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J.A. Sánchez · C.S. McFadden · S.C. France
H.R. Lasker

Molecular phylogenetic analyses of shallow-water Caribbean octocorals

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Abstract Octocorals, especially gorgonians, are conspicuous on Caribbean coral reefs, but there is no consensus regarding species relationships. Mitochondrial protein-coding genes [NADH-dehydrogenase subunits 2 (ND2) and 6 (ND6), and mutS homolog (msh1), 1633 bp] from 28 shallow-water species were sequenced to develop the first molecular phylogeny for Caribbean octocorals. The specimens were collected primarily in the Caribbean or off Brazil in 1999–2001. Morphological characters (sclerites and axial ultrastructure) were also examined in order to map them onto the molecular phylogeny. Analyses of both nucleotide and amino acid substitutions using maximum parsimony and likelihood (including maximum-likelihood and Bayesian analysis) generated very similar results, with most nodes having high levels of support. These molecular results were significantly different from the generally accepted classification. Neither Plexauridae nor Gorgoniidae were monophyletic. *Plexaurella* spp., nominal plexaurids, were basal to the gorgoniids, sharing many morphological characters with them. This corroborates previous findings using secondary metabolites and biosynthetic pathways. The sea fans, *Gorgonia* spp. and *Pacifigorgia* spp., as well as the pinnate gorgonians, *Muriceopsis flavida* and *Pseudopterogorgia* spp., did not have sea fan or pinnate relatives, suggesting there has been convergent evolution of colony form. Caribbean plexaurids appeared more derived and/or recently evolved according to both morphological and molecular data (e.g. *Eunicea* spp. and *Plexaura* spp.). Molecular phylogenetics is a promising approach for reconstructing phylogenetic relationships among octocorals as well as to understand their complex morphology.
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J.A. Sánchez (✉) · H.R. Lasker
Department of Biological Sciences,
University at Buffalo (SUNY), 109 Cooke Hall,
Buffalo, NY 14260, USA

E-mail: jsanchez@lab.si.edu
Fax: +1-202-7863043

C.S. McFadden
Department of Biology, Harvey Mudd College,
Claremont, CA 91711, USA

S.C. France
Department of Biology, College of Charleston,
Grice Marine Laboratory,
205 Fort Johnson Road, Charleston,
SC 29412, USA

Present address: J.A. Sánchez
Department of Systematic Biology and
Laboratories of Analytical Biology,
Smithsonian Institution,
P.O. Box 37012, NMNH W 329 MRC-0163,
20013-7012, Washington, D.C., USA

Introduction

Octocorals, sessile cnidarians, are found in marine habitats ranging from intertidal to abyssal waters and are distributed from the Arctic to the Antarctic (Bayer 1961). Gorgonians, octocorals containing a sclero-proteinaceous axis, are ecologically important and the dominant macrofauna on many Caribbean reefs. There can be up to 40 octocoral species on a single Caribbean coral reef (Sánchez et al. 1997, 1998; Sánchez 1999), and octocoral communities produce dense and colorful gardens, which provide three-dimensional structures for many reef dwellers and scenic value for underwater activities (Fig. 1). Despite their importance, evolutionary relationships among Caribbean octocorals have received

Fig. 1 *Plexaura* spp., *Pseudopterogorgia* spp. Caribbean gorgonian octocorals in their natural habitat. Fore-reef terrace (8 m) at Carrie Bow Cay, Belize

little attention. In the present study, we present the first phylogenetic analyses of Caribbean octocorals using mitochondrial DNA sequences.

Historically, shallow-water Caribbean octocorals were placed in the order Gorgonacea (Bayer 1961). The order has been superceded and most of the shallow-water species are now classified in the Holaxonia, which is a nominal suborder of the order Alcyonacea (Bayer 1981). However, the term Gorgonacea is still in use in several databases (e.g. GenBank-taxonomy, NCBI, and UNEP-WCMC database for animals). The term ''gorgonian'' or ''gorgonian coral'' is colloquially used throughout the literature to refer to all of the branching octocorals that contain a sclero-proteinaceous axis (gorgonin) with varying degrees of calcification (Bayer 1961; Grasshoff 1999; Sánchez et al., unpublished data). These are primarily members of Holaxonia, but also include taxa from the suborders Calcaxonia and Scleraxonia.

Most holaxonians in the Caribbean have been assigned to the families Plexauridae and Gorgoniidae. Plexaurid colonies are characterized by a thick coenenchyme (the connecting tissue between polyps), canals surrounding the axis, and large, irregularly ornate sclerites sometimes up to several millimeters long (Bayer 1961). Gorgoniidae, on the other hand, have slim branches and small sclerites, usually < 0.3 mm long, which are regularly ornate (Bayer 1961; Grasshoff and Alderslade 1997). Both family descriptions include characters that are not clearly defined at the generic level and based on those familial traits some species could be classified in either family. For instance, some descriptions of species in the Plexauridae say ''presence or not of a strong polyp sclerites armature'' and ''loculated axis or not'' (Bayer 1961). However, the absence of those characters is associated with gorgoniids. In Gorgoniidae, on the other hand, descriptions include traits such as ''tubercles arranged in whorls, but irregular in some species'' (Grasshoff and Alderslade 1997), which can correspond to some plexaurids as well. The size of the sclerites and the thickness of the coenenchyme are also quite variable, and there are many intermediate and overlapping subgroups. Species of *Muriceopsis* (Plexauridae), for instance, have thin coenenchyme and sclerites of intermediate size (Sánchez 2001); *Plexaurella* spp. (also Plexauridae) have very thick coenenchyme with small (< 0.5 mm) sclerites that are regularly ornate. Both *Muriceopsis* spp. and *Plexaurella* spp. have surface sclerites that are very different from those of other "typical" gorgoniid or plexaurid species. There is no clear division between Plexauridae and Gorgoniidae, and characters that can discern ancestry are lacking.

The first records of Caribbean reef octocorals go back to Linnaeus (e.g. *Gorgonia ventalina* Linnaeus, 1758). Numerous species have been described from the fauna, but not until Bayer (1961) was the extent of gorgonian octocoral diversity, nor its taxonomic, geographic, and ecological status, clarified and revised. There have been further additions to the fauna (e.g. Bayer 1991; Lasker et al. 1996; García-Parrado and Alcolado 1997; Sánchez 2001), but no dedicated attempts to review the fauna. Nor is there a complete and robust phylogenetic hypothesis for these octocorals. Kükenthal (1919) presented a phylogeny of some octocorals (Gorgonacea), but it relies upon untenable support (Bayer 1956). Using information from surface sclerites, Bayer (1953) proposed convergent evolution for gorgoniid colony architectures such as sea fans (*Gorgonia* spp. and *Pacifigorgia* spp.) and sea leaves (*Phyllogorgia* spp. and *Phycogorgia* spp.). Gerhart (1983) presented the first cladistic approach using presence and absence of terpenoids and their biosynthetic pathways in an analysis of 18 gorgonian species. Gerhardt identified classification issues that have not been resolved, such as the grouping of *Plexaurella* spp. with gorgoniids (*Gorgonia* spp. and *Pseudopterogorgia* spp.). Morphological characters have provided consistent phylogenetic information for within-genus cladistic relationships (*Eunicea* spp.: Sánchez 1998; *Muriceopsis* spp.: Sanchez 2001). Bayer's and Gerhart's differing hypotheses were based on mostly independent evidence, i.e. sclerites (Bayer) and secondary compounds (Gerhart). The two phylogenies present fundamentally different hypotheses that can be tested. We used coding mitochondrial DNA sequences to examine the relationships among a group of 28 species to specifically test whether *Plexaurella* spp. group with gorgoniids, and if sea fans (*Gorgonia* spp. and *Pacifigorgia* spp.) evolved independently from hypothesized non–sea fan ancestors.

Although a few species have been included in broader phylogenetic studies, molecular phylogenetic analyses of Caribbean gorgonian corals have not been undertaken. France et al. (1996: 16S lsu-rRNA) and Berntson et al. (2001: 18S ssu-rRNA; see also Berntson 1999), working on anthozoans and octocorals, respectively, report poorly resolved phylogenies for groups containing holaxonian (gorgonian) octocorals. A reanalysis of their

data, including insertions and secondary structure, has provided some evidence for two distinct clades of branching octocorals (Sánchez et al. in press). However, the resolution using nuclear and mitochondrial ribosomal gene sequences is limited due to sequence conservation. Other multicopy nuclear genes such as the internal transcribed spacers (ITS) between the 28S and 18S rRNA genes are too variable and cannot be aligned among different holaxonian families (authors' unpublished data). Therefore, analyses using different genes are necessary to discern relationships within and between octocoral families. A complete octocoral mitochondrial genome for *Sarcophyton glaucum* has been sequenced (Beaton et al. 1998; Pont-Kingdon et al. 1998), providing a variety of potential regions for phylogenetic analyses. For instance, octocorals contain a unique mitochondrial gene, msh1, which codes for a homologue of a DNA mismatch repair protein (MutS) found in eubacteria (Pont-Kingdon et al. 1995; Culligan et al. 2000). France and Hoover (2001) identified more variation in msh1 among octocoral species than in commonly sequenced mitochondrial genes such as cytochrome oxidase I (France and Hoover 2002) and 16S rDNA (France et al. 1996).

