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# Molecular assessment of the species of Gracilariaceae (Gracilariales, Rhodophyta) from the Yucatan Peninsula, Mexico, including two new records for the Mexican Atlantic

# Evaluación molecular de las especies de Gracilariaceae (Gracilariales, Rhodophyta) en la Península de Yucatán, México, incluyendo dos nuevos registros para el Atlántico mexicano

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**Abstract**
**Background.** The Gracilariaceae is one of the most diverse and abundant families of marine red algae. Most species in this family exhibit high morphological variability and overlapping of characters. In the Yucatan Peninsula 30 species have been historically recorded, but the phylogenetic identity for many of them is still unknown.
**Questions:** Is the current diversity of the family in the area overestimated?
**Studied species:** *Crassiphycus caudatus*, *C. corneus*, *C. usneoides*, *Gracilaria flabelliformis*, *G. hayi*, *G.* cf *isabellana*, *G. microcarpa*, *G. occidentalis*, *G. suzanneae*, *Gracilariopsis tenuifrons.*
**Study site and dates:** Campeche: km 33 carretera Champotón, Bahía Tortuga, Puente Xen Kan III, Punta Xen, Sabancuy, 2017, 2018; Yucatan: Puerto Sisal, Puerto Progreso, Puerto Chicxulub, Puerto Telchac, 2018; Quintana Roo: Playa 88, Xcalacoco, Punta Esmeralda, 2019.
**Methods.** Phylogenetic analysis (with COI-5P and *rbc*L sequences), supported by DNA species delimitation methods, genetic distances and morphological comparisons, allowed us to molecularly identify the specimens collected.
**Results.** The phylogenetic identity of 10 species of Gracilariaceae was confirmed, two of which are new records for the Mexican Atlantic: *G. hayi* and *G. suzanneae*.
**Conclusion.** The study demonstrated that the molecular assessment has proved to be very useful for the diversity evaluation, thus, the future phylogenetic identifies for the rest of morphospecies recorded in the area will allow a better approximation of Gracilariaceae diversity species.
**Key words:** COI-5P, diversity, morphospecies, phylogeny, *rbc*L.

**Resumen**
**Antecedentes.** Gracilariaceae es una de las familias más diversas y abundantes de algas rojas marinas, la mayoría de sus especies exhiben una alta variabilidad morfológica y superposición de caracteres. En la Península de Yucatán se han registrado 30 especies, pero aún se desconoce la identidad filogenética de muchas de ellas.
**Preguntas:** ¿Se sobreestima la diversidad actual de la familia en el área?
**Especies de estudio:** *Crassiphycus caudatus*, *C. corneus*, *C. usneoides*, *Gracilaria flabelliformis*, *G. hayi*, *G.* cf *isabellana*, *G. microcarpa*, *G. occidentalis*, *G. suzanneae*, *Gracilariopsis tenuifrons.*
**Sitios y años de estudio:** Campeche: km 33 carretera Champotón, Bahía Tortuga, Puente Xen Kan III, Punta Xen, Sabancuy, 2017, 2018; Yucatan: Puerto Sisal, Puerto Progreso, Puerto Chicxulub, Puerto Telchac, 2018; Quintana Roo: Playa 88, Xcalacoco, Punta Esmeralda, 2019.
**Métodos.** Análisis filogenéticos (con secuencias COI-5P y *rbc*L), apoyados por métodos de delimitación de especies de ADN, distancias genéticas y comparaciones morfológicas, permitió identificar molecularmente cada especie recolectada.
**Resultados.** Se confirmó la identidad filogenética de 10 especies de Gracilariaceae, dos de las cuales fueron nuevos registros para el Atlántico mexicano: *G. hayi* y *G. suzanneae*.
**Conclusión:** El presente estudio demostró que la evaluación molecular resultó ser muy útil para la estimación de la diversidad, por lo que futuras identificaciones filogenéticas para el resto de las morfoespecies registradas en el área, permitirán una mejor aproximación de la diversidad de las especies de Gracilariaceae.
**Palabras clave:** COI-5P, diversidad, filogenia, morfoespecies, *rbc*L.

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The Gracilariaceae Nägeli is one of the most diverse and abundant families of marine red algae. The family is currently composed of 242 species, grouped into nine genera, widely distributed in temperate-tropical waters throughout the world (Guiry & Guiry 2021), with the highest species richness concentrated in the subtropical and tropical regions (Dreckmann & Sentíes 2014, Lyra *et al*. 2015a). In that several of their members are commercially important and widely cultivated to produce agar (Oliveira *et al*. 2000), the systematics of the group has been relatively well studied (Gurgel & Fredericq 2004, Lyra *et al*. 2015a, 2015b, 2016, 2021, Gurgel *et al*. 2003, 2018, Guiry *et al*. 2018). However, although the monophyly of Gracilariaceae is well-supported by molecular data (Gurgel *et al*. 2018, 2020), the morphological recognition of the species continues to be a difficult task and, in some cases, almost impossible because most species exhibit high morphological variability, leading to unclear species circumscriptions and an overlapping of characters (Bird & McLachlan 1982, Santelices & Valera 1993, Lyra *et al*. 2015a, Núñez-Resendiz *et al*. 2015). This overlapping has led to the uncovering of cryptic species (Saunders 2009) and confusion in their identification and geographic distribution ranges (Byrne *et al*. 2002, Cohen *et al*. 2004, Dreckmann *et al*. 2018, Vilchis *et al*. 2019), which currently generates an underestimation or overestimation of the actual diversity of species in the family (Dreckmann 2012, Dreckmann & Sentíes 2014). Different molecular markers have been successfully employed in studies of the Gracilariales allowing for the elucidation of species boundaries, phylogenetic relationships, and the recognition of new species (Kim *et al*. 2008, Yang *et al*. 2008, 2013, Muangmai *et al*. 2014a,b, Ardito *et al*. 2017, Hardesty & Freshwater 2018, Le *et al*. 2020).

