measures, such as length and width (Lasker *et al.*, 1996; Prada *et al*., 2008; Gutierrez-Rodriguez *et al*., 2009). Among *Pseudopterogorgia* species, length of the scaphoids divides the Caribbean species into two groups (Bayer, 1961), but length or width alone cannot differentiate many species.

Elliptical Fourier analysis

Measurements such as sclerite length and width and length:width ratios have been used to quantify sclerite form (Lasker *et al.*, 1996; Prada *et al.*, 2008). Analyses of such measurements detect intra- and interspecifc variation, but those simple indices do not characterize details in form that are often the species' diagnostic traits. Quantitative analysis can characterize more detailed variation in form, and in the case of octocorals provides users with an independent characterization that does not rely on extensive experience with sclerites. Thus it has the potential of being a valuable tool in species delineation and as an adjunct in identification. Most multivariate morphologic analyses are based on landmark analysis, which requires the presence of homologous landmarks or outlines for superimposition of specimens (Crampton, 1995). Aside from the tips, sclerites do not have identifiable homologous features. Fourier analysis provides a description of form without reference to landmarks. A number of different methods can be used in Fourier shape analysis (Rohlf and Archie, 1984). Elliptical Fourier decomposition was chosen for this study because it does not require that points on the outline of the specimen be equally spaced (Crampton, 1995), thus allowing greater sampling from sections of complex shape or high variability of curvature. The method also does not require the prior definition of a biologically homologous centroid, or geometric center (Crampton, 1995).

The use of elliptical Fourier analysis on highly complex objects allows a comprehensive and complete depiction and quantification of shape that does not require prior expertise. In systems as diverse as crab carapaces (Ledesma *et al*., 2010) bivalve shells (Crampton, 1995; Gardner and Thompson, 2009), otoliths (Tracey *et al*., 2006; Stransky *et al*., 2008), leaves (Neto *et al*., 2006), mosquito wings (Rohlf

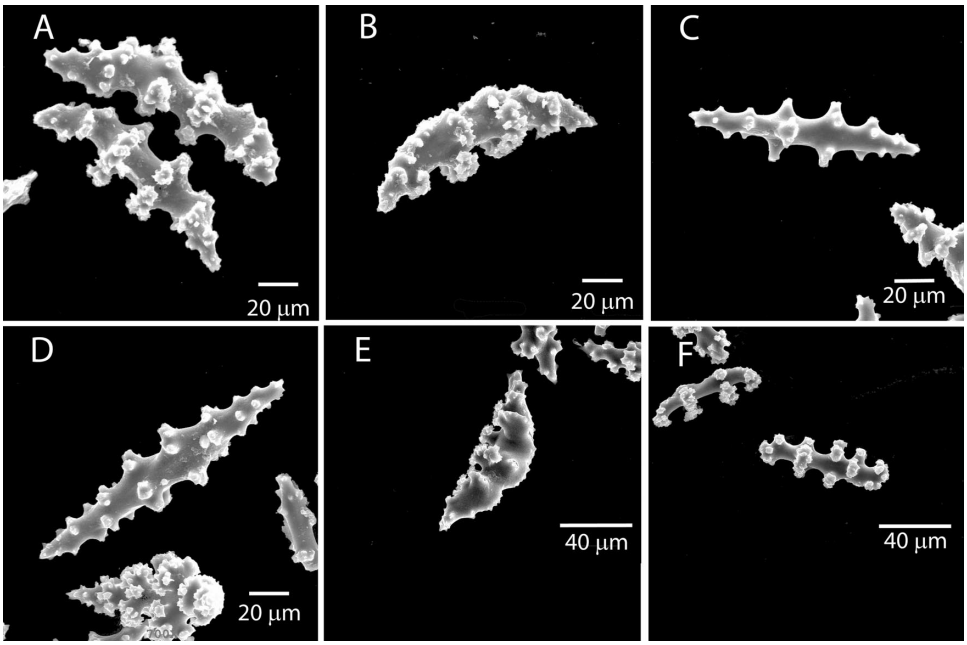

Figure 1. Sclerites from Caribbean species of *Pseudopterogorgia*. (A) Scaphoids, *P. hystrix*; (B) scaphoids, *P. kallos*; (C) spindle, *P. hystrix*; (D) spindle, *P. kallos*; (E) scaphoid, *P. humelincki*; (F) spindle, *P. rigida*.

and Archie, 1984), and human teeth (Ferrario *et al.,* 1999), researchers have consistently found that elliptical Fourier analysis can characterize shape and differentiate classes of objects.

Elliptical Fourier analysis decomposes the outline of an object into a series of closed curves (called harmonics) that vary in size, shape, and orientation and that are generated by a known mathematical function. The sum of all harmonics recreates the original outline with precision proportional to the number of harmonics used in the shape decomposition; thus the number of harmonics needed to fully describe an object is a function of the complexity of the object. Crampton (1995) suggests that eight harmonics are usually sufficient to capture most of the shape and variance in a specimen. Whereas using too few harmonics will result in the loss of morphologic details, an excessive number can add high-frequency noise to the outline. Each harmonic is described by four Fourier coefficients (elliptical Fourier descriptors, EFDs), two each for the *x*- and *y*-axes, generating a total of 4*n* coefficients labeled *an, bn, cn,* and *dn,* where *n* is the number of harmonics (Crampton, 1995). The first, largest harmonic describes the overall length of the specimen, and the following harmonics provide increasingly detailed information about its complexity.

Materials and Methods

To verify whether Fourier analysis could capture Bayer's (1961) qualitative interpretation of sclerites's shapes, we started with the taxonomy favored by that author. *Pseudopterogorgia* species have four types of sclerites (Fig. 1). We focused on the traits of the spindles and scaphoids, which are the dominant features used to identify species (Bayer, 1961). Rods and octoradiates exhibit relatively little variation across species and were not used in this study. Sclerites from one to seven colonies of eight Caribbean *Pseudopterogorgia* species (*P. americana, P. acerosa, P. bipinnata, P. elisabethae, P. hystrix, P. kallos, P. navia,* and *P. rigida*; Appendix 1) were selected and prepared. Colonies of *P. americana, P. acerosa, P. bipinnata, P. elisabethae, P. kallos,* and *P. rigida* that were used in the study were identified according to the criteria in Bayer (1961). Identification of the other specimens was based on Bayer's species descriptions and was largely based on sclerite characteristics. Thus, our analysis is not an independent assessment of the validity of the species but rather a test of how readily elliptical Fourier analysis provides a quantitative description of the sclerites that can be used to identify the specimens. Seven colonies of an initially unidentified, problematic *Pseudopterogorgia* were also included in the analyses, and in that case the analysis was used to compare the problematic specimens to the known species. Our analysis had a limited number of samples for certain species. *P. hystrix* and *P. navia* were represented by a single specimen each—a paratype and a holotype, respectively—which were the only unambiguously identified specimens we had access to. They were included because we believed our problematic specimens belonged to one of those two species. *P. rigida* was also represented by a single specimen, but its

