Gracilariopsis lemaneiformis (Gracilariaceae, Rhodophyta) in the Mexican Coasts: A Case of Disjunct Distribution?

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Abstract

Gracilariopsis lemaneiformis is a widely reported species of marine red algae, with records from around the world. Particularly in Mexico, it has been recorded along the coasts of the Gulf of Mexico and the Mexican Caribbean as well as the Tropical Mexican Pacific; so, its current distribution on the Mexican coasts is disjunct. From its molecular characterization with COI-5P and rbcL sequences of specimens collected at sites in Mexico where this species has been most frequently recorded, our aim was to re-evaluate the current distribution of Gp. lemaneiformis on the Mexican coasts and discuss the taxonomic implications. Phylogenetic analysis, supported by DNA species delimitation methods, genetic distances and morphological comparisons, showed that the current disjunct distribution of Gp. lemaneiformis in Mexico is a consequence of taxonomic misidentifications. From our results, Gp. lemaneiformis is the only species of the genus with a distribution in the Tropical Mexican Pacific, whereas Gp. tenuifrons is the only species with a distribution in the Gulf of Mexico and the Mexican Caribbean. Also, we propose that Gp. cataluziana is to be merged with Gp. tenuifrons, while Gp. costarisensis is to be merged with Gp. lemaneiformis.

Keywords

COI-5P, Cosmopolitan, Morphological Variation, rbcL, Species Delimitation

1. Introduction

Gracilariopsis E.Y. Dawson, with the type species Gp. sjoestedtii (Kylin) E.Y. Dawson, comprises 25 species currently accepted taxonomically [1]. The genus...
is widely distributed in tropical and subtropical waters through the world; it is distinguished from related genera by the absence of tubular nutritive cells connecting the gonimoblast to the pericarp, by the broad-based gonimoblast composed of small cells, and by the superficial arrangement of strictly cylindrical spermatangia [2]. Additionally, some species of this genus are commercially important because of the presence of polysaccharides, as agars, in their cell walls [3].

Gracilaropsis lemaneiformis (Bory) E. Y. Dawson, Acleto & Foldvik was originally described by Bory (as Gigartina lemaneiformis) [4] from specimens collected at Paita, Peru, the type locality. Apparently, it is a cosmopolitan marine species, widely recorded around subtropical and tropical waters of the Atlantic, Indian and Pacific oceans [1]. Particularly in Mexico, where it is very abundant, it currently presents what appears to be a disjunct distribution with numerous records, based only on the morpho-species concept [3], along the Pacific and Atlantic coasts of Mexico [5] [6]. Except for Gp. cata-luzina Gurgel, Fredericq & J.N. Norris, described on the basis of specimens from Veracruz, and a doubtful record of Gp. tenuifrons (C.J. Bird & E.C. Oliveira) Fredericq & Hommersand for Tabasco [3], Gp. lemaneiformis is essentially the only species from this genus known for Gulf of Mexico (GM) and Mexican Caribbean (MC), whereas for the tropical Mexican Pacific coast (TMP), it is the only species recorded, mainly in Oaxaca and Chiapas [5]. Morphologically, Gracilaropsis lemaneiformis is characterized by cylindrical axes, up to 100 cm tall, one to a few irregularly branched, indeterminate axes arising from a discoid holdfast; axes of 0.5 mm diameter at the base, broadening to 1.3 mm diameter and tapering toward the apices, sparsely irregularly branched; pseudoparenchymatose organization composed by one or two layers of cortical cells, three to five layers of subcortical cells, and four or five medullary cells. Spermatangia superficial in indefinite sori; Cystocarps scattered over the axes and branches, slightly constricted at the base subsurface layers below [2] [7].

As is true for most of species in the Gracilariaceae, Gracilaropsis lemaneiformis exhibits very low morphological variation and high characters overlapping with other species within the genus with which it shares a distribution [7] [8] [9]. Although molecularly all species of Gracilaropsis are clearly differentiated [2], their morphological delimitation has been complicated, leading to the uncovering of cryptic diversity or misidentifications, which has resulted in apparent cosmopolitan species [2]. Molecular-assisted taxonomy has been successfully applied to the recognition of species [10], especially in problematic groups [9] [11] [12] [13]. Particularly, in molecular studies realized from specimens previously recorded as Gp. lemaneiformis in other regions of the world, some misidentifications have been detected [2] [14].