On the coast of the Yucatan Peninsula, 30 species of Gracilariaceae, assigned to three genera, have been recorded under the morphospecies concept: *Crassiphycus caudatus* (J. Agardh) Gurgel, J.N. Norris & Fredericq, *C. corneus* (J. Agardh) Gurgel, J.N. Norris & Fredericq, *C. crassissimus* (P. Crouan & H. Crouan) Gurgel, J.N. Norris & Fredericq, *C. usneoides* (C. Agardh) Gurgel, J.N. Norris & Fredericq, *Gracilaria apiculata* P. Crouan & H. Crouan, *G. armata* (C. Agardh) Greville, *G. blodgettii* Harvey, *G. bursa-pastoris* (S.G. Gmelin) P.C. Silva, *G. cervicornis* (Turner) J. Agardh, *G. cuneata* Areschoug, *G. curtissiae* J. Agardh, *G. cylindrica* Børgesen, *G. damicornis* J. Agardh, *G. debilis* (Forsskål) Børgesen, *G. domingensis* (Kützing) Sonder ex Dickie, *G. flabelliformis* (P. Crouan & H. Crouan) Fredericq & Gurgel, *G. foliifera* (Forsskål) Børgesen, *G. gracilis* (Stackhouse) Steentoft, L.M. Irvine & Farnham, *G. mammillaris* (Montagne) M. Howe, *G. microcarpa* Dreckmann, Núñez-Resendiz & Sentíes, *G. ornata* Areschoug, *G. tikvahiae* Mc Lachlan, *G. venezuelensis* W.R. Taylor, *G. wrightii* (Turner) J. Agardh, *Gracilariopsis andersonii* (Grunow) E.Y. Dawson, Gp. cata-luziana Gurgel, Fredericq & J.N.Norris, *Gp. costaricensis* E.Y. Dawson, *Gp. longissima* (S.G. Gmelin) Steentoft, L.M. Irvine & Farnham, *Gp. tenuifrons* (C.J. Bird & E.C. Oliveira) Fredericq & Hommersand and *Gp. sjoestedtii* (Kylin) E.Y. Dawson (Gurgel *et al*. 2003, Ortega *et al*. 2001, Dreckmann 2012, Dreckmann & Sentíes 2014, García-García *et al*. 2020). Although there are detailed morphological and biogeographic studies for these species, for which an overestimation of the current diversity was already suggested (Dreckmann 2012, Dreckmann & Sentíes 2014), there is molecular support only for *Crassiphycus corneus*, *C. usneoides*, *Gracilaria macrocarpa*, and *Gracilariopsis tenuifrons* (Núñez-Resendiz *et al.* 2015, Dreckmann *et al.* 2018, Hernández *et al*. 2020). These studies have been carried out with molecular markers (*rbc*L and COI-5P), which have allowed the recognition of cryptic diversity (Núñez-Resendiz *et al*. 2015, 2016), the description of new species (Dreckmann *et al*. 2018) and the redefinition of their areas of distribution (Hernández *et al*. 2020). However, the phylogenetic identity for many of the species of Gracilariaceae recorded in the area is still unknown.

Our aim in the present study was to determine the phylogenetical identity of nine morphospecies in Gracilariaceae previously collected by authors at the Yucatan Peninsula, using molecular-assisted alpha taxonomy approaches.

## Material and methods

Fifty samples, consisting of compressed, flattened, and cylindrical specimens, identified as morphospecies of Gracilariaceae (Table S1) according to Dreckmann (2012), were collected in 12 localities along the Yucatan Peninsula, at a depth of 1.5-2.0 m, in three different years (Table S1). Apical sections for molecular analysis were preserved in silica gel until DNA extraction was done. Samples collected for morphological observations were preserved in 3 %

formaldehyde in seawater. Fresh specimens of each species were mounted on herbarium sheets and incorporated into the algal collections under a single voucher number, if they were collected in a same site and date (Table S1), at Metropolitan Herbarium UAMIZ (Herbarium abbreviations follow the online Index Herbariorum sciweb.nybg.org/science2/IndexHerbariorum.asp).

Total 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 (Saunders 2005). The chloroplast *rbc*L region was amplified using the primers F8 (Shimada *et al*. 1999), R753 (Freshwater & Rueness 1994), and F765 (Wang *et al*. 2000) R1381 (Freshwater & Rueness 1994). The PCR procedure followed Núñez-Resendiz *et al*. (2015). PCR products were purified with QIAquick Gel Extraction Kit (Qiagen, Valencia, California USA) and sequenced commercially (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 from GenBank of other species of Gracilariaceae (Table S2) was performed using Bioedit (Hall 1999). *Cryptonemia seminervis* (C. Agardh) J. Agardh and *Laurencia mutueae* Sentíes, Cassano & Dreckmann were included as outgroup in both data sets. We analysed the COI-5P and *rbc*L data sets separately. Phylogenetic analyses using maximum likelihood (ML) and Bayesian inference (BI) were performed separately with partitioned codons. The evolutionary model selected was GTR+I+G (general time reversible + invariable sites + gamma distribution) determined based on the Maximum Likelihood ratio test implemented by TOPALi version 2 software (Milne *et al*. 2009). ML analysis was performed using RAxML software (Stamatakis 2006) with the GTR+I+G model. Support for each branch was obtained from 1000 bootstrap replications. BI analysis was performed using MrBayes 3.2.2 (Ronquist *et al*. 2012). Four chains of Markov chain Monte Carlo were used, starting with a random tree, and sampling the data every 500 generations for $5 \times 10^6$ generations. 25 % of trees were discarded as burn-in. Pairwise distances values (p distance) were calculated using Mega X (Kumar *et al.* 2018).

