Molecular Phylogenetics and Evolution 98 (2016) 373–381

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Homoplasious colony morphology and mito-nuclear phylogenetic discordance among Eastern Pacific octocorals ☆

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ARTICLE INFO

Article history:
Received 2 May 2015
Revised 6 December 2015
Accepted 26 February 2016
Available online 5 March 2016

Keywords:
Molecular systematics
Octocorals
Eastern Pacific
Mito-nuclear discordance
Colony morphology
Homoplasy

ABSTRACT

Octocorals are a diverse and ecologically important group of cnidarians. However, the phylogenetic relationships of many octocoral groups are not well understood and are based mostly on mitochondrial sequence data. In addition, the discovery and description of new gorgonian species displaying unusual or intermediate morphologies and uncertain phylogenetic affinities further complicates the study of octocoral systematics and raises questions about the role played by processes such as plasticity, crypsis, and convergence in the evolution of this group of organisms. Here, we use nuclear (i.e. 28S rDNA) and mitochondrial (*mtMutS*) markers and a sample of Eastern Pacific gorgonians thought to be remarkable from a morphological point of view to shed light on the morphological diversification among these organisms. Our study reveals the loss of the anastomosed colony morphology in two unrelated lineages of the seafan genus *Pacifigorgia* and offers strong evidence for the independent evolution of a whip-like morphology in two lineages of Eastern Pacific *Leptogorgia*. Additionally, our data revealed one instance of mito-nuclear discordance in the genera *Leptogorgia* and *Eugorgia*, which may be the results of incomplete lineage sorting or ancient hybridization–introgression events. Our study stresses the importance of comprehensive taxonomic sampling and the use of independent sources of evidence to address the phylogenetic relationships and clarifying the evolution of octocorals.

1. Introduction

Homoplasious evolution, the occurrence of the same character state in distinct lineages by means of independent events (Fitch, 2000), represents an important process shaping the phenotypic evolution of corals (Class Anthozoa, Phylum Cnidaria). In both hexacorals (Fukami et al., 2004; Arrigoni et al., 2012) and octocorals (Sánchez et al., 2003a; Kim et al., 2004; McFadden et al., 2006; France, 2007; Prada et al., 2008; Dueñas and Sánchez, 2009; McFadden and van Ofwegen, 2012; Prada and Hellberg, 2013; Bilewitch et al., 2014; Rowley et al., 2015; Yasuda et al., 2015), molecular phylogenetic analyses have revealed multiple instances of morphological homoplasy at different taxonomic levels. Whether such homoplasies can be attributed to convergent or to parallel evolution remains contentious, since the distinction between these two terms is not clear-cut or it changes depending on the author (Powell, 2007; Arendt and Reznick, 2007; Scotland, 2011; Martin and Orgogozo, 2013). Terminology aside, the seemingly generalized emergence of similar phenotypes among unrelated coral taxa suggests that traits often used as diagnostic for taxonomic classification might be evolutionary labile and homoplasious (Sánchez et al., 2003a; McFadden et al., 2006, 2010; Dueñas and Sánchez, 2009; McFadden and van Ofwegen, 2013; Bryce et al., 2015; Wirshing and Baker, 2015).

Among cnidarians, members of the subclass Octocorallia are of special interest for the study of morphological evolution due to their broad environmental tolerance and wide geographic and bathymetric distribution, occurring in all of the world's oceans from zero to more than 6600 m deep (Watling et al., 2011;

☆ This paper was edited by the Associate Editor Bernd Schierwater.
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http://dx.doi.org/10.1016/j.ympev.2016.02.023
1055-7903/

Williams, 2011; Pante et al., 2012). Traditionally, the taxonomy of octocorals has been mainly based on a combination of traits derived from the analysis of the morphology of the colony (e.g. color, branching pattern) and of the study of the sclerome, that is, the inventory of calcium carbonate microskeletal elements called sclerites present in an octocoral taxon (Breedy and Guzman, 2002; Molodtsova, 2013; see also Carlo et al., 2011). The phylogenetic validity of these characters is usually taken at face value for taxonomic purposes, ignoring the evolutionary history of the traits and their potential homoplasy. For example, among Eastern Pacific gorgoniids, the genera *Leptogorgia* and *Pacifigorgia* possess similar scleromes but differ in their colony morphology. In *Pacifigorgia*, the branches anastomose to form fan-like colonies (Breedy and Guzman, 2002). *Leptogorgia* species, in contrast, generally form tree-like colonies. The genus *Eugorgia* is similar to *Leptogorgia* in its colony morphology but its sclerome, dominated by sclerites with fused warts (Breedy and Guzman, 2007; Breedy et al., 2009), is clearly different from that of *Leptogorgia* and *Pacifigorgia*. Molecular phylogenetic studies have shown a close phylogenetic relation and the monophyly of these three genera, potentially corroborating the synapomorphic status of the morphological characters used to support their taxonomy. However most phylogenetic analyses of the Eastern Pacific octocoral fauna published to date (e.g. Wirshing et al., 2005; Vargas et al., 2014) are exclusively based on mitochondrial markers and only include a very restricted taxonomic sampling of *Eugorgia* and *Leptogorgia*. Moreover, several species have been described only recently and more await identification and formal description (Breedy and Guzman, 2002, 2003, 2004, 2007, 2008, 2013; Breedy et al., 2009). Within *Leptogorgia* in particular, a number of species of unclear phylogenetic affinities, and remarkably variable morphologies—e.g. colonies resembling those of *Eugorgia* or other gorgoniid genera, forming loose anastomoses as in *Pacifigorgia*, or showing a whip-like morphology with bidirectional growth and no attachment points—occur along the Eastern Pacific, opening questions about the role played by homoplasy and lability in the morphologial evolution of these genera. *Leptogorgia* Milne-Edwards & Haime, 1857, *Pacifigorgia* Bayer, 1951 and *Eugorgia* Verrill, 1868 are important structural components of rocky habitats and high-energy environments where they can dominate the seascape. These genera account for the majority of species reported for the tropical eastern Pacific (e.g. Breedy and Guzman, 2002, 2003, 2004, 2007; Breedy et al., 2009). Thus, clarifying the phylogenetic relationships and the evolutionary processes that led to their diversification and shaped their morphologies is pivotal to our understanding of these important members of the eastern Pacific shallow water communities and will contribute to clarify octocoral phenotypic evolution in general.

