



Molecular assessment of the genus *Sargassum* (Fucales, Phaeophyceae) from the Mexican coasts of the Gulf of Mexico and Caribbean, with the description of *S. xochitlae* sp. nov.

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Abstract

The recognition of many species of *Sargassum* has been difficult because morphological characters show significant phenotypic variation depending on habitat, season, or even differences at the population level. To resolve some of these systematic issues along the Mexican Atlantic coast, molecular analyses of *Sargassum* species were carried out using DNA sequences of the COI-5P, ITS-2 and *rbcl_S* genetic markers. Of the 16 species recorded for the area, five species (*S. acinarium*, *S. mathiesonii*, *S. pteropleuron*, *S. pusillum* and *S. ramifolium*) were not found during the collections and their status remains undefined. Phylogenetic analyses of the three molecular markers showed that 10 species with their varieties (*S. bermudense*, *S. buxifolium*, *S. cymosum*, *S. filipendula*, *S. fluitans*, *S. furcatum*, *S. hystrix*, *S. natans*, *S. polyceratium*, and *S. vulgare*) were grouped into a polytomy, with low genetic diversity. If we consider these markers as suitable for proxy for species delimitation, these species should be synonymized under *S. cymosum*, the older name. Taxonomic independence was confirmed only for *S. platycarpum* and our data revealed a new morphologically and molecularly independent species within the genus, described here as *Sargassum xochitlae*.

Keywords: COI-5P, ITS-2, molecular phylogeny, *rbcl_S*, Sargassaceae, systematics

Introduction

Sargassum C. Agardh (1820) *nom. cons.* is one of the most important genera of the marine brown algae (Phaeophyceae), with 361 taxonomically accepted species in the family Sargassaceae (Guiry & Guiry 2020). In general, this genus has a worldwide distribution, especially in tropical and subtropical regions, and exhibits a species-richness gradient from the western Pacific toward the east (Yoshida 1989, Phillips 1995, Phillips & Fredericq 2000, Mattio *et al.* 2008).

Although *Sargassum* has been the subject of many taxonomic studies, such as those by Womersley (1954), Yoshida (1983, 1988, 1989), Abbott *et al.* (1988), Magruder (1988), Tsuda (1988), Tseng & Lu (1988, 2002), Trono (1992) and Noro *et al.* (1995), these have been based exclusively based on morphological characters. However, the recognition of many species in the genus has been difficult, because morphological characters show significant phenotypic variation, depending on habitat, season, and differences at the population level (De Wreede 1976, Magruder 1988, Kilar *et al.* 1992, Engelen *et al.* 2005, Mattio *et al.* 2008, Mattio & Payri 2011). Furthermore, according to Mattio *et al.* (2008), type specimens of *Sargassum* often poorly reflect infraspecific polymorphism occurring in nature because they were often described on the basis of incomplete or drift-collected material.

With the development of Molecular Assisted Alpha Taxonomy (MAAT, Cianciola *et al.* 2010), more robust classification schemes have been generated (Mattio & Payri 2011). The investigations of Phillips (1998), Phillips & Fredericq (2000), and Stiger *et al.* (2000, 2003), implementing these molecular approaches, have allowed a more objective understanding of the phylogenetic systematics of the genus, modifying previous diversity estimates. Recently, a significant number of studies that combine both morphological and molecular data, have been published for wide oceanic areas, such as the northern Gulf of Mexico (Phillips & Fredericq 2000), French Polynesia (Mattio *et al.* 2008), Western and Central Pacific Islands (Mattio *et al.* 2009), New Caledonia (Mattio & Payri 2009), Australia and New Zealand (Dixon *et al.* 2012), Mauritius and Réunion, western Indian Ocean (Mattio *et al.* 2013), Australia, New Zealand, South Africa, southern Madagascar, southern Mozambique, Japan (Dixon *et al.* 2014), Gulf of California (Andrade-Sorcia *et al.* 2014), Caribbean Colombia (Camacho, *et al.* 2015), South Africa (Mattio *et al.* 2015), and Singapore (Yip *et al.* 2018). All these studies, however, have represented a reduction in the taxonomic diversity previously recorded for *Sargassum* in those areas, since in all published phylogenies the species examined form polytomies.

On Mexican coasts, *Sargassum* species have been widely recorded in floristic checklists and have been shown as an important component of the phycological sub-tidal and intertidal communities (González-González *et al.* 1996, Dreckmann 1998, Ortega *et al.* 2001, Pedroche *et al.* 2008). Of the total 24 species of *Sargassum* known for the western Atlantic, from the Carolinas to Brazil (Wynne 2017), 16 species and six varieties have been recorded from the coasts of the Gulf of Mexico and the Mexican Caribbean (Dreckmann 1998, Littler & Littler 2000, Phillips & Fredericq 2000, Ortega *et al.* 2001, Fredericq *et al.* 2009, Wynne 2017).

Although Phillips & Fredericq (2000) carried out a molecular assessment of the *Sargassum* species for the northern part of the Gulf of Mexico (Texas to Florida), species reported from the Mexican coasts of the Atlantic were not included. Thus, the true diversity of the genus on the Mexican coasts of the Gulf of Mexico and Caribbean is still unknown. In order to obtain a more reliable diversity estimation of the species of *Sargassum* from the Mexican Atlantic, in the present study we used a combination of three molecular markers, one mitochondrial (COI-5P), one plastid (*rbcL_S*), and one nuclear (ITS2).

Materials and methods

Forty-one specimens were collected from 20 localities along the Mexican coasts of the Gulf of Mexico, Caribbean and Brazil (Fig. 1, Table S1), at depths of 0.5–1.5 m on intertidal rocks or free floating (holopelagic forms). For each specimen, an apical section was preserved in silica gel until DNA extraction, while the rest of the thallus was preserved in 4% formalin/seawater and later mounted on herbarium sheets and incorporated into the algal collections (Table S1) at the Metropolitan Herbarium UAMIZ (Herbarium abbreviations follow the online Index Herbariorum <http://sciweb.nybg.org/science2/IndexHerbariorum.asp>). The morphological identification was done using formalin-preserved specimens according to Taylor 1960, Littler & Littler 2000, Camacho *et al.* 2015, Wynne 2017. Macroscopic analysis for all specimens included examination of the size of the thallus, shape of the holdfast, shape, size and margin of blades, branching pattern, axes surface, distribution of cryptostomata in blades, blades midrib aspect, presence/absence of vesicles and morphology of the receptacles. The following literature was used for species identification: C. Agardh (1820), J. Agardh (1848), Bertossi & Ganesan (1973), Børgesen (1914a, 1914b), Camacho *et al.* 2015, Chapman (1963), Dawes & Mathieson (2008), Earle (1969), Paula (1988), and Taylor (1928, 1960, 1975). The updating of the taxonomic status was done consulting *AlgaeBase* (Guiry & Guiry 2020).