To delimit the Gracilariaceae species in the trees, we ran three DNA - based species delimitation methods for both data sets (COI-5P and *rbc*L): Automatic Barcoding Gap Discovery (ABGD) (Puillandre *et al.* 2012), Bayesian variant of Poisson Trees Processes model (bPTP) (Pons *et al.* 2006), and the General-Mixed-Yule-Coalescent (GMYC) (Zhang *et al.* 2013). The ABGD method was done in the web interface (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, 50 screening steps and 20 Nb bins. The bPTP model was done via interface web (species.h-its.org/ptp), using the ML topology (see above). 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 (Drummond *et al.* 2012), from *rbc*L 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 site 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 (Rambaut *et al.* 2014). The maximum clade credibility tree was computed using TreeAnnotator v1.8.3 (Drummond *et al.* 2012). The resulting ultrametric tree was imported into the GMYC web server (species.h-its.org/gmyc), running the single threshold.

Photographs of thalli were taken with a Nikon D7000 digital camera. Microscopic cross-sections were made by hand using a razor blade and mounted in 80 % Karo®/distilled water solution. Photomicrographs were taken using an Olympus DP12 digital camera adapted to an Olympus BX51 microscope (DIC and bright-field). Morphological measurements were obtained from micrographs using SigmaScan© Pro automated image analysis software (Jandel Scientific, Sausalito, California).

## Results

From our results with COI-5P and *rbc*L sequences, supported by DNA species delimitation methods and genetic distances, we were able to confirm the phylogenetic identity of ten species of Gracilariaceae (Figures 1, 2) distributed in the Yucatan Peninsula. Of the nine Gracilariaceae morphospecies, only *Crassiphycus corneus*, *C. usneoides* and *Gracilariopsis tenuifrons* confirmed their phylogenetic identity, while the remaining of morphospecies correspond to another identity, and are mentioned below: the samples identified as *Gracilaria blodgettii* and *G. tikvahiae* correspond to *G. microcarpa*; the samples identified as *G. damicornis* correspond to *G. flabelliformis*; the samples identified as *G. mammillaris* correspond to *G. flabelliformis*, *G. occidentalis*, *G. hayi* or *G. suzanneae*; the samples identified to *Crassiphycus caudatus* and *G.* cf. *isabellana* could be correspond to new phylogenetic identities. We have recognized eight species previously recorded in the area: *Crassiphycus caudatus*, *C. corneus*, *C. usneoides*, *Gracilaria flabelliformis*, *G.* cf. *isabellana*, *G. microcarpa*, *G. occidentalis*, and *Gracilariopsis tenuifrons*; and two species were new records for the Mexican Atlantic: *G. hayi* and *G. suzanneae*.