In a survey along the Mexican coast from TMP, GM and MC, we collected specimens morphologically related to Gp. lemaneiformis, mainly at sites where this species has been widely recorded [3] [7]. From molecular sequences of the
COI-5P and rbcL genes of these specimens, our aim was to re-evaluate the disjunct distribution of *Gp. lemaneiformis* on the Mexican coasts and discuss the taxonomic implications.

2. Material and Methods

Samples morphologically identified as *Gracilariopsis lemaneiformis* were collected from different sites of the TMP, GM and MC coasts (Table S1), where this species is abundant and widely recorded [3] [5] [6], at a depth of 0.5 - 1.5 m and sites with low waves. Apical sections for molecular analysis were preserved in silica gel until DNA extraction. Samples collected for morphological analysis were preserved in 6% formaldehyde in seawater. Fresh specimens were mounted on herbarium sheets and incorporated into the algal collections at Metropolitan Herbarium UAMIZ (Index Herbariorum [http://sweetgum.nybg.org/science/ih/]).

DNA for molecular analysis was extracted from 5 - 10 mg of dried tissue using a QIAGEN DNeasy Plant Mini Kit (Qiagen, Valencia, California USA) according to the manufacturer’s protocols. The mitochondrial COI-5P region was amplified using the primers GAZF1 and GAZR1 [15], and the chloroplast rbcL region was amplified using the primers F-rbcL-start, R753, F577, R1150, F993 and R-rbcS-start [16]. These markers were selected because of the number of sequences of *Gracilariopsis* available in GenBank, with which to compare our specimens. The PCR procedure followed Ardito et al. [12]. PCR products were purified with the QIAquick Gel Extraction (Qiagen) and commercially sequenced (Macrogen Inc., Seoul, Korea). The same set of primers was used for sequencing. The sequences generated were assembled and edited using the program Sequencher® version 5.4.5. The final alignment, with sequences of other species of *Gracilariopsis* from GenBank (Table S2 and Table S3), was performed using SeaView [17]. *Gracilaria isabellana* Gurgel, Fredericq & J.N. Norris and *G. curtissiae* J. Agardh were included as outgroups for the COI-5P data set, while *G. curtissiae* and *G. domingensis* (Kützing) Sonder ex Dickie were included as outgroups for the rbcL data set [18]. We analyzed the COI-5P and rbcL data sets separately. These DNA markers were chosen due to the high number of sequences available in GenBank. Phylogenetic analyses using Bayesian Inference (BI) and maximum likelihood (ML) were performed with codons partitioned. The evolutionary model selected for COI-5P and rbcL data sets was GTR+G+I (general time reversible + gamma distribution + invariable sites) determined by the ML ratio test implement by TOPALi v2 software [19]. ML analysis was performed using RAxML software [20] with the GTR+G+I model. Support for each branch was obtained from 1000 bootstrap replications. BI analysis was performed using Mr Bayes v3.2.2 [21]. Four chains of Markov chain Monte Carlo were used, starting with a random tree and sampling the data every 1000 generations for 5 × 10⁶ generations. 25% of trees were discarded as burn-in. Pairwise distances values (p distance) were calculated using Mega X [22].
To delimit *Gracilariopsis* species we ran three DNA-based species delimitation methods for both data sets (COI-5P and *rbcL*): Automatic Barcoding Gap Detection (ABGD) [23], the General-Mixed-Yule-Coalescent (GMYC) [24] and the Bayesian variant of Poisson Trees Processes model (bPTP) [25]. The ABGD delimited method was done via interface web (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html), with the following criteria: intraspecific variability (P) between 0.001 (Pmin) and 0.1 (Pmax), minimum gap width (X) of 0.1, Kimura-2-parameters and 50 screening steps. The bPTP model was done via interface web (https://species.h-its.org/ptp/), using the ML topology. The analysis consisted of 100,000 generations, with a thinning every 100 generations and a burn-in of 25%. For GYMC analyses, we generated an ultrametric tree for GYMC analyses in BEAST 1.8.2 software [26], from *rbcL* and COI-5P after removing identical sequences in the alignments. A coalescent constant size tree prior was set under an uncorrelated lognormal relaxed clock and GTR+G+I sites model. The analysis was set up for 10 million generations and a sampling frequency of 5000. Before performing the GMYC analyses, we checked the estimated samples size with Tracer 1.6 [27]. The maximum clade credibility tree was computed using TreeAnnotator 1.8.3 [26]. The resulting ultrametric tree was imported into the GMYC web server (https://species.h-its.org/gmyc/), running the single threshold.

