

A molecular phylogenetic analysis of the Octocorallia (Cnidaria: Anthozoa) based on mitochondrial protein-coding sequences

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Abstract

Despite their abundance and ecological importance in a wide variety of shallow and deep water marine communities, octocorals (soft corals, sea fans, and sea pens) are a group whose taxonomy and phylogenetic relationships remain poorly known and little studied. The group is currently divided into three orders (O: Alcyonacea, Pennatulacea, and Helioporacea); the large O. Alcyonacea (soft corals and sea fans) is further subdivided into six sub-ordinal groups on the basis of skeletal composition and colony growth form. We used 1429 bp of two mitochondrial protein-coding genes, *ND2* and *msh1*, to construct a phylogeny for 103 octocoral genera representing 28 families. In agreement with a previous 18S rDNA phylogeny, our results support a division of Octocorallia into two major clades plus a third, minor clade. We found one large clade (Holaxonia–Alcyoniina) comprising the sea fan sub-order Holaxonia and the majority of soft corals, and a second clade (Calcaxonia–Pennatulacea) comprising sea pens (O. Pennatulacea) and the sea fan sub-order Calcaxonia. Taxa belonging to the sea fan group Scleraxonia and the soft coral family Alcyoniidae were divided among the Holaxonia–Alcyoniina clade and a third, small clade (*Anthomastus–Corallium*) whose relationship to the two major clades was unresolved. In contrast to the previous studies, we found sea pens to be monophyletic but nested within Calcaxonia; our analyses support the sea fan family Ellisellidae as the sister taxon to the sea pens. We are unable to reject the hypothesis that the calcaxonian and holaxonian skeletal axes each arose once and suggest that the skeletal axis of sea pens is derived from that of Calcaxonia. Topology tests rejected the monophyly of sub-ordinal groups Alcyoniina, Scleraxonia, and Stolonifera, as well as 9 of 14 families for which we sampled multiple genera. The much broader taxon sampling and better phylogenetic resolution afforded by our study relative to the previous efforts greatly clarify the relationships among families and sub-ordinal groups within each of the major clades. The failure of these mitochondrial genes as well as previous 18S rDNA studies to resolve many of the deeper nodes within the tree (including its root) suggest that octocorals underwent a rapid radiation and that large amounts of sequence data will be required in order to resolve the basal relationships within the clade.

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1. Introduction

The anthozoan subclass Octocorallia includes over 3000 described species of soft corals, sea fans, and sea pens (Williams and Cairns, 2005). Octocorals are ecologically diverse and important members of a wide variety of

marine communities, from shallow tropical coral reefs to the deep sea. For example, soft corals are abundant and ecologically dominant organisms on coral reefs throughout the Indo-West Pacific, often occupying 50% or more of the available primary substrate (Tursch and Tursch, 1982; Dinesen, 1983; Dai, 1988; Riegl et al., 1995; Fabricius, 1997). Gorgonians (sea fans with a rigid scleroproteinaceous axis) dominate many Caribbean coral reefs (Sánchez et al., 1997, 1998), as well as deep sea communities

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(Heifetz, 2002; Watling and Auster, 2005), where their three-dimensional structure provides critical habitat for associated organisms (e.g., Krieger and Wing, 2002; Buhl-Mortensen and Mortensen, 2004a,b). Sea pens occupy soft sediment habitats over a wide depth range and are often abundant macrofaunal filter-feeders in the deep sea (Tyler, 2003). In contrast to other major groups of cnidarians for which there is a long and rich history of phylogenetic study (e.g., Veron et al., 1996; Collins et al., 2006), octocorals remain a poorly known and little studied group (Bayer, 1981a). Attempts to understand their taxonomy and phylogenetic relationships have been hampered by a paucity of useful morphological characters, widespread homoplasy and intraspecific variation in characters such as colony growth form and sclerite morphology, and a poor fossil record (Williams, 1997). To date, cladistic analyses based on morphological characters have been attempted only for the sea pens (Williams, 1994, 1997).

Throughout most of the 20th century, the sub-class Octocorallia was subdivided into seven orders (e.g., Hyman, 1940), two of which are clearly distinct morphologically: O. Helioporacea (blue corals), the only group of octocorals to form a massive aragonite skeleton and O. Pennatulacea (sea pens), in which a primary axial polyp (oozoid) differentiates into a bulbous peduncle, used to anchor the colony in soft substrate, and a distal rachis from which secondary polyps arise. The remaining five orders (Alcyonacea, Gorgonacea, Stolonifera, Telestacea, and Protoalcyonaria) were defined solely on the basis of differences in colony growth morphology. Recognizing that growth morphology represents a continuum and that morphologically intermediate taxa linked each of these groups, Bayer (1981b) combined these five orders into a single order, Alcyonacea, a revision that has been widely accepted by modern octocoral taxonomists (Fabricius and Alderslade, 2001).

The current classification system therefore divides Octocorallia into orders Alcyonacea (28 families of soft corals and sea fans), Pennatulacea (14 families of sea pens), and

Helioporacea (two families of blue corals). The large and morphologically diverse O. Alcyonacea is further subdivided into six sub-ordinal groups that are distinguished on the basis of colony architecture and composition of the skeletal axis, if present (Table 1). The sea fan sub-orders Holaxonia and Calcaxonia (Grasshoff, 1999) represent morphologically discrete entities, but the other four groups (Stolonifera, Alcyoniina, Scleraxonia, and Protoalcyonaria) grade into one another morphologically, and consequently have been classified loosely as sub-ordinal “groups” rather than sub-orders (Fabricius and Alderslade, 2001).

Several recent molecular phylogenetic studies of class Anthozoa using 18S rDNA or 16S mtDNA sequences have supported the monophyly of Octocorallia and have divided the sub-class into either two or three distinct clades (France et al., 1996; Berntson et al., 1999, 2001; Won et al., 2001; Sánchez et al., 2003a). The most taxonomically comprehensive of these studies found support for three major clades of Octocorallia, with one clade representing most of the sea pens, and each of the other two clades comprising a heterogeneous mix of taxa from most or all of the six sub-ordinal groups of Alcyonacea (Berntson et al., 2001). Although these data suggest a lack of phylogenetic support for the current sub-ordinal taxonomic groupings, relationships among the disparate taxa of Alcyonacea included in each major clade could not be inferred due to insufficient phylogenetic resolution.

Here, we construct a phylogeny for sub-class Octocorallia using partial sequences of two mitochondrial protein-coding genes: *ND2* and *msh1*, a *mutS* homolog that is found in the mitochondrial genome of all octocorals but no other metazoans (Pont-Kingdon et al., 1995, 1998; Culligan et al., 2000; France and Hoover, 2001). Because rates of octocoral mitochondrial gene evolution are very slow compared to other animals, these genes are informative for genus- and family-level phylogenetic analyses (France and Hoover, 2001, 2002; McFadden et al., 2004), but lack sufficient resolution to discriminate species within many genera (Sánchez et al., 2003b; Wirshing et al., 2005; Cairns and Bayer, 2005). We compare the resulting mitochondrial gene phylogeny to those obtained

Table 1
Current higher taxonomic classification of the anthozoan subclass Octocorallia

Taxonomic group	<i>N</i>	Defining characteristics
O. Pennatulacea [sea pens]	14	Axial polyp differentiated into basal peduncle and distal rachis
O. Helioporacea [blue coral]	2	Massive aragonite skeleton
O. Alcyonacea		
[soft corals—no skeletal axis]		
Grp. Protoalcyonaria	2 ^a	Solitary polyps
Grp. Stolonifera	5	Polyps united basally by simple stolons which may fuse to form ribbons
Grp. Alcyoniina	5	Polyps united within fleshy mass of coenenchyme
[sea fans—with skeletal axis]		
Grp. Scleraxonia	7	Inner axis (or axial-like layer) consisting predominantly of sclerites
SO. Holaxonia	4	Axis of scleroproteinous gorgonin, commonly with small amounts of embedded non-scleritic CaCO ₃ ; axis with hollow cross-chambered central core
SO. Calcaxonia	5	Axis of scleroproteinous gorgonin with large amounts of non-scleritic CaCO ₃ as internodes or embedded in the gorgonin; axis without hollow cross-chambered central core

N, number of described families (from Williams and Cairns, 2005).