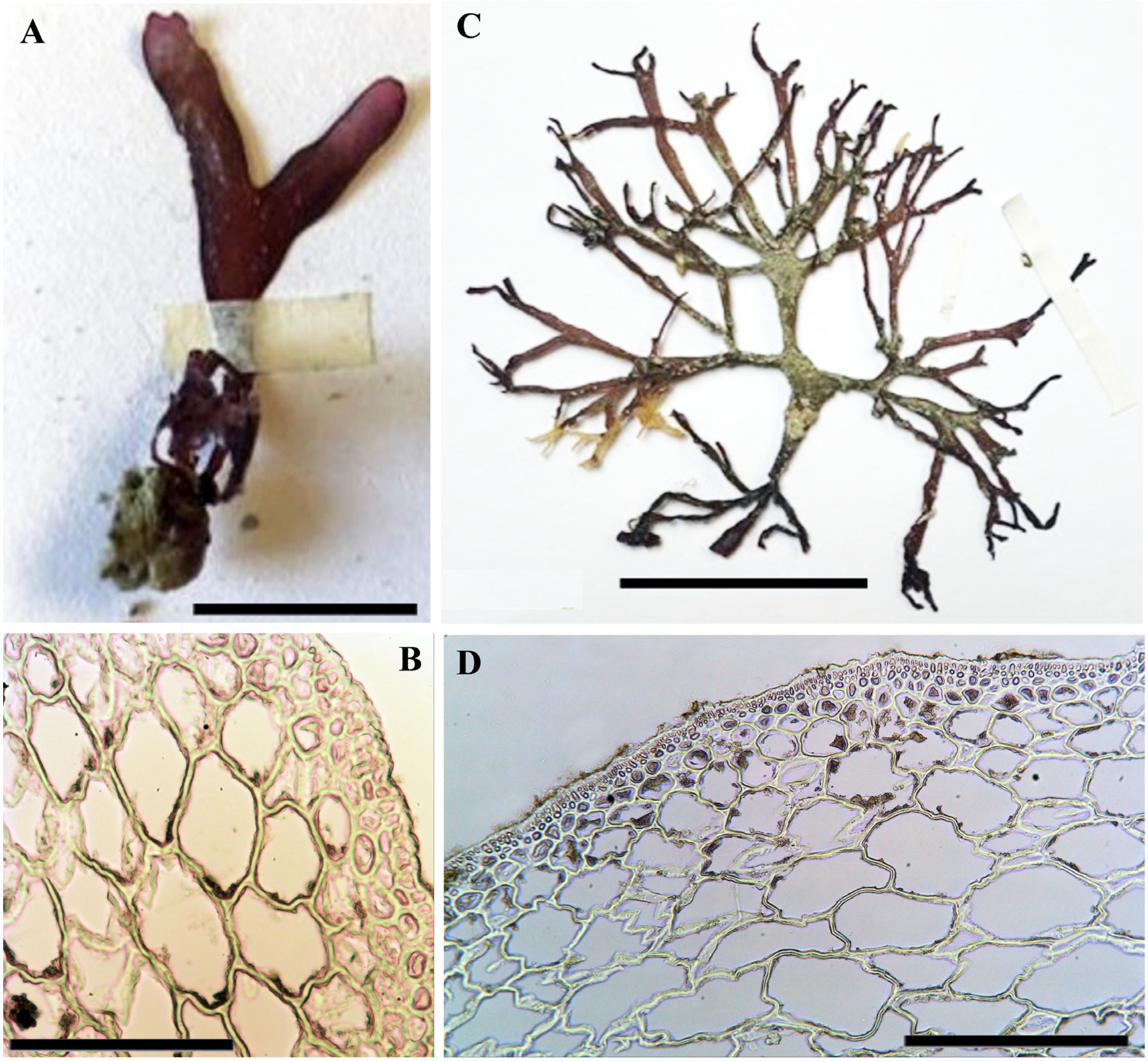

**Figure 1.** A. *G. hayi* from Bahía Tortuga. UAMIZ-1425. Scale bar = 4cm. B. Cross section of *G. hayi* showing cortical and medullary cells. UAMIZ-1425. Scale bar = 60 µm. C. *G. suzanneae* from Punta Xen. Scale bar = 4 cm. UAMIZ-1412. D. Cross section of *G. suzanneae* showing cortex and medullary cells. UAMIZ-1412. Scale bar = 60 µm.

