Latitudinal distribution and genetic divergence between shallow and mesophotic cold-water gorgonians in Chile

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Abstract

Most biodiversity studies of octocorals have focused on tropical shallow waters, particularly from Mexico to Peru, and more recently in Chile. The first description of a Chilean octocorals dates back to the H.M.S Challenger expedition in 1873–1876. Since then, only few descriptions of new soft coral species from this region have been published. In addition, the taxonomic status of most gorgonians reported from the temperate Pacific coast of South America is dubious due to the loss of the original type material for most taxa. Here, we use morphological characters and nuclear and mitochondrial markers to reevaluate the taxonomy of the Chilean gorgonians *Phycogorgia fucata* and *Leptogorgia chilensis*, and describe a new species named *Leptogorgia pichicuyensis* sp.n. We present the first description of their latitudinal distribution patterns. Our results support the monophyly of the Chilean gorgoniids investigated, and the provisional placement of *P. fucata* with other species of the genus *Leptogorgia* in the absence of further specimens of *Phycogorgia* in GenBank. Our distribution analyses reveal a clear biogeographic break congruent with the Central Chile and Araucanian marine ecoregions. Overall, our study provides the first integrative taxonomic study of Southern Eastern Pacific cold-water gorgonians suggesting a higher number of species than expected for this underexplored region.

Introduction

Gorgonian corals (Order: Alcyonacea) are a cosmopolitan group of benthic organisms in circalittoral tropical, temperate and polar seascapes (Darling et al., 2017; Gomez et al., 2014; Sánchez et al., 2019). Gorgonian occurrence and distribution is shaped by environmental factors such as temperature, substrate availability, slope, water flow and calcite saturation horizon among others (Yesson et al., 2012). According to Johnson & Hallock (2020), the optimal temperature range for gorgonians lies between 18 to 33ºC with a lower temperature tolerance between 15 to 17ºC. Thus, its distribution is restricted to tropical and temperate latitudes (Bayer, 1953; Breedy & Guzman, 2007; Sánchez et al., 2019), with only one species, *Leptogorgia lutkeni*, reported from subantarctic regions (46º S) (Williams & Lindo, 1997).

The group of cold-water gorgonians typically includes species dwelling between 50 to 200 m with temperatures ranging between 4 to 12º C (Gjerde, 2007; Roberts et al., 2006). For instance, *Acanthogorgia* sp. inhabits channels and fjords in Norway and South of Chile at ca. 50 m depth (Häussermann & Försterra, 2007; Rogers, 1999). Other cold-water gorgonians such as *Adelogorgia phyllosclera* (Gugliotti et al., 2019) or *Eunicella verrucosa* (Ransome et al., 2014) are predominantly found at 50 m depth. Yet, there are also some exceptions to these patterns occurring in shallow waters at high latitudes (Gugliotti et al., 2019). For example, rose and light orange gorgonians, including *Phycogorgia fucata*, have been observed across Central Chile and Araucanian ecoregions at depths ranging from only 3 to up to 35 m (upper mesophotic zone), thus conforming a unique subgroup of cold-water gorgonians in terms of depth distribution (Breedy et al., 2022).

The vast coastline of Chile (ca. 4200 km long) covers a large part of the Southeastern Pacific Ocean from the 18ºS to ca. 56 ºS (Lancellotti & Vasquez, 2000). From ca. 42ºS to the equator, the region is influenced by the Humboldt Current System which provides nutrient-rich, and cold waters (Echevin et al., 2012; Montecino et al., 2006). Sullivan Sealey & Bustamante (1999) recognized four distinctive ecoregions within the warm-temperate Southeastern Pacific Province: Central Peru, Humboldtian, Central Chile and Araucanian. Each ecoregion is characterized by an homogenous species assemblage of both vagile and sessile species, that is regulated by oceanographic features (Spalding et al., 2007). For instance, the species richness of Chilean littoral fishes differs across latitudinal biogeographic regions with a marked biogeographic break at ca. 40ºS (Ojeda et al., 2000). Also, Ibáñez et al. (2019) reported the presence of endemic mollusk species for the Humboldtian and Araucanian ecoregions. Although there is limited information on octocorals for the Chilean coast, the contrasting oceanographic conditions present in the region may also lead to patchiness and/ or competitive exclusion among coexisting gorgonians (Velásquez & Sánchez, 2015).

The first descriptions of Chilean gorgonians date back to the H.M.S Challenger expedition between 1873–1876 (Delgado, 2009), with spare new studies conducted during the next century (Breedy et al., 2015; Häussermann & Försterra, 2007; Verseveldt, 1967; Wells, 1972). Most gorgonians described from the Chilean coast have been reported from Patagonia, while reports from other temperate areas are uncertain due to incomplete illustrations in early taxonomic literature and loss of original type material (Breedy et al., 2022; Häussermann & Försterra, 2007). For example, with the exception of *P. fucata* (see Breedy et al., 2022), all species reported from the Araucanian ecoregion by Philippi (1892) are dubious and need of reevaluation. Regarding *P. fucata*, the species was historically assigned to other genera such as *Leptogorgia* or *Plexaura* by different authors, and the loss of Philippi's type material hampered taxonomical clarification (Breedy et al., 2022).

Currently, over 60 species of the Gorgoniidae family have been described across the world, but taxonomic identification is solely based on morphological characters, thus questioning its validity due to homoplasy of characters and phenotypic plasticity within the group (Flot et al., 2008; Gori et al., 2012; Soler-Hurtado & López-González, 2012). Molecular tools are useful to complement morphological analyses aimed at species description and identification. In this direction, considerable advances have been conducted in determining the suitability of different genetic markers to discriminate octocoral species (McFadden et al., 2010). Early studies using the *mtMutS* gene (formerly known as *msh1*) restricted to octocorals (Culligan et al., 2000), found that this marker does not have enough resolution to differentiate species although it was useful to identify groups at genus level (Bilewitch & Degnan, 2011; McFadden et al., 2010). Nuclear markers such as 28SrRNA and the Internal Transcribed Spacers (*ITS*) can provide additional species-level resolution, especially in combination with the *mtMutS* (Mcfadden & Hutchinson, 2004). Currently there is an unified view that a systematic framework based on morphology and molecular markers is required to achieve a phylogeny-based classification of octocorals (McFadden et al., 2004; Morris et al., 2012; Sánchez, 2007).Besides, phylogenetic reconstruction may allow the estimation of

divergence time among taxa groups, as suggested for the diversification of *Leptogorgia* during the Oligocene-Miocene ca. 28 – 23 Ma to 3.5–4.2 Ma (Poliseno et al., 2017; Silvestri et al., 2019) concurrent with the rise of the Panama Arc Island chain and the closing of the Central American Seaway (CAS).

In this context, the objectives of the present study are: 1) to assess the gorgonian diversity and geographical distribution in Chilean waters using both molecular and morphological analyses; 2) to clarify the phylogenetic position of Chilean gorgonian taxa with mithocondrial and nuclear markers (*COI, mtMutS, 28S*, and *ITS2*), and 3) to elucidate the evolutionary history of Chilean species within Eastern Pacific ecoregions to shed some light about possible historical events shaping biogeographic patterns in the region.

Materials and methods

Material collection

Gorgonians were collected from November 2020 to January 2021 in four sites (Pichicuy, Algarrobo, Ramuntcho and Coliumo) in the Central Chile (from 25ºS, 72ºW to 35ºS, 72ºW) and the Araucanian (from 35ºS, 72ºW to 43ºS, 72ºW) ecoregions (Fig. 1, Table 1). Different morphotypes were observed in each site, four in Pichicuy, two in Algarrobo, and one in Ramuntcho and Coliumo (Fig. 1, Table 1). The morphotypes in Pichicuy and Algarrobo were chosen mainly for the variable degrees of rose to violet colorations. Fresh gorgonian fragments of each morphotype were collected by SCUBA with the support of recreational divers between 10 to 35 m. Fragments consisted of ca. 5 cm sections from different individuals (N = 5) for molecular analyses and other 5 cm sections for taxonomic analyses (N = 5). Samples were preserved in 95% ethanol and stored at 0ºC. During sampling, living specimens were also photographed in their habitat to further document their color and shape in their natural environment.