^a One described family may not be valid.

previously using 18S rDNA and address the following specific questions: (1) What is the phylogenetic relationship between orders Alcyonacea and Pennatulacea? (2) Is there phylogenetic support for the taxonomic division of O. Alcyonacea into 6 sub-ordinal groups? (3) Is there phylogenetic support for the current family-level taxonomy of O. Alcyonacea? Although many aspects of octocoral phylogeny, including the root of the clade, remain uncertain, our study represents the most comprehensive phylogenetic treatment of this group to date, and provides a solid framework for future taxonomic revision and further testing of phylogenetic hypotheses.

2. Materials and methods

2.1. DNA extraction, amplification, and sequencing

Specimens used in this study included EtOH-preserved material from museum collections as well as fresh material collected by the authors. We obtained representatives of all three orders of Octocorallia, 5 of 6 sub-ordinal groups, 21 of 28 recognized families of O. Alcyonacea, and 7 of 14 families of O. Pennatulacea. A complete list of specimens including collection information, GenBank, and museum voucher catalog numbers is provided in [Appendix A](#).

DNA was extracted from specimens using previously published methods (e.g., [Berntson and France, 2001](#); [McFadden et al., 2001](#); [Sánchez et al., 2003b](#)). We used the primers of [McFadden et al. \(2004\)](#) to amplify the 5' end of the NADH-dehydrogenase subunit 2 gene (*ND2*) (16S647F: 5'-ACACAGCTCGGTTTCTATCTACCA-3'; ND21418R: 5'-ACATCGGGAGCCACATA-3'), and primers ND42599F (5'-GCCATTATGGTTAACTATTAC-3') ([France and Hoover, 2002](#)) or ND42625F (5'-TACGTG GYACAATTGCTG-3') ([Lepard, 2003](#)) and Mut-3458R (5'-TSGAGCAAAGCCACTCC-3') ([Sánchez et al., 2003b](#)) to amplify the 5' end of *msh1*. PCR protocols followed [Sánchez et al. \(2003b\)](#) and [McFadden et al. \(2004\)](#). For specimens that yielded no visible PCR product, we ran a second PCR reaction using an internal forward primer and 1 μ L of the original product as template (e.g., [Berntson and France, 2001](#)). For *msh1*, we used ND42625F as the internal primer; for *ND2*, initial amplification was done using forward primer 16S544F (5'-CGACCTCGATGTT GAGTTGCGG-3') and 16S647F was used as the internal primer. Negative (no DNA) controls from the first reaction were re-amplified in the second reaction to check for sample contamination. PCR products were purified using a PEG-precipitation ([Sánchez et al., 2003b](#)) or agarase-digestion protocol ([France and Hoover, 2002](#)), cycle-sequenced and run on ABI3100 (PE Applied Biosystems), CEQ8000 (Beckman Coulter) or Global IR2 DNA (Li-Cor) automated sequencers.

2.2. Data analysis

LaserGene software was used to translate nucleotide sequences using the cnidarian mitochondrial genetic code

([Pont-Kingdon et al., 1994](#)). The amino acid sequences were aligned using ClustalX v. 1.81 ([Thompson et al., 1997](#)) and adjusted by eye. Phylogenetic trees were constructed using corresponding nucleotide alignments for each gene separately as well as for the combined dataset. Bayesian phylogenetic analyses were conducted using MrBayes v. 3.04 ([Huelsenbeck and Ronquist, 2001](#)) with a GTR+I+ Γ model run for 1.5×10^6 generations (burnin = 3750 generations) and separate data partitions for *ND2* and *msh1* in the combined analysis. PAUP* v. 4.0b10 ([Swofford, 2002](#)) was used for both maximum parsimony and maximum-likelihood analyses. For maximum parsimony, we used a heuristic search with TBR branch-swapping; due to computational and time constraints, we ran 100 bootstrap replicates with a maximum of 1000 trees saved per replicate. Additional MP analyses were run with gaps coded as fifth nucleotides. Maximum-likelihood analyses were run with model parameters (TVM+I+G) chosen using Modeltest v. 3.06 ([Posada and Crandall, 1998](#)); both the Akaike Information Criterion (AIC) and hierarchical likelihood ratio test (hLRT) selected this same model. A quartet-puzzling maximum-likelihood approach with the model mtREV24 ([Adachi and Hasegawa, 1996](#)) implemented in TREE-PUZZLE 5.2 ([Schmidt et al., 2002](#)) was used to analyze amino acid alignments for the purpose of rooting the octocoral tree. We used a weighted SH (WSH) test ([Shimodaira and Hasegawa, 1999](#)) and Shimodaira's (2002) approximately unbiased (AU) test to compare the topology of the best-fit maximum-likelihood tree to alternative trees in which we constrained specified sub-ordinal groups or families to be monophyletic. Both tests were implemented using the program CONSEL run with 10 sets of 100,000 bootstrap replicates ([Shimodaira and Hasegawa, 2001](#)). We chose these topology tests over the widely used SH test ([Shimodaira and Hasegawa, 1999](#)) because the latter is overly conservative and sensitive to the number of trees being compared ([Buckley, 2002](#); [Shimodaira, 2002](#)).

3. Results

We obtained both *msh1* and *ND2* sequences for a total of 103 genera of octocorals, and sequences of one of the two genes for an additional 12 genera ([Appendix A](#)). Whenever possible we sequenced at least two representatives of each genus. In most cases, congeners had identical or very similar nucleotide sequences, so we included only a single representative species from each genus in the phylogenetic analysis. Multiple representatives were included, however, for several genera whose relationships with related taxa appeared to be paraphyletic (e.g., *Acanthogorgia*, *Alcyonium*, *Iciligorgia*, and *Pseudopterogorgia*). Several genera shared identical mtDNA sequences, including *Nephtheal* *Litophyton*, *Xenia/Heteroxenia*, and *Paralemmalia digitiformis/Lemnaia*. We included only one representative sequence from each of these pairs in the analysis.

The *ND2* fragment ranged in length from 148 to 158 amino acids. Relative to the longest sequence (*Telestula*),

most species had one or two deletions of 3–6 amino acids each near the 5' end of the gene. The *msh1* fragment ranged in length from 235 to 272 amino acids. Much of this length variation was attributable to three genera with large, unique insertions: *Lepidisis*, with three insertions of 11, 8, and 8 amino acids; *Briareum*, with insertions of 9, 4, and 4 amino acids; and *Telestula*, with a single unique 9 amino acid insertion. Despite these and other indels, nucleotide sequences of both genes maintained the correct reading frame, and we have no reason to suspect a loss of coding function for either gene. The final nucleotide alignment of the two genes combined was 1429 bp in length and included 474 bp of *ND2* and 955 bp of *msh1*. Of these 1429 nucleotides, 390 characters were invariant, and 745 of 1039 variable sites were parsimony-informative (including gaps as fifth characters).

3.1. Rooting

Because no other metazoans are known to have the mitochondrial *msh1* gene (Culligan et al., 2000), we were unable to root the *msh1* tree using a non-octocorallian outgroup. Attempts to use yeast *msh1* as an outgroup sequence were unsuccessful due to our inability to align the amino acid sequences with any certainty. Attempts to root the *ND2* tree using non-octocoral anthozoan *ND2* sequences (*Acropora tenuis*, *Metridium senile*) were impaired by length differences between octocoral and hexacoral *ND2*. Because the octocoral *ND2* is 70 amino acids longer at its 5' end, we were able to align only 85 amino acids from the 3' end of the *ND2* fragment with the *Acropora* and *Metridium* sequences. Although maximum parsimony, Bayesian, and neighbor-joining analyses of the corresponding nucleotide alignments all placed *Erythropodium* at the base of the octocoral tree, analysis of this short fragment provided no support for any internal nodes within the Octocorallia clade. A quartet-puzzling maximum-likelihood analysis of the *ND2* amino acid sequence using the mtREV24 model of evolution (Adachi and Hasegawa, 1996) provided somewhat better resolution of internal nodes and placed at the base of the tree an unresolved group of taxa that included both *Erythropodium* and *Briareum*. Because the outgroup-rooted 18S phylogeny of Berntson et al. (2001) also weakly supported placement of these taxa at the base of the octocoral clade, we have shown our phylogenetic trees rooted with *Erythropodium* as the sister taxon to the remaining

Octocorallia. Mid-point rooting yields a very similar tree topology that does not change our interpretation of clade structure and membership.