For morphological identification and comparison of the specimens, microscopic cross-sections were made by hand using a razor blade and mounted in an 80% Karo®/distilled water solution. Photomicrographs were taken using a Quasar digital camera attached to a Leica DMLB microscope (Heidelberg, Germany). Photographs were taken with a Nikon D7000 digital camera. Morphological measurements were obtained from micrographs using SigmaScan®Pro automated image analysis software (Jandel Scientific, Sausalito, California).

3. Results

The COI-5P alignment consisted of 35 sequences of 675 base pairs (bp) long, the *rbcL* alignment consisted of 48 sequences with 1241 pb long. In both analyses (Figure S1 and Figure 1), the ML and BI trees did not differ, but there were some differences among markers, due to sampling and in poorly supported branches. In both analyses, the COI-5P and *rbcL*, sequences of *Gracilariopsis* formed a monophyletic group. The COI-5P analysis showed that our sequences (identified as *Gracilariopsis lemaneiformis*) formed two clades within the genus. Clade I, with strong support (ML = 97%, BI = 1.0), was composed of our sequences from TMP and sequences from GenBank identified as *Gp. lemaneiformis* from Ecuador (Figure S1). Clade II, with the maximum support (ML = 100%, BI = 1.0), was composed of our sequences from GM and MC and sequences from GenBank identified as *Gp. tenuifrons* from Brazil and Venezuela (Figure S1). With *rbcL*, our specimens (identified as *Gp. lemaneiformis*) were grouped into two clades (Figure 1). Clade I, with strong support (ML = 99%, BI =
Figure 1. ML topology based on rbcL data. BI (left) followed by ML bootstrap values (right) on branches. Asterisks indicate maximum phylogenetic support (100% ML BP, 1.0 BI BP), a hyphen indicate values below 50. Sequences generated in this study are in boldface. Bars at the right of the tree indicate the results of the three species delimitation methods: Automatic Barcoding Gap Detection (ABGD), the General-Mixed-Yule-Coalescent (GMYC) and the Bayesian variant of Poisson Trees Processes model (bPTP). Major clades are indicated with Roman numerals. Subclades are indicated by Arabic numerals. Bar under the tree indicates Substitutions per site.

1.0), was composed of our sequences from GM and MC and four sequences from GenBank (from Brazil, Guadeloupe and Venezuela) identified as *Gp. te- nutifrons*. In turn Clade II, with strong support (ML = 82%, BI = 1.0) was differentiated into three sister subclades (subclades A, B and C), which were resolved as sister groups, also well supported (Figure 1). Subclade A was composed of our sequences from TMP and a sequence from GenBank (from Costa Rica) identified as *Gp. costaricensis* E.Y. Dawson; subclade B was composed of four sequences from GenBank (from Peru) identified as *Gp. lemaneiformis*; subclade C was composed of two sequences from GenBank (from Ecuador and Peru) identified as *Gp. lemaneiformis*.

