Molecular phylogenetic relationships reveal contrasting evolutionary patterns in Gorgoniidae (Octocorallia) in the Eastern Pacific

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Abstract

The description and delimitation of species in an evolutionary framework is essential for understanding patterns of biodiversity and distribution, and in the assessment of conservation strategies for natural resources. This study seeks to clarify the evolutionary history and genetic variation within and between closely related octocoral species that are fundamental to benthic marine ecosystems for harbouring a high diversity of associated fauna. For our study system, we focused on members of the Gorgoniidae family in the Eastern Pacific, particularly of the Ecuadorian littoral, a less studied marine ecosystem. According to our results, the diagnosis of the genus *Pacifigorgia* is here amended to include species previously considered in the genus *Leptogorgia*. The

genera *Leptogorgia* and *Eugorgia* are included within a single clade, and neither are recovered as monophyletic. In this case, according to the priority rule of the International Code of Zoological Nomenclature (ICZN), our proposal is to include the species considered in these two genera in *Leptogorgia*. In addition, we found evidence of interesting speciation patterns: morphological differentiation with no apparent genetic differentiation (in *Pacifigorgia*), and inconsistencies between mitochondrial and nuclear data that suggest a hybridisation phenomenon (in *Leptogorgia*). In the first case, recent radiation, ancient hybridisation, sympatric speciation, and in the second, reticulate evolution may have contributed to the evolutionary history of the studied taxa. Therefore, incongruences observed between morphological and molecular evidences in these octocorals, and in corals in general, may reveal the types of events/patterns that have influenced their evolution.

Introduction

Species are widely used as fundamental units in systematics, biogeography, palaeontology and ecology (Bagley et al., 2015; Barraclough and Nee, 2001), guiding research programs in many disciplines (Ardila et al., 2012; Fontdevila and Serra, 2013). Although this unit is crucial for understanding biodiversity, how best to delimit species is a hotly debated topic for many groups in which morphological characters are limited, cryptic or reduced (e.g. due to miniaturisation) (Delicado et al., 2013). An integrative taxonomic framework (Padial et al., 2010), which combines morphological and molecular studies, provides a more solid foundation for distinguishing basic units, facilitating better descriptions and the discovery of new species. However, the study of discriminant characters alone is insufficient to understand speciation patterns or to

deduce which processes may have led to the changes that gave rise to these different units.

In the last decades, evolutionary studies have not only strived to resolve species identification and ranges of genetic variation within and between closely related species (Moritz and Cicero, 2004; Wang et al., 2011), but also to clarify the speciation processes involved. However, challenges remain in resolving some of the more problematic phylogenetic questions, even in cases having significant sampling of characters and taxa (Rokas et al., 2003; Wiegmann et al., 2011). For instance, the interpretation of certain evolutionary patterns, such as those resulting from the rapid origin of lineages (explosive radiation), events of hybridisation, adaptive introgression (Hatta et al., 1999; Henning and Meyer, 2014; Torres-Suárez, 2015), and in some cases, the search for adequate molecular markers (Winkler et al., 2015), can be especially complex. Given these challenges, it is widely recognised that the best approach for establishing phylogenetic relationships among organisms is to combine as many different types of data as possible (e.g. molecular, morphological, intraspecific population ecology and evolutionary) in order to offer alternative hypotheses for different observed patterns (Eernisse and Kluge, 1993; Guil et al., 2013; López-González et al., 2014; McKinnon et al., 2004; Prada and Hellberg, 2013).

Anthozoans (in general) and octocorals (in particular) represent examples of organisms that are poorly understood phylogenetically at nearly all levels, from family to species, due to differences between morphological and molecular approaches (McFadden et al., 2006). In fact, some studies have directly shown how the traditional taxonomy (classifying based on morphology alone) of these organisms sometimes differs from results obtained by molecular phylogenetic analyses (Ardila et al., 2012; Hatta et al., 1999; Sánchez et al., 2003; Torres-Suárez, 2015). The study of these relationships has

also been hampered by both phenotypic plasticity and the use of a series of discrete and homoplastic characters to establish species in morphological descriptions (Concepcion et al., 2008; Wirshing and Baker, 2015). Some homoplastic and/or overlapping characters include colony and sclerites colouration, types and sizes of sclerites and branching patterns. In the absence of additional information, morphologically defined species can be considered as hypotheses for possible evolutionary groups; however, molecular phylogenetic reconstructions are preferred, and mitochondrial gene markers are currently most commonly used (McFadden and van Ofwegen, 2013a; Sánchez et al., 2003; Vargas et al., 2014). However, octocoral mitochondrial DNA exhibits low substitution rates: their evolution is estimated to be 10 to 100 times slower than that of other metazoans (Brockman and McFadden, 2012; Chen et al., 2009; France and Hoover, 2002; Hellberg, 2006; Huang et al., 2008; McFadden et al., 2004; Shearer et al., 2002). The presence of the octocoral-specific mtMutS gene, which facilitates mismatch repair in mitochondrial DNA, may account for the low number of observed substitutions (France and Hoover, 2002).

McFadden and colleagues (2011, 2014) showed that only ~70 % of species analysed could be discriminated using mtMutS and CoxII-Igr-CoxI (the "extended mitochondrial barcode"). Furthermore, it has been shown that if species delimitation estimates are based solely on mtDNA, boundaries between closely related octocoral species cannot be detected, and these groups would be considered identical (Vargas et al., 2014; Yasuda et al., 2015). Therefore, nuclear markers, such as 28S rRNA (28S) or internal transcribed spacers (ITS), are used to compensate for the low substitution rate of mitochondrial genes (Aguilar and Sánchez, 2007; McFadden and Hutchinson, 2004; McFadden et al., 2006, 2014; Sánchez et al., 2007). Specifically, ITS sequences have been very useful for

comparing octocoral species and indicating hybridisation events (Aguilar and Sánchez, 2007; McFadden and Hutchinson, 2004).

Gorgoniidae is one of the most diverse families of Octocorallia and includes some of the most abundant shallow-water taxa, particularly in the Eastern Pacific. Members of this family are commonly and widely distributed in this area from Baja California to Peru (Breedy et al., 2009). Within this area, the littoral of Ecuador is known to harbour a high level of biodiversity; despite this, in-depth studies, specifically of marine invertebrates and/or their phylogenetic relationships, are still lacking (Cruz et al., 2003; Soler-Hurtado and López-González, 2012; Vargas et al., 2014).

Hence, the aim of this study is to reveal the genetic diversity and relationships of members of the Gorgoniidae family in the Eastern Pacific using Ecuador as the main study area, with the ultimate goals of advancing the examination of boundaries of closely related species/genera and exploring the possible sequence of speciation events that gave rise to current gorgonian diversity.