3.2. Trees

Bayesian, maximum parsimony, and maximum-likelihood analyses all recovered very similar topologies, and a majority of the nodes that was well supported (>90%) by the Bayesian posterior probabilities also had strong support (>70%) from maximum parsimony bootstrap values (Fig. 1). Two distinct clades of octocorals were recovered in all analyses. One very large and well-supported clade included all members of the sea fan sub-order Holaxonia, a majority of the soft corals belonging to sub-ordinal group Alcyoniina, and some taxa of Scleraxonia and Stolonifera. The second clade, very well supported by the Bayesian posterior probabilities but only weakly supported by bootstrap values (Fig. 1), included all sea pens (O. Pennatulacea), the blue coral *Heliopora* (O. Helioporacea), and all members of the sea fan sub-order Calcaxonia. A third small clade, whose position relative to the two major clades remained unresolved, included several genera belonging to the soft coral family Alcyoniidae as well as the precious coral *Corallium*. To simplify, herein we refer to these three clades as Holaxonia–Alcyoniina, Calcaxonia–Pennatulacea, and *Anthomastus*–*Corallium*.

The only discrepancies among phylogenetic methods were in the levels of support for some of the deeper nodes within each of the two major clades. Bayesian analyses strongly supported the separation of a heterogeneous group of taxa at the base of the Holaxonia–Alcyoniina clade, including several stoloniferans (*Tubipora*, *Rhodolinda*, and *Telesto*), scleraxonian sea fans (*Iciligorgia*, *Solenocaulon*), nidaliid soft corals (*Nidalia*, *Chironophthya*, *Siphonogorgia*), and the aberrant alcyoniid soft coral *Mallacacanthus*. Although maximum parsimony analyses also positioned a subset of these taxa as a sister clade to the remaining Holaxonia–Alcyoniina, bootstrap values did not support this separation. Several basal nodes within the Calcaxonia–Pennatulacea clade were also more strongly supported by the Bayesian methods than maximum parsimony, in particular, the monophyly of Calcaxonia+Pennatulacea+Helioporacea. Coding gaps as fifth characters in the maximum parsimony analyses weakened support for these nodes even further, but had little effect on support for

Fig. 1. Phylogenetic relationships among 103 genera in the anthozoan sub-class Octocorallia. Left: Maximum parsimony tree, strict consensus of 38,448 equally parsimonious trees of length 3714; values at nodes are percentages from 100 bootstrap replicates with maxtrees = 1000. Right: Bayesian likelihood tree, 50% majority-rule consensus of 12,251 trees (1.5×10^6 generations; burnin = 3750); values at nodes are posterior probabilities. Circled numbers indicate the clades discussed in the text: 1, Holaxonia–Alcyoniina; 2, Calcaxonia–Pennatulacea; 3, *Anthomastus*–*Corallium*. Clades and taxon names shown in gray indicate regions of disagreement between the two trees. Colored bars indicate higher taxonomic groups: color indicates sub-ordinal group or order, letter abbreviation within bar indicates family. Light blue, Alcyoniina; green, SO. Calcaxonia; red, SO. Holaxonia; sage, Scleraxonia; yellow, Stolonifera; pink, O. Pennatulacea; dark blue, O. Helioporacea. *Family abbreviations*: Ac, Acanthogorgiidae; Al, Alcyoniidae; An, Anthothelidae; Br, Briareidae; Ce, Coelogorgiidae; Ch, Chrysogorgiidae; Cl, Clavulariidae; Co, Coralliidae; El, Ellisellidae; Go, Gorgoniidae; H, O. Helioporacea; Is, Isidiidae; Ke, Keroeidiidae; Ne, Nephthidae; Ni, Nidaliidae; Pa, Paralcyoniidae; P, O. Pennatulacea; Pl, sF. Plexaurinae (F. Plexauridae); Pr, Primnoidae; St, sF. Stenogorgiinae (F. Plexauridae); Tu, Tubiporidae; Xe, Xenidae.

any of the internal relationships within the Holaxonia–Alcyoniina clade. The position of the stoloniferan genus *Clavularia* as a sister clade to Holaxonia–Alcyoniina was well supported by all analyses.

All phylogenetic methods recovered a monophyletic O. Pennatulacea, but none of the five sub-ordinal groups of O. Alcyonacea was monophyletic. The maximum-likelihood tree ($-\ln L = 20319.07$) (Fig. 2) was significantly better than alternative trees in which the monophyly of Holaxonia, Calcaxonia, Alcyoniina, Scleraxonia, and Stolonifera was sequentially enforced (AU and WSH topology tests, $p < 0.01$) (Table 2). Representatives of groups Alcyoniina, Scleraxonia, and Stolonifera occurred both in the well-supported Holaxonia–Alcyoniina clade and among the unresolved nodes at the base of the tree. Although there was moderate support for most of the scleraxonians and stoloniferans forming a sister clade to the remaining Holaxonia–Alcyoniina, genera such as *Anthothela* (Scleraxonia) and *Coelogorgia* (Stolonifera) were nested well within clades of Alcyoniina, while *Erythropodium*, *Briareum* (both Scleraxonia), and *Telestula* (Stolonifera) fell entirely outside of Holaxonia–Alcyoniina.

The sea fan sub-order Calcaxonia was paraphyletic with O. Pennatulacea, with all analyses strongly supporting the family Ellisellidae as the sister taxon to the sea pens. A tree in which the *Anthomastus–Corallium* clade was constrained to be the sister group to the sea pens had a significantly lower likelihood (Table 2). Maximum-likelihood and Bayesian analyses also placed the blue coral *Heliopora* (O. Helioporacea) within Calcaxonia–Pennatulacea, but a constrained tree in which *Heliopora* was excluded from that clade was not significantly less likely than the maximum-likelihood tree (Table 2).

Although confined to the Holaxonia–Alcyoniina clade, members of the sea fan sub-order Holaxonia fell into a number of distinct sub-clades among which basal relationships could not be resolved. When all taxa were included in the analysis, the monophyly of Holaxonia was rejected (Table 2). However, when *Thrombophyton*, a soft coral genus that nested within a mixed clade of holaxonian sea fans, was removed from the analysis, the maximum-likelihood tree ($-\ln L = 20246.31$) was only marginally better than a tree with holaxonian monophyly enforced ($-\ln L = 20262.06$) (AU test, $p = 0.04$; WSH test, $p = 0.085$). In the absence of *Thrombophyton*, therefore, we cannot with confidence reject the monophyly of Holaxonia.

In addition to the lack of support for monophyly of most of the sub-ordinal groups of Alcyonacea, most of the families for which we sampled multiple genera were also polyphyletic (Table 2). Genera belonging to the families Alcyoniidae [Alcyoniina], Clavulariidae [Stolonifera], and Anthothelidae [Scleraxonia] were found both within Holaxonia–Alcyoniina and outside of that clade. Topology tests rejected alternative trees that enforced the monophyly of each of these families, along with the holaxonian sea fan families Plexauridae, Gorgoniidae, and Acanthogorgiidae and the soft coral families Nephtheidae, Nidaliidae, and

Xeniidae (Table 2). Only the four calcaxonian sea fan families, Ellisellidae, Isididae, Chrysogorgiidae, and Primnoidae, were monophyletic in our analysis.

Phylogenetic analyses of each gene separately produced trees that were similar in topology but only weakly supported relative to the combined tree (Fig. 3). Single-gene analyses did, however, allow us to examine the phylogenetic positions of some taxa for which we were able to sequence only one of the two genes. On the basis of analyses of *ND2* alone, two additional alcyoniid soft coral genera, *Paraminabea* and *Notodysiferus*, belonged to the small *Anthomastus–Corallium* clade. The xeniid genera *Sympodium* and *Efflatounaria* were strongly supported as members of the xeniid clade, with *Efflatounaria* positioned basal to a polytomy of seven other xeniid genera, and the sea pen genus *Anthoptilum* fell within the pennatulacean clade. *msh1* sequences placed the scleraxonian genera *Mopsella* and *Asperaxis* in a clade with scleraxonians *Iciligorgia* and *Solenocaulon*, and the plexaurid *Paracis* within the Stenogorgiinae–Acanthogorgiidae clade (weakly supported as a sister taxon to *Lepidomuricea*). The alcyoniid *Elbeenus lauramartinae* and acanthogorgiid *Cyclomuricea* both belonged to the Holaxonia–Alcyoniina clade, but neither taxon grouped with other family members. The two genera that comprise the calcaxonian family Ifalukellidae (*Ifalukella*, *Plumigorgia*) formed a well-supported clade that grouped with other calcaxonians.