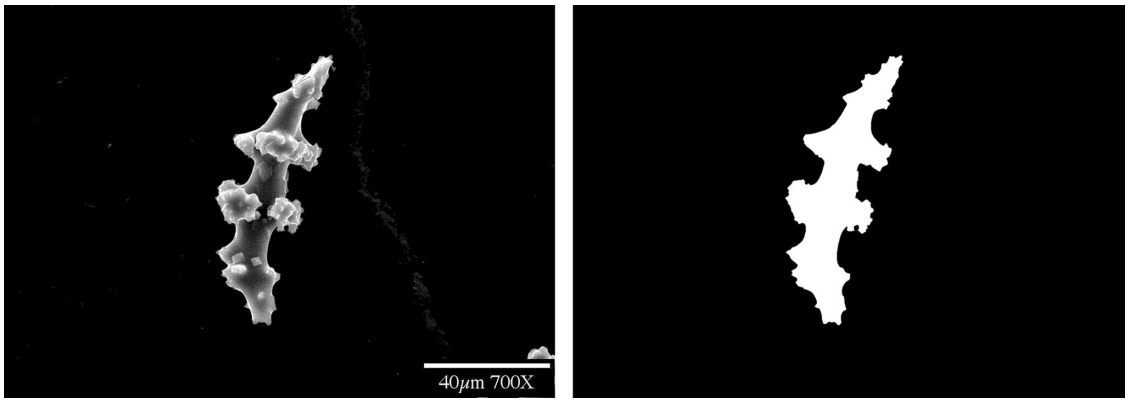

Figure 2. Scanning electron microscopy image of a scaphoid (left) and the resulting black-and-white outline image of the same scaphoid (right) that was used in the elliptical Fourier analysis.

sclerites are so distinct from the other species in the genus that we felt it important to include despite the small sample size. At least 8 and in some cases 10 each of scaphoids and spindles were analyzed from each sample. Three additional specimens representing *P. albatrossae, P. blanquillensis,* and *P. hummelincki* were included in the phylogenetic analysis (see below)—the only analysis that is not affected by sample size.

Sclerites were prepared by digesting arbitrarily selected pieces of tissue from each colony in commercial bleach solution. Sclerites were rinsed in distilled water followed by an ethanol rinse and then dried. Sclerites from a single colony were either placed on glass slides in mounting medium (Permount) for optical imaging or fixed to double-stick tape on a stud and coated with carbon for imaging with a Hitachi S-4000 field emission scanning electron microscope (SEM) operated at 25eV. Preliminary studies using images obtained with a compound microscope showed the technique to work, but somewhat higher variance along with significantly greater time to process images led us to prepare and use SEM images for the analyses. Sclerites are three-dimensional structures lying on a plane, and their orientation changes their appearance, especially for the lacunate scaphoids. Because we could not examine the same sclerites from multiple orientations we avoided obvious cases of unusual orientation. We chose sclerites in a pseudo-random fashion by scanning the stud and imaging only those that could be clearly classified as scaphoid or spindle and had minimal contact with other sclerites. The length of the sclerite was measured with ImageJ, ver. 1.44 (Rasband, 1997–2005), using the fit ellipse function. The images were then processed in Photoshop CS2 to create a black-and-white image, in which the background was white and the entire sclerite black (Fig. 2).

The black-and-white images were processed and analyzed using SHAPE, ver. 1.3 (Iwata and Ukai, 2002), a package of programs that identifies the outline of the sclerite and generates an elliptical Fourier description. Sixty harmonics were used to describe the high complexity and variability of sclerite shape. The accuracy of the overall process was qualitatively assessed by reconstructing the sclerite from the EFDs and comparing the form of the actual sclerite image and the principal component analysis reconstruction using program NEFview from the SHAPE package.

All image processing was conducted by a single investigator (JMC), but possible variation between users was tested to assess the reproducibility of the procedures. Three separate users processed six randomly selected sclerite images, starting with delineating the edge of the sclerites using Photoshop. Differences in the outlines produced by different users were few, and the image of the sclerites profiles generally differed at the scale of single pixels in Photoshop, which did not alter the resulting chain codes. Discriminant analysis of the EFDs of sclerite replicates did not differentiate the users, and a nested ANOVA showed that variance in the EFDs between users accounted for 0.25% of the variance.

The sclerite descriptions (60 harmonics $\times$ four EFDs per harmonic) were analyzed using stepwise discriminant analysis (SPSS ver. 16.0). Discriminant analysis was used to determine if analysis of the EFDs could mimic the traditional identification process, and thus all of the specimens belonged to predefined groups. Discriminant analysis selects the number of significant predictor variables through successive F-ratio testing; at the end of the analysis only descriptors that meet pre-set levels of significance, tolerance, and retention are kept in the model, reducing its dimensionality. SHAPE adjusts for size and orientation, and as a result the first harmonic does not contain morphologic information (Crampton, 1995). We standardized sclerite length for the first harmonic and conducted discriminant analyses both with and without length. A nested ANOVA of the resulting discriminant functions was used to partition the variance in EFDs among species, colonies within species, and sclerites within colonies. Because the discriminant

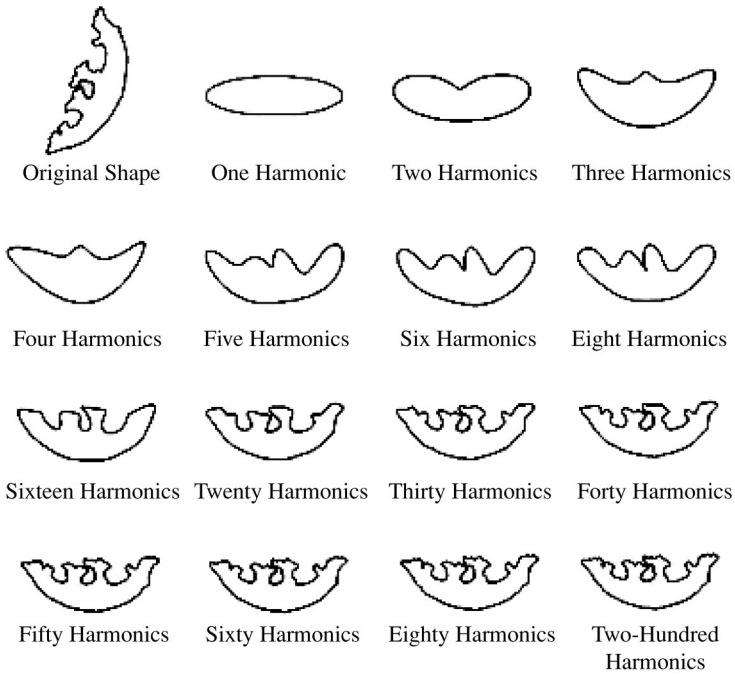

Figure 3. Progression showing characterization of sclerite shape using increasing number of harmonics. Original specimen shape (top left) and recreations of the sclerite using elliptical Fourier descriptors generated from from 1 to 200 harmonics. Species were successfully delineated with only 16 harmonics.

functions are designed to maximize differences between species, the ANOVAs are primarily useful in differentiating between within- and between-colony variation in form.