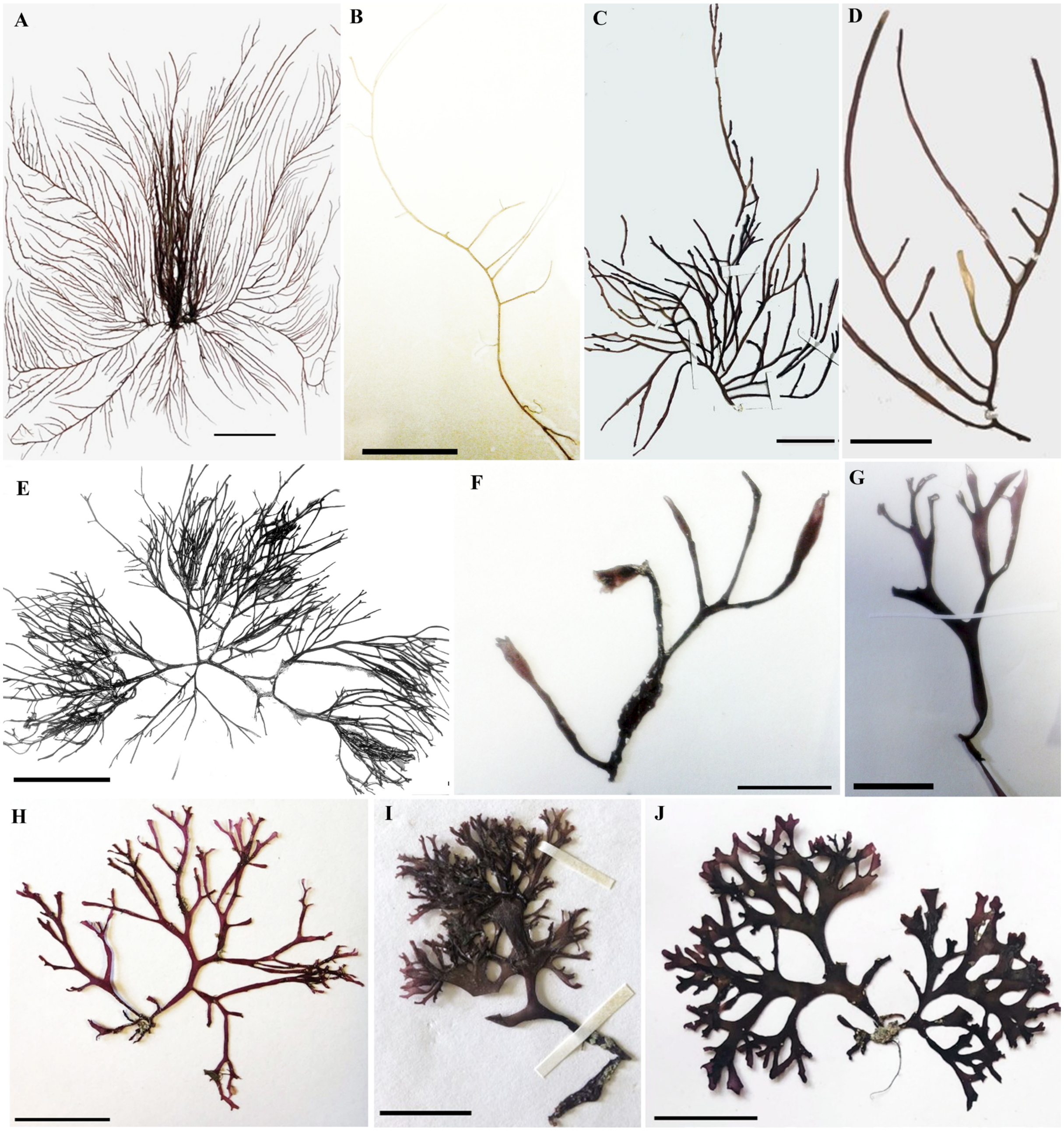

**Figure 2.** A. *Gracilariopsis tenuifrons* from Cancún. UAMIZ-1415. Scale bar = 10 cm. B. *Crassiphycus caudatus* from Bahía Tortuga. UAMIZ-1416. Scale bar = 2.5 cm. C. *corneus* from Puerto Progreso. UAMIZ-1417. Scale bar = 4 cm. D. *C. usneoides* from Puerto Progreso. UAMIZ-1420. Scale bar = 4 cm. E. *Gracilaria microcarpa* from Punta Xen. Scale bar = 4 cm. UAMIZ-1409. F-G. *G.* cf *isabellana* from Km 33 Carretera Champotón. UAMIZ-1431. Scale bar = 1 cm. H. *G. occidentalis* from Punta Xen. UAMIZ-1426. Scale bar = 4cm. I. *G. flabelliformis* from Bahía Tortuga. UAMIZ-1411. Scale bar = 4cm. I. *G. flabelliformis* from Xcalacoco. UAMIZ-1429. Scale bar = 3cm.

*Gracilaria hayi Gurgel, Fredericq & J.N.Norris in Gurgel et al. 2004; 178, figure 7 A-F (Figures 1A-B).* Thallus flattened, 5 cm long, dark red (Figure 1A). Dichotomous branching in one plane. Branches flattened up to 1 cm wide, non-constricted at the base, smooth margins, rounded apices (Figure 1A). Thalli not adhering to paper. Gradual transition between medulla and cortex. Cortex with 1-2 cell layers, cells oval to round, 5-10 µm in diameter (Figure 1B). Subcortex composed of 2 cell layers, cells oval of 26-44 µm in diameter (Figure 1B). Medulla of 2-3 cell layers, cells oval to round, 120-170 µm in diameter (Figure 1B). Reproductive characters were not observed in sequenced specimens.

*Gracilaria suzanneae L.P.Soares, C.F.D.Gurgel & M.T.Fujii in Soares et al. 2018; 346, 347, figures 1-22 (as 'suzannae') (Figures 1C-D).* Thallus flattened, 8 cm long, brown with reddish apices, coriaceous texture (Figure 1C). Trichotomous to irregular branching. Branches flattened, 2-3 cm long, 1-7 mm wide, non-constricted at the base (Figure 1C). Apical branches with a lacerate appearance. Thalli not adhering to paper. Cortex with 2-4 cell layers, cells oval to rectangular, 5-27 µm in diameter (Figure 1D). Subcortex composed of 1-2 cell layers, cells oval of 19-49 µm in diameter (Figure 1D). Medulla of 2-3 cell layers, cells oval to round, 206-250 µm in diameter (Figure 1D). Reproductive characters were not observed in sequenced specimens.

The COI-5P alignment was 614 base pairs (bp) in length and included 55 sequences, 29 newly determined; *rbc*L alignment was 1,204 bp in length and included 69 sequences, 30 newly determined. With both markers, ML and BI analyses had identical topologies; only ML tree, with BI values integrated and ML bootstrap values are shown (Figures 3, 4). The topologies resulting with both markers showed that our specimens were included in three monophyletic groups (I, II, III). Group I, with the maximum phylogenetic support in both analyses, was integrated with the species of the genus *Gracilariopsis* (Figures 3, 4). Of all the specimens collected, identified morphologically in the field as *Gracilariopsis*, only the presence of *Gp. tenuifrons* was confirmed with a distribution from Campeche to Quintana Roo. Group II, with high support (COI-P, BI = 1.0, ML = 99 %; *rbc*L, BI = 1.0, ML = 100 %), included species of the genus *Crassiphycus*, and the presence of three species was confirmed (Figures 3, 4): *C. caudatus* with distribution in Campeche; *C. corneus* and *C. usneoides* distributed along the three States of the Yucatan Peninsula. Group III, also well-supported (COI-P, BI = 1.0, ML = 97 %; *rbc*L, BI = 0.99, ML = 99 %), included the species of the genus *Gracilaria*, and six species were confirmed (Figures 3, 4): *G. flabelliformis* distributed from Campeche to Quintana Roo; *G. hayi* distributed in Campeche; *G.* cf *isabellana* distributed in Quintana Roo; *G. microcarpa* distributed from Campeche to Quintana Roo; *G. occidentalis* distributed in Campeche; and *G. suzanneae* distributed in Campeche.

Genetic distance within the species of the Gracilariaceae included in our analyses varied between the recognized genera. Species of *Gracilaria* ranged from 4.2 % (*G. occidentalis* from Mexico) to 17.6 % (*G. dentata* from Senegal) with COI-5P and from 0.8 % (*G. suzanneae* from Brazil) to 6.5 % (*G. isabellana* from Mexico) with *rbc*L. Species of *Crassiphycus* ranged from 5.2 % (*C. usneoides* from Mexico) to 10.3 % (*C. caudatus* from Brazil) with COI-5P and from 2.1 % (*C. corneus* from Venezuela) to 3.1 % (*C. usneoides* from Mexico) with *rbc*L. Species of *Gracilariopsis* ranged from 5.2 % (*Gp. andersonii* from Canada) to 6.5 % (*Gp. tenuifrons* from Brazil) with COI-5P and from 3.3 % (*Gp. lemaneiformis* from Mexico) to 3.6 % (*Gp. costaricensis* from Costa Rica) with *rbc*L.

