

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. cata-luziana* 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].

Gracilariopsis 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, *Gracilariopsis 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, *Gracilariopsis 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 *Gracilariopsis* 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 α 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-*rbcl*-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* (Kützinger) 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 implemented 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 5×10^6 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 GMYC analyses, we generated an ultrametric tree for GMYC 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 =

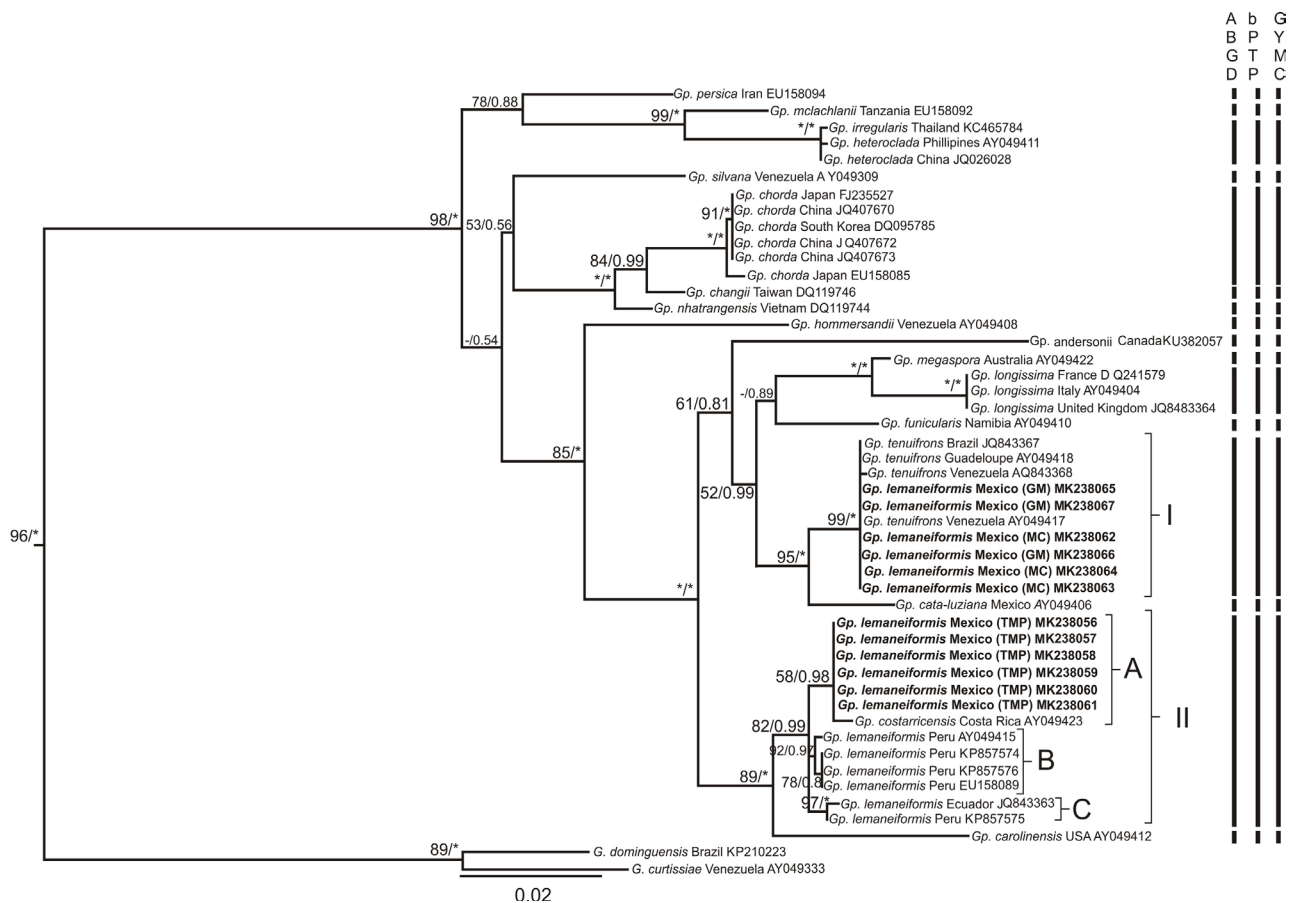


Figure 1. ML topology based on *rbcL* data. BI (left) followed by ML bootstrap values (right) on branches. Asterisks indicate maximum phylogenetic support (100% ML BP, 1.0 BI BP), a hyphen indicate values bellow 50. Sequences generated in this study are in boldface. Bars at the right of the tree indicate the results of the three species delimitations methods: Automatic 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. tenuifrons*. 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. costaricensis* 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. costaricensis* 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. costaricensis* is displayed in **Table 1**, considering only five variable vegetative characters between these species.