In shape analyses, large changes in specimen form that are limited to a few individuals can greatly increase the overall variance of the species to which the specimens belong. In landmark analysis this problem is known as the Pinocchio effect, and it can pose problems when it comes to superimposition of specimens. In landmark analysis, resistant-fit methods can be used to reduce Pinocchio effects; unfortunately there is no similar fix for elliptical Fourier analysis. To minimize such an effect, we repeated the discriminant analyses using EFDs that were averaged for each colony. Averaging the EFDs of the sclerites for each colony reduced intraspecific variance and generated more powerful data for the purposes of discriminating among different species. It also more closely tests the utility of using EFDs in species delineation, as it is the identity of the colony, not the individual sclerites, that we wish to determine.

Phylogenetic analyses of EFDs under maximum parsimony were conducted using TNT, ver. 1.1 (Goloboff *et al.,* 2008). Sclerite sizes and the 236 EFDs obtained for 59 of the 60 computed harmonics (the first harmonic was excluded because it accounts for size) were treated as continuous (additive) characters. Hence, 237 characters were obtained per sclerite type (spindle and scaphoid), resulting in a total of 474 characters. TNT allows ranges to be assigned to terminal taxa; thus, it is possible to incorporate intra-OTU (operational taxonomic unit) variation into phylogenetic inference. Each individual ($n = 43$) was treated as a terminal, and intra-individual minimum-maximum ranges were used as states. Ranges were rescaled to values between 0 and 65 (with precision of 3 decimal places), which is the maximum range and precision allowed by TNT. Parsimony searches were conducted using "new technology" algorithms (Goloboff, 1999; Nixon, 1999) with default settings and "initial level" of 100 (*i.e.,* the most aggressive and time-consuming combination of algorithms), with minimum length being found 20 times and score checked every three hits. Space was allocated for a maximum of 1000 trees to be retained during the search.

Results

Elliptical Fourier descriptors of 60 harmonics successfully captured most of the sclerite morphology (Fig. 3). Crampton (1995) found that eight harmonics should be successful at capturing the outline of specimens. However, sclerites are extremely complex, and as evident in Figure 2, higher-level harmonics were necessary to fully recreate the shape of *Pseudopterogorgia* sclerites. Although the general shape of the sclerite was generated with 12 harmonics, fine detail of the image required the introduction of higher harmonics, and some of the finest detail was still missing in the image generated with 200 harmonics (Fig. 2). Higher-level harmonics (exceeding the 16th) were necessary to

Table 1

Components of variation for discriminant scores for elliptical Fourier descriptors of Pseudopterogorgia *spp. sclerites*

Coefficient of Variation Function	Variance (%)		
	Species-level	Colony-level	Sclerite-level
SPINDLES			
1	83.0	3.8	13.2
2	67.2	10.2	22.6
3	23.1	13.4	63.5
SCAPHOIDS			
1	71.6	10.8	17.7
2	47.3	15.9	36.8
3	57.4	2.4	40.2
4	37.9	3.8	58.3

generate the fine echinulation of the sclerite edge, which is a diagnostic feature differentiating some species, but the discriminant functions separating species never included EFDs exceeding the 16th harmonic, and those EFDs described greater than 90% of the variation in shape.

A total of 319 scaphoids and 328 spindles were analyzed. When single sclerites were analyzed using discriminant analysis, correct species classifications were made for 63.0% and 52.6% of the scaphoids and spindles respectively. A nested ANOVA of discriminant function scores for sclerites indicated that as expected, most variation occurred at the species level. Variation between colonies within species was less than variation within colonies. Between-species variance accounted for 23.1% to 83.0% of the total variance in the three dominant discriminant functions for spindles, whereas variation between colonies within species only accounted for between 3.8% and 13.4% of the total variance (Table 1). Among scaphoids, variance between species accounted for 37.9% to 71.6% of the total variance in the scores for the four dominant discriminant functions, whereas between-colony variability ranged from 2.4% to 15.9% of the total variance (Table 1).

When the EFDs were averaged for each colony, correct species classification increased from 63.0% to 90.0% for scaphoids and 52.6% to 92.5% for spindles (Table 2, Appendix 2). Error rates in classification for individual species using the colony averages ranged from 0.0% to 50.0% using scaphoids and from 0.0% to 20.0% using spindles. The analysis of scaphoids generated five discriminant functions of which the first two explained 78% of the variance. There was little overlap in distribution of points for four of the species in the first two discriminant functions for the scaphoids (Fig. 4), but *P. americana* and *P. bipinnata* had very similar scores. When all five discriminant functions were

Table 2

*Species classification resulting from discriminant analysis of elliptical Fourier descriptors that were averaged for each colony**

Discriminant Analysis Species Assignment	Species										Error Rate (%)
	Pac	Pe	Pb	Pam	Pr	Ph	Pk	Pn	Unk	Total	
SCAPHOIDS											
Pac	**7**	0	0	0	0	0	0	0	0	7	0.00
Pe	0	**7**	0	0	0	0	0	0	0	7	0.00
Pb	0	0	**5**	0	0	0	0	0	1	6	16.67
Pam	0	0	0	**4**	0	0	0	0	1	5	20.00
Pr	0	0	0	0	**1**	0	0	0	0	1	0.00
Ph	0	0	0	0	0	**1**	0	0	0	1	0.00
Pk	0	0	0	0	0	0	**5**	0	0	5	0.00
Pn	0	0	0	0	0	0	0	**1**	0	1	0.00
Unk	0	0	1	0	0	1	0	0	**5**	7	28.58
SPINDLES											
Pac	**7**	0	0	0	0	0	0	0	0	7	0.00
Pe	0	**7**	0	0	0	0	0	0	0	7	0.00
Pb	0	0	**6**	0	0	0	0	0	0	6	0.00
Pam	0	0	1	**4**	0	0	0	0	0	5	20.00
Pr	0	0	0	0	**1**	0	0	0	0	1	0.005
Ph	0	0	0	0	0	**1**	0	0	0	1	0.00
Pk	0	0	0	0	0	0	**5**	0	0	5	0.0
Pn	0	0	0	0	0	0	0	**1**	0	1	0.00
Unk	0	0	0	0	0	0	0	2	**5**	7	28.58

* Numbers of correct assignments are noted in bold. (Pac, *Pseudopterogorgia acerosa*; Pam, *P. americana*; Pb, *P. bipinnata*; Pe, *P. elisabethae*; Ph, *P. hystrix*; Pk, *P. kallos*; Pn, *P. navia*; Pr, *P. rigida*).

considered, all of the species were significantly different from one another (Appendix 2). The discriminant functions included sclerite length and EFDs from the second and fourth harmonic. Most of the separation between scaphoids of the different species was along discriminant function 1, which the standardized discriminant function coefficients indicates was greatly affected by length. Scaphoid length is one of the characters used to differentiate among the *Pseudopterogorgia* spp. (Bayer, 1961), and excluding length from the discriminant analysis increased the error rate in the classifications. The discriminant functions for averaged EFDs of spindles did not include length, which reflects the similar lengths of spindles among the species. However, in the disciminant analysis of spindles, species experienced the greatest degree of separation along the discriminant function 2, which was primarily dominated by EFD d16.