In addition, for COI-5P data set, ABGD analysis defined 26 hypothetical species (nine of these from Yucatan Peninsula), and bPTP and GYMC analyses defined 24 (seven of these are from Yucatan Peninsula). For *rbc*L data set, ABGD analysis defined 22 hypothetical species (eight of these are from Yucatan Peninsula), and bPTP and GYMC analyses defined 24 (seven of these from Yucatan Peninsula).

## Discussion

Based on his review with morphological data and geographic distribution of the species of Gracilariaceae occurring on the Mexican Atlantic coast, Dreckmann (2012) concluded that the diversity of the family in the area was overestimated. Consequently, he proposed that from the 30 species that had currently been recorded in the Yucatan Peninsula, only 12 species were distributed in the area: five species of *Gracilaria* (*G. blodgettii*, *G. cervicornis*, *G. damicornis*, *G. mammillaris*, and *G. tikvahiae*), four species of *Crassiphycus* (as *Hydropuntia* Montagne) (*C. caudatus*, *C. corneus*, *C. usneoides*, and *C. crassus*), and three species of *Gracilariopsis* (*Gp. cata-luziana*, *Gp. lemaneiformis* and *Gp. tenuifrons*). These data were later confirmed in a new review of family diversity on the Mexican coasts (Dreckmann & Sentíes 2014).

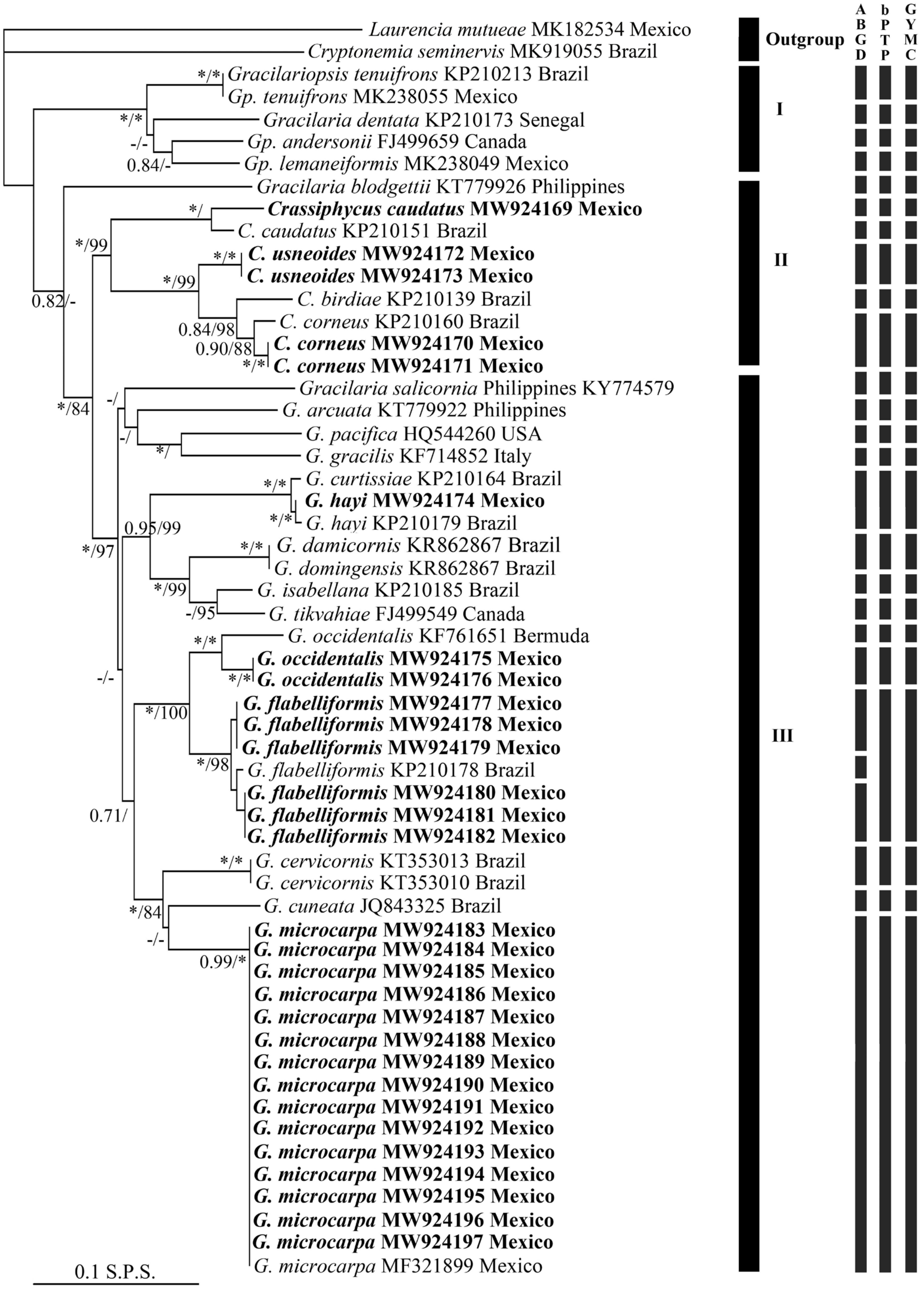

**Figure 3.** Maximum likelihood topology based on COI-5P sequences data. BI values (left) followed by ML bootstrap (right) on branches. Asterisks indicate full support (BI = 1.0, ML = 100 %), dashes indicate values below 70 %. Bars at the right of the tree indicate the results of the three species delimitations methods: Automatic Barcoding Gap Detection (ABGD), the General-Mixed-Yule-Coalescent (GMYC) and the Bayesian variant of Poisson Trees Processes model (bPTP). Sequences generated in this study are in boldface. S.P.S., substitutions per site.