The genetic p-distance values within our specimens from GM, MC and TMP ranged from 0 to 6.1% with COI-5P, and from 0 to 3.3%, with rbcL. On the contrary, there was no genetic variation with any marker (COI-5P and rbcL) between our specimens of *Gracilariopsis lemaneiformis* from GM and MC, or between specimens of TMP. However, between our specimens from MC and GM
and sequences from *Gp. tenuifrons* from GenBank, genetic distances values ranged from 0 to 0.2%, with COI-5P, and from 0 to 0.1%, with rbcL. While our specimens from GM, MC and TMP and sequence from *Gp. cata-luziana* from GenBank, genetic distance values 1.7%. On the other hand, the genetic distance between our specimens from TMP and *Gp. lemaneiformis* from GenBank was 0.1%, with COI-5P; and genetic distance between our specimens from TMP, and *Gp. costaricencis* and *Gp. lemaneiformis* from GenBank ranged from 0.3 to 0.8%, with rbcL. Finally, the genetic distance between our specimens and other *Gracilariopsis* species ranged from 4.8% to 10.7%, with COI-5P, and from 1.7 to 7.5, with rbcL.

The DNA species delimitation with both markers, based on ABGD, bPTP and GYMC analyses, showed that our specimens from TMP and sequences of *Gp. lemaneiformis* and *Gp. costaricencis* from South America, correspond to only one species, while our specimens from MC, GM and sequences of *Gp. tenuifrons* from Brazil and Antilles also correspond to a single species ([Figure S1](#) and [Figure 1](#)).

A morphological comparison between our specimens from TMP, GM and MC ([Figure 2](#)) with *Gp. tenuifrons*, *Gp. cata-luziana*, *Gp. lemaneiformis*, and *Gp. costaricencis* is displayed in [Table 1](#), considering only five variable vegetative characters between these species.

![Figure 2](#). Specimens of *Gracilariopsis lemaneiformis* collected along Mexican coast. (a) Thallus of a specimen from Gulf of Mexico (GM); (b) Cross section of the thallus of a specimen from GM showing medullary and cortical cells; (c) Thallus of a specimen form Mexican Caribbean (MC); (d) Cross section of the thallus of a specimen from MC showing medullary and cortical cells; (e) Thallus of a specimen from Tropical Mexican Pacific (TMP); (f) Cross section of the thallus of a specimen from TMP showing medullary and cortical cells. Scale bars of thalli = 10 cm. Scale bar cross-sections = 100 µm.
Table 1. Morphological comparison between our specimens of *Gracilariopsis lemaneiformis* used in this study and the species of closed related.

| Taxa                     | Thallus length (cm) | Branch diameter (mm) | Number of medullary cells | Layers of subcortical cells | Layers of cortical cells |
|--------------------------|----------------------|----------------------|---------------------------|----------------------------|-------------------------|
| *Gp. cata-luziana*²      | up to 26 (−36)       | 0.4 - 0.5            | 1 - 5                     | 1 - 2                      | 2                       |
| *Gp. costaricensis*³     | up to 25             | 0.8                  | 5 - 6                     | 1 - 2                      | 1 - 2                   |
| *Gp. lemaneiformis*³     | up to 100            | 0.5 - 1.3            | 4 - 5                     | 3 - 5                      | 1 - 2                   |
| *Gp. tenuifrons*²        | up to 40             | 1.0                  | 3 - 4                     | 1 - 3                      | 1 - 2                   |
| *Gp. lemaneiformis* (TMP)⁴ | up to 95             | 0.3 - 0.5            | 4 - 6                     | 1 - 2                      | 1 - 2                   |
| *Gp. lemaneiformis* (GM)⁴ | up to 35             | 0.5 - 0.7            | 6                         | 1 - 2                      | 2                       |
| *Gp. lemaneiformis* (MC)⁴ | up to 35             | 0.5 - 1              | 3 - 4 (−6)                | 1 - 2                      | 2                       |

TMP—Tropical Mexican Pacific; GM—Gulf of Mexico; MC—Mexican Caribbean. ²Bird and Oliveira Filho [7], ³Gurgel et al. [28], ⁴Gurgel et al. [2] and ⁵this study.

4. Discussion

Phylogenetic analysis, supported by DNA species delimitation methods, genetic distances and morphological comparisons, showed that the current disjunct distribution of *Gracilariopsis lemaneiformis* in Mexico is a consequence of taxonomic misidentifications.