In the present study we develop a molecular phylogenetic hypothesis for the major groups of Caribbean gorgonian octocorals. The particular goals of the study were: (1) to determine the phylogenetic signal in partial sequences from the mitochondrial coding genes NADH-dehydrogenase [subunits 2 (ND2) and 6 (ND6)] and mutS homologue (msh1); (2) to reconstruct phylogenetic relationships among 28 species of gorgonian octocorals from the Plexauridae and Gorgoniidae, including species from 12 genera (including *Muriceopsis* and *Plexaurella*); and (3) to compare and map major morphological characters onto the molecular hypotheses in order to identify differences and/or congruence between molecular hypotheses and commonly used taxonomic classifications.

Materials and methods

Octocoral genomic DNA

Most of the material was collected during 2000 in the Bahamas at depths of 1–30 m using SCUBA (Table 1). In the Caribbean, we sampled at least two species per genus (except *Pacifigorgia* and *Pseudoplexaura*). Since *Pacifigorgia elegans* was the only species of this genus found in the Caribbean, *P. stenobrochis* from the eastern Pacific was included in order to verify sequences at the generic level. Additional material was obtained from colleagues (see Table 1 and "Acknowledgements"). The examined material was preliminarily identified using the taxonomic work and key of Bayer (1961) based on dissection of sclerite layers, digestion of the organic matter in sodium hypochlorite, and observations of sclerites under a compound microscope (100×). Voucher specimen (Table 1) identifications were verified by direct comparison with material deposited in the National Museum of Natural History (Smithsonian Institution), which included type material and/or material identified by Bayer (1961), Lasker et al. (1996), and Sánchez (1998, 2001).

Small pieces (< 0.5 cm^3) of tissue were fixed in 95% ethanol with daily changes over 5 days and then kept at −20°C until extraction. For genomic DNA extraction we used the 2× CTAB protocol with phenol/chloroform phases and precipitation with ethanol (Coffroth et al. 1992). DNA was resuspended in 10–40 µl of TE buffer (according to pellet size) and kept at −20°C until amplification. For gene amplification we used primers designed by McFadden et al. (in press) for ND6 and ND2 (ND6-1487F: 5′-TTTGGTTAGTTATTGCCTTT-3′; ND3-2126R: 5′-CACATTCATAGACCGACACTT-3′; 16S-647F: 5′-ACACAGCTCGGTTTCTATCTACAA-3′; ND2-1418R: 5′-ACATCGGGAGCCCACATA-3′) and by France and Hoover (2001, 2002) for the 5′ end of msh1 (ND42599F: 5′-GCCATTATGGTTAACTATTAC-3′; Mut-3458R: 5′-TSGAGCAAAAGCCACTCC-3′). All the primers were anchored in an adjacent mitochondrial gene to prevent amplification of genes from nuclear or symbiont (zooxanthella) genomes (Fig. 2). All zooxanthella and blank controls were negative except for ND2, for which amplifications sometimes produced a blurry ~1,000 bp band from the symbiont that did not occur in the presence of octocoral DNA. We added ~20 bp M13 tails to all primers, a step that was necessary for sequencing with the Li-Cor Global IR2 DNA sequencing system (primers Li-Cor, M13 reverse: 5′-GGATAACAATTTCACACAGG-3′; M13 forward: 5′-CACGACGTTGTAAAACGAC-3′). Template DNA for sequencing was obtained from the combination of four PCR (polymerase chain reaction) reaction tubes containing 28 µl total (volume completed with ddH_2O) with 1 µl of DNA template (1:10 dilutions of genomic DNA extractions), ~3 U *Taq* polymerase, 3 µl of 10× buffer [10 µM Tris-HCl (pH 8.3), 50 µM KCl, 0.001% gelatin, and 3.1 µM $MgCl_2$], 0.21 µM dNTPs mix, and 0.16 µM of each primer. These reaction conditions yielded the highest template quality for cycle-sequencing. The PCR conditions for all sequenced genes and species were one initial period of 2.0 min at 94°C; followed by 35 cycles of 1.5 min at 94°C, 1.5 min at 58°C, and 1.0 min at 72°C; and a final extension step for 5.0 min at 72°C. The PCR product was precipitated with an equal volume of PEG (20% w/v PEG 8000 in 2.5 M NaCl), briefly (15 min) incubated at 37°C, and centrifuged at 14,000 rpm (15 min). Supernatant was discarded, and the DNA pellet was washed with 125 µl of 80% ethanol, followed by 5 min of centrifugation; the supernatant was again discarded, and the pellet was air dried. The pellet was resuspended in 10 µl of ddH_2O at 65°C for 5 min and finally run in a 1.5% 1× TAE-agarose gel (the TAE containing 1/10 of the normal EDTA). The DNA band in the gel was excised using a razor and separated from the agarose using a cellulose acetate membrane (Fisher-Corning Costar Spin-X columns).

PCR products were cycle-sequenced in both directions using a Global IR2 DNA sequencing system (Li-Cor). We used M13-labeled primers and the Epicentre-Li-Cor protocol (SequiTherm EXCEL II DNA sequencing kit—LC for 66 cm gels). Consensus sequences were obtained automatically by assembling the two complementary DNA chromatograms. Sequences with several ambiguities at sites other than the ends were repeated from the initial amplification step. Two to four different specimens from four species (*Pseudopterogorgia elisabethae*, *Muriceopsis flavida*, *Pterogorgia citrina*, and *Pseudopterogorgia bipinnata*) were sequenced to control for procedural error. No differences were found within species other than at the ends that were trimmed for the analyses. Several *Eunicea* spp. also had identical sequences, which indicated a low probability of procedural error. GenBank accessions for the sequenced genes and species are provided in Table 1.

Phylogenetic reconstruction

Phylogenetic analyses were conducted using coding regions and two different matrices: nucleotides and amino acids. Assembly of the three genes (ordered ND6–ND2–msh1), matrix editing, translation, and alignment were done using BioEdit (Hall 1999) and CLUSTALW (Higgins et al. 1996). Non-coding regions were removed from the analysis. Corrections for the genetic code were made according to Beaton et al. (1998), and the mold, protozoan,

Table 1 Studied species, collection sites and dates, museum voucher specimen number (USNM numbers unless otherwise indicated, National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA), and GenBank accession numbers for the three studied genes

Taxa	Collecting location	Date	Specimen	GenBank accession nos.		
Plexauridae						
Eunicea fusca Duch. and Mich., 1860	Lee Stocking I., Exuma Cays, Bahamas	Jul 2000	1007366	AY126351	AY126379	AY126407
Eunicea tourneforti M. E. and H., 1857	Lee Stocking I., Exuma Cays, Bahamas	Jul 2000	1007393	AY126350	AY126378	AY126406
Eunicea knighti Bayer, 1961	Cat Island, Bahamas	Mar 2001	1007357	AY126348	AY126376	AY126404
Eunicea sp.	San Salvador, Bahamas	Dec 1999	T#2017366	AY126349	AY126377	AY126405
Muriceopsis flavida (Lamarck, 1815)	San Salvador, Bahamas	Dec 1999	1007376	AY126371	AY126388	AY126416
Muriceopsis bayeri Sánchez, 2001	San Blas, Panama (Coll. T. Goulet)	1997	100925	AY126360	AY126389	AY126417
Plexaurella dichotoma (Esper, 1791)	Lee Stocking I., Exuma Cays, Bahamas	Jul 2000	1007506	AY126358	AY126386	AY126414
Plexaurella nutans (Duch. and Mich., 1860)	Lee Stocking I., Exuma Cays, Bahamas	Jul 2000	1007399	AY126359	AY126387	AY126415
Plexaurella grisea Kunze, 1916	Lee Stocking I., Exuma Cays, Bahamas	Jul 2000	1007394	AY126357	AY126385	AY126413
Muricea muricata (Pallas, 1766)	Lee Stocking I., Exuma Cays, Bahamas	Jul 2000	10073400	AY126352	AY126380	AY126408
Muricea pinnata Bayer, 1961	Lee Stocking I., Exuma Cays, Bahamas	Jul 2000	1007405	AY126353	AY126381	AY126409
Pseudoplexaura crucis Bayer, 1961	Lee Stocking I., Exuma Cays, Bahamas	Jul 2000	1007378	AY126345	AY126373	AY126401
Plexaura kuna Lasker et al., 1996	Lee Stocking I., Exuma Cays, Bahamas	Jul 2000	1007415	AY126356	AY126384	AY126412
Plexaura flexuosa (Lamouroux, 1821)	Lee Stocking I., Exuma Cays, Bahamas	Jul 2000	1007418	AY126355	AY126383	AY126411
Plexaura homomalla (Esper, 1792)	Florida (Coll. A. Brash)	2001	[a]	AY126354	AY126382	AY126410
Gorgoniidae						
Pseudopterogorgia bipinnata (Verrill, 1864)	San Salvador, Bahamas	Dec 1999	1007374	AY126365	AY126396	AY126424
Pseudopterogorgia acerosa (Pallas, 1766)	Lee Stocking I., Exuma Cays, Bahamas	Jul 2000	1007413	AY126372	AY126393	AY126421
Pseudopterogorgia americana (Gmelin, 1791)	San Salvador, Bahamas	Dec 1999	1007391	AY126364	AY126395	AY126423
Pseudopterogorgia elisabethae Bayer, 1961	Florida, USA (Coll. T. Snell and M. Boller)	May 2000	1007390	AY126363	AY126394	AY126422
Leptogorgia virgulata (Lamarck, 1815)	North Carolina, USA (Coll. W. O'Neal)	2000	1007414	AY126370	AY126390	AY126418
Gorgonia ventalina Linnaeus,1758	Cayo Lobo, Puerto Rico	Oct 2000	1007421	AY126366	AY126397	AY126425
Gorgonia mariae Bayer, 1961	Cayo Lobo, Puerto Rico	Oct 2000	1007505	AY126367	AY126398	AY126426
Gorgonia flabellum Linnaeus, 1758	San Salvador, Bahamas	Dec 1999	16[b]	AY126368	AY126399	AY126427
Pterogorgia citrina (Esper, 1792)	Lee Stocking I., Exuma Cays, Bahamas	Jul 2000	1007406	AY126346	AY126374	AY126402
Pterogorgia anceps Pallas, 1766	Florida, USA (aquarium shop)	2000	5[b]	AY126347	AY126375	AY126403
Pacifigorgia stenobrochis (Valenciennes, 1846)	Tropical eastern Pacific	1996	27[b]	AY126362	AY126392	AY126420
Pacifigorgia elegans (M. E. and H., 1857)	Trinidad (Coll. J.E. Randall)	1977	55684	AY126361	AY126391	AY126419
Phyllogorgia dilatata (Esper, 1806)	Rio de Janeiro, Brazil (Coll. C.R.R. Ventura)	Oct 2000	4336[c]	AY126369	AY126400	AY126428