Morphological analysis and geographical distribution

Morphological analysis and species identification was conducted using current Octocorallia taxonomic criteria (Bayer, 1951, 1953, 1961; Bayer & Grasshoff, 1983; Breedy & Guzman, 2007). Colony color was obtained using standard calibrations digital charts (https://encycolorpedia.com) from images taken during sampling. Gorgonian sclerites were extracted using an adapted protocol from Breedy & Guzman (2002). Briefly, ca. 1 cm^2 of tissue was dissolved in 10% sodium hypochlorite (NaClO) and placed in separate Eppendorf tubes. After 1h incubation the tissue was digested and the sclerites released. Then, the supernatant and debris were discarded with the help of a micropipete. Later, samples were gently rinsed 4–5 times with deionized water to remove excess of bleach. Wet preparations of isolated sclerites were mounted on slides with glycerin and examined under a LEICA DM500 microscope with a LEICA ICC50W camera to obtain photographs of the different sclerite shapes for each sampled colony. Sclerite length and width were measured using an eyepiece micrometer. Additionally, subsamples were preserved on permount medium for further evaluation with light microscopy, and others were preserved in absolute ethanol as vouchers. For scanning electron microscope (SEM), dry sclerites were attached to aluminum stubs with carbon adhesive discs, sputter-coated with gold, and examined under a JEPL 6010 PLUS/LA microscope at the *Vicerrectoría de Investigación y Desarrollo Laboratories* at the University of Concepción (Chile) and using a FESEM Zeiss Sigma 300 microscope at the Centro de Investigación en Estructuras Microscópicas, at the University of Costa Rica.

A map with theoretical geographical distribution of each of species was created using collected samples per site and photographs provided by a network of recreational divers along the entire Chilean coast that were assigned to examined specimens by external morphology of colonies.

Molecular analyses

DNA was extracted from five to ten random polyps from each ethanol preserved gorgonian specimen. Polyps were extracted using forceps and inspected under a stereo microscope. The E.Z.N.A Tissue DNA by Omega Bio-tek kit was used to extract DNA from each sample following the manufacturer's recommendations. The concentration of the extracted DNA was measured using a Quantus Fluorometer (Promega Inc.). The mitochondrial genes *COI*, and *mtMutS* were amplified by Polymerase Chain Reaction (PCR) using the following primers: COII8068F (McFadden et al., 2004) and COIOCTR (France & Hoover, 2002) for COI + Igr1, and ND42599F (France & Hoover, 2002) and MUT3458R (Sánchez et al., 2003) for *mtMutS*. Additionally, the nuclear *ITS2* and *28S* were amplified using primers ITS 2.1 and ITS 2.2 (Hugall et al., 1999) and 28S-Far and 28S-Rar, respectively (McFadden & van Ofwegen, 2013) (TableS2).

For *COI*, PCR reactions were conducted on a final volume of 30 µl containing 17.18 µl Invitrogen's nuclease free water, 6 µl Promega 5X PCR Buffer, 0.3 µl BioLabs BSA (20 mg/ml), 0.6 µl dNTP (10mM), 1.6 µl Promega MgCl2 (25mM), 1.5 µl of each primer (10 µM), 0.4 µl Promega Go TAQ DNA Polymerase (5u/ µl), and 1 µl DNA. PCR were performed in a Veriti thermocycler using the following program: 1 min initial denaturation step at 95ºC, followed by 40 cycles of 15 sec at 58ºC for annealing step, 1 min extension at 72ºC, and 7 min at 72ºC for final extension, and 3 min at 5ºC for a refrigeration step. For *mtMutS*, PCR reactions was conducted on a final volume of 30 µl containing 16.90 µl Invitrogen's nuclease free water, 6 µl Promega 5X PCR Buffer, 0.3 µl BioLabs BSA (20 mg/ml), 0.6 µl dNTP (10mM), 1.8 µl Promega MgCl2 (25mM), 1.5 µl of each primer (10 µM), 0.4 µl Promega Go TAQ DNA Polymerase (5u/ µl), and 1 µl DNA. The PCR program used was: 1 min initial denaturation step at 95ºC, followed by 40 cycles of 15 sec at 58ºC for annealing step, 1 min at 72ºC and 7 min at 72ºC for final extension, and 3 min at 5ºC for refrigeration step. For *ITS*, PCR reactions were conducted on a final volume of 30 µl containing 16.90 µl Invitrogen's nuclease free water, 6 µl Promega 5X PCR Buffer, 0.3 µl

BioLabs BSA (20 mg/ml), 0.6 µl dNTP (10mM), 1.8 µl Promega MgCl2 (25mM), 1.5 µl of each primer (10 µM), 0.4 µl Promega Go TAQ DNA Polymerase (5u/ µl), and 1 µl DNA. The PCR program used was: 1 min initial denaturation step at 95ºC, followed by 40 cycles of 15 sec at 63ºC for annealing step, 1 min at 72ºC and 7 min at 72ºC for final extension, and 3 min at 5ºC for refrigeration step. For *28S*, PCR reactions were conducted on a final volume of 30 µl containing 16.30 µl Invitrogen's nuclease free water, 6 µl Promega 5X PCR Buffer, 0.3 µl BioLabs BSA (20 mg/ml), 0.6 µl dNTP (10mM), 2.40 µl Promega MgCl2 (25mM), 1.5 µl of each primer (10 µM), 0.4 µl Promega Go TAQ DNA Polymerase (5u/ µl), and 1 µl DNA. The PCR program used was: 1 min initial denaturation step at 95ºC, followed by 40 cycles of 15 sec at 66.7ºC for annealing step, 1 min at 72ºC and 7 min at 72ºC for final extension, and 3 min at 5ºC for refrigeration step.

PCR products were visualized by electrophoresis in 1.5% agarose gels to assess PCR's product length and quality. Sequencing in both directions and purification were performed at Humanizing Genomics Macrogen Inc. (South Korea).

Phylogenetic analyses

Sequences chromatograms obtained from MACROGEN were assembled, inspected by eye, manually checked, and adjusted using GENEIOUS v. 8.1.7 (Biomatters Ltd.) (Kearse et al., 2012). Edited sequences were deposited in the NCBI GenBank under accession numbers listed in Table 1. Sequences (i.e., *COI, mtMutS, 28S and* ITS2) from other representatives of the family Gorgoniidae were downloaded from NCBI GenBank or BOLD Systems (TableS3) and aligned with the sampled nucleotide sequences here obtained using Clustal W. Translated protein-coding regions were inspected by eye in Mega v.6.0 (Tamura et al., 2013).

A preliminarily analysis of the samples was conducted using a pairwise genetic distance (*p*-distance model) with bootstrap analyses (5000 replicates) among gorgonian morphotypes of the different sites (Pichicuy, Algarrobo, Ramuntcho and Coliumo) for each molecular barcode (*COI, mtMutS, ITS and 28S*) using Mega v.6.0. Since the sampled sequences exhibited no divergence (see results section) among the five replicates of each morphotype, sites and each molecular barcode, only one representative sequence from each morphotype and site was used for phylogenetic analyses. For the phylogenetic analysis, sequences attributed to representative of the monophyletic genus *Pacifigorgia* were used as outgroup.