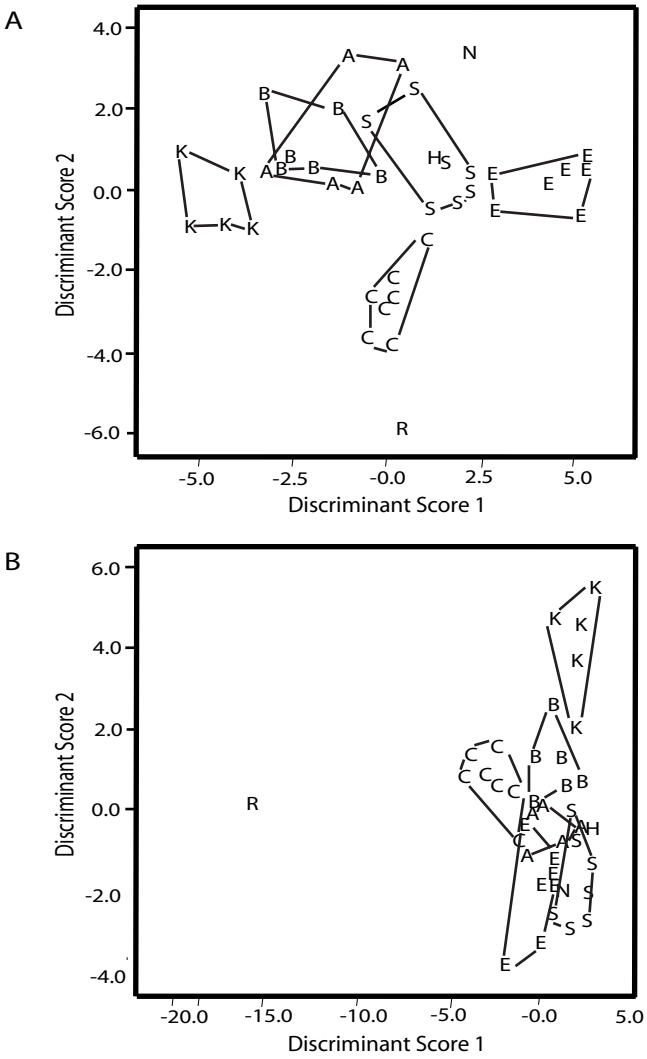

Figure 4. Plots of discriminant scores of mean numbers of scaphoids (upper) and spindles (lower) for colonies of *Pseudopterogorgia.* A, *P. americana;* B, *P. bipinnata;* C, *P. acerosa;* E, *P. elisabethae;* H, *P. hystrix,* K, *P. kallos;* N, *P. navia;* S, unidentified *Pseudopterogorgia* sp. Note the overlap between *Pseudopterogorgia hystrix* and the unidentified *Pseudopterogorgia* sp.

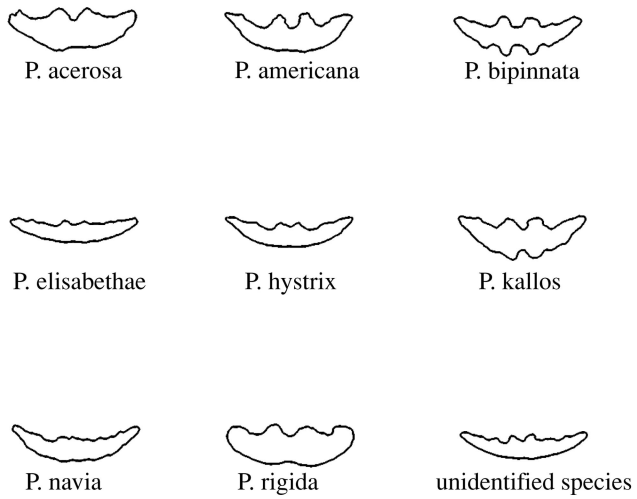

Figure 5. Reconstructions of average scaphoid morphology from elliptical Fourier descriptors for eight species of *Pseudopterogorgia* and the unidentified specimens. Size and orientation have been normalized.

Discriminant scores of the EFDs of scaphoids from the unidentified specimens were most similar to that of the *P. hystrix* holotype, and the range of discriminant scores of the spindles included the *P. hystrix* holotype. Although qualitative comparisons of the unknowns to the description in Bayer (1961) were ambiguous, the discriminant analysis suggests that the unknown colonies were *P. hystrix* (Figs. 4 and 5). When the analysis was repeated with the unidentified specimens coded as *P. hystrix*, there was no isolation or clear-cut separation between sclerites of the holotype and sclerites of the unidentified specimens.

A total of 303 trees with length of 720.995 steps were retained by the phylogenetic analysis in TNT. Figure 6 is the combinable components (semi-strict) consensus of those trees. No species was recovered as a monophyletic clade.

Discussion

Phylogenetic analysis and taxonomic implications

Sclerites have played roles in identifying specimens, in the delineation of species, and in discerning phylogenetic relationships among octocoral species. Interpretation of their form and variation in form has required extensive experience, and identification of many species is difficult and can only be made by well-trained investigators. Elliptical Fourier descriptions of the sclerites from *Pseudopterogorgia* specimens provided a quantitative assessment of form that accurately mirrored identifications based on traditional traits.

As evident in Figure 3, a large number of harmonics were needed in the analysis in order to capture all the fine details