[a]Dr. A. Brash laboratory (U. Vanderbilt Medical Center)
[b]J.A. Sánchez, personal collection
[c]MNRJ (Museu Nacional do Rio de Janeiro)

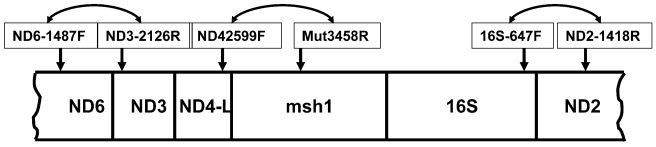


Fig. 2 Diagram of sequenced portion of octocoral mitochondrial genome and primers used (modified from Beaton et al. 1998)

and coelenterate mitochondrial code (NCBI genetic code 4) was used to translate nucleotide sequences. The complete alignments for the coding regions and amino acid positions were deposited in TREEBASE (University at Buffalo-SUNY, http://www.treebase.org; accessions S833, M1334, M1335). Both maximum-parsimony and maximum-likelihood approaches were used to analyze both nucleotide and amino acid sequences. Maximum-parsimony analyses were carried out in PAUP* for Windows (Swofford 2002). Nexus matrices were translated from NBRI/PIR format using ProSeq 2.8 (Filatov 2001). *Alcyonium digitatum* sequences (McFadden et al., unpublished data) were used as the outgroup for the analyses. We initially included six outgroup species from different orders of octocorals, but they had quite high divergence from Caribbean gorgonian octocorals, including the presence of indels in coding regions. Since the analyses did not change with inclusion of one versus six outgroups, we decided to use only the closest species that did not add indels to the alignment. Analyses of the other outgroup sequences and indels will be published elsewhere.

Tree reconstruction with maximum parsimony was conducted using the branch-and-bound search algorithm in PAUP* with default parameters. As measures of phylogenetic content the skewness of the tree-length distribution of 1,000 randomly generated trees in PAUP* was assessed with the g1-test (Hillis and Huelsenbeck 1992). To examine the noise (e.g. homoplasy) in the data sets (Fu and Murphy 1999), the permutation tail probability (PTP) was also determined using PAUP*. Character re-sampling by means of non-parametric bootstrapping (10,000 replicates) was used to visualize the branch/node support of the trees in 50%-majority-rule consensus in PAUP* (e.g. Hillis and Bull 1993).

We used several likelihood approaches for reconstructing phylogenetic relationships. Initially, we searched for the best-fit models of DNA evolution using MODELTEST (Posada and Crandall 1998), which evaluates the goodness of fit of the data alignment by a series of hierarchical likelihood-ratio tests (Posada and Crandall 2001). Using the likelihood settings recommended by MODELTEST, a maximum-likelihood tree was reconstructed using a heuristic search and random addition of sequences in PAUP*. Only 100 bootstrap replicates were done using the maximum-likelihood tree due to logistic/time constraints. Additionally, using the best-fit model from MODELTEST, Bayesian-likelihood analysis for the estimation of phylogeny was assessed using the program MrBayes (Huelsenbeck and Ronquist 2001) and the recommended block command for coding regions (Hall 2001). Finally, the program Tree-Puzzle was used to choose the best-fit model of evolution for amino acid substitution and phylogeny reconstruction using a quartet-puzzling, maximum-likelihood approach (Strimmer and von Haeseler 1996). Nucleotide/amino acid maximum-likelihood hypotheses were compared to trees reflecting the taxonomic classification scheme using the Shimodaira–Hasegawa test in PAUP* (S–H test; Goldman et al. 2000).

Morphological characters

Several morphological characters from the axis and sclerites of the studied octocorals were examined in order to map them onto the molecular phylogenies. Characters from the axis and base mineralization were mostly obtained from Bayer and McIntyre (2001). To extend those data some species were prepared using similar methods and examined under scanning electron microscopy (SEM). SEM analyses were conducted at the Instrumentation Center (Hitachi S-800), SUNY at Buffalo, and the National Museum of Natural History, SEM laboratory (AMRAY 1810), Smithsonian Institution, Washington, D.C. The sclerites were examined using the protocols of Bayer (1961, 1992) and Sánchez (2001). First we examined the complete sclerite structure (80–1,500×), and then we scanned details of warts/decorations or irregularities of the sclerites (500–5,000×). At least two sclerites of each sclerite type were observed from each species. Carbon double-sided tape was used to mount samples, and gold and carbon was used for SEM coating. Only characters that were amenable to discernable qualitative and discrete coding were retained from the initial survey. Direct measurements of prepared sclerites were made with a compound microscope in order to have at least ten measurements per species. Maximum sclerite lengths were determined from the literature (Bayer 1961; Sánchez et al. 1998; Sánchez 2001). Only measurements that could be separated by gap coding were considered informative characters (Mickevich and Johnson 1976; see also Swiderski et al. 1998).

Results

Our sequence data comprised ~366 bp of the ND6, 43 bp of the intergenic non-coding region, and the first 21 bp of the ND3 coding region (positions 1,590–2,020 compared to the sequence of *Sarcophyton glaucum*, Pont-Kingdon et al. 1998); the last ~87 bp from the 3′ end of the lsu-rRNA (16S), and ~550 bp of the ND2 (positions 722–1,358, Beaton et al. 1998); the last ~6 coding positions of ND4L, ~14 bp of non-coding intergenic spacer, and 747 bp at the 5′ end of msh1 (positions 2,644–3,411, Pont-Kingdon et al. 1998). The final database comprised 1,633 (544 AA) coding positions including, in this order, 360 bp of ND6 (1–360), 525 bp of ND2 (361–885), and 747 bp of msh1 (886–1,633). Only two gaps were present in the matrix (two and three amino acid positions in the msh1 from *Pacifigorgia stenobrochis* and the outgroup *Alcyonium digitatum*, respectively), which did not have any weight in the analysis. Since different combinations of genes, or each gene separately, yielded the same overall result we conducted the phylogenetic reconstruction using the three genes combined, which produced the best supported relationships.