DNA was extracted from 5–10 mg of dried tissue, crushed in liquid nitrogen, using NucleoSpin Plant II (Machery-Nagel, Germany) according to the manufacturer's instructions. The mitochondrial COI-5P region was amplified using the primers GazF2 and GazR2 (Saunders 2005). The *rbcL_S* region was amplified using the primers 3F (Phillips 1998) and S97R (Peters & Ramirez 2001). The ITS-2 region was amplified using the primers SARG-ITS2F and SARG-ITS2R (Yoshida *et al.* 2000). These primers were selected because they were successfully used in *Sargassum* diversity studies by Saunders (2005), Mattio *et al.* (2008), and Camacho *et al.* (2015). The PCR procedure followed Mattio *et al.* (2008). PCR products were purified with illustra GFX PCR kit (GE Healthcare, UK), according to the manufacturer's instructions, and sequenced in both directions using BigDye terminator method (ABI, USA) in ABI 3730 DNA Genetic Analyzer. Sequences were assembled and edited using the program Sequencher® 5.4.5. The final alignment included sequences of other species of Sargassaceae downloaded from GenBank (Table S2) and was performed using Bioedit (Hall 1999). *Turbinaria tricostata* E.S.Barton was used as the outgroup for COI-5P and ITS-2 data sets. *Turbinaria ornata* (Turner) J.Agardh, *T. turbinata* (Linnaeus) Kuntze, and *T. tricostata* were used as outgroups for the *rbcL_S* data

set. These outgroup species were selected according to the suggestion of Phillips *et al.* (2008). We analyzed the COI-5P, *rbcL_S* and ITS-2 data sets separately and concatenated to give a combined COI-5P-*rbcL_S*-ITS-2 data set. In the combined data set, taxa for which COI-5P, *rbcL_S* or ITS-2 sequence data were unavailable, were treated as missing data. For the combined COI-5P-*rbcL_S*-ITS-2 data set, phylogenetic analyses using Bayesian inference (BI) and maximum likelihood (ML) were performed with codons partitioned. The evolutionary model selected was GTR+G (general time reversible + gamma distribution) and was determined by the ML ratio test implemented by TOPALi v2 software (Milne *et al.* 2009). ML analysis used RAxML software (Stamatakis 2006) with the GTR+G model. Support for each branch was obtained from 1,000 bootstrap replications. BI analysis was performed using MrBayes v3.2.2 (Ronquist *et al.* 2012). Four chains of Markov chain Monte Carlo were used, starting with a random tree and sampling the data every 100 generations for 5×10^6 generations. The likelihood scores stabilized after 200,000, and we discarded the first 25% of trees as burn-in. COI-P, *rbcL_S*, and ITS-2 data sets were analyzed in Mega version 5 (Tamura *et al.* 2011) using the Neighbor-joining (NJ) algorithm and uncorrected p-distances with bootstrap resampling of 3,000 replicates. The algorithm of distance employed in these analyses was simple and did not require an evolution model (Kucera & Saunders 2008). Pairwise distance values (*p* distances) were calculated using Mega version 5 (Tamura *et al.* 2011).

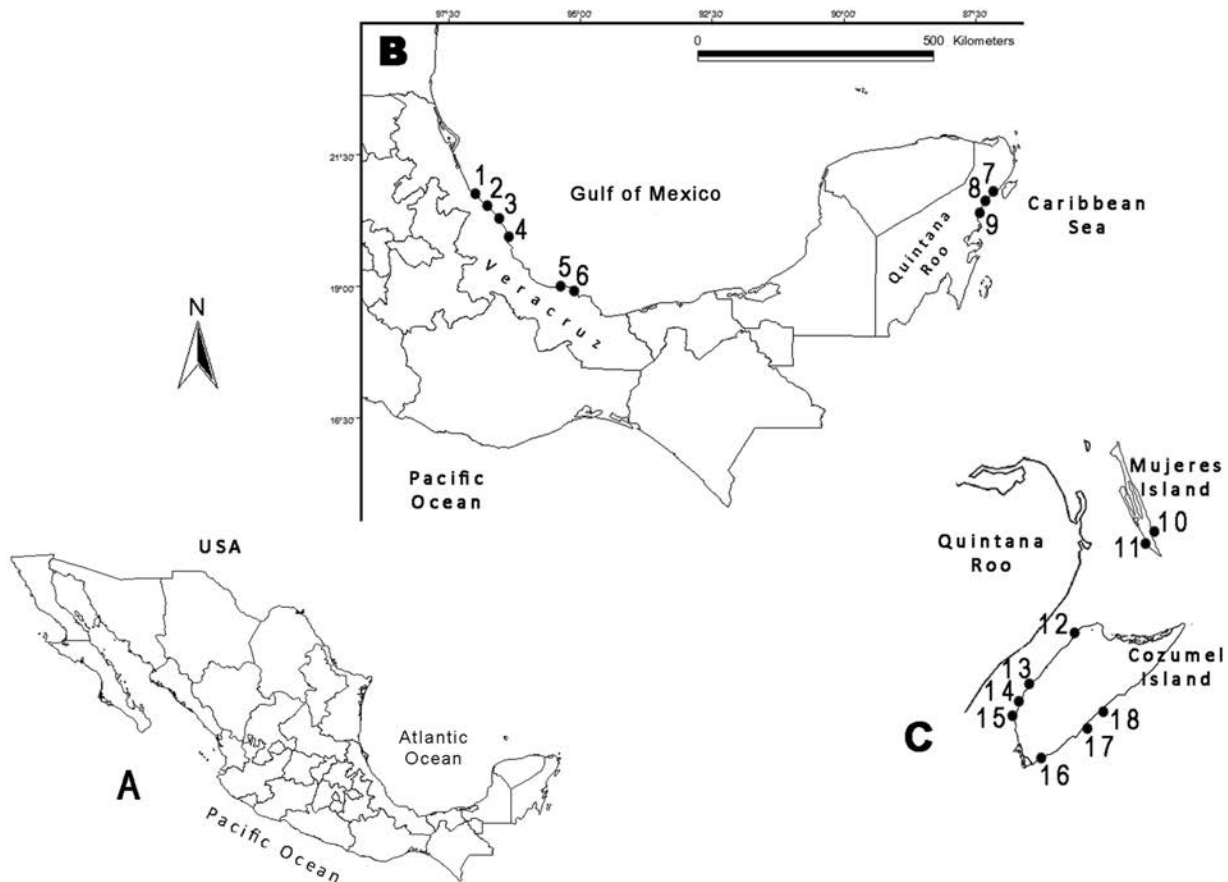


FIGURE 1. Sampling locations in Mexican coasts of the Gulf of Mexico and Caribbean. **A.** Mexico **B.** Veracruz and Quintana Roo states: 1. Punta Delgada 2. Playa Los Muñecos 3. Villa Rica 4. Playa El Morro (La Mancha) 5. Playa Hermosa 6. Playa Escondida 7. Punta Brava 8. Playa Caracol 9. Tulum **C.** Isla Mujeres and Cozumel Islands: 10. Lado Oriente 11. Playa Garrafón 12. Playa Azul 13. Hotel Cozumel 14. Chankanab 15. Playa San Francisco 16. Punta Chiquero 17. Chen Río 18. Punta Morena.