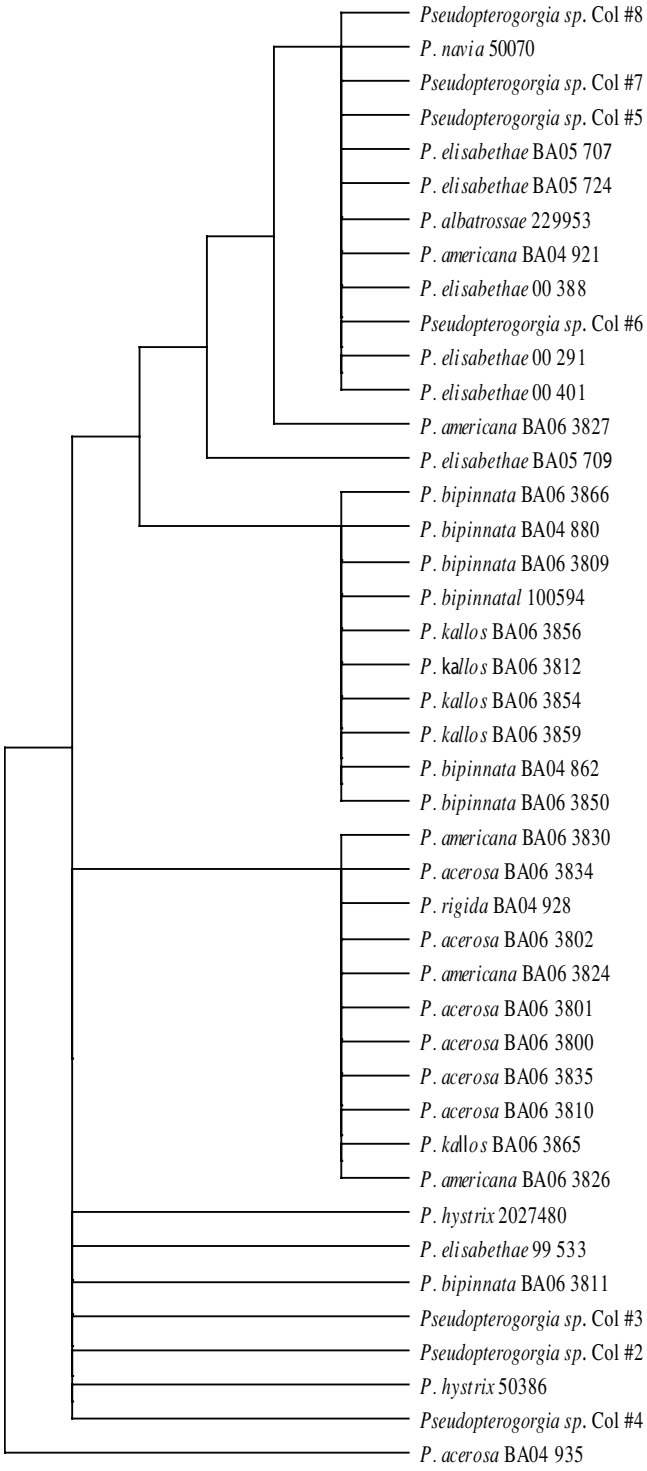

Figure 6. Combinable components (semi-strict) consensus of the 303 most parsimonious trees (720.995 step) retained by TNT employing "new technology" searching. No species were recovered as monophyletic.

of a sclerite's outline. Sixty harmonics were included in the discriminant analysis, but the resulting discriminant functions never included EFDs exceeding the 16th harmonic, and the discriminant functions for scaphoids included EFDs from only the first 4 harmonics. The lack of importance of the higher-order harmonics in the discriminant functions suggests that the general form of the sclerite was sufficient to distinguish the species. Fine details such as echinulation of the sclerites' surface, which are often important characters in species descriptions, were not described by the harmonics that were used to differentiate the species in the discriminant analysis. The discriminant analysis focused on differences in form (*i.e.,* low-order harmonics). Those differences may well be important in the overall "gestalt" of the sclerite, but they are not the differences that systematists have articulated in their descriptions.

The use of average EFDs greatly increased the proportion of correct species classifications in the discriminant analysis. Theoretically, the morphologic features that occur consistently across the sclerites of a species should be evident in the reconstructions of "average sclerites." To generate images of average scaphoids of the species, we used the average EFDs and the program NEFview (Fig. 5). Only scaphoids are presented because they contain the greatest amount of morphological information on species identity and are most easily distinguishable by sight. The images have been corrected for size so that only morphologic differences are observable. In general, the images lack much of the detail of the sketches in Bayer (1961), but more importantly, they do not always contain the specific features that Bayer used to distinguish species. For instance, in Figure 5, *P. kallos* and *P. bipinnata* share very similar features, but Bayer differentiated the species in terms of the area between the whorls, which are filled in to a greater extent in *P. kallos*. A striking difference between the images reconstructed from the EFDs and Bayer's descriptions is the absence of strongly recurved tips in the "average" scaphoid from *P. americana*. Recurved spines are the key trait in Bayer's description of the species, yet they were not obvious in the reconstructed sclerite. Similarly, echinulation is a key component in distinguishing *P. elisabethae, P. hystrix,* and *P. navia*, but it was absent in the reconstructions. This again suggests that the discriminant analysis identifies and distinguishes the species using aspects of their form different than those previously used by systematists. Although differences in EFDs themselves cannot be "translated" into morphologic features, it may be possible to develop keys on the basis of reconstructions such as those in Figure 5.

The technique may be particularly useful in analyses of problematic specimens and populations. For instance, the unidentified specimens included in this analysis had been tentatively identified as *P. hystrix*, but visual comparison with sclerites from the holotype was not, in our opinion, definitive. The discriminant score for scaphoids from the *P. hystrix* holotype fell well within the range of the scores for the unidentified *Pseudopterogorgia* colonies (Fig. 4). Al-

though the discriminant analysis maximizes differences in the discriminant scores of the different groups, both spindles and scaphoids from the unidentified specimens were not significantly different from the sclerites from the *P. hystrix* holotype. They were different from the *P. navia* sclerites, which was the alternative choice in identifying the specimens. Thus the elliptical Fourier analysis allowed us to unambiguously assign the specimens to *P. hystrix*.

The greatest utility of elliptical Fourier analysis of sclerites is in assessing differences in previously defined groups so that one may characterize the sources of variation in shape. For instance, Gardner and Thompson (2009) were able to compare the effects of genotype and geographic location in the shape of *Mytilus* spp. The technique can form the basis for rigorous tests about the variability in form that occurs between habitats and between populations, as well as the variation that may occur over an entire colony going from base to tips. EFDs cannot distinguish between genetic and environmental variation in form, but they provide a quantitative basis for comparing form. For instance, Sanchez *et al.* (2007), using ITS1-5.8S-ITS2 sequence data, suggest that *P. bipinnata* and *P. kallos* are separate morphotypes of the same species. The morphological features that Bayer (1961) used to differentiate the two species are difficult to assess in many specimens, but the Fourier analysis demonstrates that *P. kallos* sclerites are distinct from those of *P. bipinnata* (Fig. 4). This is not a surprising result, since Bayer (1961) defined the two species by differences in the sclerites. However, the colonies used in these analyses were originally identified in the field by their colony form. That finding does not argue for or against the suggested synonomy of the two groups, but it does lend credence to the presence of two morphologically distinct groups. Ascertaining whether the differences between the groups are indicative of species, population, or environmental variation will require both genetic analyses and common garden experiments that test the plasticity of sclerite form. The quantification of variance in form in concert with common garden experiments testing the effects of environment can provide a foundation for a more robust alpha taxonomy of alcyonaceans as well as for all taxa with complex morphologies that do not lend themselves to landmark-based morphometric analysis.

Phylogenetic analysis and taxonomic implications

Elliptical Fourier analysis is a promising source of characters for phylogenetic analysis. The use of Fourier coefficients yielded a number of characters that could never be achieved by a purely qualitative description of form. Given the large number of states when these characters were treated in a quantitative (additive) fashion, the complexity and phylogenetic content of datasets derived using geometric morphometrics rivals those obtained using molecular techniques. Additionally, unlike molecular characters, morphometric data can be acquired from fossil specimens. For instance, Gonzalez-Jose *et al.* (2008) used data derived from deformation analysis of skulls in apes as additive characters in successful phylogenetic reconstructions of the genus *Homo* using both parsimony and maximum-likelihood.