4. Discussion

Our mitochondrial gene phylogeny supports the results of previous 18S rDNA and 16S mtDNA studies that divide the subclass Octocorallia into two or three distinct clades (France et al., 1996; Berntson et al., 2001; Won et al., 2001; Sánchez et al., 2003a). One clade includes all members of the sea fan sub-order Holaxonia, a majority of the taxa belonging to the soft coral group Alcyoniina, and most of the taxa in groups Scleraxonia and Stolonifera. This clade corresponds well to clade C of Berntson et al.'s (2001) 18S phylogeny and the “Alcyoniina–Holaxonia” clade of Sánchez et al. (2003a). A second large clade includes all of the sea pens (O. Pennatulacea), blue corals (O. Helioporacea), and the sea fan sub-order Calcaxonia. Combined with the small *Anthomastus–Corallium* clade, this clade corresponds to the “Calcaxonia” clade of Sánchez et al. (2003a). Berntson et al.'s (2001) 18S phylogeny subdivided the Calcaxonia–Pennatulacea clade into two separate clades, one (B) corresponding to the Pennatulacea+Ellisellidae clade in our tree and the other (A) comprising three calcaxonian families plus the *Anthomastus–Corallium* clade. Our analyses of mtDNA support a monophyletic Calcaxonia+Pennatulacea that excludes *Anthomastus–Corallium*, and a tree that enforces the three clades found by Berntson et al. (2001) is significantly less likely than our best ML tree based on the AU test (but not the WSH test; Table 2).

One discrepancy between our phylogeny and the less resolved phylogeny of Berntson et al. (2001) is the position

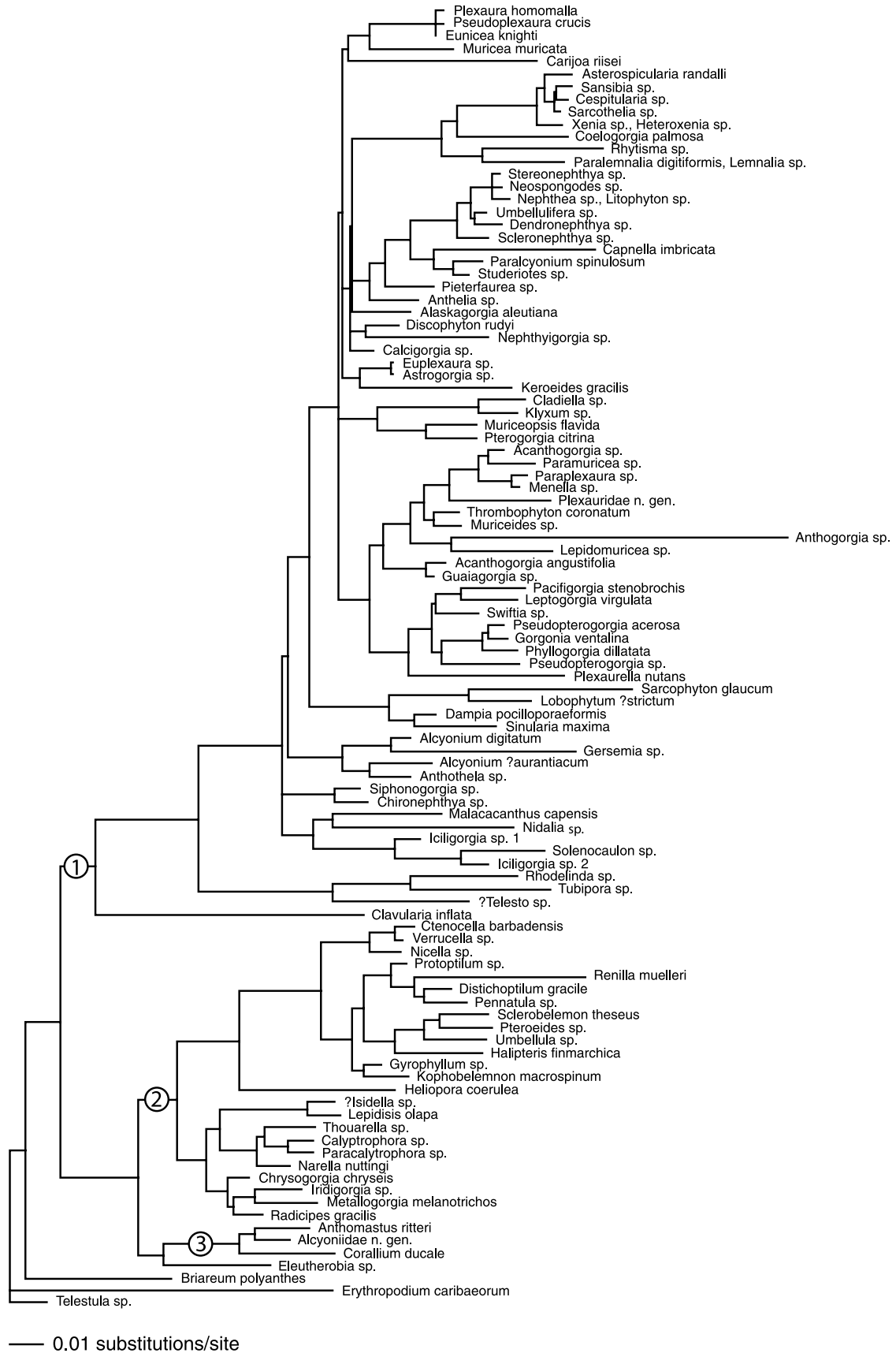


Fig. 2. Maximum-likelihood phylogram ($-\ln L = 20319.07$) of relationships among 103 genera in the anthozoan sub-class Octocorallia. Circled numbers indicate the clades discussed in the text: 1 = Holaxonia–Alcyoniina; 2 = Calcaxonia–Pennatulacea; 3 = *Anthomastus*–*Corallium*.

Table 2
Log-likelihood scores of trees with specified taxonomic groups of octocorals constrained to be monophyletic

Constrained group	–ln L	AU	WSH
None (best ML tree)	20319.07		
Holaxonia	20402.53	***	***
Calcaxonia	20388.12	***	***
Alcyoniina	20800.90	***	***
Stolonifera	20559.14	***	***
Scleraxonia	20638.85	***	***
(Pennatulacea+Calcaxonia)	20324.35	ns	ns
(Pennatulacea+Anthomastus)	20401.15	***	***
Berntson et al. (A+B+C)	20345.50	**	ns
F. Alcyoniidae	20867.73	***	***
F. Nephtheidae	20498.25	***	***
F. Nidaliidae	20352.69	**	ns
F. Xenidae	20396.59	***	***
F. Plexauridae	20693.42	***	***
F. Gorgoniidae	20520.88	***	***
F. Acanthogorgiidae	20549.58	***	***
F. Clavulariidae	20521.06	***	***
F. Anthothelidae	20431.61	***	***

Likelihood differences between the best ML tree (Fig. 2) and constrained trees were compared using Shimodaira's (2002) approximately unbiased (AU) test and a weighted Shimodaira–Hasegawa (WSH) test (Shimodaira and Hasegawa, 1999). *** $p < 0.001$; ** $p < 0.01$; ns: $p > 0.05$.

of *Heliopora* (O. Helioporacea), which they placed in the Holaxonia–Alcyoniina (clade C) as a sister taxon to *Alcyonium gracillimum*. They questioned this placement, however, because the *Heliopora* sequence used in their analyses was very incomplete. Our contrasting placement of *Heliopora* within the Calcaxonia–Pennatulacea clade was well supported by both *ND2* and *msh1* sequences. Berntson et al. also failed to recover a monophyletic Pennatulacea due to the inclusion of the sea pen *Umbellula* in a clade with *Anthomastus* and *Corallium*, a result not supported by our mitochondrial data. Finally, their phylogeny included a partial sequence for the calcaxonian genus *Plumigorgia* (Family Ifalukellidae) within Holaxonia–Alcyoniina. Our analyses of *msh1* unite both *Plumigorgia* and *Ifalukella* with the other calcaxonian families in Calcaxonia–Pennatulacea (Fig. 3A).