Since unfortunately the sequences of all the species discussed in the present study were not available with COI-5P, our discussion was particularly based on the phylogenetic analysis with rbcL, using the phylogeny with COI-5P mainly to reinforce the molecular identification of our specimens.

For GM and MC, in the phylogenetic tree all our specimens formed a monophyletic group with sequences of *Gp. tenuifrons* from Brazil and the Antilles (Figure S1 and Figure 1). This species has been widely recorded in the Caribbean Sea, but, until now, it had not been recorded on the Mexican coasts, except a doubtful record for Tabasco [3]. In turn, this clade was resolved as the sister group of *Gp. cata-luziana* from its type locality in Veracruz (AY049406), another species morphologically very similar to *Gp. tenuifrons* [28]. According to the literature, morphologically both species overlap almost completely, the only difference being the diameter of the branches, which is greater in *Gp. tenuifrons* than in *Gp. cata-luziana* [7] [28]. Our specimens from Veracruz, as described by Gurgel et al. [28] for *Gp. cata-luziana*, presented branches with smaller diameters than our specimens of the MC, whose diameters were related to what is described in the literature for *Gp. tenuifrons* (Table 1, Figure 2); however, there were no genetic differences between our GM and MC specimens. As for its distribution, the range known so far for both species was defined, for *Gp. cata-luziana* in the GM [28] and for *Gp. tenuifrons* in the MC [7]. According to Gurgel et al.
[28], the present distribution of both species was the result of a vicariant event in the area due to the emergence of the Yucatan Peninsula, which culminated in the speciation process that gave rise to both species. Although interspecific genetic distance values 1.7% between *Gp. tenuifrons* and the sequence of *Gp. cata-luziana* from GenBank support their genetic independence, we believe that *Gp. cata-luziana* should be treated as a taxonomic synonym of *Gp. tenuifrons*. This statement is supported by our results that do not support the presence of *Gp. cata-luziana* in its current distribution range. The only *rbcL* sequence available in the GenBank (AY049406) for this species presented many alignment problems with the rest of the sequences of the species of the genus, due to the presence of numerous gaps or nucleotides that were not present in others related sequences. Additionally, since its original description, this species has not been recorded again in the region.

In the case of TMP, our specimens formed a major monophyletic group that in turn was subdivided into three sister subclades, subclade A grouping to our specimens from TMP with *Gp. costaricensis* from its type locality, subclade B grouping sequences of *Gp. lemaneiformis* from its type locality in Peru, and subclade C grouping sequences of *Gp. lemaneiformis* from Ecuador and Peru. From the descriptions in the literature, morphologically *Gp. lemaneiformis* and *Gp. costaricensis* differ from each other by the thallus length, diameters of branches and number of layers of subcortical cells [2] [7]. However, according to our observations both species are not morphologically different (Table 1, Figure 2), in that it is possible to find specimens of subclade A, which would genetically correspond to *Gp. costaricensis*, with characteristics of subclade B, genetically resolved as *Gp. lemaneiformis*, and the other way around (Table 1). On the other hand, our species delimitation analysis showed that both our TMP specimens and the sequences of *Gp. costaricensis* and *Gp. lemaneiformis* are not genetically independent, but all make up a single genetic entity. As for its distribution, since *Gp. lemaneiformis* is still a cosmopolitan species in the world today [1], Gurgel et al. [28] and Arakaki et al. [14] proposed that the distribution of this species should be considered restricted to Peru and nearby sites, while records in other parts of the world should be re-evaluated. However, our results showed that our TMP specimens correspond both morphologically and genetically with *Gp. lemaneiformis*, so, the range of distribution of this species extends from Peru to Chiapas in the TMP. Although the *rbcL* interspecific genetic distance values between *Gp. costaricensis* and *Gp. lemaneiformis* were lower than interspecific values previously proposed by other authors in Gracilariaceae, namely. >2% according to Gurgel and Fredericq [29] and 1.5% - 12.8% according to Muangmai et al. [11], what is not supported is independence between both species. However, the genetic variation between three subclades could correspond to specific varieties. Yet the current evidence allows us only to propose that both species should be merged. Future studies of a phylogeographic nature in combination with detailed morphometric analysis could provide more evidence for the estab-
lishment of taxa at the infraspecific level.