In this study, we present an expanded molecular phylogeny of the Eastern Pacific gorgoniid octocorals based on nuclear (28S rDNA) and mitochondrial (*mtMutS*) markers, and including an increased taxonomic sampling of the genera *Leptogorgia* and *Eugorgia* as well as three un-described, whip-like specimens of as yet unconfirmed phylogenetic affinity. The nuclear gene 28S rDNA have been successfully used to resolve intrafamily relationships among stoloniferous octocorals (McFadden and van Ofwegen, 2012), suggesting that this marker could be of general interest for resolving the molecular phylogeny of other octocoral taxa. We re-evaluate the phylogenetic relationships and the monophyly of the three main genera of Eastern Pacific gorgoniids recently revised, namely *Leptogorgia*, *Eugorgia* and *Pacifigorgia*, and show that character lability and homoplasious colony evolution played a role on the morphological evolution and diversification of these gorgoniid genera and likely shape octocoral evolution in general at all taxonomic levels.

2. Materials and methods

2.1. Molecular procedures

All specimens used in this study (Table 1) were collected between 2008 and 2010 along the Eastern Pacific of Costa Rica (mainland and the Isla del Coco National Park) and Panama (Coiba National Park, Gulf of Chiriquí). After genomic DNA extraction (following Vargas et al., 2014), a standard three-steps PCR was used to amplify the 28S rDNA gene using the primers C2′ (forward; Chombard et al., 1998) and 28S-1260fw (reverse; Voigt et al., 2012). In case of failure, different combinations with primers 28S-NL2F (forward; Scott Nichols pers. comm., 5′-TACCGTGAGG GAAAGGTGAAA-3′), RD3a (forward; McCormack et al., 2002) and D2 (reverse; Chombard et al., 1998) were used. The PCR temperature regime was as follows: 95 °C for 3 min, 35 cycles at 95 °C for 30 s; 52–54 °C for 30 s; 72 °C for 1 min, and 72 °C for 5 min. PCR amplifications contained 5.9 μL ddH2O, 2.5 μL 5x GoTaq Flexi Buffer (Promega, Madison), 1.5 μL MgCl2 (25 mM), 0.5 μL dNTP (10 mM each), 0.5 μL of each primer (5 mM), 0.1 μL GoTaq Polymerase (5 units/μL, Promega, Madison), and 1 μL of sample DNA for a total volume of 12.5 μL. The amount of DNA used for PCR was variable, generally ranging between 20 and 150 ng.

PCR products were visualized on 1.5% agarose gels, and cleaned-up using a polyethylene–glycol precipitation. Briefly, 10 μL PCR reaction were thoroughly mixed with an equal amount of PEG solution (20% PEG 8000, 2.5 M NaCl), incubated at room temperature for 20 min, centrifuged for 15 min at maximum speed (12,000 rpm), and washed twice with 80% ethanol. The air-dried pellets were resuspended in 10 μL ddH2O. The purified products were sequenced in both directions using the BigDye Terminator 3.1 chemistry (Applied Biosystems) and the same primers used for PCR. Sequencing products were precipitated using Sodium acetate–Ethanol and analyzed in an ABI 3700 Genetic Analyser at the Department of Genetics of the Ludwig-Maximilians-Universität München, Germany. Traces were visualized and assembled using Geneious 6.1.5 (Biomatters, available from http://www.geneious.com/), and the taxonomic affiliation of each "contig" was checked using NCBI's BLAST (Johnson et al., 2008). In addition to the 28S rDNA marker, sequences of the *mtMutS* gene of families Gorgoniidae and Plexauridae were downloaded from Genbank or sequenced using protocols previously described (see Vargas et al., 2014). All sequences were deposited in the European Nucleotide Archive (see Table 1 for details).

2.2. Sequence alignment and model selection

Sequences were aligned using the MAFFT version 7 online server (Katoh et al., 2002; Katoh and Toh, 2008; http://mafft.cbrc.jp/alignment/server/) with default settings and the resulting alignments were visually inspected in Seaview version 4.5.4 (Galtier et al., 1996; Gouy et al., 2010). For the *mtMutS* alignment, the amino acid translation was used to detect and correct frameshifts. The 28S rDNA alignment contained ambiguous regions that were identified and discarded from the final matrix using the Gblocks (Castresana, 2000; Talavera and Castresana, 2007) implementation in Seaview, with the options for a less stringent filtering set. JModelTest version 2.1.3 (Darriba et al., 2012, and references therein) was used to estimate the likelihood of different substitution models, including 7 schemes, with base frequencies (+F), proportion of invariable sites (+I), and the gamma distribution with four categories of rate heterogeneity across sites (+G). The best model was chosen using both the corrected Akaike Information Criterion (AICc; Akaike, 1973; Sugiura, 1978; Hurvich and Tsai,

Table 1
Museum code and collection locality of the material sequenced in this study.