All molecular phylogenetic studies conducted to date support the division of the sub-class into at least two major clades, neither of which corresponds to the traditional ordinal divisions within sub-class Octocorallia. Both mitochondrial and nuclear 18S rDNA studies support the separation of sea pens (O. Pennatulacea) and the sea fan sub-order Calcaxonia into one major clade, and the sea fan sub-order Holaxonia plus the majority of soft corals and other groups of Alcyonacea into the other clade. Both types of molecular data also recognize the scleraxonian sea fan *Corallium*, soft coral *Anthomastus*, and several other genera as members of a third small clade whose relationship to the two major clades remains uncertain.

4.1. Relationship between Alcyonacea and Pennatulacea

On the basis of a cladistic analysis of morphological characters, Williams (1994, 1997) hypothesized that the sea

pens, a group with a highly derived colony growth morphology, evolved from a soft coral ancestor similar to the alcyoniid soft coral genus *Anthomastus*. Although our molecular analyses suggest that *Anthomastus* may be more closely related to the Calcaxonia–Pennatulacea clade than other soft corals, our data do not support a sister relationship between the *Anthomastus*–*Corallium* clade and the sea pens. Instead, our phylogeny strongly supports the calcaxonian sea fan family Ellisellidae as the sister group to O. Pennatulacea, a relationship Bayer (1955) proposed on the basis of observed similarities in the axial structure of the two groups. Observed under SEM, the structure and orientation of calcareous microcrystals in the axis of an ellisellid (*Ctenocella* sp.) and a pennatulacean (Virgulariidae) appear remarkably similar (J.A. Sánchez, unpubl. data). It is possible, however, that inclusion in our analysis of the genera *Veretillum* and *Echinoptilum*, hypothesized by Williams (1994, 1997) to be the most primitive sea pen groups, could change the inferred phylogenetic relationships within the Calcaxonia–Pennatulacea clade.

4.2. Phylogenetic support for sub-ordinal groups of Alcyonacea

Our results provide phylogenetic support for only two of the six currently recognized sub-ordinal divisions within O. Alcyonacea (Table 1). These taxonomic divisions are widely acknowledged to be problematic and likely to reflect grades of colony architecture rather than phylogenetic relationships (Fabricius and Alderslade, 2001). Of the six groups, only the sea fan sub-orders Holaxonia and Calcaxonia are morphologically discrete and defined by synapomorphies. The skeletal axis of all calcaxonians has a core of scleroproteinous gorgonin with large amounts of embedded, non-scleritic calcium carbonate. In contrast, the skeletal axis of holaxonians contains only small amounts of embedded calcium carbonate (mostly non-scleritic, although one group has sclerites in the axis) and has a hollow cross-chambered core. Our phylogeny is consistent with a single-evolutionary origin of the calcaxonian skeletal axis and with the axis of the sea pens having been derived from that of a calcaxonian ancestor. Our phylogeny is also consistent with a single-evolutionary origin of the holaxonian skeletal axis, provided we entertain the possibility that *Thrombophyton*, a genus of encrusting soft corals nested deep within a clade of holaxonians, may be a holaxonian that has secondarily lost the skeletal axis. Although the basal nodes within our Holaxonia–Alcyoniina clade remain unresolved, we are unable to reject with confidence the monophyly of Holaxonia when *Thrombophyton* is removed from the analysis. The molecular phylogeny, therefore, supports the taxonomic division of sea fans into the distinct sub-orders Calcaxonia and Holaxonia.

Our molecular data do not, however, support the phylogenetic distinction of the other three sub-ordinal groups of Alcyonacea included in our analysis (Alcyoniina, Scleraxonia, and Stolonifera). Representatives of each of these

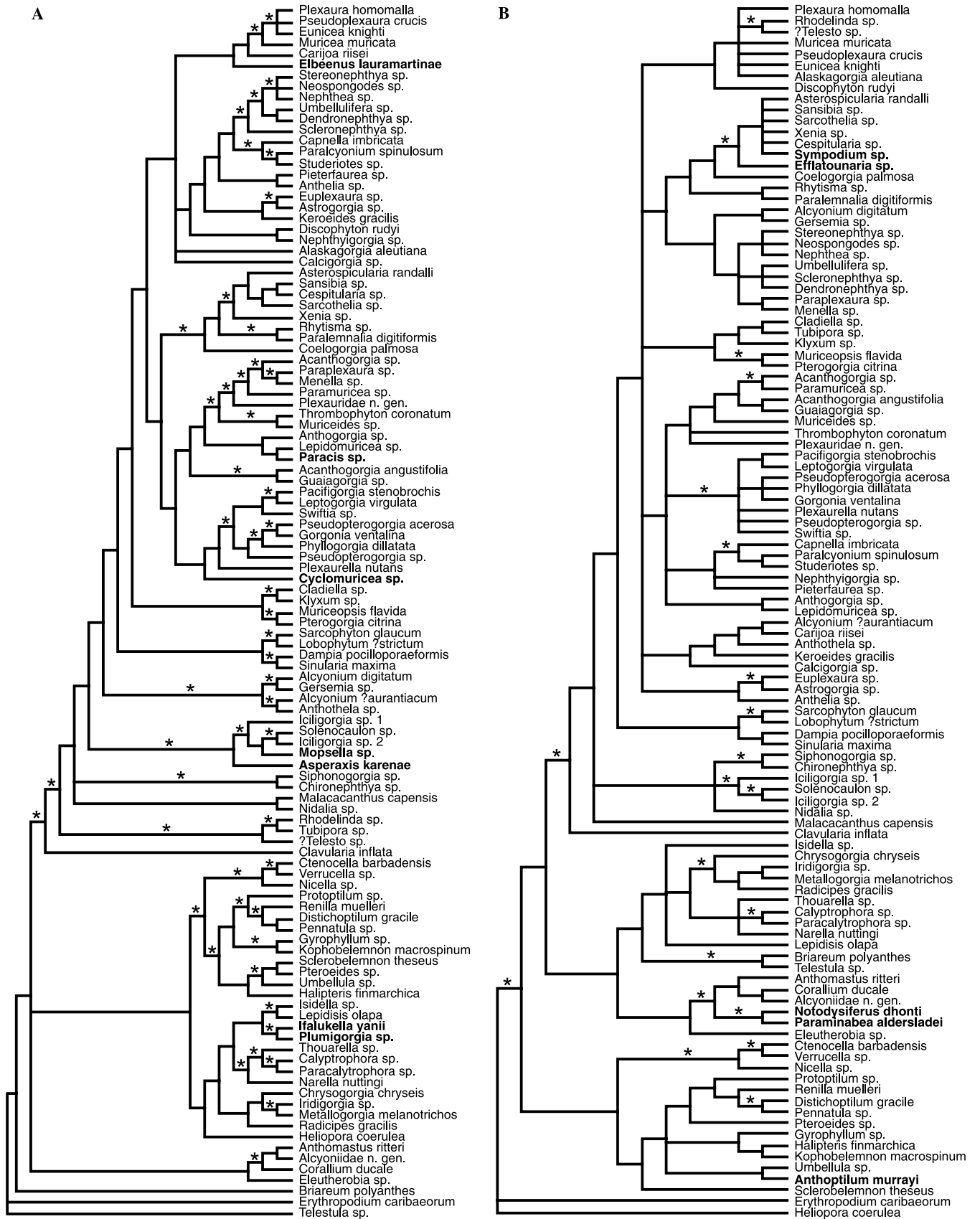


Fig. 3. Phylogenetic relationships among octocoral genera based on single-gene analyses of (A) *msh1* and (B) *ND2*. Maximum-likelihood tree topology is shown (*msh1*: $-\ln L = 16012.12$; *ND2*: $-\ln L = 5363.58$). Asterisks indicate nodes that had maximum parsimony bootstrap values >70% and Bayesian posterior probabilities >90%. The same parameters were used for all analyses as for the combined tree. Boldface indicates species that were not included in the combined analysis.

groups were distributed throughout the Holaxonia–Alcyoniina clade, the *Anthomastus*–*Corallium* clade, and the poorly resolved basal region of the octocoral tree. Our phylogeny therefore supports the assumption, evidenced by the reluctance of octocoral taxonomists to elevate them to the status of sub-orders, that these groups represent grades of morphological construction that have likely evolved repeatedly during the history of Octocorallia. For instance, although the taxa included within the group Scleraxonia share a skeletal axis or axial-like layer containing sclerites, the details of skeletal construction differ substantially among families (Fabricius and Alderslade, 2001), consistent with multiple independent derivations of this type of axis.