Although the taxonomic status of the remaining species of *Gracilariopsis* recorded in Mexico must be re-evaluated in detail, from our current results and the fact that these records are unique, doubtful and very old, or correspond to species whose distribution area has already been limited to certain regions of the world, such as *Gp. megaspora* E.Y. Dawson with its range restricted to the Indian Ocean [2], our analysis of the “disjunct distribution” of *Gp. lemaneiformis* in Mexico allows us to suppose that *Gp. tenuifrons* is the only species of the genus distributed in GM and MC, while *Gp. lemaneiformis* is the most common species distributed in TMP.

Finally, we propose the following taxonomic changes:

*Gracilariopsis lemaneiformis* (Bory) E.Y. Dawson, Acleto & Foldvik 1964: 59, pl. 56: fig. A [30].

Basionym: *Gigartina lemaneiformis*.

Heterotypic synonym: *Gp. costaricensis* E.Y. Dawson 1949 [31].

Type Locality: Paita, Peru.

*Gracilariopsis tenuifrons* (C.J. Bird et E.C. Oliveira) Fredericq & Hommersand 1989: 240 [32].

Basionym: *Gracilaria tenuifrons* C.J. Bird & E.C. Oliveira.

Heterotypic synonym: *Gp. cata-luziana* Gurgel, Fredericq & J. Norris 2003 [28].

Type Locality: Praia Avenida, near Maceio, Alagoas, Brazil.

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**Conflicts of Interest**

The authors declare no conflicts of interest regarding the publication of this paper.

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Supplementary

**Figure S1.** ML topology based on COI-5P data. BI (left) followed by ML bootstrap values (right) under branches. Asterisks indicate maximum phylogenetic support (100% ML BP, 1.0 BI BP), a hyphen indicate values below 50. Sequences generated in this study are in boldface. Bars at the right of the tree indicate the results of the three species delimitations methods: Automatic Barcode Gap Detection (ABGD), the General-Mixed-Yule-Coalescent (GMYC) and the Bayesian variant of Poisson Trees Process model (bPTP). Major clades are indicated with Roman numerals. Bar under the tree indicates Substitutions per site.

**Table S1.** Specimens identified as *Gracilariopsis lemaneiformis* collected and sequenced (*rbcL* and COI-5P) in this study.

| GenBank Accession Number | Locality | Coordinates | Collected Date | Vaucher UAMIZ Herbarium |
|--------------------------|----------|-------------|----------------|-------------------------|
| rbcL COI-5P              |          |             |                |                         |
| MK238055 MK238044        | Boca del Cielo, Chiapas | 15°51'00"N, 93°40'00"W | 25/03/2018 | UAMIZ1349#1 |
| MK238056 MK238045        | Boca del Cielo, Chiapas | 15°51'00"N, 93°40'00"W | 25/03/2018 | UAMIZ1349#2 |
| MK238058 MK238046        | Boca del Cielo, Chiapas | 15°51'00"N, 93°40'00"W | 25/03/2018 | UAMIZ1349#3 |
| MK238059 MK238047        | Boca del Cielo, Chiapas | 15°51'00"N, 93°40'00"W | 25/03/2018 | UAMIZ1350#1 |
| MK238060 MK238048        | Boca del Cielo, Chiapas | 15°51'00"N, 93°40'00"W | 25/03/2018 | UAMIZ1350#2 |
| MK238061 MK238049        | Boca del Cielo, Chiapas | 15°51'00"N, 93°40'00"W | 25/03/2018 | UAMIZ1350#3 |
| MK238062 MK238050        | Playa Tiburón, Isla Mujeres, Quintana Roo | 21°13’23"N, 86°44’00"W | 18/12/2013 | UAMIZ1351#1 |
| MK238063 MK238051        | Playa Tiburón, Isla Mujeres, Quintana Roo | 21°13’23"N, 86°44’00"W | 18/12/2013 | UAMIZ1351#2 |
| MK238064 MK238052        | Playa El Niño, Cancún, Quintana Roo | 21°11’37"N, 86°48’20"W | 20/05/2017 | UAMIZ1352 |
| MK238065 MK238053        | Costa de Oro, Veracruz | 19°09’08"N, 96°05’40"W | 02/07/2015 | UAMIZ1353#1 |
| MK238066 MK238054        | Costa de Oro Veracruz | 19°09’08"N, 96°05’40"W | 02/07/2015 | UAMIZ1353#2 |
| MK238067 MK238055        | Playa Gaviota, Veracruz | 19°08’34"N, 96°06’01"W | 20/10/2015 | UAMIZ1354 |