The phylogenetic position of the collected species was determined using different methods to contrast results and assess the support of the resulting groupings. Pairwise *p*-distances with bootstrap analyses (5000 replicates) within and among species grouped by genera were calculated and, means and SE compared among the four molecular barcodes (*COI, mtMutS, ITS and 28S*) to estimate intra- and interspecific genetic distances. Phylogenies were assessed using maximum Likelihood (ML) and Bayesian Inference (BI) using IQ-TREE (Nguyen et al., 2015) and MrBayes v.3.1 (Huelsenbeck & Ronquist, 2001), respectively. The best-fit model of nucleotide substitution for each final alignment was determined using Aikake Information Criteria (AIC) in JModeltest (Posada, 2008). The models obtained for our dataset were TIM3 + I for COI, TrN + G for mtMutS, TVM + I + G for ITS, and TPM1uf + I + G for 28S, and were used for ML and BL analyses. The best ML tree was constructed using bootstrap values (BP) from 10.000 ultrafast replicates with 10.000 iterations (Hoang et al., 2018). For BI analyses, obtained models which are not implement in MrBayes were substituted by the closest over-parameterized model (Huelsenbeck & Rannala, 2004), thus the TIM3 TVM + I + G, TrN and TPM1uf were replaced by the GTR model. The proportion of invariable sites (I) and gamma distributed rates (G) defined in jModeltest were conserved in all models. The Bayesian phylogenetic tree was constructed with 5 million generations, sampling every 1000 steps, with each run containing one cold and three heated chains. The first 25% generations were discarded as burn-in and the remaining trees were used to calculate the posterior probabilities (PP) of the clades. Also, a concatenated matrix was constructed for *COI, mtMutS and 28S* after removing taxa for which only one or two markers were available (Table S3). The concatenated matrix was used to infer ML and BI. For these analyses, TIM3 + G for *COI*, TrN + G for *mtMutS*, and TIM1 + I for *28S* models were used. For all BI analyses, the convergence of the Markov chains was assessed in Tracer v. 1.6 (Rambaut et al., 2018), and a summary of the trace plots showing evidence of convergence are provided in Supplementary Figs. S1, S2 and S3. All consensus trees were visualized, edited and rooted with sequences of species of *Pacifigorgia* in FigTree v1.4.2.

Time estimation of Chilean cold-water rose gorgonians divergence using fossil calibration

Divergence times were estimated using the *mtMutS* dataset and the program BEAST v. 2.3.2 (Bouckaert et al., 2019). The tree was calibrated based on the earliest fossil evidence for *Eunicella* (Kocurko & Kocurko, 1992) with a date of origination set to 28.4 Ma (mean = 1 and SD = 1) (Poliseno et al., 2017). Hence, two sequences of *Eunicella* mtMutS (*Eunicella verrucosa* JQ397305 and *Eunicella albicans* KY559407) obtained in GenBank were aligned with previous *mtMutS* alignment. An uncorrelated log-normal relaxed clock model and Yule model were used (Poliseno, Altuna, et al., 2017). For molecular dating analysis, the Markov chain was run for 10 million iterations, sampling every 1000 iterations. After assessing convergence of effective sample sizes (ESS) for each parameter with Tracer v. 1.6, 25% of the initial sampled iterations were omitted prior to building the calibrated tree. Mean divergence times and 95% highest posterior density interval was summarized in TreeAnnotator. A summary of the trace plots showing evidence of convergence between marginal priors and posterior densities obtained using uncorrelated log-normal relaxed clock are provided in the Supplementary Fig S4.

Results

Morphological analyses and geographic distribution

The results of this study document new distribution records and taxonomical information of *P. fucata*, with a re-description of *L. chilensis* and its neotype assignation. Also, the study provides a new species description named *L. pichicuyensis* sp. n., based on phylogenetic assessment of mitochondrial and nuclear genes as well as on morphological analyses of cold-water Chilean gorgonians. Detailed taxonomic descriptions are found in Appendix S1, and ZooBank registration for the new species is as follows: *Leptogorgia pichicuyensis* (zoobank.org.act: A93FB6FE-43D6-4BAD-92C4-788A677458FD). In regard to geographic distribution, the species *P. fucata* was found in shallow waters (5–20 m) from the coast of Talca (approx. 35ºS) to the coast of Valdivia (approx. 40ºS) (Fig. 2, TableS4A). In contrast, the species *L. chilensis* was found in the upper mesophotic zone (30–35 m) of Algarrobo (33ºS). This species was also identified in photographs provided by recreational divers in other sites of the Valparaiso Region including Quintay at 45 m depth (33ºS), Las Cruces at 35 m depth (33ºS), and Caleta Pichicuy at 37 m depth (32ºS) (Fig. 2, TableS4B). In the case of the new species *L. pichicuyensis* sp. n, its distribution is more restricted, with Caleta Pichicuy as type locality (32ºS), and some records in Papudo (32ºS) and Chañaral de Aceituno (29ºS) by local divers (Fig. 2, Table S4C).

Phylogenetic analyses

Genetic distances among samples and outgroups. A total of 35 sequences from each sampled species and each molecular barcode (*COI, mtMutS, 28S* and *ITS)* were deposited in GenBank and their accession numbers are provided in Table 2. The alignment of *COI, mtMutS, 28S* and *ITS*, had lengths of 644, 633, 548, and 592 bp, respectively. Individuals (N = 5) of the same morphotype exhibited no sequence divergence (< 0.1%) in the average of uncorrected pairwise genetic distances (*p)* in any molecular barcode except *ITS* (Table S5, S6, S7). For this *ITS* alignment, pairwise genetic distance showed enhanced variability among individuals of each morphotype (from 0 to 0.74%; Table S8).

Genetic distances *(p*-distance) among haplotypes selected in our samples (CHRAG11, CHCOG11, CHPIG11, CHPIG21, CHPIG31, CHALG11 and CHALG21) and species grouping by genera in the phylogenetic analyses showed different results for the different barcodes investigated (Tables S9 to S12). For *COI*, the haplotypes of samples assigned as *L. chilensis* from Algarrobo in the morphological analyses (CHALG11 and CHALG21) showed a close genetic distance with *Eugorgia* (0.08%) divergence among them (Table S9A). The genetic distance of the samples assigned as *P.* fucata from Ramuntcho and Coliumo (CHRAG11 and CHCOG11) showed a close genetic distance with *Eugorgia* (0.6%) compared to *Leptogorgia* (0.9%) and *Pacifigorgia* (2.3%). The new species from Pichicuy (CHPIG11, CHPIG21 and CHPIG31) showed the same pattern, and those from Algarrobo also displayed the lowest distance with *Eugorgia* (0.6%) (Table S9A). For the *mtMutS* alignment, CHRAG11 and CHGO11 were showed a close genetic distance with *Leptogorgia* (1%, Table S9B). The same pattern was also observed in CHALG11 and CHALG21 which showed the same genetic distance with *Eugorgia* and *Leptogorgia* (2 and 2%, respectively), and for CHPIG11-CHPIG21 which showed the same genetic distance with *Eugorgia* and *Leptogorgia* (2 and 2%, respectively), except for CHPIG31 which showed 1% distance with *Leptogorgia* (Table S9B). In addition, the sequences CHALG11 and CHALG 21 showed a distance of 1.3% with *L. chilensis* sequence from GenBank. For *ITS*, closest genetic distances for all haplotypes were *Eugorgia* and *Leptogorgia*, each with 3% distance (Table S9C). For *28S*, for all sampled genotypes the closest distances were both *Eugorgia* and *Leptogorgia* (4%) (Table S9D).

Molecular phylogeny of Chilean rose cold-water rose gorgonians: mitochondrial vs. nuclear data. The topology of the maximum clade credibility tree of the Bayesian analysis was similar to that of obtained with maximum likelihood method (Fig. 3). Both of them displayed two strongly supported main clades (clade I and II with BP of 100 and PP of 1). One of them corresponds to the monophyletic Genus *Pacifigorgia* (clade I) and the other to a clade including *Leptogorgia, Eugorgia* (from the Eastern Pacific and the Caribbean Sea) and our samples (clade II, Fig. 3). The phylogenetic tree showed that samples found along the different sampling locations (Pichicuy, Algarrobo, Coliumo and Ramuntcho) in the Chilean coast (Fig. 3, Table 1) were well-grouped within a single clade (clade VI, with BP of 93 and PP of 1) with two well-separated subclades (A and B) (Fig. 3). Subclade A includes *L. chilensis* from Algarrobo (CHAL11, CHALG21), forming a monophyletic group sister to subclade B including the new species *L. pichicuyensis* spn from Pichicuy (CHPIG11, CHPIG21) (Fig. 3). Both subclades were highly supported within the clade VI including Chilean samples (BP of 95 and PP of 1, and BP of 96 and PP of 0.98, respectively) (Fig. 3). However, the haplotype (CHPIG31 also corresponding to the new species from Pichicuy was located outside of the subclade B, but without taxonomic congruence (Fig. 3). In addition, the species known as *P. fucata* (CHCOG11, CHRAG11) from two different locations (Coliumo and Ramuntcho) was well separated from subclades A and B (Fig. 3). Moreover, the clade V, sister to all Chilean species, and including *L. chilensis* (?), *L. flexilis, L. alba, L. cofrini, L. cuspidata*, and *L. rigida*, was highly supported (BP of 91 and PP of 1) (Fig. 3).