Although the majority of the soft coral taxa classified as Alcyoniina belonged to the Holaxonia–Alcyoniina clade, a notable subset of genera fell into the small *Anthomastus*–*Corallium* clade. These genera all belong to the morphologically heterogeneous family Alcyoniidae and include *Anthomastus*, *Paraminabea*, *Notodysiferus*, and an undescribed genus with an encrusting growth form. The scleraxonian genus *Corallium* was united with these alcyoniids, and the 18S study of Berntson et al. (2001) also placed the scleraxonian *Paragorgia* in this same clade. Sánchez (2005) noted the similarity and possible homology of the surface sclerites in *Corallium* and the two paragorgiid genera *Paragorgia* and *Sibogorgia*, sclerite forms that also resemble those found in *Anthomastus* (Broch and Horridge, 1957). A more obvious morphological trait shared by all of these disparate genera is polyp dimorphism. Our results do not, however, support Berntson et al.'s (2001) suggestion that dimorphism has evolved only once in Octocorallia and is a synapomorphy shared by this clade and the sea pens. We find no support for a sister relationship between the sea pens and the *Anthomastus*–*Corallium* clade. Moreover, although *Corallium* and *Paragorgia* are the only dimorphic scleraxonians (Bayer, 1964), there are several other dimorphic genera of soft corals (the alcyoniids *Sarcophyton* and *Lobophytum* and the xeniid *Heteroxenia*) that do not belong to the *Anthomastus*–*Corallium* clade. Polyp dimorphism, therefore, has clearly evolved independently within multiple lineages of octocorals.

4.3. Family monophyly

Any inferences we can make regarding the monophyly of octocoral families are necessarily limited by taxon sampling, as in no case does our phylogeny include all of the genera belonging to a family (with the exception of monotypic families). Despite this limitation, we can nonetheless conclude that most families of octocorals do not represent monophyletic groups (Fig. 1; Table 2). The main exceptions are the calcaxonian sea fan families Ellisellidae, Chrysogorgiidae, Primnoidae, Isididae, and Ifalukellidae, all of which form well-supported clades in our trees. However, we have sampled representatives of only one of four described sub-families of Isididae, which additional molecular data suggest may not constitute a monophyletic group (S.C. France, unpubl. data).

The pitfalls of inferring intra-familial relationships when taxon sampling is incomplete are illustrated by our results for the holaxonian sea fan families Plexauridae and Gorgoniidae. Several previous molecular systematic studies have already addressed the relationships among taxa in these two families, concluding that they are paraphyletic and comprise at least three major clades (Gorgoniidae, Plexaurinae, and Stenogorgiinae) plus several minor clades among which the basal relationships are unclear (Sánchez et al., 2003b; Wirshing et al., 2005). Our results support those conclusions. Neither previous study, however, included representatives of the holaxonian family Acanthogorgiidae. Our results indicate that the acanthogorgiid genera *Acanthogorgia* and *Anthogorgia* also fall within Stenogorgiinae; moreover, within this clade, *Acanthogorgia* comprises two distinct lineages that are not closely related. This previously unrecognized paraphyletic relationship between Acanthogorgiidae and Stenogorgiinae further confounds the morphological arguments that have been offered as support for the resurrection of family Paramuriceidae (= Stenogorgiinae) (Wirshing et al., 2005).

4.4. Conclusions

Our phylogenetic analysis of mitochondrial protein-coding sequences supports the two-clade structure of sub-class Octocorallia seen in previous analyses of 18S rDNA and 16S mtDNA (France et al., 1996; Berntson et al., 2001; Won et al., 2001; Sánchez et al., 2003a). Our study includes the most comprehensive taxon sampling to date and provides strong support for the phylogenetic relationships among genera within many octocoral families. Most of the basal nodes in our tree, however, are still poorly resolved, and we are unable to infer the phylogenetic relationships among most families, especially within the large Holaxonia–Alcyoniina clade. The failure of both 18S rDNA (Berntson et al., 2001) and the more rapidly evolving mitochondrial markers used here to resolve these deeper nodes suggests that the Holaxonia–Alcyoniina clade underwent a rapid radiation (Page and Holmes, 1998). Unfortunately, the lack of a fossil record for the group means we are unable to estimate dates of divergence or corroborate this hypothesis with direct fossil evidence. It is likely that the basal relationships within Holaxonia–Alcyoniina will be resolved only by the eventual accumulation of large amounts of sequence data for a variety of nuclear genes.

The root of the Octocorallia phylogeny also remains uncertain, and further molecular studies using genes that are shared and can be aligned with certainty among octocorals and their sister group, Hexacorallia, will be necessary to confirm the basal topology of the clade. Although our *ND2* data and the 18S rDNA data of Berntson et al. (2001) both suggest that the scleraxonian genera *Erythropodium* and *Briareum* lie at or near the base of Octocorallia, neither study provides strong support for this root. Our conclusions regarding the division of Octocorallia into two major clades would, however, require re-interpretation only if the

root was shown to lie within either the Holaxonia–Alcyoniina or Calcaxonia–Pennatulacea clade. Neither study provides strong support for that scenario.

Despite the lack of basal resolution in our phylogeny, it is already clear that a number of families in O. Alcyonacea are in need of taxonomic revision. In particular, families Alcyoniidae, Anthothelidae, and Clavulariidae each comprise phylogenetically heterogeneous groups of genera. The holaxonian sea fan families Plexauridae, Gorgoniidae, and Acanthogorgiidae are paraphyletic, and although we recovered several distinct, well-supported holaxonian clades, their membership does not reflect current morphology-based taxonomic divisions. Likewise, the soft coral families Nephthelidae and Paraclyoniidae are paraphyletic, and several genera of Nephthelidae belong to disparate clades.

Although the molecular phylogeny does not support the division of O. Alcyonacea into the six sub-ordinal groups currently recognized on the basis of morphology, our results do not suggest any clear alternative classification scheme. The sea pens (O. Pennatulacea) and the sea fan sub-orders Calcaxonia and Holaxonia are well-defined groups supported by synapomorphies and should be retained as higher taxonomic groups. The morphological characters that loosely define the Scleraxonia, Alcyoniina, and Stolonifera (Table 1) apparently have evolved multiple times within Octocorallia, however, and those three sub-ordinal designations are clearly artificial. Although a revision of the higher taxonomic levels of Octocorallia will ideally reflect the division of the sub-class into two major

clades, the morphological similarities and distinctions among taxa belonging to each of these clades are not yet obvious. With a molecular phylogenetic framework to help guide taxonomic revision, we are hopeful that morphological synapomorphies eventually will be found that will allow us to divide Octocorallia into higher taxonomic units that reflect accurately both the underlying phylogeny and morphological diversity of the group.

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Appendix A

Octocoral specimens for which partial *msh1* and *ND2* sequences were obtained

Family [subfamily] and species	Collection locality	Catalog number	Date	<i>msh1</i>	ND2
O. ALCYONACEA					
[STOLONIFERA]					
Clavulariidae					
[Clavulariinae]					
<i>Clavularia inflata</i>	Great Barrier Reef, Qld, AUS	NTM-C011542	1991	DQ302799	DQ302873
<i>Rhodelinda</i> sp.	Kermadec Islands	NTM-C010034	1989	DQ302800	DQ302874
[Telestinae]					
<i>Carijoa riisei</i>	Rio de Janeiro, Brazil	CSM-TELCF	2003	DQ302801	DQ302875
? <i>Telesto</i> sp.	Gabo I., Victoria, AUS	NTM-C012710	2000	DQ302802	DQ302876
<i>Telestula</i> sp.	Tasman Sea, AUS	NTM-C014984	2003	DQ302803	DQ302877
Tubiporidae					
<i>Tubipora</i> sp.	Lighthouse Lagoon, Palau	UF 1811	2000	DQ302804	DQ302878
Coelogorgiidae					
<i>Coelogorgia palmosa</i>	Blue Corner East, Palau	NTM-C014914	2005	DQ302805	DQ302879
[ALCYONIINA]					
Alcyoniidae					
[Alcyoniinae]					
<i>Alcyonium digitatum</i>	Isle of Man	SBMNH 360700	1992	AY607777	AF530482
<i>Alcyonium ?aurantiacum</i>	New Zealand	NTM-C014988	1999	DQ302806	DQ302880
<i>Cladiella</i> sp.	Gunn Pt, NT, AUS	NTM-C012095	1993	DQ302807	DQ302881
<i>Dampia pocilloporaeformis</i>	Rowley Shoals, WA, AUS	NTM-C005805	1987	DQ280593	DQ302882
<i>Discophyton rudyi</i>	Tatoosh I., WA, USA	CSM-TAN14	1991	DQ302808	AF530488
<i>Elbeenus lauramartinae</i>	Uchelbeluu Reef, Koror, Palau	NTM-C013108	2001	DQ536320	N/A
<i>Eleutherobia</i> sp.	West Channel, Palau	NTM-C014902	2005	DQ302809	DQ302883
<i>Klyxum</i> sp.	Indonesia	NTM-C012417	1996?	DQ302810	DQ302884
<i>Lobophytum ?strictum</i>	Agat Bay, Guam	NTM-C014937	1998	DQ280585	AF530495