Nucleotide substitutions

Using maximum parsimony, we obtained four partially resolved most-parsimonious trees using 279 parsimony-informative characters (Fig. 3A, B; see also supplementary electronic material, Appendix 1). Most informative characters came from msh1 (176; 63%), followed by ND2 (61; 21%), and ND6 (42; 16%). The length-frequency distribution from 1,000 random trees (mean = 1,444.7, SD = 64.7) was significantly skewed ($g1 = -0.54$, $P < 0.01$) and the unpermuted most-parsimonious trees were significantly shorter than 1,000 permutation replicates (> 1,322, PTP: $P < 0.001$). Both of these analyses indicate prevalence of phylogenetic signal over noise and homoplasy. The most-parsimo-

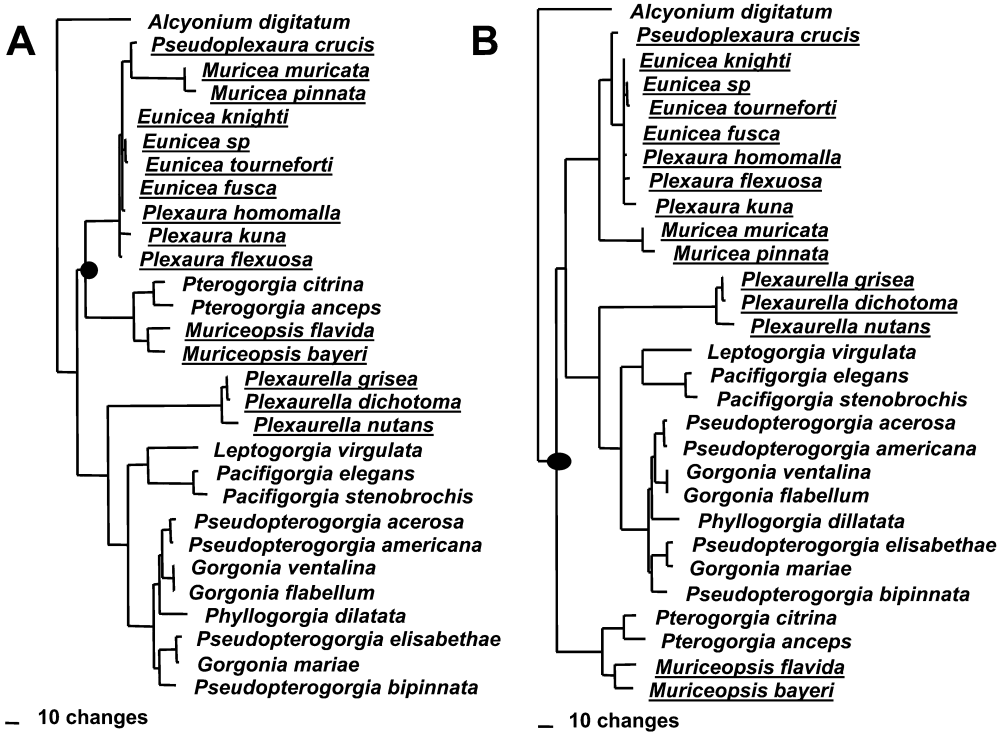


Fig. 3A, B Two of the four maximum-parsimony phylograms using nucleotide substitutions (length = 554, consistency index = 0.78, retention index = 0.89, and homoplasy index = 0.22). *Black dots* depict the main conflicting node among maximum-parsimony trees. Species classified as Plexauridae are *underlined*. See electronic supplementary material (Appendix 1) for additional trees

nious trees contained clades that mixed members of Plexauridae and Gorgoniidae (Fig. 3). Some members of Plexauridae, such as the *Plexaurella* spp., grouped basally to some gorgoniids, and some gorgoniids (*Pterogorgia* spp.) grouped with plexaurids (*Muriceopsis* spp.). The trees differed in the placement of two clades. *Muricea* spp. were either basal to *Eunicea* spp.–*Plexaura* spp.–*Pseudoplexaura* spp. (Fig. 3B) or the sister group of *Pseudoplexaura crucis* (Fig. 3A). The other was a basal node including the sister relationship *Muriceopsis* spp. and *Pterogorgia* spp. that was either basal to the whole ingroup or basal to most plexaurids (*Plexaura* spp., *Eunicea* spp., *Muricea* spp., and *Pseudoplexaura* spp.) (Fig. 3; see also supplementary electronic material, Appendix 1). Other differences among most-parsimonious trees are mainly in the placement of *Eunicea* spp. and *Plexaura* spp. (Figs. 3, 4), but the number of substitutions at those nodes is low, and the clade could be considered unresolved. Topological relationships among most gorgoniids (*Leptogorgia* spp., *Pacifigorgia* spp., *Pseudopterogorgia* spp., *Gorgonia* spp., and *Phyllogorgia* spp.) and *Plexaurella* spp. remained unchanged in all most-parsimonious trees. Bootstrap analysis showed that most basal nodes were supported with values > 94%, with the exception of *Muriceopsis* spp.–*Pterogorgia* spp. (54%), *Pseudoplexaura* spp.–*Muricea* spp. (58%) and two nodes within *Pseudopterogorgia* spp.–*Gorgonia* spp. (see supplementary electronic information, Appendix 2). There was no meaningful bootstrap support among species of *Plexaura* spp. and *Eunicea* spp. (Plexauridae), whereas most nodes within the Gorgoniidae were resolved and supported with high values. *Pseudopterogorgia* spp. and *Gorgonia* spp. exhibited mixed and polyphyletic relationships.

Likelihood-ratio tests showed that the HKY + G model ($-\ln L = 8{,}648$; Gamma shape parameter = 0.51) significantly improved ($P < 0.0001$) fit of the data in a maximum-likelihood analysis. Parameters used in the

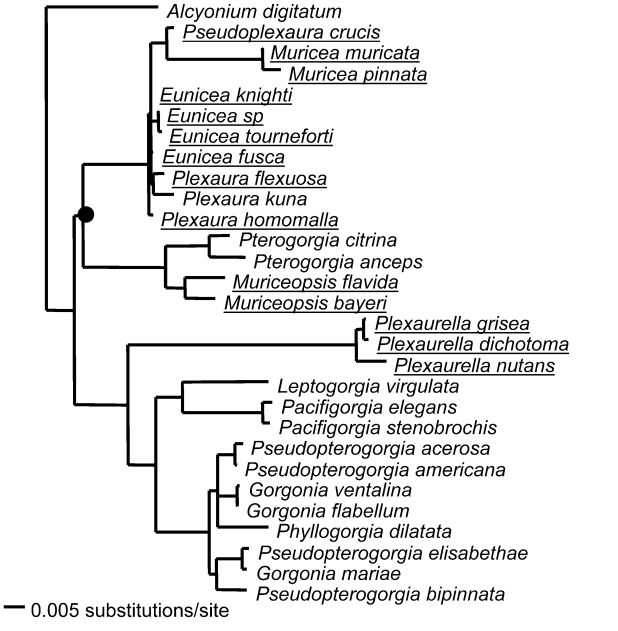


Fig. 4 Maximum-likelihood tree from best-fit model (HKY + G) selected by hLRT in MODELTEST version 3.06. *Black dot* depicts the only conflicting node among maximum-parsimony trees. Species classified as Plexauridae are *underlined*

analysis were as follows: base-frequency composition (A = 0.289, C = 0.1682, G = 0.195, T = 0.347), transition/transversion ratio (ti/tv = 3.26), and equal transition rates among sites. After 3,345 rearrangements using the heuristic search, a maximum-likelihood tree was obtained (log score = 1,765.91). The maximum-likelihood tree was identical to one of the most-parsimonious trees (Figs. 3A, 4), with the node *Muriceopsis* spp.–*Pterogorgia* spp. basal to *Eunicea* spp.–*Plexaura* spp.–*Pseudoplexaura* spp.–*Muricea* spp. It is notable in the maximum-likelihood tree that some plexaurid species, particularly in the genera *Eunicea* and *Plexaura*, had low or no substitutions per site, whereas some basal gorgoniids (*Leptogorgia* spp., *Pacifigorgia* spp., and *Pterogorgia* spp.), as well as *Plexaurella* spp., exhibited a larger number of substitutions. Bootstrap analyses from the maximum-likelihood tree (100 replicates, not shown) had nearly identical topological relationships and similar support values as the Bayesian-likelihood estimation of phylogeny (Fig. 5A). The Bayesian analyses use a different maximum-likelihood approach by assuming partitions (third codon position evolving differently). Clade support values using maximum likelihood with Bayesian analysis indicated good support for most nodes found in the maximum-likelihood tree and identified two more resolved nodes compared to maximum parsimony (Fig. 5A; see also supplementary electronic material Appendix 2). Unconstrained trees were significantly more likely than trees constrained by the classification (families and genera), indicating disagreement between the common classification and this molecular hypothesis (H–S test: diff. $-\ln L = 182.7$, $P = 0.0001$).

Amino acid substitutions

Initially, we obtained ten partially resolved most-parsimonious trees using 106 parsimony-informative characters [length = 238, consistency index (CI) = 0.798, retention index (RI) = 0.891, and homoplasy index (HI) = 0.202; trees not shown]. The lengths of 1,000 random trees had a mean of 570.8 (SD = 20.9), and the tree-length frequency distribution was significantly skewed ($g1 = -0.51$, $P < 0.01$). PTP with 1,000 replicates clearly showed that all replicates had more steps (> 485) than the unpermuted tree ($P < 0.001$). These analyses suggest low noise and homoplasy in the most-parsimonious trees. Despite the lower number of parsimony-informative characters using amino acid positions compared to base substitutions (106 and 279, respectively), the results from bootstrap analyses were nearly identical, except for three nodes comprising relationships among closely related species (see supplementary electronic material, Appendix 2).