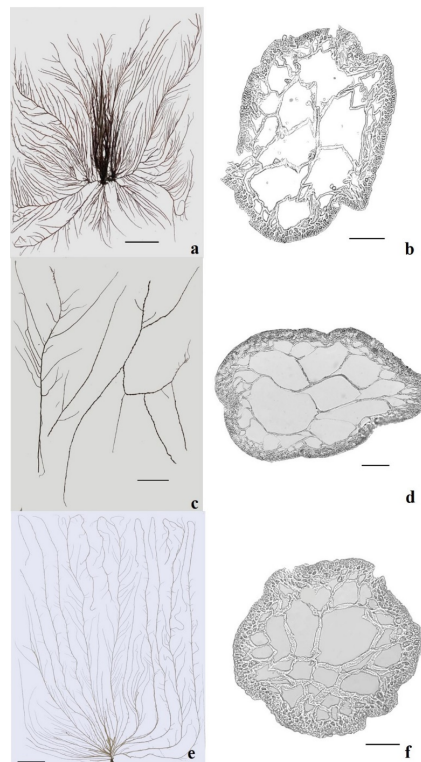


Figure 2. Specimens of *Gracilariopsis lemaneiformis* collected along Mexican coast. (a) Thallus of a specimen from Gulf of Mexico (GM); (b) Cross section of the thallus of a specimen from GM showing medullary and cortical cells; (c) Thallus of a specimen from Mexican Caribbean (MC); (d) Cross section 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 μ m.

Table 1. Morphological comparison between our specimens of *Gracilariopsis lemaneiformis* used in this study and the species of closed related.

Taxa	Characters				
	Thallus length (cm)	Branch diameter (mm)	Number of medullary cells	Layers of subcortical cells	Layers of cortical cells
<i>Gp. cata-luziana</i> ²	up to 26 (–36)	0.4 - 0.5	1 - 5	1 - 2	2
<i>Gp. costaricensis</i> ^{1,3}	up to 25	0.8	5 - 6	1 - 2	1 - 2
<i>Gp. lemaneiformis</i> ^{1,3}	up to 100	0.5 - 1.3	4 - 5	3 - 5	1 - 2
<i>Gp. tenuifrons</i> ¹	up to 40	1.0	3 - 4	1 - 3	1 - 2
<i>Gp. lemaneiformis</i> (TMP) ⁴	up to 95	0.3 - 0.5	4 - 6	1 - 2	1 - 2
<i>Gp. lemaneiformis</i> (GM) ⁴	up to 35	0.5 - 0.7	6	1 - 2	2
<i>Gp. lemaneiformis</i> (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. ¹Bird and Oliveira Filho [7], ²Gurgel *et al.* [28], ³Gurgel *et al.* [2] and ⁴this 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