Species	Museum code	Locality	Accession number	
			mtMutS	28S
Eugorgia daniana	HMG93	Roca Hacha, Coiba Island, Panamá		LT221083
Eugorgia daniana	MZUCR OCT0003	Islas Galápagos, Ecuador	LT221110	LT221085
Eugorgia beebei	SV52	Bahía Santa Elena, Costa Rica		LT221088
Eugorgia mutabilis	MZUCR 2297	Golfo Dulce, Costa Rica	LT221112	LT221084
Eugorgia siedenburgae	MZUCR 2281	Bahía Santa Elena, Costa Rica	LT221096	LT221087
Eugorgia siedenburgae	MZUCR 2272	Bahía Santa Elena, Costa Rica	LT221097	LT221086
Eugorgia siedenburgae	MZUCR 2278	Bahía Santa Elena, Costa Rica	LT221094	LT221089
Leptogorgia alba	HMG71	Golfo de Chiriquí, Panamá		LT221064
Leptogorgia alba	SV05	Cocos Island National Park, Costa Rica	LT221108	
*Leptogorgia alba**	MZUCR OCT0005	Isla del Coco National Park, Costa Rica	LT221113	LT221065
Leptogorgia cofrini	HMG32	Jicarita, Coiba Island, Panamá		LT221061
Leptogorgia cofrini	HMG17	Catedrales, Coiba Island, Panamá		LT221060
Leptogorgia cofrini	HMG62	Golfo de Chiriquí, Panamá		LT221062
Leptogorgia cuspidata	HMG97	Catedrales, Coiba Island, Panamá		LT221066
Leptogorgia cortesi	MZUCR 2128	Golfo Dulce, south Pacific Costa Rica	LT221105	
Leptogorgia pumila	HMG79	Piedra Hacha, Coiba Island, Panamá		LT221059
Leptogorgia pumila	HMG80	Piedra Hacha, Coiba Island, Panamá	LT221116	LT221058
Leptogorgia regis	MZUCR OCT0010	Islas Murciélago, Costa Rica	LT221100	
Leptogorgia regis	MZUCR 1593	Bahía Santa Elena, Costa Rica	LT221098	LT221082
Leptogorgia regis	MZUCR 1563	Bahía Santa Elena, Costa Rica	LT221101	LT221080
Leptogorgia regis	MZUCR OCT0012	Bajo La Mota, Bahía Salinas, Costa Rica	LT221099	LT221081
*Leptogorgia sp. (white whip)**	MZUCR OCT0002	Bahía Santa Elena, Costa Rica	LT221114	LT221063
Leptogorgia sp. (red whip)	MZUCR OCT0004	Bahía Santa Elena, Costa Rica	LT221115	
*Leptogorgia sp. (red whip)**	MZUCR OCT0006	Bahía Santa Elena, Costa Rica	LT221106	LT221069
*Leptogorgia sp. (Yellow whip)**	SV10	Bahía Santa Elena, Costa Rica		LT221067
*Leptogorgia sp. (red whip)**	SV11	Bahía Santa Elena, Costa Rica		LT221068
Leptogorgia taboguillae	MZUCR 1239	Islotes, Golfo Dulce, Costa Rica	LT221102	LT221075
Leptogorgia taboguillae	MZUCR 1436	Golfo de Chiriquí, Panamá	LT221104	
Leptogorgia taboguillae	MZUCR 1242	Golfo de Chiriquí, Panamá	LT221103	
Leptogorgia taboguillae	MZUCR 1663	Bahía Salinas, Guanacaste, Costa Rica	LT221093	
Leptogorgia taboguillae	MZUCR 1243	Golfo de Chiriquí, Panamá		LT221076
Leptogorgia taboguillae	MZUCR 1247	Golfo de Chiriquí, Panamá		LT221073
Leptogorgia taboguillae	MZUCR 1244	Golfo de Chiriquí, Panamá		LT221072
Leptogorgia taboguillae	MZUCR 1240	Golfo de Chiriquí, Panamá		LT221074
Leptogorgia taboguillae	MZUCR OCT0013	Golfo de Chiriquí, Panamá		LT221079
Leptogorgia taboguillae	MZUCR 1352	Golfo de Chiriquí, Panamá		LT221078
Leptogorgia taboguillae	MZUCR 1238	Golfo de Chiriquí, Panamá		LT221071
Leptogorgia taboguillae	MZUCR 1088	Golfo de Chiriquí, Panamá		LT221077
Leptogorgia taboguillae	HMG47	Piedra Hacha, Coiba Island, Panamá		LT221070
*Leptogorgia tricorata**	MZUCR 1836	Isla del Coco National Park, Costa Rica	LT221109	LT221055
*Leptogorgia tricorata**	MZUCR 1836	Isla del Coco National Park, Costa Rica	LT221111	LT221056
*Leptogorgia tricorata**	MZUCR 1833A	Isla del Coco National Park, Costa Rica		LT221057
Pacifigorgia cairnsi	HMG23	Piedra Hacha, Coiba Island, Panamá		LT221047
Pacifigorgia catedralensis	HMG112	Catedrales, Coiba Island, Panamá		LT221054
Pacifigorgia catedralensis	HMG20	Catedrales, Coiba Island, Panamá		LT221046
Pacifigorgia catedralensis	HMG109	Catedrales, Coiba Island, Panamá		LT221053
Pacifigorgia firma	HMG53	Golfo de Chiriquí, Panamá		LT221049
Pacifigorgia firma	HMG103	Catedrales, Coiba Island, Panamá		LT221052
Pacifigorgia irene	HMG65	Golfo de Chiriquí, Panamá		LT221051
Pacifigorgia irene	HMG10	Octavios, Panamá		LT221045
Pacifigorgia irene	HMG26	Jicarita, Coiba Island, Panamá		LT221048
Pacifigorgia rubicunda	HMG29	Jicarita, Coiba Island, Panamá		LT221040
Pacifigorgia rubicunda	HMG01	Octavios, Panamá		LT221039
Pacifigorgia rubicunda	HMG74	Catedrales, Coiba Island, Panamá		LT221041
Pacifigorgia cf. *senta*	MZUCR OCT0001	Islas Murciélago, Bahía Santa Elena, Costa Rica	LT221107	LT221044
Pacifigorgia smithsoniana	HMG59	Catedrales, Coiba Island, Panamá		LT221050
Pacifigorgia stenobrochis	HMG04	Octavios, Panamá		LT221042
Pacifigorgia stenobrochis	HMG41	Piedra Hacha, Coiba Island, Panamá		LT221043
Psammogorgia cf. *arbuscula*	HMG38	Piedra Hacha, Coiba Island, Panamá		LT221092
Psammogorgia cf. *arbuscula*	HMG15	Octavios, Panamá		LT221091
Heterogorgia verrucosa	HMG13	Panamá		LT221090
Muricea sp.	MZUCR OCT0023	Costa Rica	LT221095	

Note: vouchers of all specimens with museum codes HMGXX are available from SV on request. Specimens tagged with a * where amplified using a different pair of primers for the 28S marker; see Section 2.