Material and Methods

Samples collection, species and study area

Between February 2010 and June 2014, fresh gorgonian octocoral material was sampled from eleven stations located in Ecuador. Gorgonians were collected by SCUBA diving between depths of 5 and 30 metres. Samples were fixed in absolute ethanol for subsequent molecular analyses.

We also examined samples of type specimens and additional materials deposited at the Museum of Comparative Zoology (MCZ), Harvard University, Cambridge, MA;

Muséum National d'Histoire Naturelle (MNHN), Paris; Natural History Museum (NHM), London; Yale Peabody Museum of Natural History, New Haven, CT and the Smithsonian National Museum of Natural History, (NMNH), Washington D.C. (Table 1).

Each sample was identified using morphological characters according to current taxonomy (Bayer et al., 1983; Breedy et al., 2009; Breedy and Guzman, 2007, 2002). In the case of *Pacifigorgia*, some samples were not definitively identified due to the lack of clearly distinguishable morphological characters. These specimens have been labelled *Pacifigorgia* sp1 to sp7.

DNA extraction, PCR amplification, and sequencing

DNA was extracted from 20-30 mg of tissue using the DNeasy extraction Kit (Qiagen, Inc.) according to the manufacturer's protocol. Two mitochondrial (CoxII+Igr+CoxI, hereafter referred to as Cox, and mtMutS) and two nuclear (ITS1 + 5.8S + ITS2, hereafter ITS, and 28S) regions were amplified by PCR using the following primers: COII8068F (McFadden et al., 2004), COIOCTR (France and Hoover, 2002), the newly developed COI-Gorg1-R3 (5' AGAGAAGGTGGTAATAACCAGAAA-3') and COI-Gorg2-F2 (5'-GATTCGGAAATTGGTTTGTG-3') for Cox; ND42599F (France and Hoover, 2002) and MUT3458R (J. A. Sánchez et al., 2003) for mtMutS; ITS 2.1 and ITS 2.2 for ITS (Hugall et al., 1999); 28S-Far, 28S-Rar (McFadden and van Ofwegen, 2013b) and the newly developed 28S-R3 (ACTGCATRTATGAACTCCA) for 28S.

Amplifications were carried out in 50 µl final volume reactions containing 5 µl of 10x buffer (containing 10x 2 mM MgCl₂), 1 µl dNTPs mix (10 mM), 0.8 µl of each primer (10 µM), 0.5 µl of Taq DNA polymerase (5U/µl) (Biotools) and 2 µl of genomic DNA.

Thermocycler parameters for all amplifications included an initial 4 min denaturation step at 94 °C. Subsequent cycling parameters for each region are as follows: for Cox, 40 cycles of 45 s at 94 °C, 1 min at 58 °C and 1 min at 72 °C with a final extension step of 10 min at 72 °C; for mtMutS, 35 cycles of 90 s at 94 °C, 90 s at 58 °C and 1 min at 72 °C with a final extension step of 5 min at 72 °C; for ITS, 35 cycles of 60 s at 94 °C, 90 s at 56 °C and 1 min at 72 °C with a final extension step of 7 min at 72 °C; for 28S, 30 cycles of 90 s at 94 °C, 90 s at 50 °C and 1 min at 72 °C with a final extension step of 5 min at 72 °C. As the ITS region is a multicopy marker, intra-individual variations occur (Álvarez and Wendel, 2003; Concepcion et al., 2008); therefore amplicons with polymorphic positions were subsequently cloned using competent TOPO® bacterial cells (Invitrogen, Thermo Fisher Scientific) and the pGEM-T Vector System (Promega). After plasmid isolation using the Wizard Minipreps DNA Purification System (Promega), up to 10 positive clones were sequenced for both strands with M13 universal primers. The amplification products were purified by ethanol precipitation and with 0.5 U Gelase (Epicentre Biotechnologies).

The amplicons were sequenced using BigDye Terminator in an ABI 3730 genetic analyzer (Applied Biosystems). The obtained sequences were edited using Sequencher v.4.6 (Gene Code Corporation, Ann Arbor, MI, USA). In the case of ITS, only the consensus sequence was used. The complete molecular data matrix also included published sequences available in GenBank (Table 1). New sequences were deposited in GenBank (Table 1).

Collapse 1.2 (Posada, 2004) was used to identify different haplo/genotypes among all the Gorgoniidae sequences (excluding outgroups) for each molecular marker. For these analyses, some sequences (e.g. GenBank sequences of longer fragments) were trimmed to avoid artificial haplo/genotypes.

jModelTest was used to determine the best-fit model for nucleotide evolution for each final alignment (Posada, 2008). Phylogenetic analyses were performed using PhyML v3.0 for Maximum Likelihood (ML) (Guindon and Gascuel, 2003). Maximum Parsimony (MP) was calculated in PAUP 4b10 (Swofford, 2002). MP parameters included a heuristic search with tree bisection-reconnection (TBR) branch swapping. The ML and MP supports were performed with 1000 bootstrap replicates (bootstrap values = bv). Bayesian inference of phylogenetic relationships was performed with MrBayes v3.1. (Huelsenbeck and Ronquist, 2001): two parallel runs of 5 million generations, with each run containing one cold and three heated Markov Chains Monte Carlo (MCMC), were carried out, sampling every 1000 generations. The initial 10% of sampled trees were discarded as the burn-in and the support values determined by posterior probabilities (pp).

Results

Four matrices were obtained for the four analysed genes: (1) mtMutS (143 specimens, 832 characters); (2) Cox (117 specimens, 990 characters); (3) ITS (64 specimens, 697 characters); and (4) 28S (86 specimens, 886 characters). The number of informative characters were unequal among the genes, with the ITS region having higher numbers of parsimony informative characters and single-species genotypes (Table 2). A concatenated matrix was also generated using the alignment of specimens with data for all four markers: mtMutS+Cox+ITS+28S (46 specimens, 3371 characters).

The best-fit models of nucleotide substitution obtained for our data were HKY+G for mtMutS, HKY+I+G for Cox, K80+I+G for ITS and 010023+I+G for 28S. For the concatenated data, the best-fit model was HKY+I+G.

Overall, phylogenetic analysis of the concatenated matrix, in which 265 sites were considered parsimony informative, gave the most supported and resolved nodes (Fig. 1). The inter-species relationships in some groups were different for the nuclear and mitochondrial genes; however, for each gene, all three approaches (BI, MP and ML) resulted in the same topologies.