Results

The COI-5P alignment consisted of 51 sequences, 627 bp in length, of which 38 were newly generated in this study; the *rbcL_S* alignment consisted of 82 sequences, 755 bp in length, of which 35 were newly generated; the ITS-2 alignment consisted of 69 sequences, 534 bp in length, of which 37 were newly generated; and the combined COI-5P-*rbcL_S*-ITS-2 alignment consisted of 82 sequences and was 1,916 bp in length.

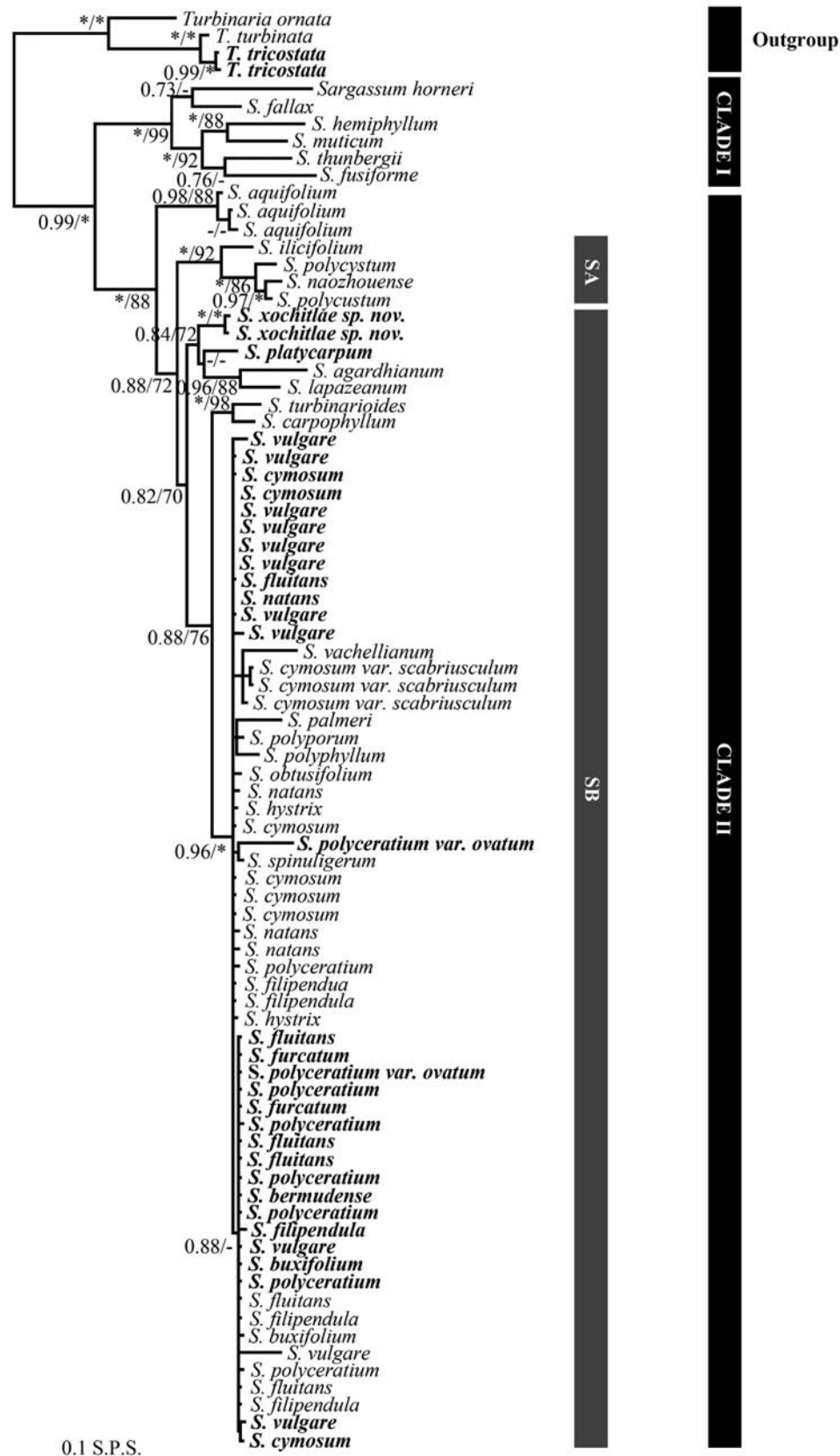


FIGURE 2. Bayesian topology based on combined COI-5P_rbcL_S-ITS-2 sequences data (1,916 bp). GenBank accession numbers and collection sites are shown in Table S1 for samples analyzed in this work and in Table S2 for sequences obtained in GenBank. Posterior probability (BI, left) followed by ML bootstrap (right) values are shown on the branches. Asterisks indicate full support (1.00 BI, 100% ML) and hyphens indicate values below 70. SA and SB refer to subclades (see results). Sequences generated in this study are in boldface. S.P.S. = substitutions per site.