1989) and the Bayesian Information Criterion (BIC; Schwartz, 1978).

2.3. Assessing substitution saturation

Nucleotide substitution saturation has the potential to erode the phylogenetic information present in a marker (Salemi, 2009). In extreme cases, saturation can lead to sequence convergence due to similarities in nucleotide frequencies (Xia and Lemey, 2009) and result in "false" phylogenetic relationships. Therefore, we applied the information entropy-based method of Xia et al. (2003) available in DAMBE version 5.3.48 (Xia, 2001, 2013; Xia and Xie, 2001) to test weather the *mtMutS* or the 28S rDNA sequences were subject to substitution saturation. For the *mtMutS*

we further evaluated the codon positions separately. This method requires an a priori estimate of the proportion of invariable sites, which was likewise calculated in DAMBE using three models of nucleotide substitution (K80, MLCompositeTN93, and GTR). We additionally produced plots of the number of transitions and transversions vs. corrected genetic distance using the model suggested by JModelTest2.

2.4. Phylogenetic analyses

For phylogenetic reconstruction, the final alignments were used to infer a Maximum Likelihood (ML) tree and a Bayesian phylogeny using RAxML 7.2.8 (Stamatakis, 2006) and MrBayes 3.2.4 x64 (Ronquist et al., 2012), respectively. The GTR model of sequence evolution (Tavaré, 1986) was used for all datasets, and among-site rate variation was modeled using a discrete approximation to a gamma distribution with four rate categories (Yang, 1994). Additionally, the online version of PhyML 3.0 (http://atgc.lirmm.fr/phyml/; Guindon et al., 2010) was used to estimate a ML tree implementing the substitution models suggested by JModelTest. The PhyML search was set to start from five random trees and from a BIONJ tree, and both the SPR & NNI heuristics were used for tree search. Branch support in the ML analyses was assessed using 1000 boostrap pseudo-replicates in both RAxML 7.2.8 (rapid boostrap; Stamatakis et al., 2008) and PhyML 3.0. For the Bayesian analysis, we used the default priors (including a compound Dirichlet distribution that draws from a gamma distribution for branch lenghts, or Unconstrained:GammaDir (1.0, 0.100, 1.0, 1.0); Rannala et al., 2012), except for a change in the prior of the shape parameter of the Gamma distribution for among-site rate variation to Uniform (0.1, 50). We explored four temperature values (0.2, 0.1, 0.05, and 0.01) to ensure mixing and appropriate swapping rates among chains. For each temperature, we started four independent runs with four chains (one cold, three heated) each, sampling every 500 generations during 10 million generations. Changes in likelihood scores during Markov chains were assessed using Tracer v. 1.6 (Rambaut et al., 2014), while topology convergence was evaluated with the online server of AWTY (Nylander et al., 2008; http://king2.scs.fsu.edu/CEBProjects/awty/awty_start.php). The first 25% generations were discarded as burn-in and the remaining trees were used to calculate the posterior probabilities (PP) of the clades. For those individuals that had sequences of both markers available, a concatenated matrix was also used to infer ML and Bayesian trees using the aforementioned settings but allowing each marker to have its own set of model parameters. Likewise, trees for the individual markers using this restricted dataset were produced for comparison. Trees of *mtMutS* were rooted using *Alcyonium* spp. following Vargas et al. (2014) and Sánchez et al. (2003b), while those of 28S rDNA were rooted with *Rumphella*.

3. Results

3.1. Data matrices, substitution models and phylogenetic inference

The 28S rDNA was amplified and sequenced for 54 individuals of the genera *Pacifigorgia*, *Leptogorgia*, *Eugorgia*, *Heterogorgia* and *Psammogorgia*. Together with publicly available accessions, a matrix of 73 sequences was assembled (54 unique sequences). The final 28S rDNA matrix contained 705 sites, including 229 variable sites (140 parsimony-informative). For the final *mtMutS* matrix, 168 sequences (119 unique sequences) were aligned covering 660 sites (348 variable, 268 informative). Both markers were recovered for 40 individuals, from which a concatenated matrix of 1365 sites (177 variable, 127 informative) was constructed. Using AICc and BIC as implemented in JModelTest, the GTR + I + G and HKY + G models of substitution were consistently selected for 28S rDNA and *mtMutS*, respectively. In the case of the concatenated data matrix, AICc selected either GTR + I or GTR + G as the best model, while BIC preferred K80 + I or K80 + G. The topologies produced using the GTR + G model (RAxML) and the K80 + G + I (PhyML) were nearly identical, and henceforth only the GTR + G trees are considered.

For the Bayesian analyses, we obtained good mixing (with acceptance rates between 15% and 60%) using a temperature of 0.01 for *mtMutS*, and of 0.1 for 28S rDNA and the concatenated data set. We report the results of those temperatures, but other values resulted in identical topologies and equivalent PPs. In all cases we obtained an average standard deviation of split frequencies below 0.009, along with EES > 5000 and PSRF of 1.000 for all parameters, suggesting convergence. Similarly, the graphics obtained with Tracer and AWTY gave no indication of a lack of convergence (Supp. Figs. S1–S12). In general, MrBayes topologies were highly congruent with ML inferences. Hence only RAxML topologies are reported, mapping the support of both ML and Bayesian inference analyses on them.

No evidence of substitution saturation was found using plots of transitions and transversions vs. corrected genetic distance (Suppl. Fig. S13) or the method of Xia et al. (2003). This result was independent of the starting model of nucleotide substitution used to calculate the proportion of invariant sites ($p < 0.0000$ in all cases).

3.2. Phylogenetic relationships: mitochondrial vs. nuclear data

The inferred phylogenetic relationships using the *mtMutS* gene are identical to those previously reported (Vargas et al., 2014; see also Wirshing et al., 2005), including the nesting within Plexauridae of some genera traditionally assigned to Gorgoniidae, such as *Pterogorgia* and *Pinnigorgia* (Suppl. Fig. S14), and the plexaurid *Plexaurella* sister to a large clade composed of *Psammogorgia*, *Swiftia*, *Thesea* as well as most members of the family Gorgoniidae (excluding *Pterogorgia*). The 28S rDNA phylogeny also recovered a clade containing Gorgoniidae, *Psammogorgia* and *Plexaurella*, although with less internal support and resolution (Suppl. Fig. S15).