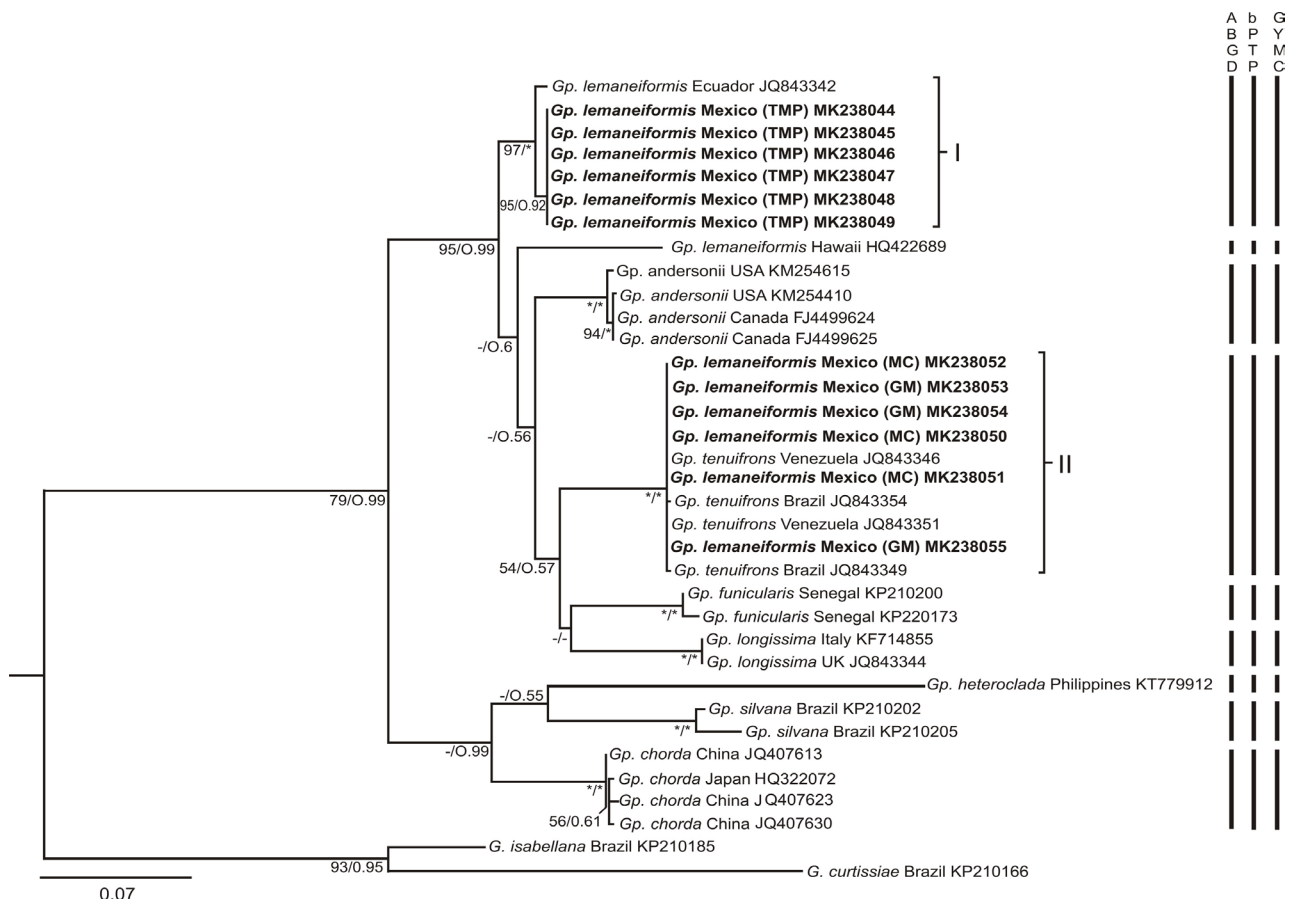


Figure S1. ML topology based on COI-5P data. BI (left) followed by ML bootstrap values (right) under branches. Asterisks indicate maximum phylogenetic support (100% ML BP, 1.0 BI BP), a hyphen indicate values below 50. Sequences generated in this study are in boldface. Bars at the right of the tree indicate the results of the three species delimitations methods: Automatic 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. 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
<i>rbcl</i>	COI-5P				
MK238056	MK238044	Boca del Cielo, Chiapas	15°51'00"N, 93°40'00"W	25/03/2018	UAMIZ1349#1
MK238057	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