In spite of the large number of characters, our phylogenetic analysis of sclerites from *Pseudopterogorgia* spp. did not yield a consensus tree with satisfactory resolution of the relationships within the genus. This suggests that either there is little phylogenetic signal in the sclerites, the *status quo* taxonomy does not reflect phylogeny, or most likely, some combination of both. The integration of intra-specific variation into the analysis through the utilization of ranges may have introduced noise due to ecophenotypic plasticity. Sclerites are thought to serve multiple functions, including support and defense. Lewis and Wallis (1991) showed how the form and placement of the sclerites limit flexion of colonies. Intraspecific variation is also common, such as that reported by Gutierrez-Rodriguez *et al.* (2009) in the length of *Pseudopterogorgia elisabethae* sclerites at multiple sites in the Bahamas and Florida. A number of studies have found plasticity in sclerite size correlated with environmental conditions. Studies by West (1998) found that colonies of *Briareum asbestinum* in deep-water habitats, which are less subject to wave action, have larger sclerites and are less dense relative to shallow-water morphotypes. Koehl (1996) suggested that increasing sclerite densities correlate with increasing colony toughness, and field assays by West (1997) indicate that colonies with smaller sclerites in high densities are more resistant to tearing than colonies with larger sclerites in lower densities. Velimirov (1976) reported that variation in colony growth form and sclerites of *Eunicella cayolinii* correlates with intensity of water movement, and Kim *et al.* (2004) and Prada *et al.* (2008) noted numerous morphological differences, including length and width of sclerites, among shallow- and deep-water forms of *Eunicea flexuosa*. However, environmental plasticity should be a minor issue for higher-level reconstructions.

The failure of the phylogenetic analysis to recover monophyletic species may also reflect problems within the *Pseudopterogoria* and Alcyonacea in general that go beyond the scope of our study. Analyses using mitochondrial data demonstrated that the genus *Pseudopterogorgia* is paraphyletic with respect to species of *Gorgonia* and to *Phyllogorgia dilatata* (Sanchez *et al.*, 2003). Maximum-likelihood analysis employing nuclear data (ITS1, 5.8S, and ITS2) (Sanchez *et al.*, 2007) including several individuals of *P. bipinnata* and *P. kalos* recovered these two species as being paraphyletic to each other. The same results were obtained using the mitochondrial gene *MSH1*. This latter analysis also included two individuals of *P. elisabethae*, one from

the Bahamas and the other from Carrie Bow Key, Belize, which formed a clade paraphyletic to *Gorgonia mariae.*

The use of elliptical Fourier analysis on highly complex objects allows a comprehensive and complete depiction and quantification of shape, which does not require prior expertise. The technique can form the basis for rigorous tests about the variability in form that occurs between habitats and between populations or over the astogeny of the colony, and the combination of EFDs and multivariate analysis can be useful in studies addressing genetic and environmental variation. Multivariate analysis of the sclerites does not distinguish between primitive and derived characters, and the EFDs were not useful in developing a phylogeny for these *Pseudopterogorgia* spp. It is unclear if the failure of this approach to the species of *Pseudopterogorgia* stems from the complex evolutionary history of the genus, the integration of the sclerites' phenotypic plasticity into the analysis, artificial taxonomy, or a combination of all these factors. Nevertheless, this approach provides a methodological framework for phylogenetic analysis using sclerites, and insofar as these structures remain important diagnostic characters, it should be useful elsewhere in the Octocorallia. The use of elliptical Fourier analysis should also prove valuable in analyses of other taxa with complex morphologies that do not lend themselves to landmark-based morphometric analysis.

Acknowledgments

This research was supported by National Science Foundation grants OCE-0327129 and OCE- 0825852 and a grant from the National Undersea Research Center at the University of North Carolina. Collection of many of the samples was facilitated by the use of equipment supplied by Sherwood Scuba. Our early attempts at analyzing sclerite form were aided by the use of SEM images provided by Raphael Ritson-Williams from the Smithsonian Institution station at Fort Pierce, Florida. Mary Alice Coffroth, Charles Mitchell, and two anonymous reviewers provided constructive criticism of both the project and this manuscript, and discussions about elliptical Fourier techniques with David Sheets were particularly important. We especially thank Peter Bush at the University at Buffalo South Campus Instrument Center for his assistance in obtaining the SEM images.

Literature Cited

Bayer, F. M. 1961. *The Shallow Water Octocorallia of the West Indian Region. A Manual for Marine Biologists.* Martinus Nijhoff, The Hague.

Bayer, F. M., M. Grasshoff, and J. Verseveldt. 1983. *Illustrated Trilingual Glossary of Morphological and Anatomical Terms Applied to Octocorallia.* E. J. Brill, Leiden.

Crampton, J. S. 1995. Elliptic Fourier shape analysis of fossil bivalves: some practical considerations. *Lethaia* **28:** 179–186.

Darwin, C. 1859. *Origin of Species.* [Online]. Project Gutenberg. Available: http://www.gutenberg.org/files/2009/2009-h/2009-h.htm [2010, October 18].

De Queiroz, K. 2007. Species concepts and species delimitation. *Syst. Biol.* **56:** 879–886.

Dunkelberger, D. G., and N. Watabe. 1974. Synthesis and transport of the organic matrix of the spicules in the gorgonian *Leptogorgia virgulata* (Lamarck) (Coelenterata: Gorgonacea). *Cell Tissue Res.* **6:** 573–586.

Ferrario, V. F., C. Sforza, G. M. Tartaglia, A. Colombo, and G. Serrao. 1999. Size and shape of the human first permanent molar: A Fourier analysis of the occlusal and equatorial outlines. *Am. J. Phys. Anthropol.* **108:** 281–294.

Gardner, J. P. A., and R. J. Thompson. 2009. Influence of genotype and geography on shell shape and morphometric trait variation among North Atlantic blue mussel (*Mytilus* spp.) populations. *Biol. J. Linn. Soc.* **96:** 875–897.

Goldberg, W. M. 1976. A comparative study of the chemistry and structure of gorgonian and antipatharian coral skeletons. *Mar. Biol.* **35:** 253–267.

Goloboff, P. A. 1999. Analyzing large data sets in reasonable times: solutions for composite optima. *Cladistics* **15(4):** 774–786.

Goloboff, P. A., J. S. Farris, and K. C. Nixon. 2008. TNT, a free program for phylogenetic analysis. *Cladistics* **24(5):** 774–786.

Gonzalez-Jose, R., I. Escapa, W. A. Neves, R. Cúneo, and H. M. Pucciarelli. 2008. Cladistic analysis of continuous modularized traits provides phylogenetic signals in *Homo* evolution. *Nature* **453:** 775–778.