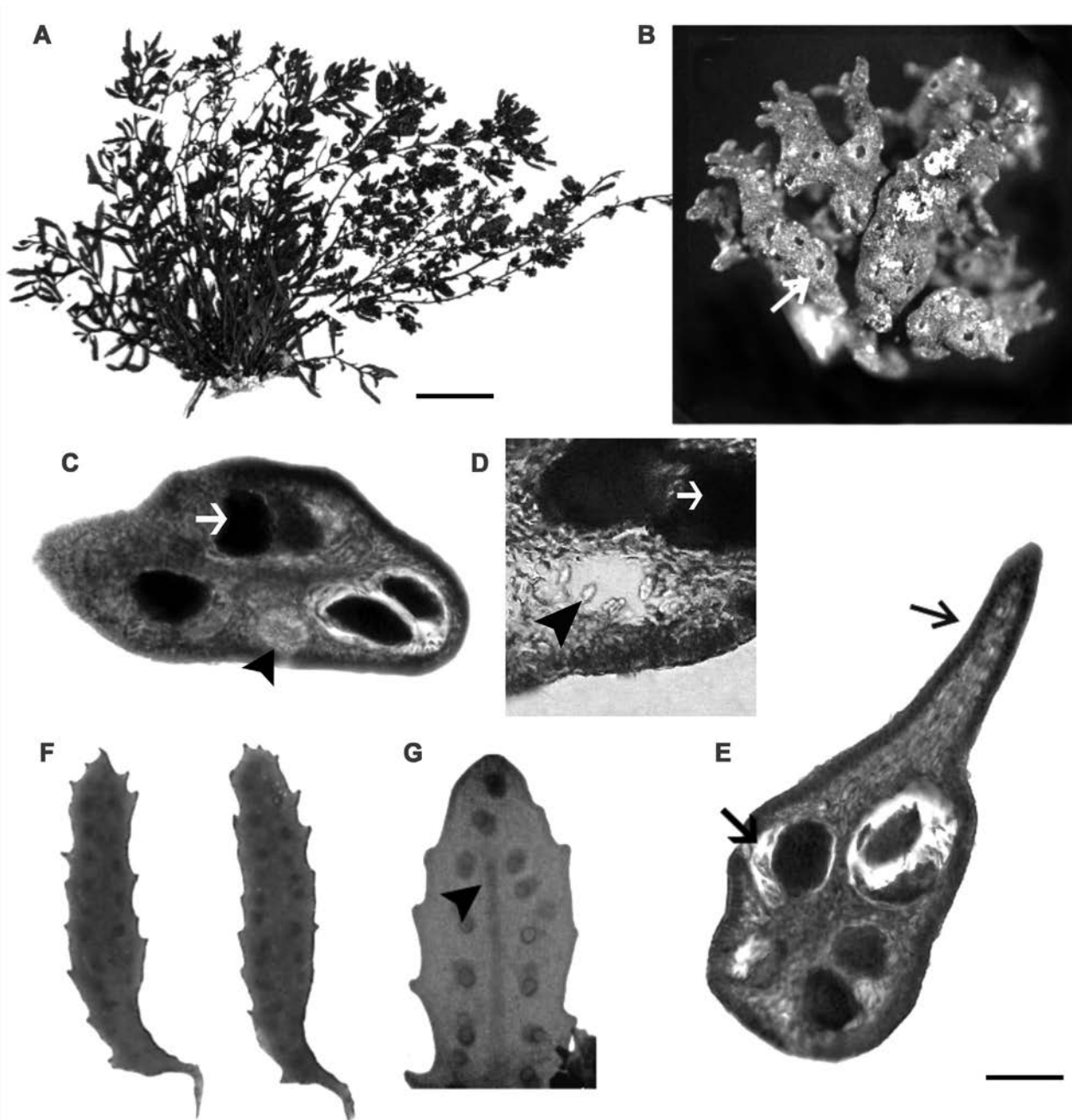


FIGURE 3. Morphology of *Sargassum xochitlae* sp. nov. **A.** Holotype, UAMIZ 1055, Habit. **B.** Morphology of receptacles showing apical pores (arrow). **C.** Transversal section of bisexual receptacle with female conceptacles (arrow), and male conceptacle (arrowhead). **D.** Detail of male conceptacle (arrowhead) and zygote (arrow). **E.** Transversal section of receptacle showing a prominent spine (thin arrow), female conceptacle with zygote (thick arrow). **F.** Blades. **G.** Blade showing cryptostomata and midrib (arrowhead). Scale bars represent: Figs. A= 4.5 cm, B = 0.8 mm, C-E= 250 μ m, D= 100 μ m, F-G= 1.5 mm.

The topologies resulting from the combined COI-5P-*rbcL*_S-ITS-2 (Fig. 2) and all single analysis (COI-5P, Fig. S1; *rbcL*_S, Fig. S2; ITS-2, Fig. S3) were similar and inferred identical phylogenetic hypotheses. Therefore, the phylogenetic relationships of the Mexican *Sargassum* specimens will be described from the combined analysis, in which the three markers used were integrated (Fig. 2). Species of *Sargassum* formed a monophyletic group, with strong phylogenetic support. Two major clades were observed for *Sargassum* included in these analyses (Clade I and II). Clade I was formed by species occurring mostly in Asia and in the Pacific Ocean (*S. fallax*, *S. horneri*, *S. fusiforme*, *S. thunbergii*, *S. muticum* and *S. hemiphyllum*). Clade II, was formed by *S. aquifolium* occurring in the Pacific and Indian Oceans as sister species to a larger clade, which was subdivided into two sister subclades (SA and SB). SA was formed by *S. ilicifolium*, *S. polycystum* and *S. naozhouense*, species occurring in Asia or in the Pacific and Indian Oceans. SB, which grouped all the Mexican and Brazilian specimens, was formed by a clade integrated by a molecularly and

morphologically different unidentified *Sargassum* species from Mexico, which was resolved as the sister species of *S. platycarpum* from the Atlantic and Caribbean, *S. agardhianum* and *S. lapazeanum*, both from the Pacific region. The other clade within SB contained *S. turbinarioides* and *S. carpophyllum*, both occurring in the Indo-Pacific region as sister to a well-supported clade composed of a big polytomy. This polytomy grouped most of the Mexican specimens and the one specimen we sequenced from Brazil (type locality from *S. cymosum*), this included the following species: *S. bermudense*, *S. buxifolium*, *S. cymosum*, *S. filipendula*, *S. fluitans*, *S. furcatum*, *S. hystrix*, *S. natans*, *S. polyceratium*, and *S. vulgare* and several sequences from GenBank (*S. obtusifolium*, *S. palmeri*, *S. polyporum*, *S. spinuligerum*, and *S. vachellianum*), most of the Caribbean area but also a couple of sequences from Australia and Japan.

The genetic distances within specimens in the polytomy ranged from 0 to 0.3% for COI-5P; 0 to 0.8% for *rbcL*_S; and 0 to 0.4% for ITS-2. The interspecific distances between *Sargassum* species outside the polytomy ranged from 2.3% to 11.4% for COI-5P; 1.7% to 4.6% for *rbcL*_S; 1.7% to 4.9% for ITS-2. The genetic distances between *S. platycarpum* and the Mexican specimens for the new, unidentified species of *Sargassum* was 2.3% for COI-5P; 1.7% for *rbcL*_S; 2.0% for ITS-2. The combination of the unique morphology (described below, using four specimens collected in two localities: eastern side of Isla Mujeres and Punta Morena, Isla Cozumel, Q. Roo) and the molecular data support the following new species proposal:

Sargassum xochitlae González-Nieto, M.C. Oliveira & Mateo-Cid *sp. nov.* (Fig. 3A-G).

Type Locality:—Isla Mujeres, Quintana Roo, Mexico. 21° 13' 16" N, 86°43' 20" W; 14 March 2012, coll. D. González-Nieto, (**Holotype:** UAMIZ 1055). GenBank accession numbers for *rbcL* MT470853; COI- 5P MT470873 and ITS2 MT465424.

Etymology:—The species epithet is dedicated to the memory of *Xochitl*, sister of the first author (DGN).

Description:—Thallus generally with pinnately or irregularly arranged laterals, 12–20 cm high, main axes arising from holdfast, which is small and conical; axes always terete and smooth, blades linear or lanceolate 1.5–3.0 cm width and 0.6–2.5 cm long, pedicels short almost sessile, margins with tiny spines sometimes entire, apex acute; base of blade cuneate or attenuate, midrib thin, not prominent and not percurrent; cryptostomata big 180–190 µm arranged in one row on each side of midrib, vesicles absent. Thalli monoecious, bisexual receptacles compressed, terete in base, clearly spiny, ramose of 2–4 mm long and 500–800 µm thick; conceptacles with apical pores.