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Table S2. Sequences of *rbcL* from GenBank used in the alignment.

| Specie | Geographic Information | Accession number |
|--------|------------------------|------------------|
| **Outgroup** | | |
| *G. curtissiae* J. Agardh | La Encrucijada, Venezuela | AY049333 |
| *G. dominguensis* (Kützing) Sonder ex Dickie | Bahia, Urucura, Serra Grande, Brazil | KP210223 |
| **Ingroup** | | |
| *Gracilariopsis andersonii* (Grunow) E. Y. Dawson | Gwaii Haanas, Ramsey Island, British Island, Canada | KU382057 |
| *Gp. carolinensis* L. M. Liao & Hommersand | Wilmington, North Carolina, USA | AY094940 |
| *Gp. cata-luziana* Gurgel, Fredericq & J. N. Norris | Anton Lizardo, Veracruz, Gulf of Mexico, Mexico | AY094940 |
| *Gp. changii* S.-M. Lin | Sail Rock, Kenting National Park, Taiwan | DQ119746 |
| *Gp. chorde* (Holmes) Ohmi | Awaji Island, Japan | FJ235527 |
| *Gp. chorde* | Jindo, Hoidong, South Korea | DQ995785 |
| *Gp. chorde* | Shimoda, Shizouka | EU567347 |
| *Gp. costaricensis* E. Y. Dawson | Guanacaste, Nicoya Peninsula, southern of Playa Tamarindo, Costa Rica | AY094943 |
| *Gp. funicularis* Iyer, Bolton & Coyne | Swakopmund, Namibia | AY094941 |
| *Gp. heteroclada* J.-F. Zhang & B.-M. Xia | Dapdap, Bulusan, Luzon, Phillipines | AY094941 |
| *Gp. heteroclada* | Hainan, China | JQ206028 |
| *Gp. hommersandi* Gurgel, Fredericq & J. N. Norris | Los Roques Archipelago, Los Francisky Island, Venezuela | AY094948 |
| *Gp. irregularis* (I. A. Abbott) N. Muangmai, A. Chirapat & A. Lewmanomont | Ao Len, Trat Province, Thailand | KC465784 |
| *Gp. lemaneiformis* (Bory de Saint-Vincent) E. Y. Dawson, Acleto & Foldvik | Guandong, China | JQ407670 |
| *Gp. lemaneiformis* | Guandong, China | JQ407672 |
| *Gp. lemaneiformis* | Guandong, China | JQ407673 |
| *Gp. lemaneiformis* | Eten, Chiclayo, Lambayeque, Peru | KP857574 |
| *Gp. lemaneiformis* | Ancon, Lima, Peru | KP857576 |
| *Gp. lemaneiformis* | Ancon, Lima, Peru | EU158089 |
| *Gp. lemaneiformis* | Yacilla, Piata, Piura, Peru | AY094915 |
| *Gp. lemaneiformis* | San Andres, Pisco, Ica, Peru | KP857575 |
| *Gp. lemaneiformis* | Ecuador | JQ843363 |
| *Gp. longissima* (S. G. Gmelin) Steentoft, L. M. Irvine & Farrham | Le Theven, Santec, France | DQ241579 |
| *Gp. longissima* | Venetian Lagoon, Italy | AY094940 |
| *Gp. longissima* | United Kingdom | JQ843364 |
| *Gp. mclachlanii* Buriyo, Bellorin & M. C. Oliveira | Pangamkungu, Tanzania | EU158092 |
| *Gp. megaspore* E. Y. Dawson | South Australia, Robe, Lake Buttler, Australia | AY094942 |
| *Gp. nhatrangensis* NhuHau Le & S.-M. Lin | Nhatrang City, Vietnam | DQ119744 |
| *Gp. persica* Bellorin, Sohrabipour & E. C. Oliveira | Bandarabbas, Persian Gulf, Iran | EU158094 |
| *Gp. silvana* Gurgel, Fredericq & J. N. Norris | La Vela de Coro, Falcon State, Venezuela | AY094930 |
| *Gp. tenulifrons* (C. J. Bird & E. C. Oliveira) Fredericq & Hommersand | La Pena, Araya, Venezuela | JQ843368 |
| *Gp. tenulifrons* | Alagoas, Maceio, Lago de Mundaú, Brazil | JQ843367 |
| *Gp. tenulifrons* | Ilet Caret, Guadeloupe, French West Indies | AY0949418 |
| *Gp. tenulifrons* | Arya Peninsula, Sucre, Venezuela | AY0949417 |
### Table S3. Sequences of COI-5P from GenBank used in the alignment.