The reconstruction obtained from the concatenated dataset (Fig. 1) showed two well-supported ($pp \geq 0.95$; $bv \geq 70$) clades: the first representing the genus *Pacifigorgia* (Clade I) ($pp = 1$; $bv = 100$), and the second including both *Eugorgia* and *Leptogorgia* representatives (Clade II) ($pp = 1$; $bv \geq 98$). The taxa belonging to *Eugorgia* comprised two lineages (Clade II.C), one composed of *E. ahorcadensis* and *E. nobilis* ($pp = 1$; $bv \geq 99$) (Clade II.C.1), and the other by *E. daniana* ($pp = 1$; $bv = 100$) (Clade II.C.2), as the sister species of a subset of *Leptogorgia* taxa; however, there was no support for the node joining these lineages. This subset included the species *L. alba* (in different subclades), *L. cofrini*, *L. flexilis* (in two different branches) and *L. cuspidata* (Clade II.A). Finally, *L. diffusa*, *L. obscura* (sister species to *L. diffusa*) and *L. mariarosae* (Clade II.B) completed this main clade ($pp = 1$; $bv \geq 99$).

In the combined reconstruction, Clade II.A is divided into two subclades (Fig. 1). Subclade II.A.1 grouped *L. alba* (1), *L. alba* (2), *L. cofrini*, *L. alba* (3) and *L. alba* (4), as the sister group of *L. flexilis* (1) ($pp \geq 0.81$; $bv \geq 100$); subclade II.A.2 grouped individuals of *L. cuspidata*, *L. flexilis* (2) and *L. alba* (5) ($pp \geq 1$; $bv \geq 100$).

The *Pacifigorgia* representatives were recovered in three monophyletic groups (Fig. 1). Within Clade I.A, *P. irene*, *P. machalilla*, *P. rubripunctata*, *P. rubinoffi* and four of the unidentified species (sp1 - 4) grouped together ($pp = 1$; $bv \geq 99$). The well-supported subclade consisting of *P. irene*, *P. machalilla* and *Pacifigorgia* sp1, *Pacifigorgia* sp2

and *Pacifigorgia* sp3 showed no differentiation among the putative species. Clade I.B, sister to Clade I.A, is comprised of *P. cribrum*, *P. media*, *P. rubicunda* and two other unidentified species (sp5 and 6) (pp = 1; bv $\geq$ 87). Clade I.C contained *P. flavimaculata*, *P. stenobrochis*, *P. sculpta* and the final unidentified species (sp7) (pp = 1; bv $\geq$ 93).

As only specimens with complete datasets were used in the concatenated matrix, results from analyses of single gene matrices, in which more samples and species were represented, was used to provide additional evidence about the evolutionary history of these taxa.

In some of the individual analyses, *L. chilensis*, *L. styx*, *L. ramulus*, *L. cortesi* (mtMutS tree) (Fig. 2), and *L. rigida* (both mtMutS and 28S trees) (Fig. 2) were also included in Clade II.A. However, in the mtMutS reconstruction, the two samples belonging to *L. ramulus* (no type material) grouped with two different clades, one in Clade II.A, and the other with *L. labiata* and its sister clade of *E. daniana*, *E. multifida* and *E. mutabilis* (Clade II.C.2) (Fig. 2). In the 28S tree, the clade formed by *E. daniana*, *E. multifida* and *E. mutabilis* was the sister clade of *E. beebei* and *E. siedenburgae*, in contrast to the mtMutS tree (Fig. 2). In both the mtMutS and 28S trees, *L. toboguilla* and *L. regis* were also included in Clade II.B; *L. rubra* was also included in this clade with high support based on ITS (Fig. 2).

Eugorgia ahorcadensis and *E. nobilis*, which constituted the lineages within Clade II.C.1, were closely related to *E. ampla* (Cox, mtMutS and ITS trees) and *E. querciformis* (mtMutS tree) (Fig. 2).

Analysis of each gene individually also provided information about phylogenetic relationships for *Pacifigorgia*. In the mtMutS analysis, Clade I.A also included *P. adamsii*, *P. agassizi*, *P. cairnsi*, *P. elegans*, *P. smithsoniana*, *P. bayeri*, *P. catedralensis*,

P. ferruginea, *P. firma* and *L. tricolorata* (Fig. 2). For 28S, the clade was completed with
P. cairnsi, *P. catedralensis*, *P. firma*, *P. smithsoniana* and *L. tricolorata* (Fig. 2).
Polytomies in *Pacifigorgia* and *Leptogorgia* were more frequent in the gene trees,
especially in the case of the mitochondrial genes. Clade I.B also included *P. exilis* and
P. samarensis in mtMutS, Cox and ITS trees (Fig. 2) but this relationship was only
strongly support for mtMutS (Fig. 2). Finally, Clade I.C, which also included *P. senta*
and *L. pumila* in the 28S and mtMutS trees, was well supported in the concatenated tree
but not in individual gene trees (Fig. 2). The relationships among Clades I.A, I.B and
I.C differed in the single gene trees.

The results showed some contrasting patterns. In Subclades II.A.1 and II.A.2,
specimens of the same species grouped together in the mitochondrial gene analyses,
having species specific mtDNA haplotypes (see mtMutS and Cox trees) (Fig. 2,
Appendices 1 and 2). In the nuclear gene analyses, these specimens showed differences
and did not always group according to species (see ITS and 28S trees). The topology of
Subclade II.A.2 was the same in both the concatenated and the ITS trees, except for *L.*
flexilis (1). In the ITS tree, this specimen was included in the clade with *L. alba* (3) and
L. alba (4) rather than being its sister group. In the 28S tree, *L. flexilis* (1) instead
grouped with Subclade II.A.2, together with *L. cuspidata*, *L. alba* (5) and *L. flexilis* (2).

Similar to the case of *L. ramulus* in the mtMutS analysis, in which specimens were split
between two different clades, some other species were divided according to sample
origin (i.e. type locality or material from elsewhere). For example, samples of *P. firma*
(including type material MZC 51918) in the case of Cox and *P. cairnsi* or *P. cf. cairnsi*
(including type material MCZ 51915) in the case of mtMutS were found in different
lineages.

After including taxa from the single gene analyses and summarising all the information, the trees and phylogenetic relationships obtained from the concatenated dataset and the individual genes are incongruent. At the mitochondrial level, clades I.A and I.C clustered together instead of being distinct sister-group clades as in the concatenated tree. For Clade II, the incongruences are more complex. In mtMutS, Clade II.A was mixed with the species belonging to Clade II.C.1, and this group was the sister clade to the clade consisting of species from clades II.B and II.C.2. Relationships based on Cox showed Clade II.B as the sister group to the rest of the *Leptogorgia* taxa. Furthermore, clades II.A and II.C.2 were clustered together with Clade II.C.1 as its sister group, and the three aforementioned clades together were sister group of Clade I (Figs. 1 and 2).

For the nuclear gene reconstructions, there were no subdivisions within Clade I. The same topology for Clade II was obtained for ITS and the concatenated data. In the 28S analysis, Clade II was composed of three subclusters in a polytomy: II.C.1, II.C.2 and a clade composed of II.A, sister of II.B (Figs. 1 and 2).