Habitat:—On rocks, intertidal, exposed.

Distribution:—Known only from Isla Mujeres and Isla Cozumel, Quintana Roo.

Discussion

The evidence shown for the three markers used (one mitochondrial, one plastid and one nuclear) in the present study revealed that of the 16 species previously recorded in the Gulf of Mexico and the Mexican Caribbean, 10 of the species formed a single, large polytomy with low genetic divergence for all 3 markers. This indicates that they belong to the same species. Likewise, our morphological and molecular evidence also supported the establishment of a new species for the Atlantic coast of Mexico, *Sargassum xochitlae* a species known so far only to Isla Mujeres and Isla Cozumel, Quintana Roo.

In addition to the genetic evidence that delimits *Sargassum xochitlae sp. nov.* from the rest of the species, morphologically it displays a set of diagnostic characters that allow its recognition among other morphologically and/or molecularly closely related species (Table 1). Phylogenetically the new species is more closely related to two species in the Pacific Ocean and to *S. platycarpum*, but in terms of the morphology it is similar to *S. hystrix*, which is not closely related to *S. xochitlae* based on the phylogenetic analyses.

One of the most significant results in our phylogenetic analyses is the large polytomy that groups most of the specimens analyzed in this work and some others with available sequences from GenBank. These specimens show remarkable morphological differences and diagnostic characters that were used to assign species' epithets to each specimen (Fig. 3A-K). However, the interspecific divergence values between them did not support their genetic independence, because these values were much lower (COI-5P: 0–0.3%; *rbcL*_S: 0–0.8%; ITS-2: 0–0.4%) than those found among the species outside the polytomy (COI-5P: 2.3–11.4%; *rbcL*_S: 1.7–4.6%; ITS-2: 1.7–4.9%). Similar results using only ITS2 were found by Sissini *et al.* (2017) for benthic and holopelagic specimens of *Sargassum* from Brazil, Caribbean and other regions in the West Atlantic. These authors argued that, further investigation using other molecular markers should be done in order to clarify the taxonomic status of those species.

These data show that at least 10 of the 16 species, including their varieties previously recorded on the Mexican

coasts of the Gulf of Mexico and the Caribbean as distinct species, correspond to a single phenotypically highly variable species, including the two holopelagic species *S. fluitans* and *S. natans*, which consequently leads to a considerable reduction in the diversity of *Sargassum* in this area. However, these results are not the first to report a dramatic reduction in the true diversity of the genus, as they are consistent with what was previously found worldwide for *Sargassum* species. For example: Andrade-Sorcía *et al.* (2014), after a molecular assessment of the genus in the Gulf of California, recognized six out of the 42 taxa (infraspecific names included) previously recorded for the region (five already known, plus a new one); from the 17 species of *Sargassum* previously recorded from the northern Chinese coasts (Yellow Sea and East China Sea), Huang *et al.* (2017) confirmed only eight species; although 18 different specific epithets were attributed to French Polynesian *Sargassum*, only three species are considered valid by Mattio *et al.* (2008); from 45 specific and infraspecific names belonging to genus *Sargassum*, previously recorded for New Caledonia, only 12 are recognized by Mattio & Payri (2009); historical records list 41 species of *Sargassum* in Singapore, but only five was confirmed by Yip *et al.* (2018); finally, a total of 27 different *Sargassum* epithets are listed in the literature for South Africa, but only seven taxa were identified molecularly by Mattio *et al.* (2015). In all these studies, it is clear that *Sargassum* is a phenotypically plastic genus, which is why the identification of species based solely on morphology led to the overestimation of their diversity worldwide.