| Specie | Geographic Information | Number Accession |
|--------|------------------------|------------------|
| **Outgroup** | | |
| *G. curtissiae* J. Agardh | Rio Grande do Norte, Diogo Lopes, Brazil | KP210166 |
| *G. isabellana* Gurgel, Fredericq & J. N. Norris | Sao Paulo, Ubatuba, Brazil | KP210185 |
| **Ingroup** | | |
| *Gp. andersonii* (Grunow) E. Y. Dawson | California, Bird Rock, Pacific Grove, USA | KM254615 |
| *Gp. andersonii* | California, Santa Cruz, USA | KM254410 |
| *Gp. andersonii* | British Columbia, Canada | FJ499624 |
| *Gp. andersonii* | British Columbia, Canada | FJ499625 |
| *Gp. chorda* (Holmes) Ohmi | Misaki, Japan | HQ322072 |
| *Gp. chouae* Zhang & B. M. Xia | Guangdong, China | JQ407613 |
| *Gp. funicularis* Iyer, Bolton & Coyne | Senegal | KP210200 |
| *Gp. funicularis* | Senegal | KP210201 |
| *Gp. heteroclada* J.-F. Zhang & B.-M. Xia | Prieto Diaz, Sorsogon, Philippines | KT779912 |
| *Gp. lemaneiformis* (Bory de Saint-Vincent) E. Y. Dawson, Acleto & Foldvik | Guangdong, China | JQ407623 |
| *Gp. lemaneiformis* | Guangdong, China | JQ407630 |
| *Gp. lemaneiformis* | Ecuador | JQ843342 |
| *Gp. lemaneiformis* | Hawaii | HQ422689 |
| *Gp. longissima* (S. G. Gmelin) Steentoft, L. M. Irvine & Farnham | United Kingdom | JQ843344 |
| *Gp. longissima* | Lake Ganzirri, Messina, Italy | KF714855 |
| *Gp. silvana* Gurgel, Fredericq & J. N. Norris | Rio Grande do Norte, Praia de Ponta Negra, Brazil | KP210202 |
| *Gp. silvana* | Espírito Santo, Meaipe, Brazil | KP210205 |
| *Gp. tenuifrons* (C. J. Bird & E. C. Oliveira) Fredericq & Hommersand | La Pena, Arraya, Venezuela | JQ843351 |
| *Gp. tenuifrons* | La Pena, Arraya, Venezuela | JQ843346 |
| *Gp. tenuifrons* | Sao Paulo, Itanhaem, Brazil | JQ843349 |
| *Gp. tenuifrons* | Rio de Janeiro, Cabo Frio, Brazil | JQ843354 |