Within Gorgoniidae, however, nuclear and mitochondrial data exhibit a previously unidentified conflict (Fig. 1). While the 28S rDNA topology is consistent with current taxonomic definitions of *Eugorgia* and *Leptogorgia*, the *mtMutS* phylogeny with extended taxonomic sampling resulted in a non-monophyletic *Eugorgia* and *Leptogorgia*. In the case of *Eugorgia*, both markers supported the subdivision of the genus in two clades consisting of *Eugorgia siedenburgae* and *Eugorgia daniana* + *Eugorgia multifida* + *Eugorgia mutabilis*, respectively. The positions of these clades, however, was not congruent between different analyses. In the 28S rDNA, *Eugorgia* is monophyletic with the lineage of *E. siedenburgae* (plus a sample of *Eugorgia beebei* from which the *mtMutS* sequence did not amplify) being sister to the *E. daniana* + *E. multifida* + *Eugorgia mutabilis* clade. A monophyletic *Eugorgia* is supported marginally by both ML (BS of 78%) and Bayesian (PP of 0.95) analyses. In contrast, in the *mtMutS* phylogeny the two subclades of *Eugorgia* are included within different lineages of *Leptogorgia*: *E. siedenburgae* is sister to a clade of Eastern Pacific and Caribbean *Leptogorgia* while *E. daniana* + *E. multifida* + *Eugorgia mutabilis* is sister to an undescribed whip-like species of *Leptogorgia* in a clade that also includes *Leptogorgia regis* and *Leptogorgia taboguillae*. Restricting the analyses to those individuals that had both markers sequenced resulted in identical conclusions for the *mtMutS*. In the case of the 28S rDNA, a less resolved tree was recovered where *Leptogorgia* was monophyletic, but formed a polytomy with the two clades of *Eugorgia* (Supp. Fig. S16).

Both markers supported the division of Eastern Pacific *Leptogorgia* in several clades roughly corresponding to morphological