Incongruences between markers in this group may reflect possible evolutionary processes influencing speciation patterns of Clade II.A species. For example, although some specimens of *L. alba*, *L. cuspidata* and *L. flexilis* presented mitochondrial haplotypes consistent with their taxonomic adscription, their nuclear genotypes (based on ITS and 28S) were shared with those of other species, pointing to possible hybridisation.

Discussion

Molecular markers

Many phylogenetic studies still rely on single markers or markers of limited utility to provide phylogenetic inferences. The use of individual genes has been criticised as this method seldom recovers precise topologies (Rokas et al., 2003; Salichos and Rokas, 2013). Due to this limitation, the current trend in phylogenetic analysis is based on the idea of “more is better” (Winkler et al., 2015). In previous studies, mtMutS, both alone and in combination with other mitochondrial genes, has been used to examine inter- and intrageneric relationships among shallow-water Caribbean holaxonians (Sánchez et al., 2003; Vargas et al., 2010b; Wirshing and Baker, 2015), Indo-Pacific alcyoniid soft corals (McFadden et al., 2006) and deep-sea gorgonians (France, 2007; Thoma et al., 2009). Indeed, mtMutS and ITS2 are two of the most commonly used and variable regions for analysis. Hence, they represent important genetic markers for phylogenetic reconstructions (Aguilar and Sánchez, 2007; Herrera et al., 2010; McFadden et al., 2010; Yasuda et al., 2015). Similarly, the incorporation of 28S and Cox has further potentiated such phylogenetic analyses (Brockman and McFadden, 2012; France and Hoover, 2002; McFadden and van Ofwegen, 2013a), whereas the mitochondrial genes ND2 and ND6 (also evaluated in this study however not shown) show equal or less resolution at the species level compared with other mitochondrial genes, as has been highlighted previously (McFadden et al., 2006, 2004; Sánchez et al., 2003).

In this study, the analysed phylogenetic resolution at the species level indicate that the nuclear regions ITS and 28S showed the greatest number of informative characters (53.9%), with ITS being the principle contributor (34.57%, even including the conserved 5.8S). The mitochondrial region contributed greatly to the phylogenetic signal in the concatenated analysis (almost 46%), with equal contributions from mtMutS (22.3%) and Cox (23.79%). Although not all of the species analysed (64 in total) could be identified by molecular markers, analysis of the nuclear regions

distinguished 18 species, while the mitochondrial analysis distinguished an additional 5 species. Another 18 species were distinguished by both nuclear and mitochondrial haplotypes. The usefulness of the ITS sequence in octocoral species comparisons, and as an indicator of hybridisation events, has been previously shown (Aguilar and Sánchez, 2007; Calderón et al., 2006; McFadden et al., 2001; McFadden and Hutchinson, 2004). Moreover, the results presented here further demonstrate the benefits of a combined phylogenetic analysis of nuclear genes with concatenated mitochondrial genes (Aguilar and Sánchez, 2007; McFadden et al., 2004; McFadden and van Ofwegen, 2013b). As the number of characters analysed per region increases, so do the number of variable sites and well supported gene trees, leading to higher confidence levels in the relationships (Herrera et al., 2010; Winkler et al., 2015). However, in our results, even with the combination of the four genes, variation at the species level was insufficient for determining the relationships of some complexes.

Phylogenetic relationships, evolutionary patterns and nomenclatural consequences

Until recently, the monophyly of *Pacifigorgia* within Gorgoniidae was well supported by morphological and molecular data (Breedy & Guzman, 2002, 2003, Vargas et al., 2010, 2014). However, in a recent phylogeny by Ament-Velásquez et al. (2016), two *Leptogorgia* species, *L. pumila* and *L. tricolorata*, were included in the *Pacifigorgia* clade based on analyses of 28S and mtMutS. *Leptogorgia pumila* sometimes shows the diagnostic character of the genus *Pacifigorgia* (see Ament-Velásquez et al. 2016: 378). However, according to the original description of *L. tricolorata* by Breedy and Cortés (2011), anastomosis is absent in this species, in contrast to *Pacifigorgia*. In the phylogeny obtained here (also based on 28S and mtMutS, the only available genes for

these two species), which includes eight additional gorgoniid species, both *L. pumila* and *L. tricolorata* are included within the *Pacifigorgia* clade (Fig. 2), consistent with the findings of Ament-Velásquez et al. (2016). As a result, there are several nomenclatural consequences for these related genera. On the one hand, the genus *Leptogorgia* can no longer be maintained as polyphyletic, as is the case when *L. pumila* and *L. tricolorata* are included in the genus *Pacifigorgia*. On the other hand, the morphologic diagnosis of the genus *Pacifigorgia* cannot be restricted to anastomosed forms, as open branched forms (and intermediate forms like *L. pumila*) are clearly closely related to characteristic *Pacifigorgia* species.

Leptogorgia pumila (originally described as *Gorgonia (Litigorgia) pumila* Verrill, 1868: 415) and *L. tricolorata* Breedy and Guzman, 2011 should now be considered derived forms in the genus *Pacifigorgia*, and the diagnosis of this genus should be amended and extended as follows: gorgoniid with attached erect colonies, branching pattern often anastomosed, although occasionally, open ramification is observed, in single or multiple planes (parallel or perpendicular); polyps usually small in size, fully retractile into coenenchymal mounds (flat, slightly raised or prominent); coenenchymal sclerites in the form of tuberculate spindles (acute and blunt) and capstans; anthocodial sclerites relatively scarce as platelets and/or flat rods, at the base of tentacles; sclerites usually coloured, with bicoloured forms sometimes present.

In the phylogenies obtained here, *Leptogorgia* and *Eugorgia* representatives also did not form monophyletic clades according to species. Clearly this disagrees with current taxonomic designations, which are exclusively based on morphological characters (Bayer et al., 1983; Breedy et al., 2009; Breedy and Guzman, 2007). These characters include the presence of modified sclerites (disc-spindles, double-disc) mainly in *Eugorgia*; but disc-spindles have also been found in some species of *Leptogorgia* from