(continued on next page)

Appendix A (continued)

Family [subfamily] and species	Collection locality	Catalog number	Date	<i>mshI</i>	ND2
<i>Malacacanthus capensis</i>	Algoa Bay, South Africa	CSM-SAFR155.2	1998	DQ302811	DQ302885
<i>Notodysiferus d'hondti</i>	King George Sound, WA, AUS	NTM-C014221	1989	N/A	DQ302886
<i>Paraminabea aldersladei</i>	Chuuk, Micronesia	NTM-C012053	1993	N/A	DQ302887
<i>Rhytisma</i> sp.	Swain Reefs, Qld, AUS	NTM-C001942	1980	DQ302812	DQ302888
<i>Sarcophyton glaucum</i>	Rowley Shoals, WA, AUS	NTM-C010771	1987	DQ280525	DQ302889
<i>Simularia maxima</i>	Piti Bay, Guam	UF 3500	1998	DQ302813	DQ302890
<i>Thrombophyton coronatum</i>	Santa Catalina I., CA, USA	SBMNH 145123	1992	DQ302814	DQ302891
Alcyoniidae n. gen.	North West Cape, WA, AUS	WAMZ 13105	2002	DQ302815	DQ302892
[Anthomastinae]					
<i>Anthomastus ritteri</i>	Pebble Beach, CA, USA	CSM-ANRI	1998	DQ302816	DQ302893
Nephtheidae					
<i>Capnella imbricata</i>	GBR, Qld, AUS	NTM-C012235	1992	DQ302817	DQ302894
<i>Dendronephthya</i> sp.	Darwin Harbor, NT, AUS	NTM-C012655	2000	DQ302818	DQ302895
<i>Gersemia</i> sp.	Balsfjorden, Norway	RMNH-Coel.14708	1978	DQ302819	DQ302896
<i>Lemnalia</i> sp.	Ashmore Reef, WA, AUS	NTM-C011720	1986	DQ302820	DQ302897
<i>Litophyton</i> sp.	Cartier I., NT, AUS	NTM-C011318	1992	DQ302821	DQ302898
<i>Nephthea</i> sp.	Andaman & Nicobar I.	NTM-C012400	1995?	DQ302822	DQ302899
<i>Neospongodes</i> sp.	Darwin Harbor, NT, AUS	NTM-C013130	2001	DQ302823	DQ302900
<i>Paralemnalia digitiformis</i>	GBR, Qld, AUS	NTM-C012309	1994?	DQ302824	DQ302901
<i>Scletonephthya</i> sp.	Palau	NTM-C011489	1990	DQ302825	DQ302902
<i>Stereonephthya</i> sp.	Cartier I., NT, AUS	NTM-C011307	1992	DQ302826	DQ302903
<i>Umbellulifera</i> sp.	Arafura Sea, NT, AUS	NTM-C011063	1990	DQ302827	DQ302904
Nidaliidae					
[Nidaliinae]					
<i>Nidalia</i> sp.	West Channel, Palau	NTM-C014876	2005	DQ302828	DQ302905
<i>Pieterfaurea</i> sp.	Algoa Bay, South Africa	NTM-C013943	1998	DQ302829	DQ302906
[Siphonogorgiae]					
<i>Chironephthya</i> sp.	Arafura Sea, NT, AUS	NTM-C012426	1995	DQ302830	DQ302907
<i>Nephtyigorgia</i> sp.	Caledon Bay, NT, AUS	NTM-C011345	1991	DQ302831	DQ302908
<i>Siphonogorgia</i> sp.	Rowley Shoals, WA, AUS	NTM-C011159	1987	DQ302832	DQ302909
Paralcyoniidae					
<i>Paralcyonium spinulosum</i>	Sesimbra, Portugal	CSM-PASP	2001	DQ302833	DQ302910
<i>Studeriotis</i> sp.	Darwin, NT, AUS	NTM-C011441	1982	DQ302834	DQ302911
Xeniidae					
<i>Anthelia</i> sp.	Tasman Sea, AUS	NTM-C013050	1997	DQ302835	DQ302912
<i>Asterospicularia randalli</i>	Double Reef, Guam	CSM-JS10.12.98.05	1998	DQ302836	AF530497
<i>Cespitularia</i> sp.	Semporna I., Malaysia	NTM-C013542	1999	DQ302837	DQ302913
<i>Efflatounaria</i> sp.	Central GBR, AUS	NTM-C012311	1994	DQ302838	DQ302914
<i>Heteroxenia</i> sp.	Rowley Shoals, WA, AUS	NTM-C010897c	1987	DQ302839	DQ302915
<i>Sansibia</i> sp.	Darwin Harbor, NT, AUS	NTM-C012955	2001	DQ302840	DQ302916
<i>Sarcothelia</i> sp.	Oahu, Hawaii	NTM-C015151	2000	DQ302841	DQ302917
<i>Sympodium</i> sp.	Central GBR, AUS	NTM-C012271	1994	N/A	DQ302918
<i>Xenia</i> sp.	Ngederak, Palau	CSM-XESP	2000	DQ302842	AF530496
[SCLERAXONIA]					
Briareidae					
<i>Briareum polyanthes</i>				AY533653	AY534734
Anthothelidae					
[Anthothelinae]					
<i>Anthothela</i> sp.	Oceanographer Cyn, NW Atl	USNM 1014917	2001	DQ297415	DQ297434
<i>Erythropodium caribaeorum</i>	Bocas del Toro, Panama		2003	DQ302843	DQ302919
[Semperinae]					
<i>Iciligorgia</i> sp. 1	Tasman Sea, AUS	NTM-C014376	2003	DQ302844	DQ302920
<i>Iciligorgia</i> sp. 2	NE Kalimantan, Indonesia	RMNH Coel. 33331	2003	DQ302845	DQ302921
<i>Solenocaulon</i> sp.	NE Kalimantan, Indonesia	RMNH Coel. 33332	2003	DQ302846	DQ302922
Coralliidae					
<i>Corallium ducale</i>	Cross Seamount, Hawaii	USNM 94456	1993	DQ297416	DQ297435
Melithaeidae					
[Asperaxinae]					
<i>Asperaxis karenae</i>	Port Davey, Tasmania, AUS	NTM-C013575	2002	DQ302847	N/A
[Melithaeinae]					
<i>Mopsella</i> sp.	Gulf of Carpentaria, NT, AUS	NTM-C014468	2003	DQ302848	N/A
[HOLAXONIA]					
Acanthogorgiae					
<i>Acanthogorgia</i> sp.	Bishop Seamount, Hawaii	USNM 94442	1993	AY268461	DQ297436

Appendix A (continued)