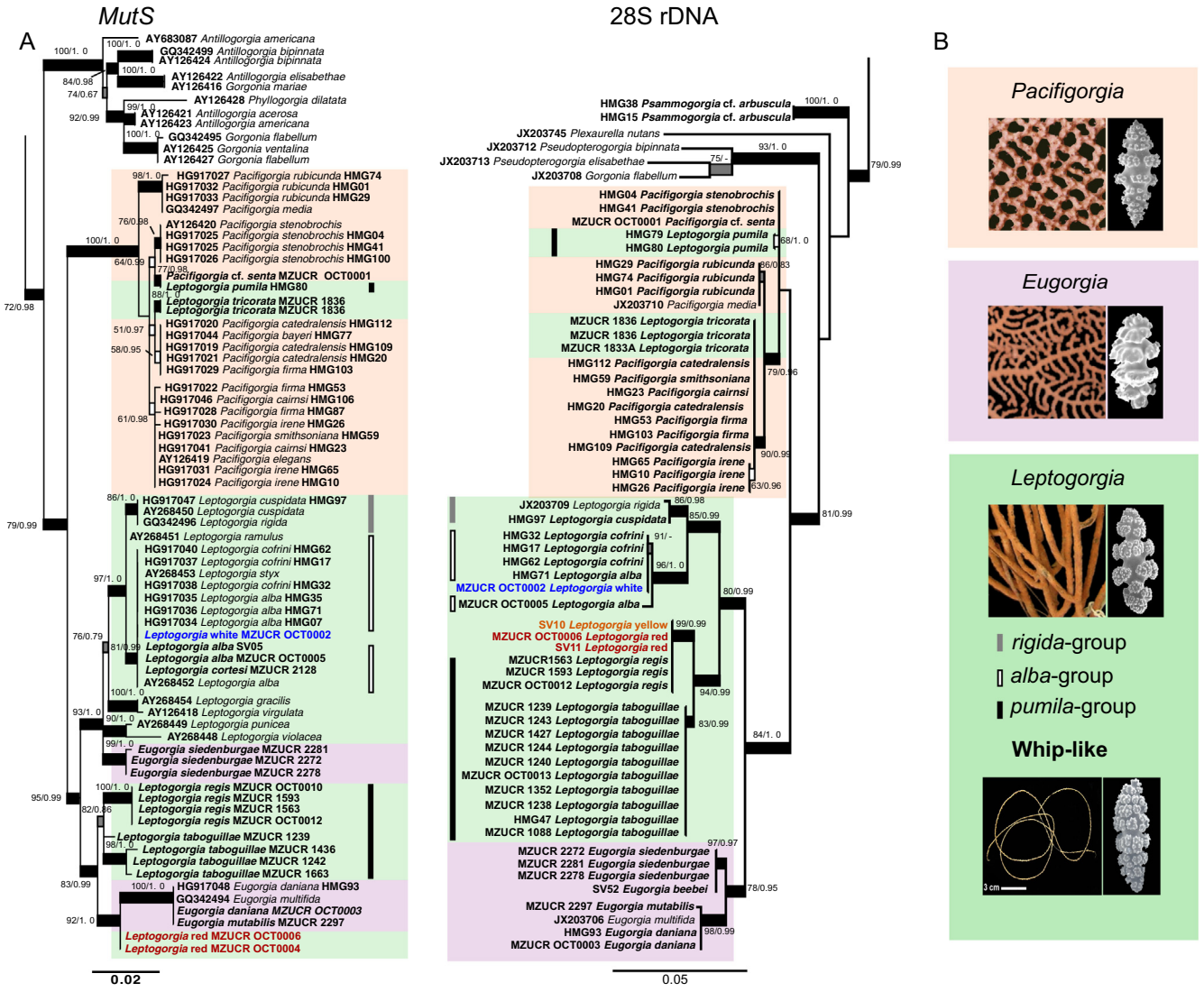

Fig. 1. Comparison of the *mtMutS* and 28S rDNA phylogenies (A), stressing the taxonomic definitions of *Pacifigorgia*, *Leptogorgia* and *Eugorgia* based on colony branching pattern and sclerome characters (B). New sequences generated by us are in bold. Tree branches with high support (BS values larger than 70% and PP higher than 0.95) in either of the phylogenetic analysis are thickened, while specific support values are shown near each branch in a "BS/PP" pattern. Black thick branches are supported in both ML and Bayesian inference. Grey and white thick branches are supported only in ML or Bayesian inference analyses, respectively. The colored boxes delimit the three main genera. *Pacifigorgia* (orange box) has branches that anastomose into a mesh and its sclerites have whorls of tubercles that never fuse completely. *Eugorgia*'s (purple box) branches never anastomose and its sclerome has a dominance of double-disk sclerites. *Leptogorgia* (green box) also has branches that (normally) do not anastomose but its sclerome resembles that of *Pacifigorgia*. Within *Leptogorgia*, the three morphological groups defined by Breedy and Guzman (2007) and Guzman and Breedy (2008) are shown with adjacent white, grey or black bars. Also within *Leptogorgia*, whip-like individuals are shown with bold letters and either blue, red or orange color. The comparison between markers shows that there is supported conflict between nuclear and mitochondrial data, which has taxonomic implications. Photographs by O. Breedy. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

groups defined by Breedy and Guzman (2007) and Guzman and Breedy (2008), namely the "*pumila*-group", the "*alba*-group", and the "*rigida*-group". Some newly sequenced species belonging to *Leptogorgia* were nested in the otherwise monophyletic *Pacifigorgia*. Specifically, *Leptogorgia pumila* and *Leptogorgia tricorata* were found to be sister to different species of *Pacifigorgia*, not forming a monophyletic group. In addition, and somewhat surprisingly, some whip-like undescribed specimens of *Leptogorgia* did not form a clade but were included in different morphological groups within *Leptogorgia*. In this respect, the white phenotype was consistently included by both markers within the "*alba*-group" (i.e. the white species of *Leptogorgia*) while additional mito-nuclear conflict was detected in the position of the red whip-like phenotype, which was included in one of the *Eugorgia* clades in the *mtMutS* phylogeny but formed a clade with *Leptogorgia regis* in the 28S rDNA phylogeny. No *mtMutS* sequence was available for a third whip-like yellow phenotype, but the 28S rDNA haplotype of the sequenced specimen was identical to the haplotypes found in red whip-like individuals.

Except for the branches defining *Eugorgia* and *Leptogorgia* (excluding *L. pumila* and *L. tricorata*), the phylogeny inferred from the concatenated matrix is well resolved (Fig. 2) and some supported internal structure can be observed within each genus. *Eugorgia* and *Leptogorgia* have two main subclades each, separated by fairly long branches. Within *Leptogorgia*, the white whip-like individual was included in the "*alba*-group", whereas the red specimen was sister to *L. regis* in agreement with the results obtained using the 28S rDNA marker. The genus *Pacifigorgia* has three main subclades, with relatively short branches and poor internal phylogenetic structure.

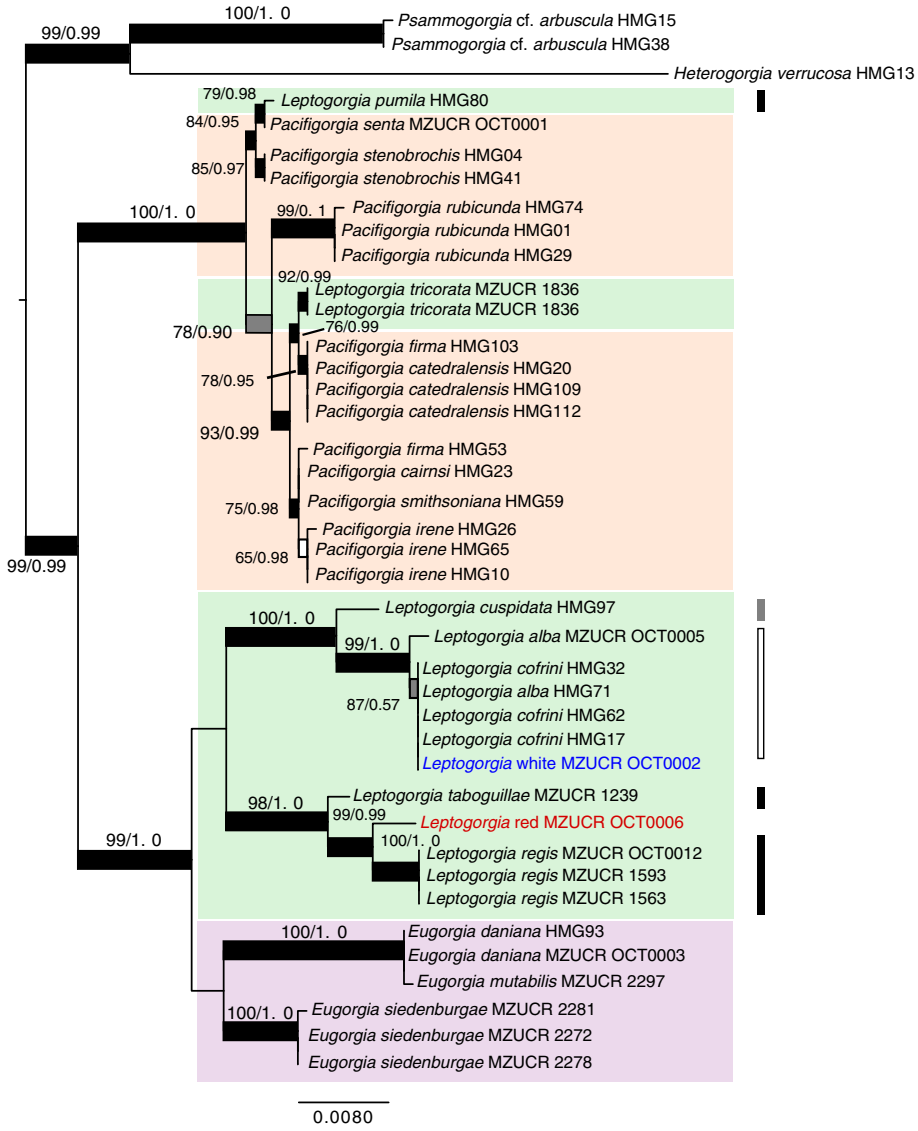

Fig. 2. Phylogeny of the combined *mtMutS* + 28S rDNA dataset. Despite the lack of support in the branches defining *Eugorgia* and *Leptogorgia*, the topology is highly congruent with the 28S rDNA tree. Colors and symbols as in Fig. 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