Gutierrez-Rodriguez, C., M. S. Barbeitos, J. A. Sanchez, and H. R. Lasker. 2009. Phylogeography and morphological variation of the branching octocoral *Pseudopterogorgia elisabethae*. *Mol. Phylogenet. Evol.* **50:** 1–15.

Iwata, H., and Y. Ukai. 2002. SHAPE: A computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors. *J. Hered.* **93:** 384–385.

Kim, E., H. R. Lasker, M. A. Coffroth, and K. Kim. 2004. Morphological and genetic variation across reef habitats in a broadcast-spawning octocoral. *Hydrobiologia* **530–531:** 423–432.

Kingsley, R. J., and N. Watabe. 1982. Ultrastructural investigation of spicule formation in the gorgonian *Leptogorgia virgulata* (Lamarck) (Coelenterata: Gorgonacea). *Cell Tissue Res.* **223:** 325–334.

Knowlton, N., and J. B. C. Jackson. 1994. New taxonomy and niche partitioning on coral reeds—jack of all trades or master of some. *Trends Ecol. Evol.* **9:** 7–9.

Koehl, M. A. R. 1996. When does morphology matter? *Annu. Rev. Ecol. Syst.* **27:** 501–542.

Lasker, H. R., K. Kim, and M. A. Coffroth. 1996. Reproductive and genetic variation among Caribbean gorgonians: the differentiation of *Plexaura kuna*, new species. *Bull. Mar. Sci.* **58:** 277–288.

Ledesma, F. M., S. Van der Molen, and P. J. Barón. 2010. Sex identification of *Carcinus maenas* by analysis of carapace geometrical morphometry. *J. Sea Res.* **63:** 213–216.

Lewis, J. C., and E. V. Wallis. 1991. The function of surface sclerites in gorgonians (Coelenterata, Octocorallia). *Biol. Bull.* **181:** 275–288.

Mayr, E. 1970. *Populations, Species and Evolution*. Harvard University Press, Cambridge, MA.

McFadden, C. S., S. C. France, J. A. Sanchez, and P. Alderslade. 2006. A molecular phylogenetic analysis of the Octocorallia (Cnidaria : Anthozoa) based on mitochondrial protein-coding sequences. *Mol. Phylogenet. Evol.* **41:** 513–527.

Neto, J. C., G. E. Meyer, D. D. Jones, and A. K. Samal. 2006. Plant species identification using Elliptic Fourier leaf shape analysis. *Comput. Electron. Agric.* **50:** 121–134.

Nixon, K. C. 1999. The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics* **15(4):** 407–414.

Prada, C., N. V. Schizas, and P. M. Yoshioka. 2008. Phenotypic

plasticity of speciation? A case from a clonal marine organism. *BMC Evol. Biol*. **8:** 47.

Rasband, W. S. 1997–2005. ImageJ. [Online]. U.S. National Institutes of Health, Bethesda, MD. Available: http://rsb.info.nih.gov/ij/ [2011, May 10].

Rohlf, F. J., and J. W. Archie. 1984. A comparison of Fourier methods for the description of wing shape in mosquitoes (*Ritera culicidae*). *Syst. Zool*. **33:** 302–317.

Sanchez, J. A., C. S. McFadden, S. C. France, and H. R. Lasker. 2003. Molecular phylogenetic analyses of shallow-water Caribbean octocorals. *Mar. Biol.* **142:** 975–987.

Sanchez, J. A., C. Afular, D. Dorado, and N. Manrique. 2007. Phenotypic plasticity and morphological integration in a marine modular invertebrate. *BMC Evol. Biol*. **7:** 122.

Sethmann, I., U. Helbig, and G. Worheide. 2007. Octocoral sclerite ultrastructures and experimental approach to underlying biomineralisation principles. *CrysEngComm*. **9:** 1262–1268.

Stransky, C., H. Baumann, S. E. Fevolden, A. Harbitz, H. Hoie, K. H. Nedreaas, A. B. Salberg, and T. H. Skarstein. 2008. Separation of Norwegian coastal cod and Northeast Arctic cod by outer otolith shape analysis. *Fish. Res.* **90:** 26–35.

Tracey, S. R., J. M. Lyle, and G. Duhamel. 2006. Application of elliptical Fourier analysis of otolith form as a tool for stock identification. *Fish. Res*. **77:** 138–147.

Velimirov, B. 1976. Variation in growth forms of *Eunicella cavolinii* Koch (Octocorallia) related to intensity of water movement. *J. Exp. Mar. Biol. Ecol*. **21:** 109–117.

West, J. M. 1997. Plasticity in the sclerites of a gorgonian coral: tests of water motion, light level, and damage cues. *Biol. Bull*. **192:** 279–289.

West, J. M. 1998. The dual role in a gorgonian coral: conflicting functions of support and defense. *Evol. Ecol*. **12:** 803–821.

Wilkins, J. S. 2009. *Species: A History of the Idea. Species and Systematics,* Vol.1. University of California Press, Berkeley. 305 pp.

Appendix 1
Specimens of *Pseudopterogorgia* used in the analyses

Species	Colony ID Number	Location	Latitude	Longitude
P. acerosa	BA06-3834	Florida Keys	24°59.083′N	80°24.958′W
P. acerosa	BA06-3810	Florida Keys	24°59.083′N	80°24.958′W
P. acerosa	BA06-3801	Florida Keys	24°59.083′N	80°24.958′W
P. acerosa	BA06-3802	Florida Keys	24°59.083′N	80°24.958′W
P. acerosa	BA06-3800	Florida Keys	24°59.083′N	80°24.958′W
P. acerosa	BA06-3835	Florida Keys	24°59.083′N	80°24.958′W
P. acerosa	BA04-935	Bahamas	25°56.936′N	77°20.403′W
*P. albatrossae**[1]	USNM51750 / 229953	St. John		
P. americana	BA06-3826	Florida Keys	24°59.083′N	80°24.958′W
P. americana	BA06-3827	Florida Keys	24°59.083′N	80°24.958′W
P. americana	BA06-3824	Florida Keys	24°59.083′N	80°24.958′W
P. americana	BA06-3830	Florida Keys	24°59.083′N	80°24.958′W
P. americana	BA04-921	Bahamas	25°56.936′N	77°20.403′W
P. americana	BA04-922	Bahamas	25°56.936′N	77°20.403′W
P. bipinnata	BA06-3850	Florida Keys	24°59.016′N	80°24.832′W
P. bipinnata	BA06-3811	Florida Keys	24°59.083′N	80°24.958′W
P. bipinnata	BA06-3809	Florida Keys	24°59.083′N	80°24.958′W
P. bipinnata	BA06-3866	Florida Keys	24°59.016′N	80°24.832′W
P. bipinnata	BA04-880	Bahamas	25°56.936′N	77°20.403′W
P. bipinnata	BA04-862	Bahamas	25°56.936′N	77°20.403′W
*P. blanquillensis**	USNM 100594	Navasssa		
P. elisabethae	BA05-709	Bahamas	26°20.957′N	77°45.034′W
P. elisabethae	BA05-707	Bahamas	26°20.957′N	77°45.034′W
P. elisabethae	BA05-724	Bahamas	26°23.506′N	77°46.028′W
P. elisabethae	00-388	Bahamas	25°56.94′N	77°20.40′W
P. elisabethae	00-401	Bahamas	25°56.94′N	77°20.40′W
P. elisabethae	00-291	Bahamas	25°56.94′N	77°20.40′W
P. elisabethae	99-533	Bahamas	25°56.94′N	77°20.40′W
*P. hummelincki**	USNM1018805 / 2027480	Tobago		
P. hystrix	Psp Col 2	Bahamas	25°56.936′N	77°20.403′W
P. hystrix	Psp Col 3	Bahamas	25°56.936′N	77°20.403′W
P. hystrix	Psp Col 4	Bahamas	25°56.936′N	77°20.403′W
P. hystrix	Psp Col 5	Bahamas	25°56.936′N	77°20.403′W
P. hystrix	Psp Col 6	Bahamas	25°56.936′N	77°20.403′W
P. hystrix	Psp Col 7	Bahamas	25°56.936′N	77°20.403′W
P. hystrix	Psp Col 8	Bahamas	25°56.936′N	77°20.403′W
P. hystrix[1]	USNM 50386	Bahamas	23°34′00″N	76°33′00″W
P. kallos	BA06-3812	Florida Keys	24°59.083′N	80°24.958′W
P. kallos	BA06-3856	Florida Keys	24°59.016′N	80°24.832′W
P. kallos	BA06-3854	Florida Keys	24°59.016′N	80°24.832′W
P. kallos	BA06-3865	Florida Keys	24°59.016′N	80°24.832′W
P. kallos	BA06-3859	Florida Keys	24°59.016′N	80°24.832′W
P. navia[2]	USNM 50070	Hispaniola	19°10′35″N	69°20′45″W
P. rigida	BA04-928	Bahamas	25°56.936′N	77°20.403′W