The *28s* (Fig. 4a) and the concatenated of *COI + mtMutS + 28S* r*DNA* alignment (Fig. 4b) analysis were generally congruent with the *mtMutS* phylogeny (Fig. 3). These phylogenies further supported the sister relationship between the *Leptogorgia* clade (clade 5) and the Chilean clade (clade 6) observed in the *mtMutS* alignment (Fig. 4a, b). Although the *28S* gene showed poor internal resolution to separate congeneric species, it supports the monophyly of our Chilean samples (clade VII, Fig. 4a). The matrix of the concatenated analysis evidenced higher support values for subclades A and B (BP of 99 and PP of 1, and BP of 76 and PP of 0.96, respectively) (Fig. 4b) also present in the *mtMutS* tree (Fig. 3).

For the CHPIG31 haplotype, its sequence was identical to other sequences from Pichicuy but the higher variability of ITS allowed the identification of punctual mutations (CHPIG31- CHPIG12, with no mutation; CHPIG31- CHPIG32 and CHPIG33, one mutation step; CHPIG31- CHPIG15, two mutations steps; CHPIG31 and all the other samples, 3 mutation steps) and caused the segregation in the phylogenic tree.

Time estimation of Chilean cold-water gorgonians divergence

The fossil-calibrated phylogenetic reconstruction based on *mtMutS* resulted in moderately supported nodes (e.g. BF < 70, PP < 0.90) (Fig. 5). Nevertheless, the topology of the tree was similar to the Bayesian and maximum likelihood analyses, although with some slight differences. From the root, emerge two main clades that roughly correspond to clade I and II in the *mtMutS* tree. The divergence time of these clades is estimated at 21.14 Ma (Fig. 5). According to our results, Genus *Pacifigorgia* (clade 1) diverged more recently (8.87 Ma) than *Eugorgia* and *Leptogorgia* (clade 2). Following, divergence time of the clade that contains *Eugorgia* and *Leptogorgia* from the Eastern Pacific is 10.60 Ma (clade 4, Fig. 5). Besides, the divergence time between the clade containing our Chilean samples and the sister clade with *Leptogorgia* is estimated at 9.32 Ma (Fig. 5). Finally, the Chilean clade diverged later at 3.67 Ma (Fig. 5).

Discussion

Taxonomy and mito-nuclear phylogenetic relationships

The present study provides new insights into Chilean gorgonian diversity, clarifying their taxonomic status and phylogenetic relationships with species from other regions. In all, we describe a new local species, named *L.pichicuyensis* sp. n., provide new geographic records and taxonomical information for *P. fucata*, and offer a re-description of *L. chilensis.*

The flat gorgonian described in our study showed the same morphology, color, and type of sclerites than the lectotype of *P. fucata* assigned in the review conducted by Breedy et al., (2022). The dominant sclerites were spindles and capstans in similar proportions to those observed in the lectotype (0.45 vs. 0.48, and 0.34 vs. 0.47, respectively in Breedy et al. (2022) and the present study). Here, we present new geographic distribution sites such as Punta Lavapie, Cobequecura and/or Talca, all in the Araucanian ecoregion, and the first genetic analyses of this species. The phylogenetic trees supported the inclusion of *P. fucata* in Genus *Leptogorgia* resulted in a polyphyletic assemblage, although without taxonomic coherence. As described by Breedy et al., (2022), the flat axes or expansion of coenenchyme of *Phycogorgia* are characters that separate the two genera. Incomplete lineage sorting or hybridization may explain the lack of congruence of the taxonomic and molecular data (Soler-Hurtado et al., 2017). In addition, it is known that multicopy of rDNA may produce polyphyletic species/genera, producing the opposite situation that we find taxonomically (Ament-Velásquez et al., 2016).

Leptogorgia chilensis, together with *P. fucata*, was first described by Philippi (1866) from Algarrobo (Chile). However, Philippi's type material for this species is also lost and no lectotype is neither available. For this reason, Breedy & Guzman (2007) used a similar specimen from California to describe the species, although it could be possible that said material belongs to another species. In fact, our sample material was collected at the type locality described by Philippi (1866), in Algarrobo, and for this reason we designated the specimen MZUCR 3492 as the neotype to clarify the species' taxonomic status (Article 75, ICZN). In addition, although Philippi's illustration was very schematic and lacked taxonomic details, the rose gorgonian he collected displayed a dichotomous branching in one plane and was wider than taller and he described that the color of the colonies varied between pale/light pink to pale orange (Philippi 1866) as observed in our specimens. Our study suggests that from the morphological point of view the species from California corresponds to another species different from that found in Chile and its taxonomy deserved to be reconsidered. In this sense, the spindles and capstans of the Chilean samples are larger than those of the California specimen (0.17 mm vs. 0.12 mm, and 0.16 mm vs. 0.08 mm, respectively). Also, anthocodial sclerites were observed in Californian specimens, but not in those from Algarrobo specimens, and the color of the colonies from California are very orange, which is a character considered an important feature to differentiate *Leptogorgia* species in the Eastern Pacific (Breedy et al., 2021). The reconstructed *mtMutS* phylogeny indicated that our samples and the nucleotide sequences from California and described as *L. chilensis* are not closely related. Indeed, genetic distances analyses shows a divergence of 1.3% between them, reinforcing the idea that specimens from California attributed to *L. chilensis* should be assigned to a different *Leptogorgia* species.

The description of the new species, *L. pichicuyensis* sp. n. in Caleta Pichicuy increases the number of *Leptogorgia* in the Chilean coast. The species was observed in high density (29.9 to 36.5 ind. per m^2) at shallow depths (3 to 25 m), and its three-dimensional complexity provides an important habitat for a diversity of sessile and vagile organisms (in review Camps-Castellà et al., 2022). This *Leptogorgia* species is placed within the highly supported Chilean clade and showed a coherent phylogeny across investigated trees including the concatenated one (*COI, mtMutS* and *28S*). From the morphological point of view, the dark shade of magenta-pink tonality of *L. pichicuyensis* sp. n. clearly differs from those ranging from pink to pale rose in *L. chilensis* and *P. fucata*. In addition, the proportion of capstans in the new species is much lower than in the other two species (0.03 vs. 0.77 in *L. chilensis* and 0.45 in *P. fucata*). The phylogenetic tree based on *mtMutS* incorporates the subsamples CHPIG11 and CHPIG21, corresponding to the new species, into a clade sister of the other Chilean species. However, the haplotype CHPIG31 is placed out of the clade being incongruent with taxonomic results. In fact, *ITS* results showed a different punctual mutation between the haplotypes (CHPIG31- CHPIG12, with no mutation; CHPIG31- CHPIG32 and CHPIG33, one mutation step; CHPIG31- CHPIG15, two mutations steps; CHPIG31 and all the other samples, 3 mutation steps) which may caused the segregation in the phylogenic tree.

Time estimation of Chilean cold-water gorgonians divergence and biogeographical distribution

The reconstructed *mtMutS* phylogenetic tree and divergence time are similar to those found in previous studies which pointed to the separation of Leptogorgia and *Eugorgia* during the early Miocene (median = 21.4 Ma, and 15 Ma in Poliseno et al. (2017)). According to Poliseno et al. (2017), using the same fossil calibration, the divergence between Eastern Pacific and Western Atlantic species occurred about 28 Ma. However using complete mitochondrial genomes, Silvestri et al. (2019) estimated a more recent divergence, between 11 and 20 Ma. Even so, our time estimates and those of previous studies point to occurrence in the Miocene (5–23 Ma), an epoch of global warmth and increased sea level (Steinthorsdottir et al., 2021). Our results also evidence low genetic distance within *Leptogorgia* being difficult to discriminate among species and suggesting a great diversification during this period. According to O'Dea et al. (2016), seawater exchange between Eastern Pacific and Western Atlantic occurred until the late Miocene (10 Ma), thus allowing gametes and larvae flow. Our results also advise the separation of the Chilean clade from the remaining *Leptogorgia* species from the Tropical East Pacific during the late Miocene (median = 9.32 Ma), suggesting the end of the connection with the Atlantic Ocean, but is not until late Pliocene (3.67 Ma) that the Chilean clade emerges (see also Thiercelin 2016 for similar findings for other taxa). The closure of the Isthmus of Panama affected the Caribbean and Atlantic oceans might have acted modifying the oceanographic regime, especially in warm, oligotrophic Caribbean waters (Johnson et al., 2007). Hence, the progressive closure represents a recent vicariant event for marine taxa due to the isolation of population by habitat fragmentation (Thiercelin, 2015). All this could have resulted in a strong pattern of endemism in the Chilean clade, separating this group of gorgonian species from the rest in the East Pacific.