The program Tree-Puzzle chose the model for amino acid substitutions "mtREV24," which is a model for amino acid substitution in mitochondrial genes (Adachi and Hasegawa 1996). Amino acid frequencies ranged from 1.4% (Cys) to 9.9% (Ser), with a notable bias for leucine (17.4%). Quartet puzzling was used to choose from possible maximum-likelihood trees using amino acid substitutions (mtREV24) and simultaneously calculate branch support values (Strimmer and von Haeseler 1996). The phylogenetic reconstruction included 10,000 puzzling steps and 23,751 analyzed quartets (738, 3.1% unresolved quartets). Species topological rela-

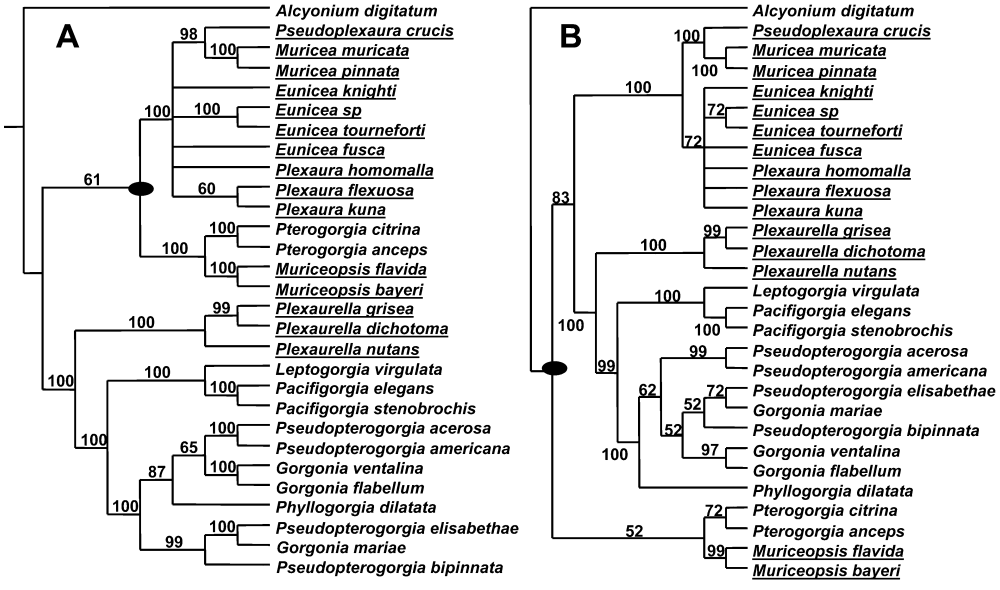


Fig. 5A, B Branch/node support from different likelihood phylogenetic analyses. **A** Bayesian-estimated likelihood tree (HKY) using nucleotide partition by positions, 1,000,000 Monte Carlo Markovian chain generations (Bayesian–Monte Carlo simulation by MrBayes sampling every 100 simulations). Above node support is the 50%-majority-rule consensus from the 10,001 sampled trees generated by PAUP*. **B** Maximum-likelihood tree using the model mtREV24 for mitochondrial amino acid substitution in the program Tree-Puzzle, showing support from 10,000 puzzling quartets. *Black dots* depict the only conflicting node among maximum-parsimony trees. Species classified as Plexauridae are *underlined*

Table 2 Morphological characters and states for Caribbean gorgonian corals (for species distribution see Fig. 7)

Characters		States					
A	Polyp sclerites (Fig. 6C)	(1) Robust, bent, and ornate rods, forming a strong armature	(2) Small, thin, and smooth rods, not forming an armature				
B	Sclerite warts (or tubercles) (Fig. 6A, B)	(1) Radial trend with long lateral processes, sometimes fused, usually > 20 μm in radius	(2) Complex warts (many modular sub-warts), disposed in transverse girdles, usually < 20 μm wide				
C	Base/holdfast mineralization[a]	(1) Aragonite	(2) CHAp				
D	Maximum spindle length (middle or internal cortex layers) (Fig. 6L–N)	(1) < 0.3 mm (Fig. 6L)	(2) 0.4–0.7 mm (Fig. 6N)		(3) > 0.8 mm up to several millimeters (Fig. 66M)		
E	Axis mineralization (Fig. 6D)	(1) Absent	(2) Carbonate hydroxiapatite (CHAp)		(3) Calcite-$MgCO_3$		
F	Surface layer cortex sclerites (Fig. 6E–K)	(1) Club (Fig. 6K)	(2) Scaphoids (Fig. 6F)	(3) Antler (Fig. 6E)	(4) Blunt and short spindles (double heads) (Fig. 6I)	(5) Disk-spindles (Fig. 6H)	(6) Unilaterally spinose (Fig. 6G)	(7) Spiny-tip (Fig. 6J)

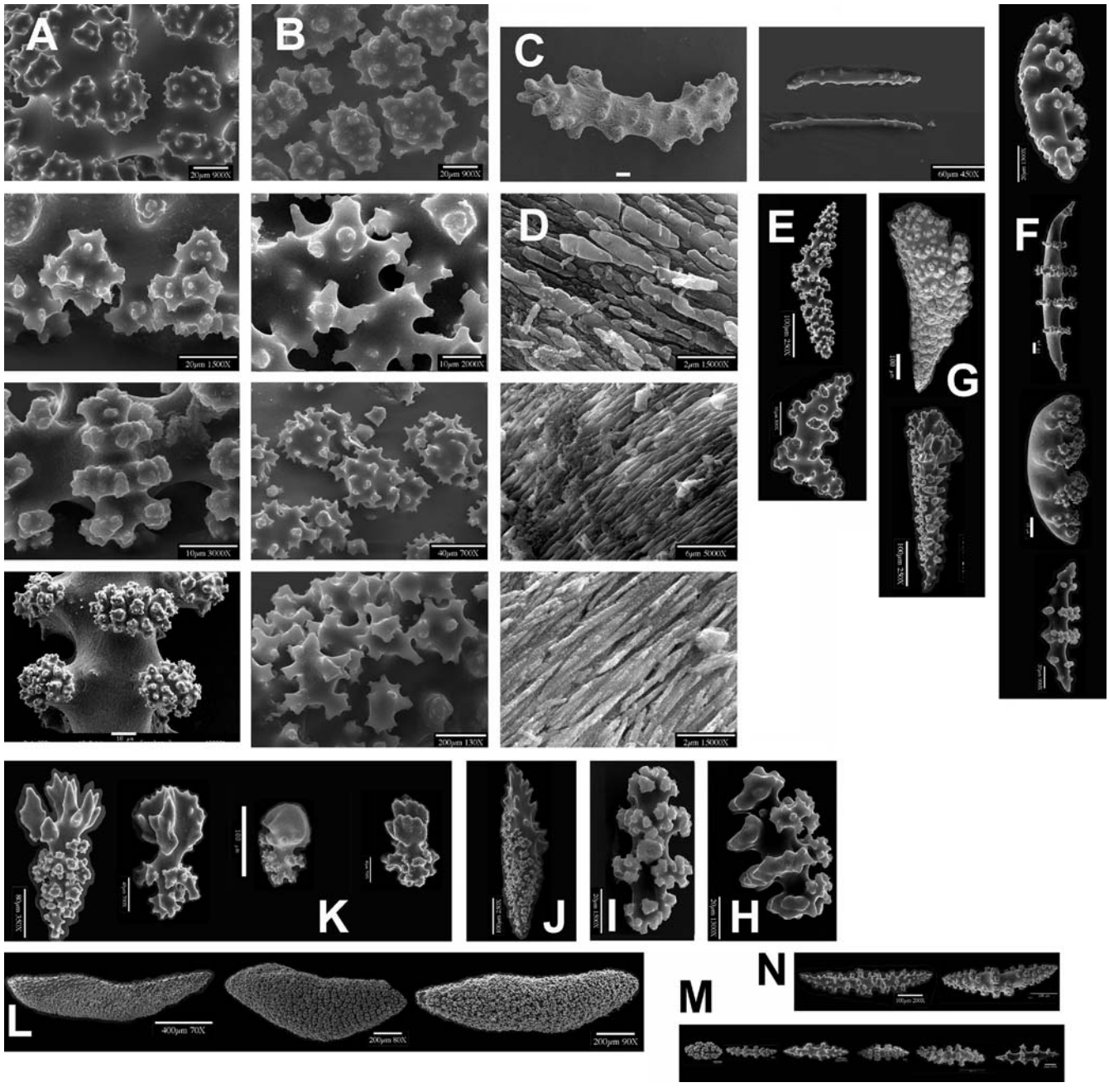
[a]See also Bayer and MacIntyre (2001)