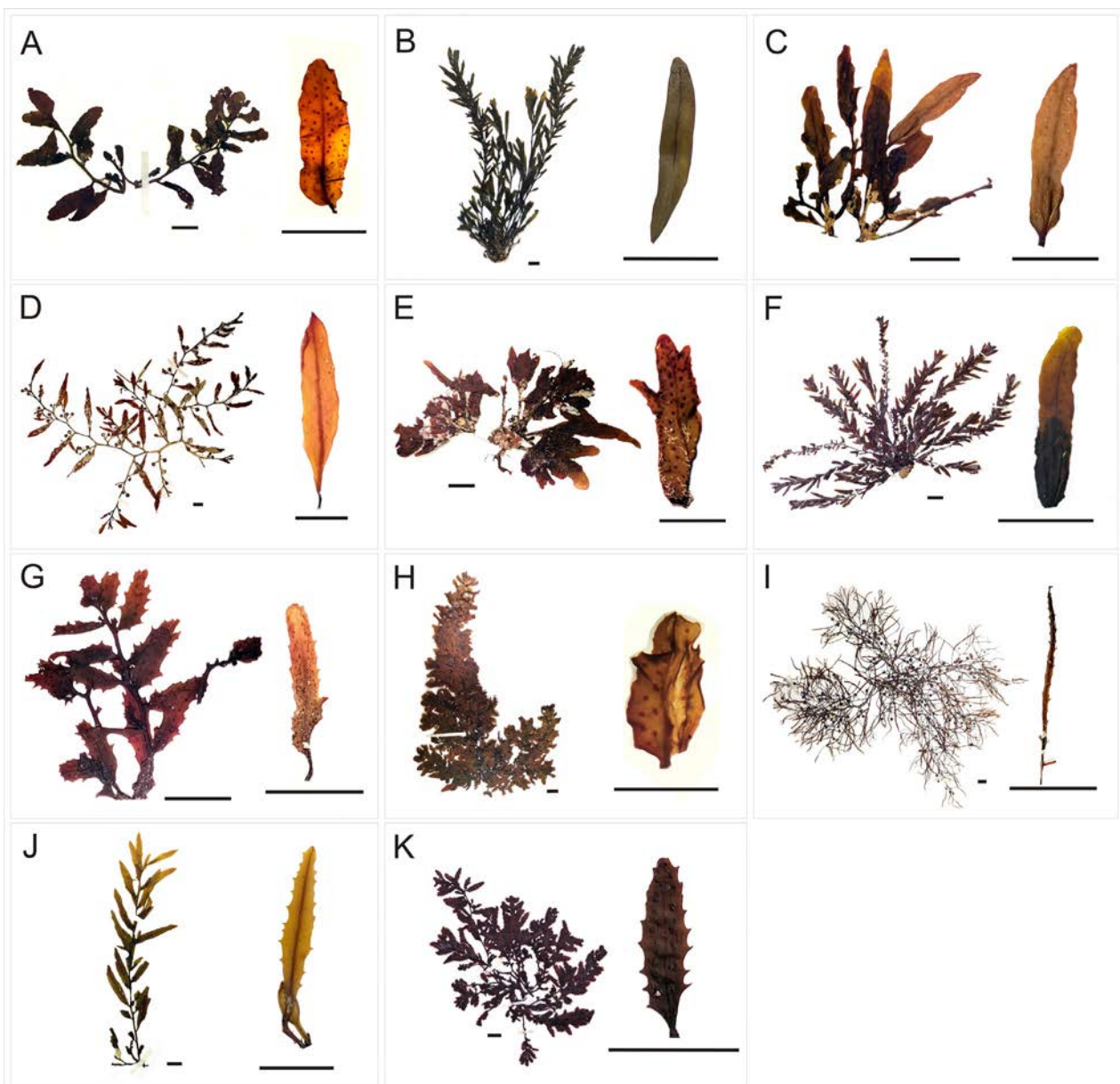


FIGURE 4. Morphology (habit and blade) of specimens identified in this work. **A.** *Sargassum bermudense*, (UAMIZ-1073), **B.** *S. buxifolium*, (UAMIZ-1051), **C.** *S. cymosum*, (UAMIZ-2512), **D.** *S. fluitans*, (UAMIZ-1053), **E.** *S. furcatum*, (UAMIZ-1061), **F.** *S. hystrix*, (UAMIZ-1077) **G.** *S. filipendula*, (UAMIZ-1048), **H.** *S. polyceratium*, (UAMIZ-1068), **I.** *S. natans*, (UAMIZ-1034), **J.** *S. vulgare*, (UAMIZ-1028), and **K.** *S. platycarpum*, (UAMIZ-1050). Scale bars = 1.0 cm.

TABLE 1. Morphological comparison between *Sargassum xochitilae* and related species.

Character	<i>Sargassum cf. cymosum</i> (Gulf of Mexico, Colombia) ¹	<i>Sargassum platycarpum</i> (Martinique) ^{2,3}	<i>Sargassum platycarpum</i> var. <i>bermudensis</i> (Bermuda) ⁴	<i>Sargassum platycarpum</i> var. <i>lenormandii</i> ⁴ (Cape Verde) ⁴	<i>Sargassum xochitilae</i> sp. nov.
Thallus/Size	Up to 40 cm	Up to 36 cm	ND	ND	Up to 20 cm
Holdfast	Discoid	Small conical	ND	ND	Small conical
Axis	Terete or compressed, smooth to muricate	Terete, smooth	Terete, muricate	Terete, smooth	Terete, smooth
Blades shape	Oblong-elliptical to lanceolate or linear	Linear- lanceolate	Linear	Lanceolate	Linear
Length/width (mm)	10-35/3-8	18-36/2-6	15/1	6-25/4-15	0.6-25/15-30
Margins	Irregularly scattered teeth, rarely serrate	Coarsely dentate	Finely serrated	Coarsely dentate	Finely dentate
Midrib	Prominent	Prominent	Prominent	Prominent	Inconspicuous
Pediceal	Short and terete	Short and terete	ND	Short and terete	Short and terete
Basis blades	Cuneate to rounded	Cuneate	ND	Cuneate	Cuneate or attenuate
Apex blades	Obtuse to acute	Acute	Acute	Acute	Obtuse to acute
Cryptostomata diameter	50-140 µm	ND	ND	ND	180-190 µm
Vesicles / Shape	Elliptical to spherical	Absent	Spherical	Sub spherical	Absent
Pediceal	Terete	Absent	Compressed	Compressed	Absent
Reproduction	Diocious, occasionally Monoecious	Monoecious	ND	Monoecious	Monoecious
Receptacles margin	Spiny-like protuberances	Strongly spiny	Sparingly spiny	Spiny	Spiny
Receptacles in transverse section	Terete	Compressed	Subterete	Subterete	Compressed, terete in base
Receptacles height	12-20 mm	ND	3 mm	Up 5 mm	2-4 mm
Receptacles wide	1.2 mm	ND	ND	ND	500-800 µm

1. Camacho *et al.* (2015); 2. Montagne (1842, 1856); 3. Herbarium MNHN *Sargassum platycarpum* of Martinique: Holotype <http://dsipphoto.mmhn.fr/sonnerat/Montagne/PC0045042.jpg>. 4. Grunow (1915); ND: Data not available

Based on this evidence and considering the divergence of the molecular markers used as a suitable proxy for species delimitation, all of the names represented in the polytomy, identified under different species names (*S. bermudense*, *S. buxifolium*, *S. cymosum*, *S. filipendula*, *S. fluitans*, *S. furcatum*, *S. hystrix*, *S. natans*, *S. polyceratium*, and *S. vulgare*), should be considered one species. This leads to another question, which of those names better represent this highly plastic and widespread Atlantic species. To be able to address this question, further data on type locality sequencing for each of those species and detailed analysis of the type specimens is necessary. Of those names, *S. cymosum* for which we have sequenced a specimen from the type locality (Brazil) is the oldest name. Although *S. vulgare* was also described in the same manuscript as *S. cymosum*, its current nomenclatural status is *nom. illeg.* (Guiry & Guiry 2020). Therefore, the nomenclatural priority corresponds to *S. cymosum*, which is the second in order of appearance in Agardh's work (1820). Therefore, until further data is available, we propose to refer to this highly variable and widespread species in the Western Atlantic and Caribbean as *Sargassum cf. cymosum* C. Agardh (1820: 20), and complemented by Camacho *et al.* (2015), with the following description (See Fig. 4A-K; Table 1): Thallus erect, tough, leathery, to 40 cm high, dark brown, holdfast strong, disc-like, main axes one to several, elongate, slender, with spines few 0.3–0.7 mm long. Blades numerous, 3–8 mm wide, 1.0–3.5 cm long; upper blades elliptical, oblong-lanceolate or linear; basal blades sometimes sparingly pinnately divided; apex rounded to slightly notched; margins with irregularly scattered teeth; stipe to 1 mm long. Cryptostomata 50–140 µm diam, small. Vesicles elliptical to spherical, few, often mucronate, on stalks about equal to the diameter. Thalli dioecious, occasionally monoecious. Receptacles terete, 2 to 4 times dichotomously branched; female conceptacles with prominent wart-like surface up to 6 mm long and 0.7 mm diam.; male conceptacles with wart-like surface up to 10 mm long and 0.5 mm in diam.

Habitat: -on hard substrates (rocks or coral fragments), low intertidal or free-floating (holopelagic).

Regarding the GenBank sequences that were part of the polytomy, but whose distribution does not correspond to the Gulf of Mexico and the Mexican Caribbean (*S. obtusifolium* (from USA and French Polynesia), *S. palmeri* (from USA and Colombia), *S. polyporum* (from Japan), *S. spinuligerum* (from France), and *S. vachellianum*), morphological features used to identify them were not available, so we are uncertain of those identifications.