In regard to geographical distribution of gorgonian samples that occurred during the last 3Ma, we observe a largely segregated along the latitudinal gradient. First, *L. pichicuyensis* sp. n. is present in the type locality, Caleta Pichicuy (32 ºS), and in adjacent localities of Papudo (32ºS) and Chañaral de Aceituno (29ºS), in the Chilean Central ecoregion. *L. chilensis* is also present from Caleta Pichicuy to Las Cruces (33ºS), in Araucanian and Chilean Central ecoregions. In contrast, *P. fucata* has a more restricted distribution area, and is only present in the Araucanian ecoregion, between Talca (35º S) to Valdivia (40 ºS). According to Moreno et al. (2006), there is a biogeographic break between latitudes of 36º-41ºS, corresponding to the separation between Central Chile and Araucanian ecoregions. These ecoregions feature distinctive abiotic agents such as upwelling, marine currents or coastal topography that may contribute to a higher level of endemism (Spalding et al., 2007). The main differences between Central Chile and Araucanian ecoregions are the humid climate with greater rainfall and the high productive salt marshes in Araucanian ecoregion compared to the other ecoregion that is less productive and has isolated upwelling foci and is less productive (Valparaíso and Coquimbo) (Sullivan Sealey & Bustamante, 1999). For instance, similar patterns of endemism between these ecoregions have been observed for polychaete communities (Moreno et al., 2006). Since a genetic discontinuity is also observed at similar latitudes for kelp communities (Tellier et al., 2009), we hypothesize that our results along the Chilean coast are the result of regional speciation factors.

Conclusions

Our study provides the first integrative taxonomic study of Southern Eastern Pacific cold-water gorgonians evidencing a higher number of species than expected for this underexplored region. The Chilean continental platform has been scarcely explored, and most original gorgonian descriptions are dubious, exposing the need for further intensive research in shallow and mesophotic habitats. The descriptions of a new species using molecular tools as a complement to morphological identification shows the relevance of integrative taxonomic analyses. This study also highlights the strong endemic character of Chilean gorgonians, although further research is needed to better understand the potential processes underlying the patterns of endemism in marine organisms on the southeastern Pacific coast.

Declarations

Competing interests: The authors declare no competing interests.

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Tables

Table 1: Sample information including sample ID in field, dive spot, collection locations, region, ecoregion, date of collection, depth, lat/long, environmental parameters (if known), and their corresponding museum catalogue (Museum voucher). MNHNCL: Museo Nacional de Historia Natural de Chile, Chile; MZUCR: Museo de Zoología, Universidad de Costa Rica, colección húmeda, Costa Rica.

Sample ID	Dive spot	Location	Region	Ecoregion	Collection date	Depth (m)	Lat./long.(dms)	Museum voucher
CHPIG11	La Isla	Pichicuy	Valparaiso	Central Chile	12/12/2020	20	32°20’47.50”S/71°27’59.25”W	MZUCR 3489 (fragment) / MNHNCL CNID-15080 (whole colony)
CHPIG12	La Isla	Pichicuy	Valparaiso	Central Chile	12/12/2020	20	32°20’47.50”S/71°27’59.25”W	MNHNCL CNID-15077
CHPIG13	La Isla	Pichicuy	Valparaiso	Central Chile	12/12/2020	20	32°20’47.50”S/71°27’59.25”W	MNHNCL CNID-15078
CHPIG14	La Isla	Pichicuy	Valparaiso	Central Chile	12/12/2020	20	32°20’47.50”S/71°27’59.25”W	MNHNCL CNID-15079
CHPIG15	La Isla	Pichicuy	Valparaiso	Central Chile	12/12/2020	20	32°20’47.50”S/71°27’59.25”W	-
CHPIG21	Jardin del Gato	Pichicuy	Valparaiso	Central Chile	21/11/2020	25	32°20’55.10”S/71°28’11.82”W	MZUCR 3490 (fragment)
CHPIG22	Jardin del Gato	Pichicuy	Valparaiso	Central Chile	21/11/2020	25	32°20’55.10”S/71°28’11.82”W	MNHNCL CNID-15073
CHPIG23	Jardin del Gato	Pichicuy	Valparaiso	Central Chile	21/11/2020	25	32°20’55.10”S/71°28’11.82”W	MNHNCL CNID-15074
CHPIG24	Jardin del Gato	Pichicuy	Valparaiso	Central Chile	21/11/2020	25	32°20’55.10”S/71°28’11.82”W	MNHNCL CNID-15075
CHPIG25	Jardin del Gato	Pichicuy	Valparaiso	Central Chile	21/11/2020	25	32°20’55.10”S/71°28’11.82”W	MNHNCL CNID-15076
CHPIG31	Jardin del Gato	Pichicuy	Valparaiso	Central Chile	11/12/2020	25	32°20’55.10”S/71°28’11.82”W	MZUCR 3491 (fragment)
CHPIG32	Jardin del Gato	Pichicuy	Valparaiso	Central Chile	11/12/2020	25	32°20’55.10”S/71°28’11.82”W	MNHNCL CNID-15070
CHPIG33	Jardin del Gato	Pichicuy	Valparaiso	Central Chile	11/12/2020	25	32°20’55.10”S/71°28’11.82”W	MNHNCL CNID-15071
CHPIG34	Jardin del Gato	Pichicuy	Valparaiso	Central Chile	11/12/2020	25	32°20’55.10”S/71°28’11.82”W	MNHNCL CNID-15072
CHPIG35	Jardin del Gato	Pichicuy	Valparaiso	Central Chile	11/12/2020	25	32°20’55.10”S/71°28’11.82”W	-
CHALG11	Bajo Ivar	Algarrobo	Valparaiso	Central Chile	5/2/2021	35	33°21’10.59”S/71°41’59.70”W	MZUCR 3492 (fragment)/ MNHNCL CNID-15064 (whole colony)
CHALG12	Bajo Ivar	Algarrobo	Valparaiso	Central Chile	5/2/2021	35	33°21’10.59”S/71°41’59.70”W	MNHNCL CNID-15061
CHALG13	Bajo Ivar	Algarrobo	Valparaiso	Central Chile	5/2/2021	35	33°21’10.59”S/71°41’59.70”W	-