the Atlantic (Breedy et al., 2009). Moreover, the external morphology and characters used for diagnostic descriptions, such as colony shape, branching type, polyp mound and colony colour variability, are very similar in these two genera (Breedy et al., 2009; Breedy and Guzman, 2007). The discrepancy between the phylogenetic study showing *Leptogorgia* and *Eugorgia* as monophyletic clades, with *Eugorgia* as the sister group to *Leptogorgia* (Vargas et al., 2014), and this study is certainly due to differences in the source and sampling of species included in the analyses. The addition of other *Eugorgia* species and increased sampling of both taxa revealed differing relationships and the lack of monophyly of both genera. To have a taxonomy based on evolutionary units, species assigned to, at least, Eastern Pacific *Leptogorgia* and *Eugorgia* should be considered as belonging to the same taxon. According to the ICZN (1999), the name of such a taxon should be *Leptogorgia*, by the Principle of Priority (art. 23), as it was described before *Eugorgia*. This evolutionary pattern among Eastern Pacific *Leptogorgia* species implies the emendation and extension of the morphological-molecular diagnosis of the genus. The diagnosis of *Leptogorgia* can be described as follows: gorgoniid attached colonies, with open branching pattern (e.g. dichotomous, pinnate and bushy), in single or multiple planes; anastomosis patterns are absent although basal branches can occasionally fuse; polyps usually small in size, fully retractile into coenenchymal mounds; coenenchymal sclerites in the form of tuberculate spindles, capstans, but also disc spindles and double disc; anthocodial sclerites relatively scarce, as platelets and flat rods, at the base of tentacles; sclerites usually coloured, with bicoloured forms sometimes present.

In the combined and single gene analyses, certain relationships among species within each genus remained unresolved. Incongruent patterns were found mostly for *Leptogorgia* and *Pacifigorgia*, pointing to two different, but not mutually exclusive, evolutionary scenarios.

For the genus *Leptogorgia*, hybridisation processes are likely driving species evolution. These processes have been suggested to have played an important role in coral evolution (van Oppen et al., 2001; Vollmer and Palumbi, 2004; Willis et al., 2006). Indeed, the obtained data indicated possible hybridisation from inconsistencies observed among individual gene trees (particularly between mtDNA and nDNA) involving the species *L. alba*, *L. cuspidata*, *L. flexilis* and probably *L. cofrini* (Clade II.A). These inconsistencies could have arisen from gene and/or organism evolution. In the first case, incomplete lineage sorting of each marker and/or their different evolutionary rates can coalesce at different times of an organism's history. In the second case, the inconsistencies might be explained by hybridisation/reticulate evolution (Seehausen, 2004; Vollmer and Palumbi, 2004; Wirshing and Baker, 2015). Evolutionary outcomes of hybrid formation could be dependent on the strengths of reproductive barriers (Li et al., 2016). Evidence of complex inter-specific gene introgression is observed in this study and may involve both "pure" parental species and hybrids, thus demonstrating hybrid fertility. In fact, cross-fertilisation in mass spawning processes have been reported in *Acropora* and *Madracis* (Diekmann et al., 2001; Hatta et al., 1999), in which a significant number of hybrids may be produced (Diekmann et al., 2001; Fukami et al., 2003; Hatta et al., 1999; van Oppen et al., 2001). In this scenario, a sort of species complex or syngameon (Lotsy, 1925) that likely lacks effective reproductive barriers forms, leading to repeated rounds of separation and fusion of species, ultimately forming a reticulate pattern of many interconnecting lineages (Veron, 1995). Thus, difficulties may arise in distinguishing between origins from a single pure species or from hybrid swarms when one parental phenotype becomes fixed (Seehausen, 2004). The second evolutionary scenario involves conspicuous morphological differentiation with minimal to no molecular differentiation. In the combined phylogeny, a polytomy,

likely due to the lack of informative positions, was observed among certain *Pacifigorgia* species (Clade I.A), such as *P. irene*, *P. machalilla*, *Pacifigorgia* sp1, *Pacifigorgia* sp2 and *Pacifigorgia* sp3. However, these species are sympatric (often living side by side) and can be identified by consistent morphological characters (Breedy and Guzman, 2003, 2002; Soler-Hurtado and Guerra-García, 2016; Williams and Breedy, 2004). In the individual gene analyses, species such as *P. adamsi*, *P. agassizii*, *P. irene* and *P. machalilla*, also did not present any genetic differentiation. Several possible explanations may account for the lack of genetic differentiation in these cases. Explosive or rapid radiation (Henning and Meyer, 2014; Kocher, 2004; Winkler et al., 2015) is one possibility. If this were the case, each species would have a fixed species-specific morphology, and would be reproductively isolated from a single common ancestral species that was morphologically highly polymorphic (Hatta et al., 1999). However, it is difficult to conceive how several of these gorgonian species could have arisen simultaneously from the ancestral species sympatrically, and have fixed and different morphologies without apparent ecological diversification. These relationships are difficult to ascertain as little difference between the forms of successive common ancestors would exist (Hoelzer and Melnick, 1994). For example, rapid radiation could have given rise to the clade formed by the species *P. irene* and *P. machalilla* (among others): each species shows clear diagnostic characters related to their morphology, such as the network anastomosis and branching shapes (Soler-Hurtado et al., 2016), yet they lack molecular evidence of their distinctiveness. Moreover, if speciation occurred very recently, common ancestral alleles at some loci would remain in the species, and incomplete lineage sorting would be observed, hindering reciprocal monophyly (Yasuda et al., 2015).

The species in this study share a common ecological niche, thus it is necessary to know the contribution of other biotic and abiotic factors influencing the divergence and speciation of these organisms. Sympatric speciation with reproductive isolation may result from several different situations (Bolnick and Fitzpatrick, 2007; Levitan et al., 2004). However, explosive radiation is generally seen as an adaptive radiation; that is, the rapid adaptation to different ecological niches, as seen in the classic example of cichlid fishes. These fishes exploit a broad range of ecological niches accompanied by the evolution of a suite of morphological specialisations (Fryer and Iles, 1972; Henning and Meyer, 2014). Under an adaptive radiation scenario, differentiation in peri-, para- or allopatric areas followed by a secondary contact should be considered. Determining if an adaptive radiation event likely occurred would require several types of new experiments and evidence. For example, a deeper knowledge of the gametogenic cycle and the duration of spawning events, as well as hybridisation experiments that reveal if (even in overlapping spawns) pre- or postzygotic reproductive incompatibility exists, and if so, whether such incompatibility could be associated with recent adaptation (see the case of *Cryptasterina*; Puritz et al., 2012).

Unfortunately, the relative frequency of sympatric speciation is difficult to resolve because biogeographic changes can obscure underlying geographical patterns of past speciation events (Bolnick and Fitzpatrick, 2007). In fact, taxa with good fossil records indicate that any reconstruction of divergence across the tropical Eastern Pacific should consider the possibility that modern-day patterns have been shaped by different processes, such as extinction (Guzmán and Cortés, 1993; Lessios, 2008). Pulses of coral extinction and species origination in the Western Atlantic since the Pliocene are well documented, but in comparison, the record from the Eastern Pacific is poor (Lessios, 2008).