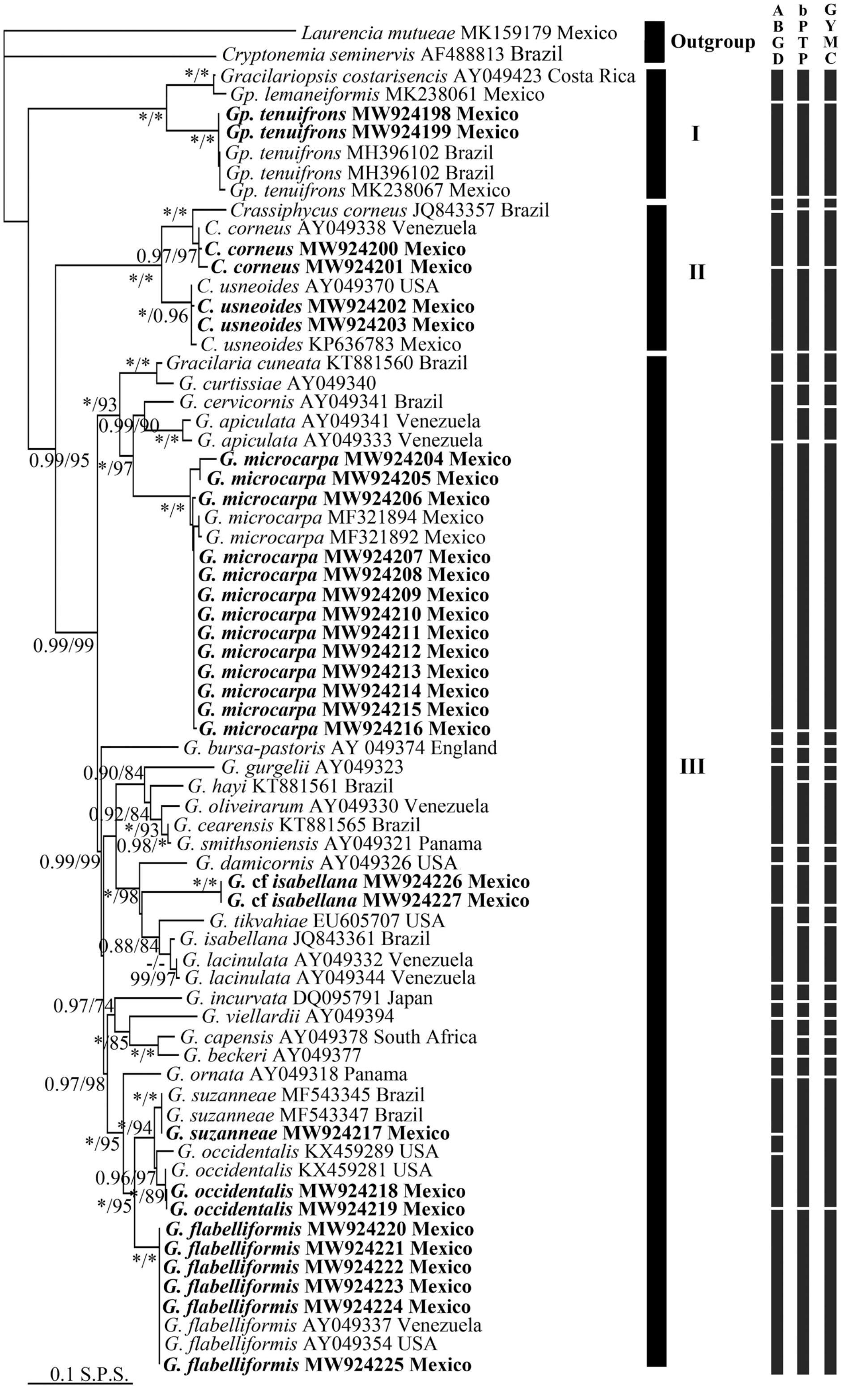

**Figure 4.** Maximum likelihood topology based on *rbc*L sequences data. BI values (left) followed by ML bootstrap (right) on branches. Asterisks indicate full support (BI = 1.0, ML = 100 %), dashes indicate values below 70 %. Bars at the right of the tree indicate the results of the three species delimitations methods: Automatic Barcoding Gap Detection (ABGD), the General-Mixed-Yule-Coalescent (GMYC) and the Bayesian variant of Poisson Trees Processes model (bPTP). Sequences generated in this study are in boldface. S.P.S., substitutions per site.

Recently, Hernández *et al.* (2020) demonstrated, with molecular evidence, that specimens morphologically identified as *Gp. lemaneiformis* from Yucatan Peninsula correspond to *Gp. tenuifrons* and it is distributed along Atlantic Mexican coast, and probably this species is the only distributed in the area.