CHALG14	Bajo Ivar	Algarrobo	Valparaiso	Central Chile	5/2/2021	35	33°21’10.59”S/71°41’59.70”W	MNHNCL CNID-15062
CHALG15	Bajo Ivar	Algarrobo	Valparaiso	Central Chile	5/2/2021	35	33°21’10.59”S/71°41’59.70”W	MNHNCL CNID-15063
CHALG21	La Pared	Algarrobo	Valparaiso	Central Chile	5/2/2021	30	33°21’24.72”S/71°42’40.50”W	MZUCR 3493 (fragment)
CHALG22	La Pared	Algarrobo	Valparaiso	Central Chile	5/2/2021	30	33°21’24.72”S/71°42’40.50”W	MNHNCL CNID-15065
CHALG23	La Pared	Algarrobo	Valparaiso	Central Chile	5/2/2021	30	33°21’24.72”S/71°42’40.50”W	MNHNCL CNID-15066
CHALG24	La Pared	Algarrobo	Valparaiso	Central Chile	5/2/2021	30	33°21’24.72”S/71°42’40.50”W	MNHNCL CNID-15067
CHALG25	La Pared	Algarrobo	Valparaiso	Central Chile	5/2/2021	30	33°21’24.72”S/71°42’40.50”W	MNHNCL CNID-15068
CHRAG11	Ramuntcho	Ramuntcho	Bío Bío	Araucanian	3/12/2020	15	36°44’36.11”S/73°11’49.55”W	MZUCR 3494 (fragment)
CHRAG12	Ramuntcho	Ramuntcho	Bío Bío	Araucanian	3/12/2020	15	36°44’36.11”S/73°11’49.55”W	MNHNCL CNID-15086
CHRAG13	Ramuntcho	Ramuntcho	Bío Bío	Araucanian	3/12/2020	15	36°44’36.11”S/73°11’49.55”W	MNHNCL CNID-15087
CHRAG14	Ramuntcho	Ramuntcho	Bío Bío	Araucanian	3/12/2020	15	36°44’36.11”S/73°11’49.55”W	MNHNCL CNID-15088
CHRAG15	Ramuntcho	Ramuntcho	Bío Bío	Araucanian	3/12/2020	15	36°44’36.11”S/73°11’49.55”W	MNHNCL CNID-15089
CHCOG11	Coliumo	Coliumo	Bío Bío	Araucanian	17/12/2020	10	36°31’13.49”S/72°57’21.01”W	MNHNCL CNID-15081
CHCOG12	Coliumo	Coliumo	Bío Bío	Araucanian	17/12/2020	10	36°31’13.49”S/72°57’21.01”W	MNHNCL CNID-15082
CHCOG13	Coliumo	Coliumo	Bío Bío	Araucanian	17/12/2020	10	36°31’13.49”S/72°57’21.01”W	MNHNCL CNID-15083
CHCOG14	Coliumo	Coliumo	Bío Bío	Araucanian	17/12/2020	10	36°31’13.49”S/72°57’21.01”W	MNHNCL CNID-15084
CHCOG15	Coliumo	Coliumo	Bío Bío	Araucanian	17/12/2020	10	36°31’13.49”S/72°57’21.01”W	MNHNCL CNID-15085

Table 2: Sample information and their corresponding GenBank accession numbers for each gene

Sample ID	Genbank. Acc. No.			
	COI	*mtMutS*	*ITS*	*28S*
CHPIG11	OQ427231	OQ928108	OQ377614	OQ377594
CHPIG12	OQ427230	-	OQ377613	OQ377593
CHPIG13	OQ427229	OQ928107	OQ377612	OQ377592
CHPIG14	OQ427228	OQ928106	OQ377611	OQ377591
CHPIG15	OQ427227	OQ928105	OQ377610	OQ377590
CHPIG21	OQ427241	OQ928118	OQ377624	OQ377589
CHPIG22	OQ427240	OQ928117	OQ377623	OQ377588
CHPIG23	OQ427239	OQ928116	OQ377622	OQ377587
CHPIG24	OQ427238	OQ928115	OQ377621	OQ377586
CHPIG25	OQ427237	OQ928114	OQ377620	OQ377585
CHPIG31	OQ427236	OQ928113	OQ377619	OQ377584
CHPIG32	OQ427235	OQ928112	OQ377618	OQ377583
CHPIG33	OQ427234	OQ928111	OQ377617	OQ377582
CHPIG34	OQ427233	OQ928110	OQ377616	OQ377581
CHPIG35	OQ427232	OQ928109	OQ377615	OQ377580
CHALG11	OQ427256	OQ928132	OQ377638	OQ377604
CHALG12	OQ427255	OQ928131	OQ377637	OQ377603
CHALG13	OQ427254	OQ928130	-	OQ377602
CHALG14	OQ427253	OQ928129	OQ377636	OQ377601
CHALG15	OQ427252	OQ928128	OQ377635	OQ377600
CHALG21	OQ427226	OQ928104	OQ377609	OQ377599
CHALG22	OQ427225	OQ928103	OQ377608	OQ377598
CHALG23	OQ427224	OQ928102	OQ377607	OQ377597
CHALG24	OQ427223	OQ928101	OQ377606	OQ377596
CHALG25	OQ427222	OQ928100	OQ377605	OQ377595
CHRAG11	OQ427251	OQ928127	OQ377634	OQ377574
CHRAG12	OQ427250	OQ928126	OQ377633	OQ377573
CHRAG13	OQ427249	-	OQ377632	OQ377572
CHRAG14	OQ427248	OQ928125	OQ377631	OQ377571
CHRAG15	OQ427247	OQ928124	OQ377630	OQ377570
CHCOG11	OQ427246	OQ928123	OQ377629	OQ377579
CHCOG12	OQ427245	OQ928122	OQ377628	OQ377578
CHCOG13	OQ427244	OQ928121	OQ377627	OQ377577
CHCOG14	OQ427243	OQ928120	OQ377626	OQ377576
CHCOG15	OQ427242	OQ928119	OQ377625	OQ377575

Figures

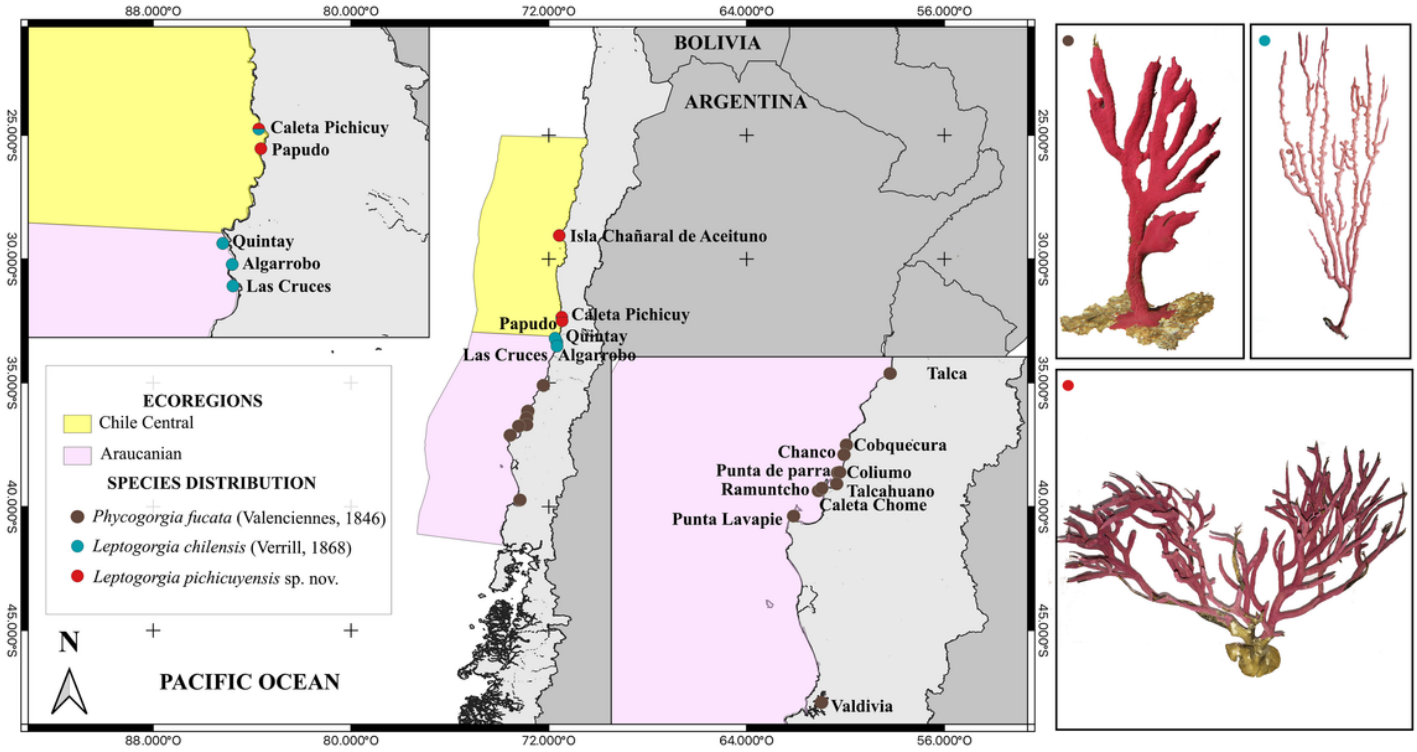

Figure 1

Distribution of five sampling sites along two ecoregions (Chile Central and Araucanian) (Spalding et al. 2007) that Chilean cold-water rose gorgonians has been previously observed (recreational divers) without identification. 1: Caleta Pichiuy, Valparaíso region; 2: Algarrobo, Valparaíso región; 3: Coliumo, Bío Bío region; and 4: Ramuntcho, Bío Bío region.