4.1. Colony morphology is highly homoplasious in Eastern Pacific octocorals

The phylogenetic hypotheses here inferred (Figs. 1 and 2, S14–S16) using the nuclear 28S rDNA and mitochondrial *mtMutS* markers revealed a complex evolutionary scenario for the investigated Eastern Pacific octocoral taxa. Previous phylogenetic studies on these fauna concluded that *Leptogorgia* (the Eastern Pacific species), *Pacifigorgia*, and *Eugorgia* were monophyletic (Vargas et al., 2014). Incorporating new taxa into the *mtMutS* tree suggests that the Eastern Pacific *Leptogorgia* species are not monophyletic, since *L. taboguillae* and *L. regis* are part of a divergent clade including *E. daniana* and allied taxa. Breedy and Guzman (2007) noted that both *L. taboguillae* and *L. regis* have a very distinct morphology among *Leptogorgia* species, with the branching pattern of *L. regis* resembling that of some *Eugorgia* species. On the other hand, the sampled Caribbean *Leptogorgia* taxa formed a supported clade with the remaining Eastern Pacific species and *Eugorgia siedenburgae* in the *mtMutS* phylogeny. In contrast, Eastern Pacific *Leptogorgia* is recovered as monophyletic in the 28S rDNA tree (with some exceptions, see below), yet a larger sampling of markers or species could change or corroborate this conclusion.

Some species morphologically classified as *Leptogorgia* (*L. pumila* and *L. tricorata*) nested within *Pacifigorgia* with high support in all analyses, making the current taxonomic definition of *Pacifigorgia* paraphyletic. The only morphological distinction between *Pacifigorgia* and *Leptogorgia* is the anastomosis of branches into meshes in the first genus. A few species of *Leptogorgia* have been reported to occasionally form loose anastomoses in the Eastern Pacific (Breedy and Cortés, 2011), and among the species nested in the *Pacifigorgia* clade only *L. pumila* shows branch anastomoses, albeit very rarely. The other species (*L. tricorata*) has never been observed to anastomose (Breedy and Cortés, 2011). The inclusion of these species within *Pacifigorgia* indicates that a character reversal to the ancestral state (i.e. no-anastomosed branches; Vargas et al., 2014) has occurred independently in at least two lineages within *Pacifigorgia*. The ability to form anastomoses has evolved multiple times in different,

unrelated octocoral lineages (e.g. *Gorgonia*) suggesting that this character is evolutionary labile (Sánchez et al., 2003b; Sánchez, 2004). Our results further point towards the evolutionary lability of branch anastomosis among octocorals and show that this trait can be labile at shallow phylogenetic levels (i.e. within genera).

Despite the lack of congruence between the mitochondrial and nuclear data, it seems clear that the two whip-like specimens sampled are not closely related, with the red specimen being related to *L. regis* and *L. taboguillae* and the white phenotype to the "*alba*-group". Whip-like colonies have been only recently found in Eastern Pacific shallow waters, despite their common occurrence among Western Atlantic–Caribbean and Eastern Atlantic species of *Leptogorgia*. In the Caribbean, Bayer (1961) noted four *Leptogorgia* species that show a whip-like morphology (i.e. *Leptogorgia stheno*, *L. medusa*, *L. euryale* and *L. setacea*). In West Africa, some species show the same whip-like morphology observed in our samples, e.g. the *Leptogorgia riodouroi* group *sensu* Grasshoff (1988). All these species have similar morphology: whip-like colonies without or with very scarce branching, with occasional holdfasts but normally laying or floating on the substrate. Yet, there are differences in color, polyp-mounds (prominent, slightly raised), and especially sclerites compared to the Eastern Pacific species. Western Atlantic–Caribbean species have long, bent spindles with asymmetrical tubercles and incomplete disk-spindles that are not present in the African and Eastern Pacific species, and the West Africa taxa possess characteristic long and spiny spindles without any prominent ornamentation. Using ITS2 sequence data and secondary structure models, Sánchez (2007) placed the West African taxa in the genus *Filigorgia* Stiasny. The analyses of Aguilar and Sánchez (2007) using ITS2 and of the sclerome of *Filigorgia* suggest that this genus is not related to the Eastern Pacific or Western Atlantic–Caribbean *Leptogorgia* species, making this a clear example of independent evolution of the whip-like colony morphology. The recently described Mexican species *Leptogorgia filicrispa* Horvath, 2011 closely resembles the whip-like specimens included in our phylogenetic analyses. This species contains both red ("salmon pink"' in the original description) and white individuals (Horvath, 2011). Our results demonstrate that, in the eastern Pacific, whip-like *Leptogorgia* specimens of different colors are not closely related. Thus, a re-evaluation of the taxonomy of *L. filicrispa* including the analysis of molecular markers seems necessary to corroborate its identity or assign valid names to the different color morphs included in its current concept if they are not closely related lineages. In general, our findings clearly indicate that the whip-like colony morphology is a homoplasious state among Eastern Pacific *Leptogorgia* and more generally within this genus. In addition, the multiple occurrences of the whip-like phenotype suggest a role of natural selection in favoring unbranched and detached colonies among species of *Leptogorgia*. The ecological and evolutionary significance of this phenotype deserves further investigation.