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

Specie	Geographic Information	Accession number
Outgroup		
<i>G. curtissiae</i> J. Agardh	La Encrucijada, Venezuela	AY049333
<i>G. dominguensis</i> (Kützing) Sonder ex Dickie	Bahia, Urucuca, Serra Grande, Brazil	KP210223
Ingroup		
<i>Gracilariopsis andersonii</i> (Grunow) E. Y. Dawson	Gwaii Haanas, Ramsey Island, British Island, Canada	KU382057
<i>Gp. carolinensis</i> L. M. Liao & Hommersand	Wilmington, North Carolina, USA	AY049412
<i>Gp. cata-luziana</i> Gurgel, Fredericq & J. N. Norris	Anton Lizardo, Veracruz, Gulf of Mexico, Mexico	AY049406
<i>Gp. changii</i> S.-M. Lin	Sail Rock, Kenting National Park, Taiwan	DQ119746
<i>Gp. chorda</i> (Holmes) Ohmi	Awaji Island, Japan	FJ235527
<i>Gp. chorda</i>	Jindo, Hoidong, South Korea	DQ095785
<i>Gp. chorda</i>	Shimoda, Shizouka	EU567347
<i>Gp. costaricensis</i> E. Y. Dawson	Guanacaste, Nicoya Peninsula, southern of Playa Tamarindo, Costa Rica	AY049423
<i>Gp. funicularis</i> Iyer, Bolton & Coyne	Swakopmund, Namibia	AY049410
<i>Gp. heteroclada</i> J.-F. Zhang & B.-M. Xia	Dapdap, Bulusan, Luzon, Phillipines	AY049411
<i>Gp. heteroclada</i>	Hainan, China	JQ026028
<i>Gp. hommersandii</i> Gurgel, Fredericq & J. N. Norris	Los Roques Archipelago, Los Francisky Island, Venezuela	AY049408
<i>Gp. irregularis</i> (I. A. Abbott) N. Muangmai, A. Chirapart & A. Lewmanomont	Ao Len, Trat Province, Thailand	KC465784
<i>Gp. lemaneiformis</i> (Bory de Saint-Vincent) E. Y. Dawson, Acleto & Foldvik	Guandong, China	JQ407670
<i>Gp. lemaneiformis</i>	Guandong, China	JQ407672
<i>Gp. lemaneiformis</i>	Guandong, China	JQ407673
<i>Gp. lemaneiformis</i>	Eten, Chiclayo, Lambayeque, Peru	KP857574
<i>Gp. lemaneiformis</i>	Ancon, Lima, Peru	KP857576
<i>Gp. lemaneiformis</i>	Ancon, Lima, Peru	EU158089
<i>Gp. lemaneiformis</i>	Yacilla, Paita, Piura, Peru	AY049415
<i>Gp. lemaneiformis</i>	San Andres, Pisco, Ica, Peru	KP857575
<i>Gp. lemaneiformis</i>	Ecuador	JQ843363
<i>Gp. longissima</i> (S. G. Gmelin) Steentoft, L. M. Irvine & Farnham	Le Theven, Santec, France	DQ241579
<i>Gp. longissima</i>	Venetian Lagoon, Italy	AY049404
<i>Gp. longissima</i>	United Kingdom	JQ843364
<i>Gp. mclachlanii</i> Buriyo, Bellorin & M. C. Oliveira	Pangamkungu, Tanzania	EU158092
<i>Gp. megaspore</i> E. Y. Dawson	South Australia, Robe, Lake Buttler, Australia	AY049422
<i>Gp. nhatrangensis</i> NhuHau Le & S.-M. Lin	Nhatrang City, Vietnam	DQ119744
<i>Gp. persica</i> Bellorin, Sohrabipour & E. C. Oliveira	Bandarabbas, Persian Gulf, Iran	EU158094
<i>Gp. silvana</i> Gurgel, Fredericq & J. N. Norris	La Vela de Coro, Falcon State, Venezuela	AY049309
<i>Gp. tenuifrons</i> (C. J. Bird & E. C. Oliveira) Fredericq & Hommersand	La Pena, Araya, Venezuela	JQ843368
<i>Gp. tenuifrons</i>	Alagoas, Maceio, Lago de Mundau, Brazil	JQ843367
<i>Gp. tenuifrons</i>	Ilet Caret, Guadeloupe, French West Indies	AY049418
<i>Gp. tenuifrons</i>	Arya Peninsula, Sucre, Venezuela	AY049417

Table S3. Sequences of COI-5P from GenBank used in the alignment.

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