An ancient hybridisation between differentiated species, provoking a re-homogenisation of genomes, might have also shaped this pattern of morphological differentiation versus molecular identity. If the hybrids were fertile and backcrossed with their parental species, then the transmission of genes from one species to another served as a channel for interspecific gene flow (Hatta et al., 1999). The hybrids may not have necessarily expressed intermediate phenotypes, as it occurs, for example, in some *Acropora* hybrids that sometimes exhibit unexpected morphologies, which have been recognised as independent species with their own morphology (Hatta et al., 1999; Wolstenholme et al., 2003).

Other inconsistencies were revealed in our phylogenetic analyses. Materials identified as *L. ramulus*, *P. firma* and *P. cairnsi* were split into different clades (see mtMutS and Cox trees, and Appendices 1, 2), suggesting misidentification or undetected cryptic speciation. The inclusion in this study of type material for the latter two species suggests that the specimens studied by Vargas *et al.* (2014) either were misidentified or, as only mitochondrial genes were analysed, consist of hybrids with the morphological appearance of *P. firma* or *P. cairnsi* but with paternal contributions from species such as *P. irene*, *P. adamsi* or *P. elegans*, and *P. bayeri*, *P. catredalensis* or *P. ferruginea*, respectively. However, in the study by Vargas *et al.* (2014), there were differences between *P. cairnsi* and *P. cf. cairnsi*. In the case of *L. ramulus*, the correct taxonomic designation has yet to be confirmed with the type material. Given these inconsistencies, it is necessary, at least in this group of species (and recommended for the study of all octocorals), to incorporate molecular characters in the morphological descriptions. This is especially important for nomenclatural proposals, and should be included not only in the original descriptions of new taxa (genera and species), but also in any subsequent proposals of synonymization. For example, the proposed synonymy of *Eugorgia*

ahorcadensis and *E. nobilis* (Breedy et al., 2013), justified by the possible wide variability of *E. nobilis*, should be corrected given that, in the present study, both types of materials were compared and were recovered phylogenetically as clearly distinct sister taxa.

In summary, the phylogenetic patterns observed in this study may reflect multiple and possibly overlapping causes as hybridisation, rapid radiation, sympatric differentiation and secondary contact can model these patterns. The different relationships found in the species of both genera (*Leptogorgia* and *Pacifigorgia*) suggest that these interesting speciation processes are occurring in the Eastern Pacific. Different evolutionary events, such as a recent radiation (explosive speciation), an ancient hybridisation or a sympatric speciation, may have influenced the evolutionary history of the genus *Pacifigorgia*. Complex speciation histories, including a reticulate process, may have shaped the evolution of the genus *Leptogorgia* and produced a complex of hybrid individuals, with a possible constant gene flow connecting them.

These results strengthen both the role of phylogenetic analysis in understanding the homoplastic and continuous characters used for the morphological taxonomy of octocorals (Dueñas and Sánchez, 2009; Sánchez, 2007; Vargas et al., 2010a), and the need for a major revision of the morphological descriptions to also include molecular analyses, particularly of “pure” species in order to lineage trace characters, combine genes (Vargas et al., 2014; Yasuda et al., 2015) and take advantage of new sequencing tools.

This study highlights the complexity of inferring evolutionary patterns and demonstrating processes that explain adaptation and speciation in some organisms such as corals. As more species studies are conducted, these types of speciation events (such

as rapid radiations or reticulate evolution) will likely be considered more as key processes (Bilewitch et al., 2014; Brockman and McFadden, 2012; van Oppen et al., 2001; Wirshing and Baker, 2015) that will help clarify or answer unresolved questions about the evolutionary histories of certain species groups.

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Tables

Table 1. Gorgoniidae species included in the molecular analyses. Materials in bold are species sequenced for this study. Note that the names of previously published sequences are as they appear in GenBank and their original publications (including numbers or letters). Numbers after species names indicate different specimens analysed. Type material is marked with an asterisk (*). *Leptogorgia* and *Pacifigorgia* specimens from the Atlantic Ocean are indicated with an (A).

Table 2. Informative characters and haplo/genotypes provided by each molecular marker for the Gorgoniidae phylogenetic analyses. N: number of specimens analysed.

Figure captions

Figure 1. Phylogenetic relationships among taxa from the Gorgoniidae family. Tree topology was inferred by Bayesian analysis, based on combined mitochondrial (coxII+Igr+coxI and mtMutS) and nuclear (ITS and 28S) genes. The number of stars above the branches indicate the number of phylogenetic methods that supported each clade (BI, pp $\geq$ 0.95; ML and MP, bootstrap $\geq$ 70). Numbers after species names indicate different specimens analysed, and an asterisk (*) indicates it is the type material. The orange circle indicates the nodes containing the *Eugorgia* species included in the analysis. Representative images of the typical external morphology of a colony for each genus, *Pacifigorgia* (including a magnified view), *Eugorgia* (including an image of its coenenchymal sclerites) and *Leptogorgia*

Figure 2. Phylogenetic relationships based on separate mitochondrial (coxII+Igr+coxI and mtMutS) and nuclear (ITS and 28S) genes. The number of stars above the branches indicate the number of phylogenetic methods that supported each clade (BI, pp $\geq$ 0.95; ML and MP, bootstrap $\geq$ 70). Note that the names of previously published sequences are

as they appear in GenBank and their original publications (including numbers or letters). Numbers after species names indicate different specimens analysed. *Leptogorgia* and *Pacifigorgia* specimens from Atlantic Ocean are indicated with an (A).

Appendices

Appendix 1. Detailed phylogenetic relationship based on mitochondrial mtMutS. An asterisk (*) indicates it is the type material. For symbols explanation see legend of Figure 2.

Appendix 2. Detailed phylogenetic relationship based on mitochondrial coxI+Igr+coxII. An asterisk (*) indicates it is the type material. For symbols explanation see legend of Figure 2.

Appendix 3. Detailed phylogenetic relationship based on nuclear ITS. An asterisk (*) indicates it is the type material. For symbols explanation see legend of Figure 2.

Appendix 4. Detailed phylogenetic relationship based on nuclear 28S. An asterisk (*) indicates it is the type material. For symbols explanation see legend of Figure 2.

Figure 1

Figure 2

Table 1. Gorgoniidae species included in the molecular analyses. Materials in bold are species sequenced for this study. Note that the names of previously published sequences are as they appear in GenBank and their original publications (including numbers or letters). Numbers after species names indicate different specimens analysed. Type material is marked with an asterisk (*). *Leptogorgia* and *Pacifigorgia* specimens from the Atlantic Ocean are indicated with an (A).