Of the six additional species of *Sargassum* recorded for the region, we only confirmed the genetic independence for *S. platycarpum* (Fig. 4K), but the other five (*S. acinarium*, *S. mathiesonii*, *S. pteropleuron*, *S. pusillum*, and *S. ramifolium*) were not found in the field, consequently their status remain uncertain for the Mexican coast. The absence of those species may be due in part to misidentifications as they also represent older records in the area (Ortega *et al.* 2001). Although, *S. pteropleuron* was found in the Caribbean and Western Atlantic in a recent study (Camacho *et al.* 2015).

In conclusion, of the 16 species previously reported from the Gulf of Mexico and the Mexican Caribbean, we confirmed the existence of 1) *S. platycarpum*, 2) a highly variable and widely distributed species, yet to be correctly named, but that we will refer to as *S. cf. cymosum*, and 3) describe a new species based on morphology and molecular data *Sargassum xochitlae*. Thus, as in many other regions of the world, the true diversity of *Sargassum* in the Atlantic coast of Mexico seems to be much lower than previously understood.

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FIGURE S1. Neighbor-joining (NJ) tree based on COI-5P sequences data. Bootstrap values on branches, asterisks indicate full support (100) and hyphens indicate values below 70. Sequences generated in this study are in boldface. Species names are followed by GenBank accession numbers and, when available, the country where it was collected.

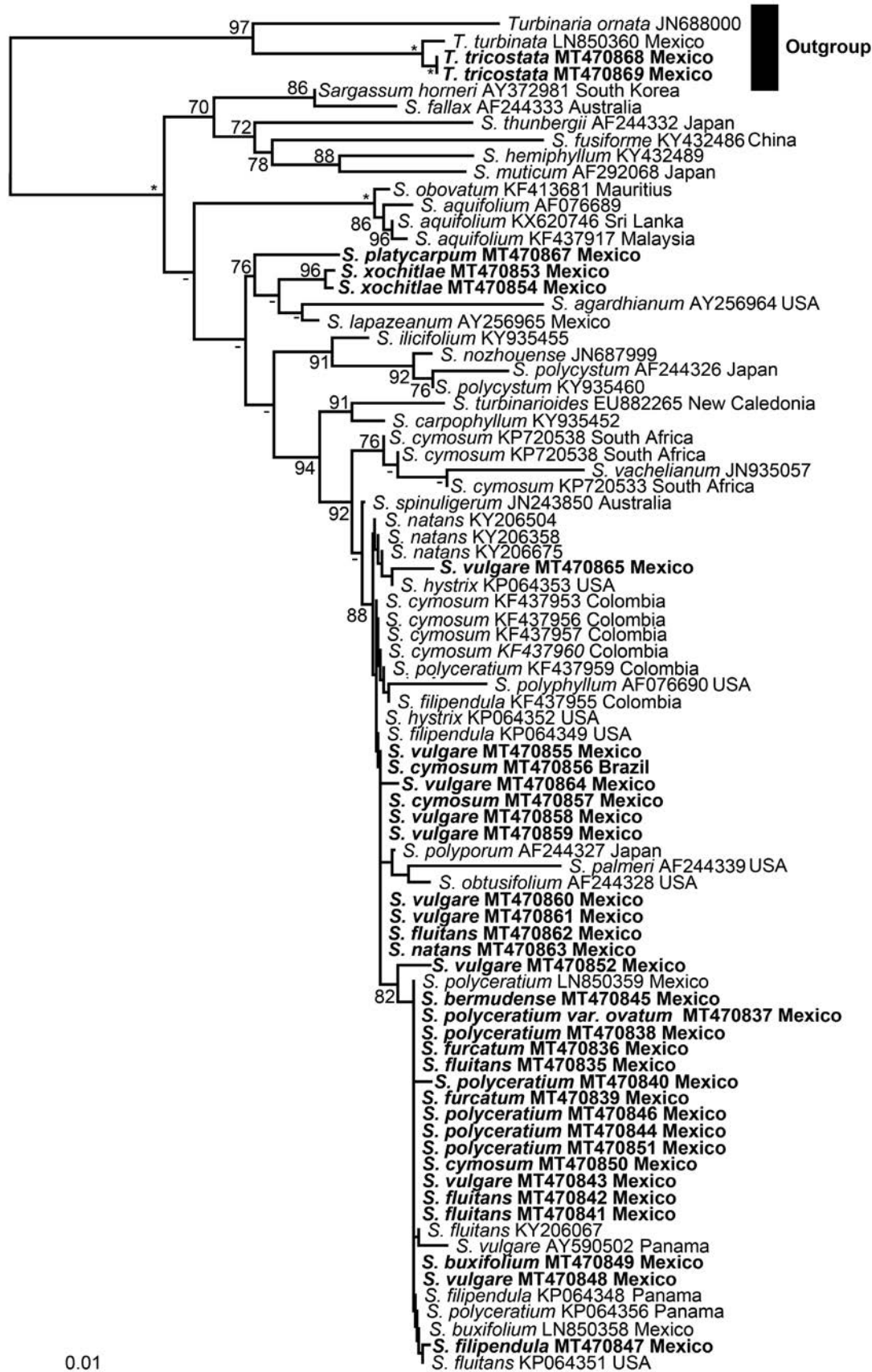


FIGURE S2. Neighbor-joining (NJ) tree based on *rbcL_S* sequences data. Bootstrap values on branches, asterisks indicate full support (100) and hyphens indicate values below 70. Sequences generated in this study are in boldface. Species names are followed by GenBank accession numbers and, when available, the country where it was collected.