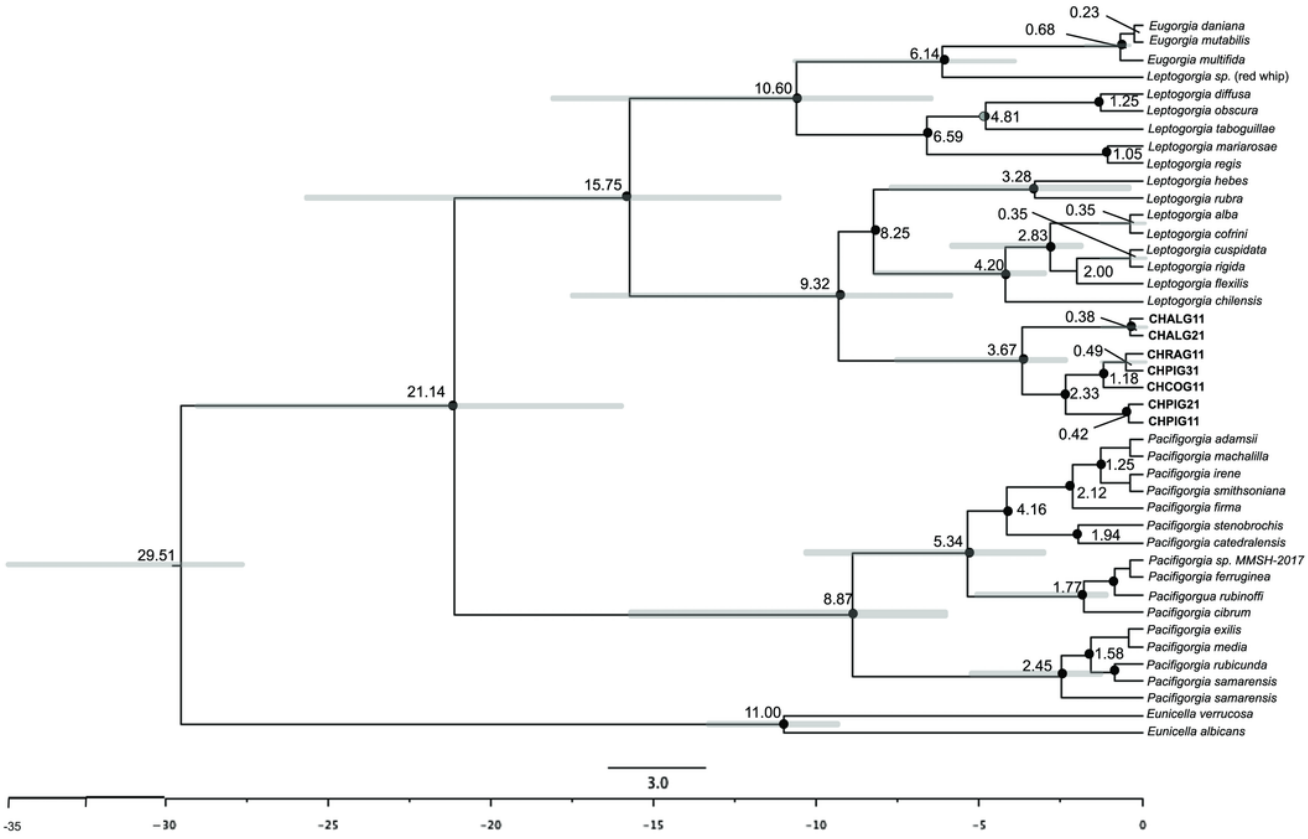

Figure 2

Geographical distribution of the Chilean gorgonian species (*Phycogorgia fucata, Leptogorgia chilensis,* and *L. pichicuyensis* sp. n.). The solid circle with respective color shows the position of the localities where were they are present. The polygons show the two ecoregions where the gorgonians are present (Chile Central and Araucanian).

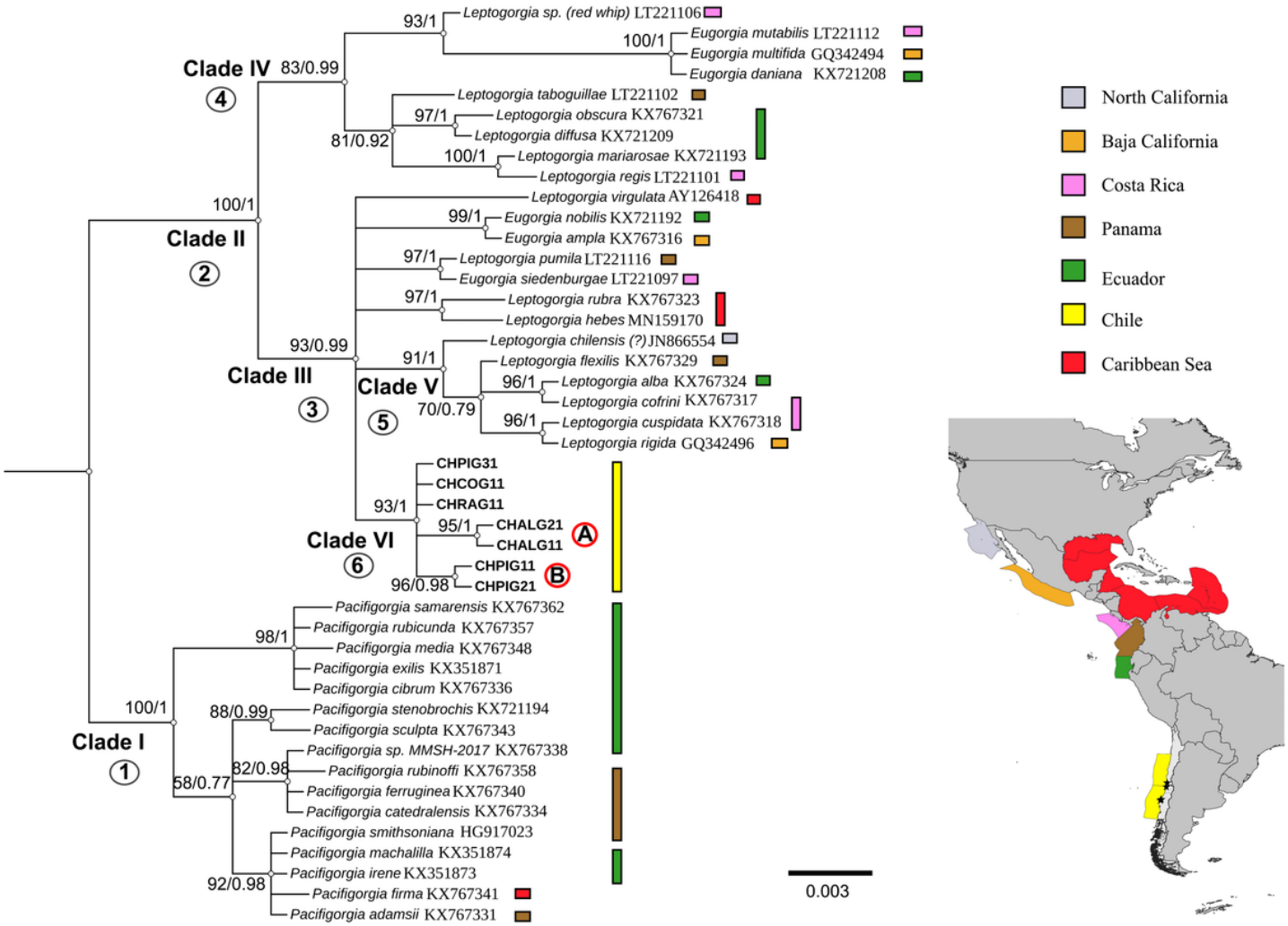

Figure 3

Phylogenetic placement of our sampled sequences (CHPIG11, CHPIG21, CHPIG31, CHALG11, CHALG21, CHCOG11, CHRAG11) in the collection locations (Caleta Pichicuy, Algarrobo, Coliumo and Ramuntcho) in Bayesian consensus phylogeny based on *mtMutS*gene. The numbers indicate clade supports for ML/BI (bootstrap and posterior probability, respectively). New sequences of our samples are in *boldface*. Color indicated geographic area of collected species. Important clades are labeled with roman numbers I-VI. Red circles with letters refer to the subclades within the clade VI.