Our results suggest a dramatic reduction of the floristic diversity known for the family in the Yucatan Peninsula. Of the 30 species currently recorded, our investigation can only support the phylogenetic identity of ten species, including two new records, despite our having sequenced specimens with the morphological characteristics corresponding to most of the reported species, especially those described by Dreckmann (2012). Of the confirmed species in our study, only the species of *Crassiphycus* (except *C. crassissimus)* and *Gp. tenuifrons* were consistent with those proposed by Dreckmann & Sentíes (2014) and Hernández *et al.* (2020), respectively. Of the species of *Gracilaria*, none of those proposed by Dreckmann & Sentíes (2014) was confirmed by our results. In fact, all these morphospecies turned out to be morphotypes of *G. flabelliformis* or *G. microcarpa* (recently described by Dreckmann *et al*. 2018). Both species are very abundant in the area, the first of flattened thalli and the second with cylindrical thalli. Except for *G. suzanneae* and *G. hayi*, originally described from Brazil (Soares *et al*. 2018) and Panama (Gurgel *et al*. 2004), respectively, which constitute new records for the Mexican Atlantic coasts; the rest of these *Gracilaria* species had already been previously recorded in the area. *Gracilaria isabellana* has been previously recorded from the coast of Veracruz, but although we found at the Yucatan Peninsula specimens morphologically very similar to what we had called *G.* cf *isabellana*, phylogenetic analysis and the results with DNA species delimitation methods suggested that these specimens may consist of an undescribed species. Additional morphological observations and sequence data are required to confirm its true identity.

For the rest of the morphospecies (García-García *et al*. 2020) of Gracilariaceae recorded for the Yucatan Peninsula and not confirmed in our study, eleven of them have type localities outside the Western Atlantic. *G. armata*, *G. bursa-pastoris*, *G. gracilis*, *G. multipartite*, and *Gp. longissima* have type localities in the European Atlantic and Mediterranean Sea; *G. pacifica* I.A. Abbott*, Gp. andersonii*, *Gp. costaricensis* and *Gp. sjoestedtii* in the American Pacific; and *G. debilis* and *G. follifera* in the Indian Ocean; which present a disjunct distribution, that is, an area of distribution interrupted by a geographical barrier (oceanic or continental). This type of distribution has been of great interest because geographic barriers can be the cause of evolutionary divergence processes between the populations involved, causing speciation. For example, regarding the *Gracilariopsis* species, Hernández *et al.* (2020) showed that the above-mentioned species are not present in the Mexican Atlantic, and that these records probably correspond molecularly to *Gp. tenuifrons*. So, the disjunct distribution of these species was ruled out. The morphological similarity between *Gracilariopsis* species is the main cause of misidentifications, which in turn leads to the wrong definition of their alleged disjunct distribution ranges and the overestimation of specific richness. This is a problem that may be occurring with *Gracilaria* species whose records in the American Atlantic do not correspond to their areas of distribution. However, another problem that could be the explanation of disjunct distribution for some species of Gracilariaceae family is the introduction of non-native species. Thus, the phylogenetic identified of these records not only help us clarify their taxonomic status, also their biogeographic processes.

Another important factor to consider in the molecular identification of the species found in this study is that the values of interspecific distance between them. In some cases, such as *Gracilaria hayi*, *G. suzanneae*, and *G. curtissiae*, the values of the interspecific distances ranged between 0.8 and 1.5 %, which is less than the range of interspecific values proposed by Gurgel *et al.* (2004) to delimit species of Gracilariaceae (from 2 to 3.4 %). From our results, a morphometic study of these species could provide additional evidence to support their phylogenetic independence, as it was in the case of *Crassiphycus* species, previously considered as cryptic species and currently discriminated by morphometric analysis (Vilchis *et al.* 2019, 2020).

Considering the values of the interspecific distances, the morphological similarity between several species in the Gracilariaceae, and the results using DNA species delimitation methods, it seems that some species in the family have been wrongly described as new diversity; consequently, they must be critically reviewed. For example, in a very recent study (Lyra *et al*. 2021), the floristic diversity for the Gracilariaceae species from Brazil was discussed under morphological and molecular evidence. Consequently, genera previously described as independent from *Gracilaria* due to their paraphyletic position in the phylogeny (Gurgel *et al.* 2018, 2020), have been merged back into a single genus (Lyra *et al.* 2021). *Crassiphycus* was one of the genera included in a broadly circumscribed *Gracilaria sensu lato* proposed by Lyra *et al*. (2021). However, from our phylogenetic analysis, we recognize *Crassiphycus* as an independent genus of *Gracilaria*. Likewise, in the study by Lyra *et al.* (2021), *Crassiphycus usneoides* was not included within the diversity of Gracilariaceae in Brazil, because they did not molecularly confirm its presence and discussed, in agreement with Núñez-Resendiz *et al.* (2015), the difficulty of recognizing them morphologically. Previously, *C. usneoides* was included as part of a complex of cryptic species along with *C. corneus* by Núñez-Resendiz *et al.* (2015), both species with type localities in Brazil. In their study they described that within the morphological spectrum of the two species included in the complex, there were two genetically independent entities, although morphologically indistinguishable. Recently, Vilchis *et al*. (2019, 2020) was able to discriminate both genetic entities, by applying morphometric techniques, both by the diameter in the middle and apical portion of the thallus (significantly larger in *C. usneoides* than in *C. corneus*) and by the shape of the cells (narrower in *C. usneoides*). Given that *C. corneus* and *C. usneoides* were circumscribed only with morphological evidence, the characters that were used for discriminated them overlap between the two species, so it may be that in Brazil only *C. corneus* is distributed. However, our morphological and molecular results do support the presence of both species in the Yucatan Peninsula.

In conclusion, based on our current results, we could detect the phylogenetic identity to 10 species from the molecular analysis of nine morphospecies. These identities correspond to species previously recorded and two new records for the Yucatan Peninsula. The study demonstrated that the molecular assessment has proved to be very useful for the diversity evaluation, thus, the future phylogenetic identifies for the rest of morphospecies recorded in the area will allow a better approximation of Gracilariaceae diversity species.

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## Supplementary material

Supplemental data for this article can be accessed here: https://doi.org/10.17129/botsci.2915

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