* Colonies used only in phylogenetic analysis.

1 Paratype.

2 Holotype.

Appendix 2
Results of Discriminant Analyses

A2-1a

Characteristics of dominant eigenvectors for scaphoids

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	9.261	53.6	53.6	0.950
2	3.815	22.1	75.7	0.890
3	2.795	16.2	91.9	0.858
4	1.162	6.7	98.6	0.733
5	0.236	1.4	100.0	0.437

Table A2-1b

Canonical discriminant function coefficients for scaphoids

	Function				
Character/EFD	1	2	3	4	5
Length (mm)	67.918	66.154	–40.859	15.361	19.803
a2	–51.372	–68.765	32.796	–40.210	79.405
d2	68.882	–27.506	–25.011	83.258	22.714
a4	–61.814	189.503	–79.910	21.024	–100.992
d4	–25.657	–2.811	89.973	–37.789	–53.585
(Constant)	–2.647	–6.756	5.484	2.639	–8.605

Table A2-1c

Pairwise comparisons of discriminant scores of Pseudopterogorgia *spp. scaphoids*

Species		Pe	Pb	Pam	Pr	Ph	Pk	Pn	Unk
Pac	F	18.852	14.022	12.828	3.951	2.903	17.164	7.722	9.125
	Sig.	0.000	0.000	0.000	0.008	0.032	0.000	0.000	0.000
Pe	F		23.733	25.139	9.126	2.720	41.356	3.431	7.932
	Sig.		0.000	0.000	0.000	0.041	0.000	0.016	0.000
Pb	F			9.169	9.114	3.199	4.917	4.698	8.665
	Sig.			0.000	0.000	0.021	0.003	0.003	0.000
Pam	F				11.253	4.769	16.928	4.962	11.971
	Sig.				0.000	0.003	0.000	0.002	0.000
Pr	F					6.342	10.066	8.506	9.496
	Sig.					0.001	0.000	0.000	0.000
Ph	F						5.663	2.280	**0.249**
	Sig.						0.001	0.075	**0.937**
Pk	F							9.961	18.569
	Sig.							0.000	0.000
Pn	F								3.163
	Sig.								0.022

Pac, *Pseudopterogorgia acerosa*; Pam, *P. americana*; Pb, *P. bipinnata*; Pe, *P. elisabethae*; Ph, *P. hystrix*; Pk, *P. kallos*; Pn, *P. navia*; Pr, *P. rigida;* Unk, field specimens that could not readily be identified. Note that Unk specimens were not significantly different from *P. hystrix*.

Table A2-2a

Characteristics of dominant eigenvectors for spindles

Function	Eigenvalue	% of variance	Cumulative %	Cannonical Correlation
1	11.715	55.7	55.7	0.96
2	4.727	22.5	78.1	0.909
3	2.897	13.8	91.9	0.862
4	1.15	5.5	97.3	0.731
5	0.503	2.4	99.7	0.579
6	0.058	0.3	100	0.234

Table A2-2b

Canonical discriminant function coefficients for spindles

Character/ EFD	Function					
	1	2	3	4	5	6
Length (mm)	–7.678	–71.763	–32.623	37.009	–38.599	47.011
a2	–136.059	–37.915	–28.200	98.461	42.791	0.419
d4	–76.351	106.415	192.446	–115.014	–25.684	160.601
a8	151.689	117.596	–131.529	170.330	164.826	60.528
d8	148.398	37.745	56.390	39.210	108.924	–131.145
d16	148.576	238.996	168.017	196.862	–282.922	22.665
(Constant)	3.802	8.469	6.406	–8.368	4.454	–11.614

Table A2-2c

Pairwise comparisons of discriminant scores of Pseudopterogorgia *spp. for spindles*

Species		Pac	Pe	Pb	Pam	Pr	Ph	Pk	Pn	Unk
Pac	F		18.794	13.993	11.600	3.936	3.032	15.978	7.688	9.307
	Sig.		0.000	0.000	0.000	0.008	0.027	0.000	0.000	0.000
Pe	F			23.313	24.501	9.127	2.679	39.294	3.337	7.777
	Sig.			0.000	0.000	0.000	0.043	0.00	0.018	0.000
Pb	F				7.297	8.974	3.263	4.287	4.806	8.637
	Sig.				0.000	0.000	0.020	0.005	0.003	0.000
Pam	F					11.111	4.286	12.641	4.281	10.587
	Sig.					0.000	0.000	0.000	0.005	0.000
Pr	F						6.286	9.410	8.451	9.429
	Sig.						0.001	0.000	0.000	0.000
Ph	F							5.530	2.485	**0.266**
	Sig.							0.001	0.056	**0.928**
Pk	F								9.982	17.509
	Sig.								0.000	0.000
Pn	F									3.295
	Sig.									0.019

Pac, *Pseudopterogorgia acerosa*; Pam, *P. americana*; Pb, *P. bipinnata*; Pe, *P. elisabethae*; Ph, *P. hystrix*; Pk, *P. kallos*; Pn, *P. navia*; Pr, *P. rigida;* Unk, field specimens that could not readily be identified). Note that Unk specimens were not significantly different from *P. hystrix*.