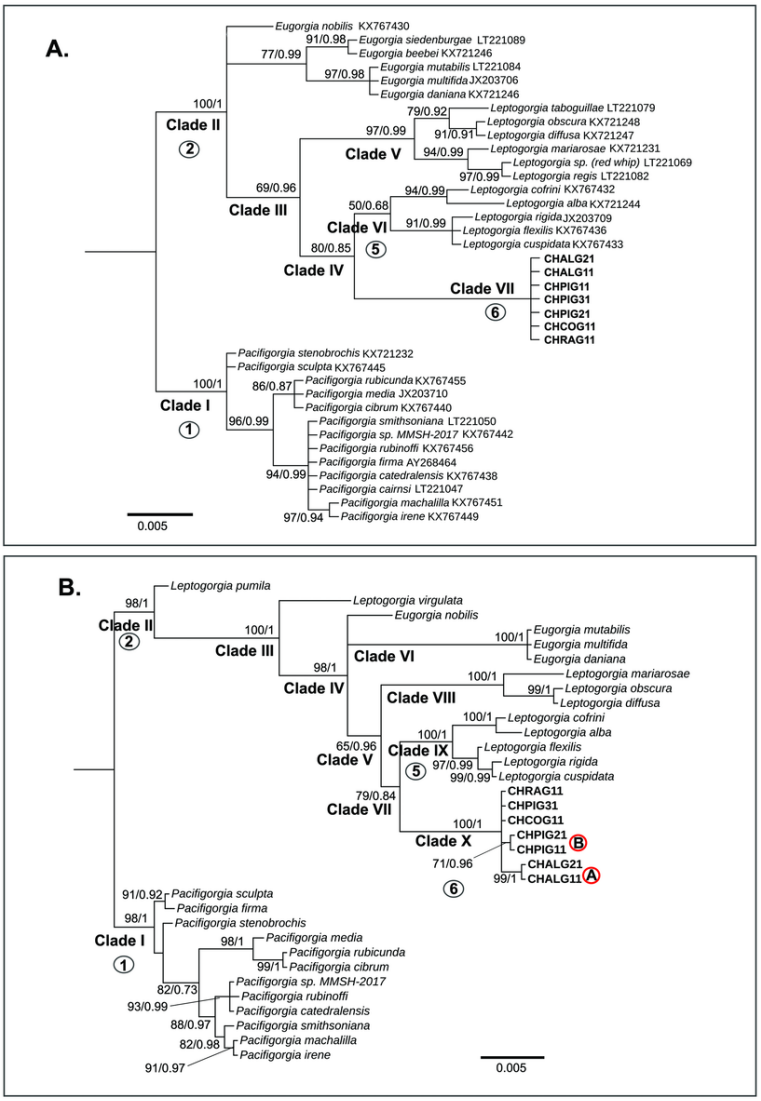

Figure 4

Phylogenetic placement of our sampled sequences (CHPIG11, CHPIG21, CHPIG31, CHALG11, CHALG21, CHCOG11, CHRAG11) in the collection locations (Caleta PIchicuy, Algarrobo, Coliumo and Ramuntcho) in Bayesian consensus phylogeny based on: A) *28S* gene, and B) phylogeny of the concatenated alignment of *COI+mtMutS+28S* rDNA dataset. The numbers indicate clade supports for ML/BI (bootstrap and posterior probability, respectively). New sequences of our samples are in *boldface*. Important clades are labeled with roman numbers I-VI. Red circles with letters refer to the subclades within the clade VI. Grey circles with numbers correspond to clades defined in the phylogenetic reconstruction based on *mtMutS*.

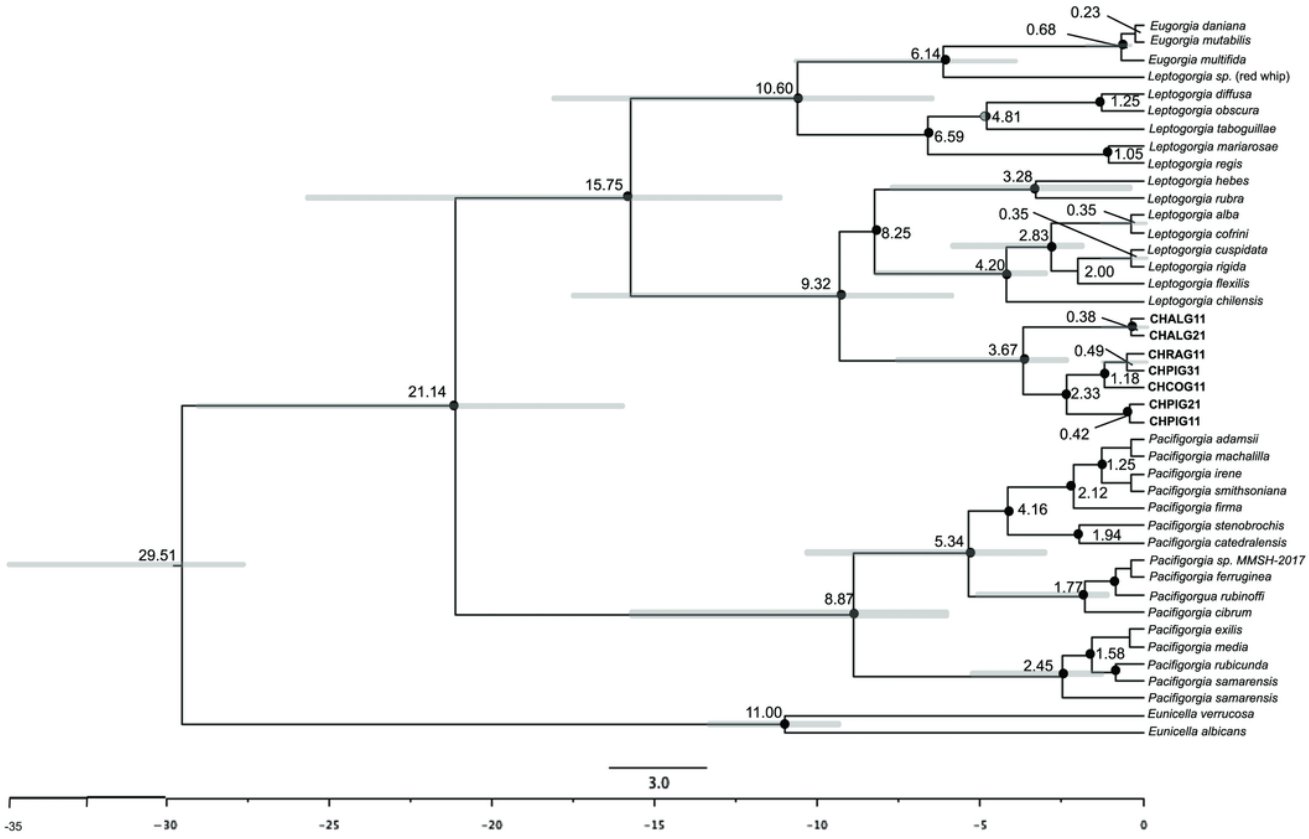

Figure 5

A) Chronogram of the maximum clade credibility tree constructed using BEAST based on *mtMutS*gene. Node bars show 95 % highest posterior density intervals (HPD). Black circles at the nodes indicate strong support from ML (BS >70), and Bayesian (PP > 0.95). Split circles indicate low support from one analysis only (left: ML). Numbers on the branches indicate node age estimates in million years. Scale bar of the tree refers to million years. New sequences of our samples are in *boldface*, B) Geographic distribution of *Leptogorgia*, *Eugorgia* and *Pacifigorgia* based on literature (Breedy & Guzman 2002, 2007a; Breedy et al. 2009).

Supplementary Files

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