Fig. 6A–N Examples of some of the morphological characters using scanning electron microscopy that were mapped on the molecular hypotheses (see character states in Table 2). **A, B** Sclerite ornamentation (warts). **A** Details of warts from (*top to bottom*): *Plexaurella grisea* (*scale bar*: 20 μm), *Muriceopsis flavida* (20 μm), *Pacifigorgia stenobrochis* (10 μm), and *Pterogorgia citrina* (10 μm). **B** Details for (*top to bottom*): *Plexaura kuna* (20 μm), *P. flexuosa* (10 μm), *Eunicea* sp. (40 μm), and *Muricea muricata* (200 μm). **C** Polyp rod sclerite from *Eunicea* sp. (*left*) and rods from *Pseudopterogorgia elisabethae* (*right*, 60 μm). **D** Examples of axial/base mineralization from (*top to bottom*): *P. elisabethae* holdfast showing carbonate hydroxiapatite (CHAp) (2 μm), calcite-$MgCO_3$ from *Plexaurella nutans* axial loculi (6 μm), and aragonite from *P. flexuosa* base (2 μm). **E–H** Different surface sclerite types. **E** Surface sclerites from (*top to bottom*): *P. nutans* (100 μm) and *P. grisea* (antler-like, 60 μm); **F** sclerites (scaphoids) from (*top to bottom*): *Phyllogorgia dilatata* (20 μm), *Pseudopterogorgia acerosa* (10 μm), *Gorgonia ventalina* (10 μm), and *Pseudopterogorgia bipinnata* (10 μm); **G** sclerites from (*top to bottom*): *Muriceopsis bayeri* (100 μm) and *M. flavida* (100 μm); **H** sclerite (disk-spindle) from *Leptogorgia virgulata* (20 μm); **I** sclerite from *Pacifigorgia elegans* (20 μm); **J** sclerite from *M. muricata* (100 μm); **K** club-sclerites from (*left to right*): *Plexaura homomalla* (80 μm), *P. flexuosa* (40 μm), *Pseudoplexaura crucis* (100 μm), and *P. kuna* (40 μm). **L–N** Examples showing size differences among three size-classes of spindle sclerites. **L** Spindles from (*left to right*): *Muricea pinnata* (400 μm), *P. kuna* (200 μm), and *P. flexuosa* (200 μm); **M** spindles from (*left to right*): *Pacifigorgia stenobrochis* (20 μm), *P. acerosa* (10 μm), *Pseudopterogorgia americana* (20 μm), *Gorgonia mariae* (10 μm), *Pterogorgia anceps* (100 μm), and *P. bipinnata* (20 μm); **N** spindles from (*left to right*): *M. flavida* (100 μm) and *P. grisea* (100 μm). See Fig. 7 and Table 2 for character state descriptions and species distribution

tionships were very similar to the maximum-parsimony results, with the exception of two additional nodes that were well supported with this analysis (Fig. 5B; supplementary electronic material, Appendix 2). Nonetheless, there was no significant difference between the mtREV24 hypothesis (amino acids) and the HKY + G model using nucleotide substitutions (S–H test: diff. $-\ln L = 5.93$, $P = 0.187$).

Morphological characters and the molecular hypotheses

A limited number of homologous morphological characters (6) were found among the axis/base mineralization and sclerite characteristics (Table 2). Mineral axial composition (if present) is discrete with a clear species distribution (Figs. 6D, 7C, E; see also supplementary electronic material, Appendix 3). Though sclerites exhibit many morphological characters, only a few were clearly different among species. Only one character (base mineralization) supported the Plexauridae and Gorgoniidae classification (Fig. 7C). Five characters were congruent with the molecular phylogenetic hypotheses presented here. Length, CI, and RI were not different for the six morphological characters when mapping them onto the maximum-likelihood trees from nucleotide and amino acid substitutions (Fig. 7). Most character state changes occurred in basal clades and/or gorgoniids, which agreed with the distances and substitution rates



found with the molecular analyses. Interestingly, all characters supported the position of *Plexaurella* spp. as basal to gorgoniids. *Plexaurella* spp. have unique axis mineralization characters (calcite and $MgCO_3$, Fig. 6D) and surface sclerites (antler-like spindles, Fig. 6E). *Plexaurella* spp. have intermediate-sized sclerites, between the tiny sclerites of some gorgoniids (< 0.3 mm) and the large spindles of some plexaurids (up to several millimeters, Fig. 6N). *Plexaurella* spp. shared important characters with Gorgoniidae, such as small polyp rods and the absence of armature in the polyps (Figs. 6C, 7A, B), and complex (many internal geometrical/fractal subwarts) warts (or tubercles) disposed in transverse girdles, usually < 20 μm wide (Figs. 6A, 7A, B). These traits were also present in *Muriceopsis* spp., suggesting a separation from Plexauridae where they have been classified.

Discussion

Phylogenetic analyses of partial sequences from the NADH-dehydrogenase (subunits 6 and 2) and the 5′ end of the mutS homologue (msh1) produced well-supported phylogenetic relationships for Caribbean gorgonian octocorals. Maximum-parsimony and -likelihood analyses of both base and amino acid substitutions produced very similar results, with high supporting values for most

nodes. The only conflicting node was the clade *Pterogorgia* spp.–*Muriceopsis* spp., which had low support values in all of the analyses. These molecular results differ significantly from the common classification scheme, particularly regarding separation of the families Plexauridae and Gorgoniidae. *Plexaurella* spp., nominal plexaurids, were basal with respect to gorgoniids and shared many morphological characters with the gorgoniids. This result supports Gerhart's (1983) hypothesis that *Plexaurella* spp. are related to gorgoniids rather than to plexaurids. Similarly, *Muriceopsis* spp. also grouped with gorgoniids (*Pterogorgia* spp.) instead of plexaurids.

The sea fans *Gorgonia* spp. and *Pacifigorgia* spp. had non-sea fan relatives, supporting Bayer's (1953) hypothesis of convergent evolution. Convergent evolution in colony form was also observed among the pinnate gorgonians *Muriceopsis* spp. and *Pseudopterogorgia* spp. The pattern of convergent evolution observed among these gorgonian families seems to follow the overall pattern of convergence in branching observed among higher taxa of octocorals. Sánchez et al. (in press) hypothesize that branching in octocorals has evolved at least twice among suborders of octocorals, particularly Calcaxonia and Holaxonia.

Mapping morphological characters onto molecular results improves our understanding of evolutionary relationships among Caribbean octocorals. Ancestral characters (e.g. placed basal in the phylogenetic tree) seemed to be present in Gorgoniidae (e.g. CHAp mineralization, smallest spindles, and complex warts). Plexaurids, excluding *Plexaurella* spp. and *Muriceopsis* spp., all had large spindle sizes, lacked branch mineralization, contained aragonite in the base, and had club-like sclerites in the surface cortex layer. The morphologically intermediate genus *Plexaurella* (traditionally placed in the Plexauridae), was more closely related and occupied a basal position relative to gorgoniids. These molecular results made Gorgoniidae paraphyletic. Alternatively, *Plexaurella* spp. could be reassigned to a different family with respect to Plexauridae and Gorgoniidae. Independent sources of phylogenetic information, such as single-copy nuclear genes, as well as additional non-Caribbean species are needed to resolve the placement of the clade *Muriceopsis* spp.–*Pterogorgia* spp. because it had two possible topological positions, both with low branch support, in all the analyses.

Caribbean gorgonian octocorals: presence of recently evolved species?

Among Caribbean octocorals mitochondrial DNA (mtDNA) divergence was lower among some plexaurids (*Eunicea* spp. and *Plexaura* spp.) compared to gorgoniids and other plexaurids. The biogeography of the groups present in the Caribbean may provide an explanation for this. For instance, the genus with the lowest divergence, *Eunicea*, is distributed only in the Caribbean Sea. Gorgoniids, on the other hand, are distributed worldwide (Bayer 1953). The genus *Leptogorgia* has many species in the Pacific Ocean (Bayer 2000) as well as on the African Atlantic coast (Grasshoff 1988). *Pacifigorgia* spp. are mostly distributed in the eastern Pacific (e.g. Breedy 2001), but *P. elegans* occurs in the Carib-

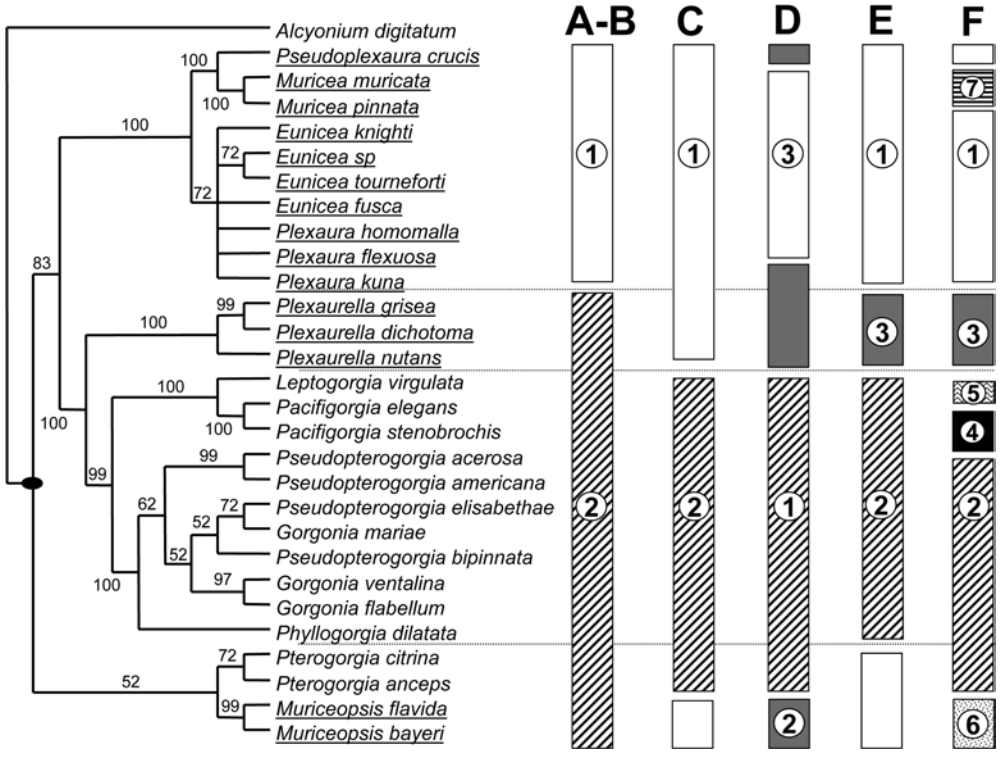


Fig. 7A–F Morphological characters mapped onto Bayesian-estimated likelihood tree (as in Fig. 5A). Characters (*A–F*) and character states (indicated by *numbers* and *shading*) defined in Table 2 [*A* polyp sclerites (Fig. 6C); *B* sclerite warts (or tubercles) (Fig. 6A, B); *C* base/holdfast mineralization; *D* maximum spindle length (middle or internal cortex layers) (Fig. 6L–N); *E* axis mineralization (Fig. 6D); *F* surface layer cortex sclerites (Fig. 6E–K)]. Mapping characters on the tree: length = 14, consistency index = 0.78, and retention index = 0.94. Species classified as Plexauridae are *underlined*