Species	Igr + COI	mtMutS	ITS	28S
<i>Gorgoniidae</i>				
<i>Adelogorgia phyllosclera</i>		JN866558		
<i>Eugorgia ahorcadensis</i> (*)	KX721173	KX721192	KX721211	KX721230
<i>Eugorgia ampla</i> (*)	KX767378	KX767316	KX767467	
<i>Eugorgia daniana</i>	HG917080	LT221110		LT221083
<i>Eugorgia daniana</i> (1)	KX721188	KX721207	KX721226	KX721245
<i>Eugorgia daniana</i> (2)	KX721189	KX721208	KX721227	KX721246
<i>Eugorgia multifida</i>		GQ342494		JX203706
<i>Eugorgia mutabilis</i>		LT221112		LT221084
<i>Eugorgia nobilis</i> (*)	KX767373	KX767311	KX767465	KX767430
<i>Eugorgia pumila</i>	KX767374	KX767312		
<i>Eugorgia querciformis</i>		KX767313		
<i>Eugorgia rubens</i>	KF874216	JN866557		
<i>Eugorgia siedenburgae</i>		LT221096		LT221087
<i>Eugorgia siedenburgae</i>		LT221097		
<i>Eugorgia beebei</i>	KX767375		KX767466	LT221088
<i>Eunicea tourneforti</i>	GQ342445	AY126406		
<i>Eunicella tricornata</i>				JX203707
<i>Gorgonia flabellum</i>	GQ342418	GQ342495		JX203708
<i>Leptogorgia alba</i>	HG917081	HG917034		LT221064
<i>Leptogorgia alba</i>	HG917083	HG917035		
<i>Leptogorgia alba</i> (1)	KX721176	KX721195	KX721214	KX721233
<i>Leptogorgia alba</i> (2)	KX721182	KX721201	KX721220	KX721239
<i>Leptogorgia alba</i> (3)	KX721183	KX721202	KX721221	KX721240
<i>Leptogorgia alba</i> (4)	KX721184	KX721203	KX721222	KX721241
<i>Leptogorgia alba</i> (5)	KX721186	KX721205	KX721224	KX721243
<i>Leptogorgia alba</i> (6)	KX721187	KX721206	KX721225	KX721244
<i>Leptogorgia alba</i> (8)	KX767376	KX767314		
<i>Leptogorgia alba</i> (9)	KX767377	KX767315		
<i>Leptogorgia capverdensis</i> (A)				FJ642931
<i>Leptogorgia chilensis</i>	KF874214	AY268460		
<i>Leptogorgia cofrini</i>	HG917084	HG917037		LT221061
<i>Leptogorgia cofrini</i>	HG917086	HG917040		LT221060
<i>Leptogorgia cofrini</i>	HG917085			
<i>Leptogorgia cofrini</i>	HG917087			
<i>Leptogorgia cofrini</i> (1)	KX767379	KX767317	KX767468	KX767432
<i>Leptogorgia cortesi</i>	LT221105			
<i>Leptogorgia cuspidata</i>		AY268450		LT221066
<i>Leptogorgia cuspidata</i> (1)	KX767380	KX767318	KX767469	KX767433
<i>Leptogorgia diffusa</i> (1)	KX721190	KX721209	KX721228	KX127247
<i>Leptogorgia diffusa</i> (2)	KX737681	KX767319	KX767470	KX767431
<i>Leptogorgia flexilis</i> (1)	KX767385	KX767325	KX767479	
<i>Leptogorgia flexilis</i> (2)	KX767386	KX767326	KX767477	
<i>Leptogorgia flexilis</i> (3)	KX767387	KX767327	KX767476	
<i>Leptogorgia flexilis</i> (4)	KX767388	KX762328	KX767478	KX767435
<i>Leptogorgia flexilis</i> (5)	KX767389	KX767329	KX767479	KX767436
<i>Leptogorgia pumila</i>				LT221059
<i>Leptogorgia pumila</i>	LT221116			LT221058
<i>Leptogorgia gracilis</i>		AY268454		
<i>Leptogorgia hebes</i> (A)		AY268459		
<i>Leptogorgia labiata</i>		AY268447		
<i>Leptogorgia mariarosae</i> (*)	KX721174	KX721193	KX721212	KX721231
<i>Leptogorgia obscura</i> (1)	KX721191	KX721210	KX721229	KX721248
<i>Leptogorgia obscura</i> (2)	KX767382	KX767320		
<i>Leptogorgia obscura</i> (3)	KX767383	KX767321		
<i>Leptogorgia ramulus</i>		KX767322		
<i>Leptogorgia ramulus</i>		AY268451		
<i>Leptogorgia regis</i>		LT221100		LT221081
<i>Leptogorgia regis</i>		LT221198		LT221082
<i>Leptogorgia rigida</i>		GQ342496		
<i>Leptogorgia rubra</i> (A)		KX767323	KX767471	
<i>Leptogorgia styx</i>		AY268453		

Table 2. Informative characters and haplo/genotypes provided by each molecular marker for the Gorgoniidae phylogenetic analyses. N: number of specimens analysed.

Molecular markers	N	n° informative characters/total	n° total haplo/genotypes	n° diagnostic haplo/genotypes	n° haplo/genotypes shared by different species
mtMutS	125	83/614	38	26	12
Cox	105	63/909	27	19	8
ITS	64	96/669	46	41	5
28S	78	55/734	44	35	9