Family [subfamily] and species	Collection locality	Catalog number	Date	<i>msh1</i>	ND2
<i>Acanthogorgia angustifolia</i>	Bear Seamount	USNM 100895	2000	DQ297418	DQ297437
<i>Anthogorgia</i> sp.	Tasman Sea, AUS	NTM-C014983	2003	DQ302849	DQ302923
<i>Calcigorgia</i> sp.	Bobrof Is., Alaska, USA	SCF-BOI09131	2003	DQ297419	DQ297438
<i>Cyclomuricea</i> sp.	Tasman Sea, AUS	NTM-C014571	2003	DQ302850	N/A
Gorgoniidae					
<i>Gorgonia ventalina</i>	Cayo Lobo, Puerto Rico	USNM 1007421	2000	AY126425	AY126397
<i>Guaigorgia</i> sp.	Gulf of Carpentaria, NT, AUS	NTM-C014545	2003	DQ302851	DQ302924
<i>Leptogorgia virgulata</i>	North Carolina, USA	USNM 1007414	2000	AY126418	AY126390
<i>Pacificgorgia stenobrochis</i>	E Pacific	JAS-27	1996	AY126420	AY126392
<i>Phyllogorgia dilatata</i>	Rio de Janeiro, Brazil	MNRJ-4336	2000	AY126428	AY126400
<i>Pseudopterogorgia acerosa</i>	Lee Stocking I., Bahamas	USNM 1007413	2000	AY126421	AY126393
<i>Pseudopterogorgia</i> sp.	Gulf of Carpentaria, NT, AUS	NTM-C014541	2003	DQ302852	DQ302925
<i>Pterogorgia citrina</i>	Lee Stocking I., Bahamas	USNM 1007406	2000	AY126402	AY126374
Keroeidae					
<i>Keroeides gracilis</i>	Tasman Sea, AUS	NTM-C014573	2003	DQ302853	DQ302926
Plexauridae					
[Plexaurinae]					
<i>Alaskagorgia aleutiana</i>	Aleutian Islands, AK, USA	USNM 1007125	1994	AY533649	AY534738
<i>Eunicea knighti</i>	Cat Island, Bahamas	USNM 1007366	2001	AY126404	AY126376
<i>Euplexaura</i> sp.	Gulf of Carpentaria, NT, AUS	NTM-C014536	2003	DQ302854	DQ302927
<i>Muricea muricata</i>	Lee Stocking I., Bahamas	USNM 1007340	2000	AY126408	AY126380
<i>Muriceopsis flavida</i>	San Salvador, Bahamas	USNM 1007376	1999	AY126416	AY126388
<i>Plexaura homomalla</i>	Florida, USA		2001	AY126410	AY126382
<i>Plexaurella nutans</i>	Lee Stocking I., Bahamas	USNM 1007399	2000	AY126415	AY126387
<i>Pseudoplexaura crucis</i>	Lee Stocking I., Bahamas	USNM 1007378	2000	AY126401	AY126373
[Stenogorgiinae]					
Plexauridae n. gen.	Tasman Sea, AUS	NTM-C014562	2003	DQ302855	DQ302928
<i>Astrogorgia</i> sp.	Tasman Sea, AUS	NTM-C014408	2003	DQ302856	DQ302929
<i>Lepidomuricea</i> sp.	Tasman Sea, AUS	NTM-C014578	2003	DQ302857	DQ302930
<i>Menella</i> sp.	Gulf of Carpentaria, NT, AUS	NTM-C014493	2003	DQ302858	DQ302931
<i>Muriceides</i> sp.	Tasman Sea, AUS	NTM-C014445	2003	DQ302859	DQ302932
<i>Paracis</i> sp.	Tasman Sea, AUS	NTM-C014576	2003	DQ302860	N/A
<i>Paramuricea</i> sp.	Muir Seamount	YPM 28867	2003	DQ297420	DQ297439
<i>Paraplexaura</i> sp.	Gulf of Carpentaria, NT, AUS	NTM-C014494	2003	DQ302861	DQ302933
<i>Swiftia</i> sp.	Tasman Sea, AUS	NTM-C014396	2003	DQ302862	DQ302934
[CALCAXONIA]					
Chrysogorgiidae					
<i>Chrysogorgia chryseis</i>	Cross Seamount	SCF-CR106-2	1993	DQ297421	DQ297440
<i>Iridogorgia</i> sp.	Muir Seamount	YPM 28866	2003	DQ297422	DQ297441
<i>Metallogorgia melanotrichos</i>	Manning Seamount	SCF-MAN306-1	2003	DQ297423	DQ297442
<i>Radicipes gracilis</i>	Bear Seamount	USNM 100900	2000	DQ297424	DQ297443
Ellisellidae					
<i>Ctenocella barbadensis</i>				AY533651	AY534736
<i>Nicella</i> sp.	Tasman Sea, AUS	NTM-C014406	2003	DQ302863	DQ302935
<i>Verrucella</i> sp.	Tasman Sea, AUS	NTM-C014982	2003	DQ302864	DQ302936
Ifalukellidae					
<i>Ifalukella yanii</i>	Yap, Micronesia	0CDN 3387-Q	1992	DQ536319	N/A
<i>Plumigorgia</i> sp.	Palawan, Philippines	0CDN 3101-G	1995	DQ536318	N/A
Isididae					
[Keratoisidinae]					
<i>?Isidella</i> sp.	Lanai Is., Hawaii, USA	SCF-LAD25	1996	DQ297425	DQ297444
<i>Lepidisis olapa</i>	Cross Seamount, Hawaii, USA	SCF-CR206-4	1993	DQ297426	DQ297445
Primnoidae					
<i>Calyptraphora</i> sp.	Lanai Is., Hawaii, USA	SCF-LAD36	1996	DQ297427	DQ297446
<i>Narella nuttingi</i>	Pensacola Seamount, Hawaii	USNM 94424	1993	DQ297428	DQ297447
<i>Paracalyptraphora</i> sp.	Penguin Bank, Hawaii, USA	SCF-PBS09	1996	DQ297429	DQ297448
<i>Thouarella</i> sp.	Oceanographer Cyn, NW Atl	USNM 1014915	2001	DQ297430	DQ297449
O. PENNATULACEA					
[SESSILIFLORAE]					
Kophobelemnidae					
<i>Kophobelemnion macrospinum</i>	Tasman Sea, AUS	NTM-C014985	2003	DQ302865	DQ302937
<i>Sclerobelemnion theseus</i>	Colombia	INVEMAR		DQ311678	DQ311679

(continued on next page)

Appendix A (continued)

Family [subfamily] and species	Collection locality	Catalog number	Date	<i>msh1</i>	ND2
Anthoptilidae					
<i>Anthoptilum murrayi</i>	Tasman Sea, AUS	NTM-C014385	2003	N/A	DQ302938
Protoptilidae					
<i>Protoptilum</i> sp.	Bishop Seamount, Hawaii	USNM 94465	1993	DQ297431	DQ297450
<i>Distichoptilum gracile</i>	Tasman Sea, AUS	NTM-C014561	2003	DQ302866	DQ302939
Renillidae					
<i>Renilla muelleri</i>	Gulf of Mexico, Florida, USA	SCF-FLA	2001	DQ297432	DQ297451
Umbellulidae					
<i>Umbellula</i> sp.	Tasman Sea, AUS	NTM-C014384	2003	DQ302867	DQ302940
[SUBSESSILIFLORAE]					
Halopteridae					
<i>Halopteris finmarchica</i>	Tasman Sea, AUS	NTM-C014596	2003	DQ302868	DQ302941
Pennatulidae					
<i>Gyrophyllum</i> sp.	Tasman Sea, AUS	NTM-C014392	2003	DQ302869	DQ302942
<i>Pennatula</i> sp.	Tasman Sea, AUS	NTM-C014415	2003	DQ302870	DQ302943
<i>Pteroeides</i> sp.	Tasman Sea, AUS	NTM-C014391	2003	DQ302871	DQ302944
O. HELIOPORACEA					
Helioporidae					
<i>Heliopora coerulea</i>	Blue Corner East, Palau	CRCNI 577	2005	DQ302872	DQ302945

Sub-ordinal group is indicated in uppercase boldface and square brackets, family in lowercase boldface. Catalog numbers are indicated for voucher specimens, GenBank Accession Numbers for *msh1* and *ND2* sequences. CSM, Collection of C.S. McFadden; CRCNI, OCDN: Coral Reef Research Foundation, Palau; JAS, Collection of J.A. Sánchez; MNRJ, Museu Nacional do Rio de Janeiro, Brazil; NTM, Museum and Art Gallery of the Northern Territory, Australia; RMNH, Nationaal Natuurhistorisch Museum, formerly Rijksmuseum van Natuurlijke Historie, Leiden, The Netherlands; UF, Florida Natural History Museum, FL, USA; USNM, National Museum of Natural History, Smithsonian Institution, USA; SBMNH, Santa Barbara Museum of Natural History, CA, USA; SCF, Collection of S.C. France; WAMZ, Western Australian Museum; YPM, Yale Peabody Museum, New Haven, CT, USA.

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