bean. *Muriceopsis* spp., which grouped with gorgoniids in this analysis (*Pterogorgia* spp.), are also distributed in the western and eastern Atlantic, and the phylogeny of this genus seems to be related to a post-Pangean distribution pattern (Sánchez 2001). *Pseudopterogorgia* spp. are also present in Indo-Pacific reefs, although the Atlantic species are likely an endemic clade (Williams and Vennam 2001).

Plexaura spp. and *Eunicea* spp., on the other hand, exhibit a strikingly different biogeographic pattern. *Eunicea* is one of the more diverse genera in the Caribbean, and all 16 species occur sympatrically (Sánchez 1998). Closely related species within the genera *Eunicea* and *Plexaura* are reproductively isolated, and their genetics and morphology agree with the hypothesized species boundaries (Lasker et al. 1996; Sánchez 1998). Neither genus is found on Brazilian Atlantic reefs, where many gorgoniids occur (Bayer 1961). *Eunicea* and *Plexaura* appear to have undergone rapid speciation, as indicated by the low genetic divergence among species within their restricted geographical range.

Mitochondrial DNA sequences as phylogenetic characters for octocorals

MtDNA regions, such as genes for cytochrome oxidase I and cytochrome *b*, have been uninformative for low-level phylogenetic studies (e.g. intraspecific and closely related species) in other anthozoans such as scleractinian corals (e.g. Medina et al. 1999; Van Oppen et al. 1999). These genes also have low variation among octocorals (France and Hoover 2001, 2002). However, sequences from the NADH-dehydrogenase subunits and msh1 had sufficient variability to reconstruct holaxonian relationships. Comparisons of these Caribbean octocoral sequences with species from suborders Calcaxonia and Alcyoniina and the order Pennatulacea identified many indels in coding regions (authors' unpublished data), which suggests a great deal of phylogenetic information is also present in these genes for comparison of octocoral orders. The use of NADH-dehydrogenase subunits also provides the opportunity to include octocorals in broader phylogenies, since this gene is present in most metazoan mtDNA (Boore 1999). It is worth noting that although NADH-dehydrogenase and msh1 are among the most variable genes in the octocoral mtDNA, they provided limited information for presumably recently evolved species, such as *Eunicea* spp. and *Plexaura* spp. These genes, in fact, are invariant within species populations (LePard and France, unpublished data). Though regrettable for population and phylogeographic analyses, this low variation could be considered an advantage for delineating species boundaries. Saturation in these genes is very unlikely due to the low levels of mutation (France and Hoover 2001), which may be related to the function of the msh1 gene, which codes for a DNA mismatch repair protein (Pont-Kingdon et al. 1995; Culligan et al. 2000; Malik and Henikoff 2000).

Morphological characters and the molecular hypotheses

Using maximum parsimony, the distribution of morphological characters, such as polyp sclerites, sclerite warts, and axis mineralization, was completely consistent with the two more likely molecular phylogenetic hypotheses. Base mineralization, surface layer sclerites, and maximum spindle length, however, all required at least one homoplasy when mapped onto the molecular tree (Fig. 7, see also supplementary electronic material, Appendix 3). Sclerites and axial structures have been the most important characters for the taxonomy and classification of octocorals (e.g. Bayer et al. 1983), but an evaluation of their homology among species, genera, and families has not been made. Mapping sclerite characters onto the molecular results was consistent with genus but not family boundaries. The most variable character found was the type of surface sclerite. This character coincided with seven different clades with both phylogenetic and taxonomic meaning (*Plexaurella* spp., *Leptogorgia* spp., *Pacifigorgia* spp., *Pseudopterogorgia* spp.–*Gorgonia* spp.–*Phyllogorgia* spp.–*Pterogorgia* spp., *Muriceopsis* spp., *Muricea* spp., and *Eunicea* spp.–*Plexaura* spp.–*Pseudoplexaura* spp.). The phylogenetic utility of this character was first realized by Bayer (1953), and based on differences in surface sclerites he suggested convergent evolution for the sea fan forms of *Pacifigorgia* spp. and *Gorgonia* spp. Within each type there are also sub-types of sclerites. For instance, gorgoniid genera harboring scaphoids can be readily distinguished by modifications of the girdles and ornaments of these structures (Bayer 1961).

The explanation for such diversity of sclerite forms could be due to their functional morphology. Surface sclerites modify colony flexion by preventing compressibility of the outer cortex when they contact each other, which helps the colony to resist waves and flow forces (Lewis and Von Wallis 1991). This strong interaction with the environment could select for differences in sclerite type as a function of colony morphology. Since internal sclerites such as axial spindles are more conserved among species, it could be hypothesized that external sclerites are more likely to represent adaptive features.

Other sclerite characters such as sclerite warts and degree of polyp armature provide additional support for the molecular phylogenetic hypotheses. These sclerite traits had the same distribution among species and provided a clear delineation between derived plexaurids (*Muricea* spp.–*Pseudoplexaura* spp.–*Eunicea* spp.–*Plexaura* spp.) and basal "gorgoniids" (*Plexaurella* spp.–*Muriceopsis* spp.–gorgoniids). Derived plexaurids harbor enormous spindles that can exceed 1 mm in length. Ornamentations on their surfaces were also characteristic. Sclerites within the polyps of these species

were also robust, ornamented, and frequently formed a sclerite armature in the base of the polyp. Basal "gorgoniids," on the other hand, had the tiniest spindles and polyp rods. Their ornamentation was more regular, and the wart girdles were very symmetrical, and they often exhibited smaller, modular warts. Differences in these characters may be explained by the nature of sclerite growth in the two groups. Sclerite construction mechanisms are only known for a few species, but sclerite characters appear to be correlated to extra- versus intracellular stages of formation. Sclerite formation in plexaurids involves both intra- and extra-cellular mechanisms as well as crystallization stages that interact with other cellular structures (Goldberg and Benayahu 1987). In contrast, gorgoniid octocoral sclerites mature within single scleroblasts (Kinsley and Watabe 1982). Gorgoniid sclerites are about an order of magnitude smaller than plexaurid sclerites. Although more work is necessary to understand the complex morphological evolution of octocorals, a combination of molecular phylogenetics and morphology (sclerites and axial structures) is a very promising approach for determining phylogenetic relationships among octocoral families and, perhaps, sub-orders.

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References

Adachi J, Hasegawa M (1996) Model of amino acid substitution in proteins encoded by mitochondrial DNA. J Mol Evol 42:459–468

Bayer FM (1953) Zoogeography and evolution in the octocorallian family Gorgoniidae. Bull Mar Sci Gulf Caribb 3:100–119

Bayer FM (1956) Octocorallia. In: Moore RC (ed) Treatise of invertebrate paleontology, part F. Coelenterata. Geological Society of America and University of Kansas Press, Lawrence, pp 167–231

Bayer FM (1961) The shallow water Octocorallia of the West Indian region. Stud Fauna Curacao Other Caribb Isl 12:1-373

Bayer FM (1981) Key to the genera of Octocorallia exclusive of Pennatulacea (Coelenterata: Anthozoa), with diagnosis of new taxa. Proc Biol Soc Wash 94:902–947

Bayer FM (1991) *Thelogorgia* new-genus of gorgonacean octocorals with descriptions of four new species from the western Atlantic. Bull Mar Sci 49:506–537

Bayer FM (1992) The heloporacean octocoral *Ephiphaxum*, recent and fossil: a monographic iconography. Stud Trop Oceanogr 15:1–76

Bayer FM (2000) A new species of *Leptogorgia* from the eastern Pacific (Coelenterata: Octocorallia: Holaxonia). Proc Biol Soc Wash 113:609–616

Bayer FM, MacIntyre IG (2001) The mineral component of the axis and holdfast of some gorgonacean octocorals (Coelenterata: Anthozoa), with special reference to the family Gorgoniidae. Proc Biol Soc Wash 114:309–345

Bayer FM, Grasshoff M, Verseveldt J (eds) (1983) An illustrated trilingual glossary of morphological and anatomical terms applied to Octocorallia. Brill, Leiden, The Netherlands