4.2. Possible explanations for mito-nuclear phylogenetic discordance among Eastern Pacific gorgonians

Mito-nuclear discordance is defined as a significant difference in the patterns of differentiation between the two marker types that results in the reconstruction of different evolutionary relationships among organisms (Toews and Brelsford, 2012). From a methodological viewpoint, an important concern with the use of ribosomal nuclear markers for phylogenetic inference is the presence of intragenomic variation. Incomplete gene conversion can result in different lineages of rDNA within a single individual that may confound phylogenetic reconstructions (O'Donnell and Cigelnik, 1997). An example of this is the scleractinian genus *Acropora*, which has extremely high levels of ITS variability (Wei et al., 2006). However, assessments of ITS2 intragenomic divergence in gorgonians related to the Eastern Pacific taxa showed relatively low levels of such variation (Dorado and Sánchez, 2009; Torres-Suárez, 2014). For example, while *Acropora palmata* has an ITS2 average pairwise sequence divergence of around 1.5% (Vollmer and Palumbi, 2004), Torres-Suárez (2014) found a divergence of only 0.7% for a number of gorgonian species. Our own preliminary explorations of ITS2 variation through cloning in Eastern Pacific species showed even lower levels of maximum divergence (Vargas, unpublished): 0.3% pairwise divergence in *Pacifigorgia rubicunda* (5 clones), *Leptogorgia alba* (4 clones), and *Eugorgia daniana* (6 clones). Considering that *A. palmata* has lower levels of intragenomic divergence in the 28S rDNA than in the ITS2 (Vollmer and Palumbi, 2004) and assuming gene conversion occurs at similar rates across the entire rDNA operon, we would expect little interference of such variation in our phylogenetic reconstructions. More importantly, biases introduced by the multicopy nature of rDNA would typically produce polyphyletic species/genera, which is the opposite situation to what we find. However, it is clear that while informative, the 28S rDNA still has limited polymorphism and other nuclear markers should be considered.

From a biological perspective, the usual explanation for mito-nuclear discordance is based on the expected differences in inheritance and effective population size of mitochondrial vs. nuclear markers (Zink and Barrowclough, 2008; Toews and Brelsford, 2012). These disparities may lead to incomplete lineage sorting (and thus to para- or polyphyly) more often in the nucleus compared to the mitochondria (Hudson and Turelli, 2003; Toews and Brelsford, 2012). Interestingly, we found the Eastern Pacific *Leptogorgia* and *Eugorgia* to be non-monophyletic when analyzing the mitochondrial marker. Arguably, the fact that morphological evidence (that is, taxonomy) is consistent with the nuclear tree suggests that the cause of disparity may be related to the mitochondrial evolutionary dynamics among octocorals. Indeed, cnidarian mitochondrial genomes seem to be unusual in many ways, including the presence of introns, structural rearrangements, and extremely low substitution rates (Bilewitch and Degnan, 2011). Specifically, it has been suggested that the cnidarian mitochondrial genome evolves 10–20 times slower than its vertebrate counterpart (van Oppen and Willis, 1999; Shearer et al., 2002). Yet, the *mtMutS* gene has the highest levels of variation among octocoral mitochondrial protein-coding genes (McFadden et al., 2010; Bilewitch and Degnan, 2011) and seems to evolve as fast as the nuclear 28S rDNA as judged by the branch lengths of the phylogenetic trees inferred here.

Additional biological processes could be responsible for the observed mito-nuclear conflict, among them homoplasy, unrecognized paralogy, and hybridization (Funk and Omland, 2003). In our case, the lack of substitution saturation and of frame-shifts or stop codons (which would suggest the presence of pseudogenes) in the *mtMutS* gene argues against homoplasy or unrecognized (fairly ancient) paralogy as the causes of the mito-nuclear conflict detected. Recent paralogs that retain coding structure are more likely to occur among different related species, not between different genera, although we cannot fully exclude that possibility. On the other hand, genes of maternally inherited cytoplasmic elements (i.e. mitochondria and chloroplasts) frequently spread from one population or species to another (i.e. they introgress) more rapidly than biparentally or paternally inherited components (Martinsen et al., 2001; Chan and Levin, 2005; Gompert et al., 2008). Mito-nuclear conflict in which the nuclear tree is in agreement with morphology and shows monophyly while mitochondrial trees do not has been observed in recent radiations with potentially high levels of hybridization and mitochondrial introgression (e.g. Shaw, 2002; Sullivan et al., 2004; Fontenot et al., 2011). Modular sessile marine animals, such as scleractinian and

octocoral species, have reproductive traits (e.g. broadcast spawning) that may predispose them to hybridize (McFadden and Hutchinson, 2004; Willis et al., 2006). Hybridization and introgression have been shown to occur in species of *Acropora* (Vollmer and Palumbi, 2002) and possibly in the octocoral *Alcyonium* (although without introgression; McFadden and Hutchinson, 2004), and in general may play an important role in the diversification of coral reefs species (Vollmer and Palumbi, 2002; Willis et al., 2006; see Willis et al., 2006 for other examples). Ancient hybridization events during the early divergence of *Eugorgia* and *Leptogorgia* could explain the detected mito-nuclear conflict, but more data is needed to explore this and other scenarios.

5. Conclusions

The use of nuclear and mitochondrial genetic markers revealed contrasting phylogenetic patterns between the three main genera of Eastern Pacific octocorals, stressing the importance of applying independent sources of information for phylogenetic inference. Moreover, expanding the taxonomic sampling further showed that *Leptogorgia* and *Pacifigorgia* are not monophyletic in their current definition. Future taxonomic actions should transfer *L. pumila* and *L. tricorata* to *Pacifigorgia*. The description of this genus requires emendation to reconcile the morphological and molecular data.

Convergence (*sensu* Arendt and Reznick, 2007) of morphological complexity in axial structures and sclerites has been established at large phylogenetic scales among octocorals, for instance between the Calcaxonia and Alcyoniina–Holaxonia (Sánchez et al., 2003a). Similarly, independent evolution of anastomosed and pinnate colonies has been detected between different clades of Gorgoniidae and Plexauridae in the Caribbean Sea (Sánchez et al., 2003b). This study shows lability (that is, the property of evolutionary changeability in a trait; Silvertown et al., 2006) of the colony morphology among closely related gorgonian species within genera, providing further evidence for the generalized homoplasic nature of morphology among Octocorallia.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2016.02.023.

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