<i>Leptogorgia toboguillae</i>		LT221102		LT221075
<i>Leptogorgia toboguillae</i>		LT221104		LT221076
<i>Leptogorgia tricolorata</i>		LT221109		LT221055
<i>Leptogorgia tricolorata</i>		LT221111		LT221057
<i>Leptogorgia virgulata</i> (A)		AY268458	AY268463	
<i>Pacifigorgia adamsi</i> (*)	KX767391	KX767331		
<i>Pacifigorgia agassizii</i> (*)	KX767392	KX767332		
<i>Pacifigorgia bayeri</i>	HG917061	HG917044		
<i>Pacifigorgia cairnsi</i> (*)	KX767393	KX767333		
<i>Pacifigorgia cairnsi</i>	HG917062			LT221047
<i>Pacifigorgia catedralensis</i>	HG917065	HG917019		LT221054
<i>Pacifigorgia catedralensis</i>	HG917066	HG917020		
<i>Pacifigorgia catedralensis</i>(1)(*)	KX767394	KX767334		KX767438
<i>Pacifigorgia</i> cf. <i>cairnsi</i>	HG917063	HG917046		
<i>Pacifigorgia</i> cf. <i>cairnsi</i>	HG917064	HG917021		
<i>Pacifigorgia cribrum</i> (1)	KX767395	KX767335	KX767481	KX767439
<i>Pacifigorgia cribrum</i> (2)	KX767396	KX767336	KX767482	KX767440
<i>Pacifigorgia curta</i>			KX767483	
<i>Pacifigorgia elegans</i> (A)		AY126419		
<i>Pacifigorgia exilis</i> (*)	KX351878	KX351871	KX767487	
<i>Pacifigorgia ferruginea</i> (*)	KX767400	KX767340	KX767488	
<i>Pacifigorgia firma</i>	HG917068			LT221049
<i>Pacifigorgia firma</i>	HG917069	HG917028		LT221052
<i>Pacifigorgia firma</i>	HG917067			
<i>Pacifigorgia firma</i> (1) (*)	KX351879	KX351872		
<i>Pacifigorgia flavimaculata</i> (1)	KX767401	KX767341	KX767489	KX767443
<i>Pacifigorgia flavimaculata</i> (2)	KX767402	KX767342	KX767490	KX767444
<i>Pacifigorgia irene</i>	HG917071	HG917024		LT221051
<i>Pacifigorgia irene</i>	HG917072	HG917022		LT221045
<i>Pacifigorgia irene</i> (1)	KX351880	KX351873	KX767495	KX767449
<i>Pacifigorgia irene</i> (2)	KX767406	KX767346	KX767494	KX767448
<i>Pacifigorgia machalilla</i> (1) (*)	KX351881	KX351874	KX767496	KX767450
<i>Pacifigorgia machalilla</i> (2) (*)	KX351882	KX351875	KX767497	KX767451
<i>Pacifigorgia machalilla</i> (3) (*)	KX351883	KX351876	KX767498	
<i>Pacifigorgia media</i>	GQ342421	GQ342497		
<i>Pacifigorgia media</i> (*)		KX767347		
<i>Pacifigorgia media</i> (1) (*)	KX767407	KX767348	KX767499	
<i>Pacifigorgia media</i> (2)	KX767408	KX767349		
<i>Pacifigorgia rubicunda</i>	HG917074	HG917033		LT221040
<i>Pacifigorgia rubicunda</i>	HG917073	HG917032		LT221039
<i>Pacifigorgia rubicunda</i> (1)	KX767414	KX767356	KX767504	KX767454
<i>Pacifigorgia rubicunda</i> (2)	KX767415	KX767357	KX767505	KX767455
<i>Pacifigorgia rubicunda</i> (3) (*)	KX767413	KX767355		
<i>Pacifigorgia rubinoffi</i> (1) (*)	KX767417	KX767359	KX767506	KX767456
<i>Pacifigorgia rubinoffi</i> (2) (*)	KX767416	KX767358	KX767507	
<i>Pacifigorgia rubripunctata</i> (1)	KX767418	KX767360	KX767508	KX767457
<i>Pacifigorgia rubripunctata</i> (2)	KX767419	KX767361		
<i>Pacifigorgia samarensis</i> (*)	KX767420	KX767362	KX767509	
<i>Pacifigorgia</i> cf. <i>senta</i>		LT221107		LT221044
<i>Pacifigorgia sculpta</i> (1) (*)	KX351884	KX351877	KX767510	
<i>Pacifigorgia sculpta</i> (2)	KX767403	KX767343	KX767491	KX767445
<i>Pacifigorgia sculpta</i> (3)	KX767404	KX767344	KX767492	KX767446
<i>Pacifigorgia sculpta</i> (4)		KX767345		
<i>Pacifigorgia smithsoniana</i>	HG917076	HG917023		LT221050
<i>Pacifigorgia</i> sp1	KX767390	KX767330	KX767480	KX767437
<i>Pacifigorgia</i> sp2 (1)	KX767409	KX767351	KX767501	KX767452
<i>Pacifigorgia</i> sp2 (2)	KX767411	KX767353	KX767502	KX767453
<i>Pacifigorgia</i> sp2 (3)	KX767412	KX767354		
<i>Pacifigorgia</i> sp2 (4)	KX767410	KX767352		
<i>Pacifigorgia</i> sp2 (5)		KX767350		
<i>Pacifigorgia</i> sp3 (1)	KX767423	KX767365	KX767513	
<i>Pacifigorgia</i> sp3 (2)	KX767424	KX767366	KX767514	KX767460
<i>Pacifigorgia</i> sp4 (1)	KX767397	KX767337	KX767484	KX767441
<i>Pacifigorgia</i> sp4 (2)	KX767398	KX767338	KX767485	KX767442
<i>Pacifigorgia</i> sp4 (3)	KX767399	KX767339	KX767486	KX767462
<i>Pacifigorgia</i> sp4 (4)	KX767427	KX767369	KX767517	
<i>Pacifigorgia</i> sp4 (5)		KX767370	KX767518	
<i>Pacifigorgia</i> sp5 (1)	KX767425	KX767367	KX767515	KX767461
<i>Pacifigorgia</i> sp5 (2)	KX767426	KX767368	KX767516	
<i>Pacifigorgia</i> sp6	KX767428	KX767371	KX767519	KX767463
<i>Pacifigorgia</i> sp7 (1)	KX767405	KX767345	KX767493	KX767447
<i>Pacifigorgia</i> sp7 (2)	KX767421	KX767363	KX767511	KX767458
<i>Pacifigorgia</i> sp7 (3)	KX767422	KX767364	KX767512	KX767459
<i>Pacifigorgia stenobrochis</i>	HG917078	AY126420		LT221042
<i>Pacifigorgia stenobrochis</i>	HG917079	HG917018		
<i>Pacifigorgia stenobrochis</i> (1)	KX737629	KX737672	KX767520	KX767464

<i>Pacifigorgia stenobrochis</i> (2)	KX721175		KX767521	
<i>Phyllogorgia dilatata</i>		AY126428		
<i>Pseudopterogorgia bipinnata</i>			EU043122	JX203712
<i>Pseudopterogorgia elisabethae</i>			EU017561	
<i>Pseudopterogorgia rubrotincta</i>		JX152768		
<i>Pterogorgia anceps</i>	GQ342424	AY126403		JX203714
 <u><i>Plexauridae</i></u>				
<i>Anthomuricea</i> sp.		DQ302855		
<i>Heterogorgia verrucosa</i>		HG917052		
<i>Paramuricea</i> sp.	FJ264913			
<i>Muricea laxa</i>		AY683064		
<i>Muricea purpurea</i>		GQ293304		
<i>Muricea</i> sp.	HG917060	HG917015		
<i>Muriceopsis flavida</i>	GQ342449	AY126416		JX203744
<i>Plexaura flexuosa</i>		AY126411		
<i>Plexaurella nutans</i>	GQ342451	AY126415		JX203745
<i>Scleracis guadalupensis</i>	KC984626	KC984590		
<i>Swiftia pacifica</i>	KF874210	KF856083		
<i>Swiftia pallida</i>		JQ241247		KC788259
 <u><i>Alcyoniidae</i></u>				
<i>Alcyonium haddoni</i>				JX203642
<i>Alcyonium verzeveldti</i>	GU355978			
<i>Sarcophyton glaucum</i>	AF064823	AB759499		