Beaton MJ, Roger AJ, Cavalier-Smith T (1998) Sequence analysis of the mitochondrial genome of *Sarcophyton glaucum*: conserved gene order among octocorals. J Mol Evol 47:697–708

Berntson EA (1999) Evolutionary patterns within Anthozoa (phylum Cnidaria) reflected in ribosomal gene sequences. PhD thesis, MIT and Woods Hole Oceanographic Institution, Boston

Berntson EA, Bayer FM, McArthur AG, France SC (2001) Phylogenetic relationships within the Octocorallia (Cnidaria: Anthozoa) based on nuclear 18S rRNA sequences. Mar Biol 138:235–246

Boore JL (1999) Animal mitochondrial genomes. Nucleic Acids Res 27:1767–1780

Breedy O (2001) A new species of *Pacifigorgia* from the eastern Pacific (Coelenterata: Octocorallia: Gorgoniidae). Bull Biol Soc Wash 10:181–187

Coffroth MA, Lasker HR, Diamond ME, Bruenn JA, Bermingham E (1992) DNA fingerprints of a gorgonian coral: a method for detecting clonal structure in a vegetative species. Mar Biol 114:317–325

Culligan KM, Meyer-Gauen G, Lyons-Weiler J, Hays JB (2000) Evolutionary origin, diversification and specialization of eukaryotic MutS homolog mismatch repair proteins. Nucleic Acids Res 28:463–471

Filatov D (2001) Processor of sequences manual. University of Birmingham. Available at http://www.biosciences.bham.ac.uk/labs/filatov/proseq.html

France SC, Hoover LL (2001) Analysis of variation in mitochondrial DNA sequences (ND3, ND4L, MSH) among Octocorallia (=Alcyonaria) (Cnidaria: Anthozoa). Bull Biol Soc Wash 10:110–118

France SC, Hoover LL (2002) DNA sequences of the mitochondrial COI gene have low levels of divergence among deep-sea octocorals (Cnidaria: Anthozoa). Hydrobiologia 471:149–155

France SC, Rosel PE, Agenbroad JE, Mullineaux LS, Kocher TD (1996) DNA sequence variation of mitochondrial large-subunit rRNA provides support for a two-subclass organization of the Anthozoa (Cnidaria). Mol Mar Biol Biotechnol 5:15–28

Fu J, Murphy RW (1999) Discriminating and locating covariance: an application of permutation tail probability (PTP) analyses. Syst Biol 48:380–395

García-Parrado P, Alcolado P (1997) Nueva especie de *Eunicea* Lamouroux, 1816 (Plexauridae: Octocorallia) encontrada en Cuba. Avicennia 4/5:46–50

Gerhart DJ (1983) The chemical systematics of colonial marine animals: an estimated phylogeny of the order Gorgonacea based on terpenoid characters. Biol Bull (Woods Hole) 164:71–81

Goldberg W, Benayahu Y (1987) Spicule formation in the gorgonian coral *Pseudoplexaura flagellosa*. 1. Demonstration of intracellular and extracellular growth and the effect of ruthenium red during decalcification. Bull Mar Sci 40:287–303

Goldman N, Anderson JP, Rodrigo AG (2000) Likelihood-based tests of topologies in phylogenetics. Syst Biol 49:652–670

Grasshoff M (1988) The genus *Leptogorgia* (Octocorallia: Gorgoniidae) in West Africa. Atl Rep 14:91–147

Grasshoff M (1999) The shallow water gorgonians of New Caledonia and adjacent islands (Coelenterata: Octocorallia). Senkenb Biol 78:1–121

Grasshoff M, Alderslade P (1997) Gorgoniidae of Indo-Pacific reefs with description of two new genera (Coelenterata: Octocorallia). Senckenb Biol 77:23–35

Hall BG (2001) Phylogenetic trees made easy. Sinauer, Sunderland, Mass.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Higgins DG, Thompson JD, Gibson TJ (1996) Using CLUSTAL for multiple sequence alignments. Meth Enzymol 266:383–402

Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst Biol 142:182–192

Hillis DM, Huelsenbeck JP (1992) Signal, noise, and reliability in molecular phylogenetic analysis. J Hered 83:189–195

Huelsenbeck JP, Ronquist F (2001) MrBayes: a program for the Bayesian inference of phylogeny. Available from http://morphbank.ebc.uu.se/mrbayes/manual.pdf

Kingsley RJ, Watabe N (1982) Ultrastructural investigation of spicule formation in the gorgonian *Leptogorgia virgulata* (Lamarck) (Coelenterata: Gorgonacea). Cell Tissue Res 239:305–310

Kükenthal W (1919) Gorgonaria. Wiss Ergebn Dt Tiefsee-Exped "Valdivia" 13

Lasker HR, Kim K, Coffroth MA (1996) Reproductive and genetic variation among gorgonians: the differentiations of *Plexaura kuna*, new species. Bull Mar Sci 58:277–288

Lewis JC, Von Wallis E (1991) The function of surface sclerites in gorgonians (Coelenterata, Octocorallia). Biol Bull (Woods Hole) 181:275–288

Linnaeus C (1758) Systema Naturae. I. Editio decima, reformata. Holmiae

Macintyre IG, Bayer FM, Logan MAV, Skinner HCW (2000) Possible vestige of early phosphatic biomineralization in gorgonian octocorals (Coelenterata). Geology 28:455–458

Malik HS, Henikoff S (2000) Dual recognition-incision enzymes might be involved in mismatch repair and meiosis. Trends Biochem Sci 25:414–418

McFadden CS, Tullis ID, Hutchinson MB, Winner K, Sohm JA (in press) Variation in coding (NADH dehydrogenase subunits 2, 3 and 6) and non-coding intergenic spacer regions of the mitochondrial genome in Octocorallia (Cnidaria: Anthozoa). Mar Biotechnol

Medina M, Weil E, Szmant AM (1999) Examination of the *Montastraea annularis* species complex (Cnidaria: Scleractinia) using ITS and COI sequences. Mar Biotechnol 1:89–97

Mickevich MF, Johnson MF (1976) Congruence between morphological and allozyme data. Syst Zool 25:260–270

Pont-Kingdon GA, Okada NA, Macfarlane JL, Beagley CT, Wolstenholme DR, Cavalier-Smith T, Clark-Walker GD (1995) A coral mitochondrial mutS gene. Nature 375:109–111

Pont-Kingdon G, Okada N, Macfarlane AJL, Beagley CT, Watkins-Sims CD, Cavalier-Smith T, Clark-Walker GD, Wolstenholme DR (1998) Mitochondrial DNA of the coral *Sarcophyton glaucum* contains a gene for a homologue of bacterial MutS: a possible case of gene transfer from the nucleus to the mitochondrion. J Mol Evol 46:419–431

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. Bioinformatics 14:817–818

Posada D, Crandall KA (2001) Selecting the best-fit model of nucleotide substitution. Syst Biol 50:580–601

Sánchez JA (1998) Systematics of the Caribbean candelabrum octocorals (*Eunicea* Lamouroux, 1816; Cnidaria; Octocorallia; Gorgonacea; Plexauridae) with description of new species and some aspects of their natural history. MS thesis, National University of Colombia, Bogotá

Sánchez JA (1999) Black coral–octocoral distribution patterns on a deep-water reef, Imelda bank, Caribbean Sea, Colombia. Bull Mar Sci 65:215–225

Sánchez JA (2001) Systematics of the southwestern Caribbean *Muriceopsis* Aurivillius (Cnidaria: Octocorallia) with description of a new species. Bull Biol Soc Wash 10:160–180

Sánchez JA, Zea S, Diaz JM (1997) Gorgonian communities of two contrasting environments from oceanic Caribbean atolls. Bull Mar Sci 61:61–72

Sánchez JA, Diaz JM, Zea S (1998) Octocoral and black coral distribution patterns on the barrier reef-complex of Providencia Island, southwestern Caribbean. Caribb J Sci 34:250–264

Sánchez JA, Lasker HR, Taylor DJ (in press) Phylogenetic analyses among octocorals (Cnidaria) according to mitochondrial and nuclear DNA sequences (lsu-rRNA 16S, and ssu-rRNA 18S) support two convergent clades of branching gorgonians. Mol Phylogenet Evol

Strimmer K, von Haeseler A (1996) Quartet puzzling: a quartet maximum likelihood method for reconstructing tree topologies. Mol Biol Evol 13:964–969

Swiderski DL, Zelditch ML, Fink WL (1998) Why morphometrics is not special: coding quantitative data for phylogenetic analysis. Syst Biol 47:508–519

Swofford DL (2002) PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sinauer, Sunderland, Mass.

Van Oppen MJH, Willis BL, Miller DJ (1999) Atypically low rate of cytochrome *b* evolution in the scleractinian coral genus *Acropora*. Proc R Soc Lond B Biol Sci 266:179–179

Williams GC, Vennam JS (2001) A revision of the Indo–West Pacific taxa of the gorgonian genus *Pseudopterogorgia* (Octocorallia: Gorgoniidae), with the description of a new species from western India. Bull Biol Soc Wash 10:71–95