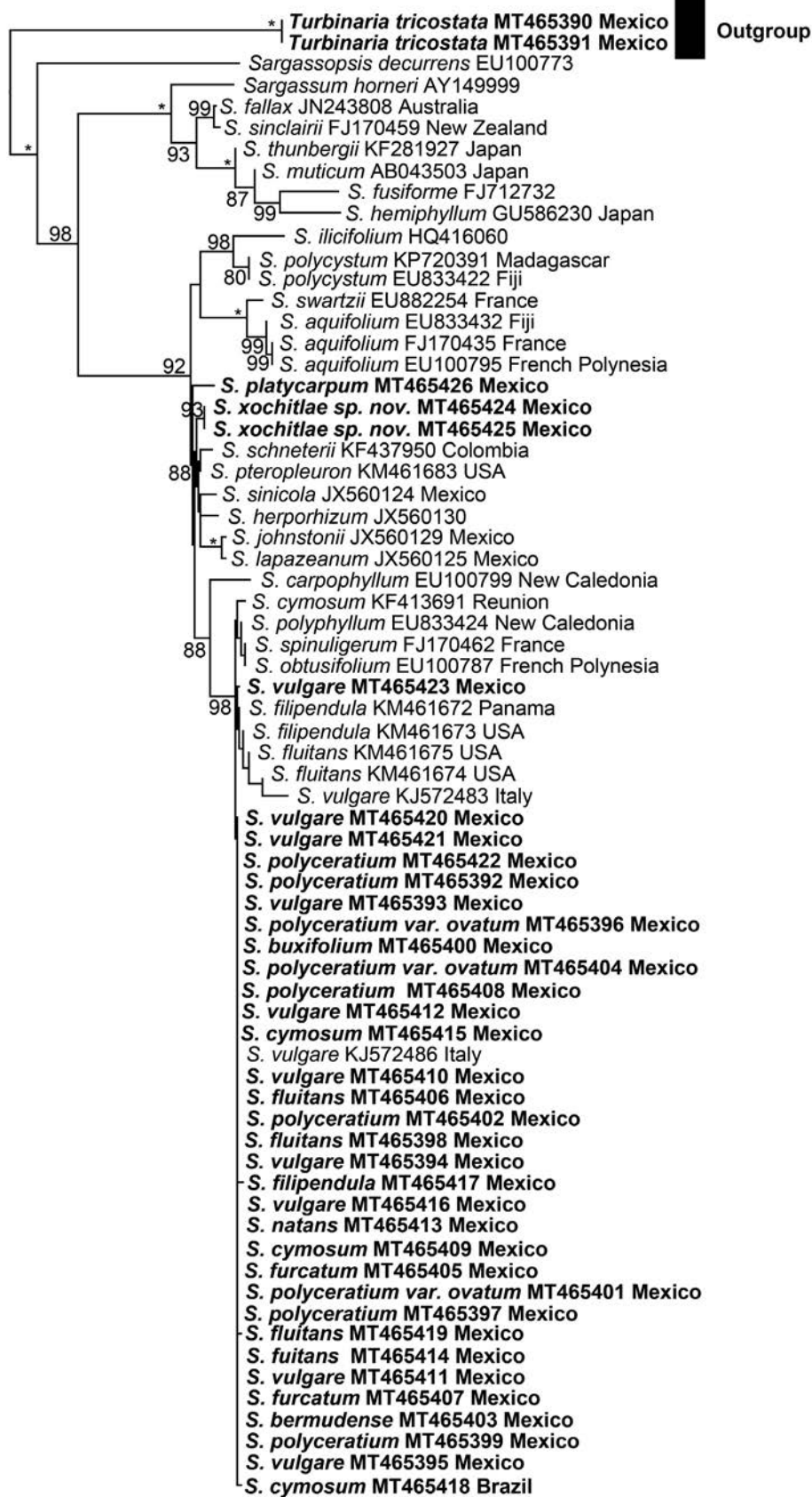


FIGURE S3. Neighbor-joining (NJ) tree based on ITS-2 sequences data. Bootstrap values on branches, asterisks indicate full support (100) and hyphens indicate values below 70. Sequences generated in this study are in boldface. Species names are followed by GenBank accession numbers and, when available, the country where it was collected.

TABLE S1. Specimens of *Sargassum* and *Turbinaria* collected for this study, with locality, GPS coordinates, voucher number (UAMIZ), and GenBank accession numbers of the sequences generated. All samples, except one (indicated below) were collected on the Atlantic Mexican coast.

Taxa name	Locality. Date, collector	GPS coordinates	Voucher number UAMIZ	GeneBank accession numbers		
				COI-5P	<i>rbcL_S</i>	ITS2
<i>S. bermudense</i>	Playa Azul, Cozumel, Quintana Roo. July 2011, D. González Nieto & L.E. Mateo-Cid	20.54535 N 86.9325 W	1073	MT470877	-	MT465403
<i>S. bermudense</i>	Chen Río, Cozumel, Quintana Roo. May 2013, D. González Nieto & L.E. Mateo-Cid	20.34136 LN 86.90600 LW	1064	MT470892	MT470845	MT465393
<i>S. buxifolium</i>	Punta Morena, Cozumel, Quintana Roo. May 2013, D. González Nieto & L.E. Mateo-Cid	20.40861 N 86.85333 W	1051	MT470891	MT470849	MT465400
<i>S. cymosum</i>	Playa Los Muñecos, Veracruz. Sep. 2011, D. González Nieto	19.74380 N 96.40687 W	1036	MT470899	MT470857	MT465415
<i>S. cymosum</i>	Playa Caracol, Quintana Roo. March 2012, D. González Nieto & A. Senties	20.28472 N 86.96751 W	1054	MT470894	MT470850	MT465409
<i>S. cymosum</i>	Praia Domingas Dias, Ubatuba, SP, Brazil. June 2014, A. Senties	23.49658 S 45.14329 E	2512	MT470906	MT470856	MT465418
<i>S. flipendula</i>	Punta Morena, Cozumel, Quintana Roo. May 2013, D. González Nieto & L.E. Mateo-Cid	20.40861 N 86.85333 W	1048	-	MT470866	
<i>S. fluitans</i>	Playa Hermosa, Veracruz. Sep. 2011, D. González Nieto	18.66447 N 95.13006 W	1033	MT470904	MT470862	MT465419
<i>S. fluitans</i>	Playa Los Muñecos, Veracruz. Sep. 2011, D. González Nieto	19.74380 N 96.40687 W	1038	MT470885	MT470841	MT465414
<i>S. fluitans</i>	Tulum, Quintana Roo. March 2012, D. González Nieto & A. Senties	20.16273 N 87.45231 W	1053	MT470887	MT470835	MT465406
<i>S. fluitans</i>	Punta Chiquero, Cozumel, Quintana Roo. May 2013, D. González Nieto & L.E. Mateo-Cid	20.50788 N 86.96038 W	1052	MT470880	MT470842	MT465398
<i>S. furcatum</i>	Playa Garrafón, Isla Mujeres, Quintana Roo. March 2012, D. González Nieto & A. Senties	21.20985 N 86.72255 W	1061	MT470888	MT470839	MT465405
<i>S. furcatum</i>	Punta Brava, Quintana Roo. March 2012, D. González Nieto & A. Senties	20.44714 N 87.27863 W	1060	MT470884	MT470836	MT465407
<i>S. natans</i>	Playa Hermosa, Veracruz. Sep. 2011, D. González Nieto	18.66447 N 95.13006 W	1034	MT470905	MT470863	MT465413
<i>S. platycarpum</i>	Punta Chiquero, Cozumel, Quintana Roo. May 2013, D. González Nieto & L.E. Mateo-Cid	20.50788 N 86.96038 W	1050	MT470875	MT470867	MT465426

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TABLE S1 (Continued)

Taxa name	Locality. Date, collector	GPS coordinates	Voucher number UAMIZ	GeneBank accession numbers		
				COI-5P	<i>rbcL_S</i>	ITS2
<i>S. polyceratium</i>	Playa Caracol, Quintana Roo. March 2012, D. González Nieto & A. Senties	20.28472 N 86.96751 W	1065	MT470886	-	MT465408
<i>S. polyceratium</i>	Chen Río, Cozumel, Quintana Roo. May 2013, D. González Nieto & L.E. Mateo-Cid	20.34136 LN 86.90600 LW	1068	MT470881	MT470846	MT465392
<i>S. polyceratium</i>	Recorrido del hotel Cozumel al muelle, Cozumel, Quintana Roo. July 2011, D. González Nieto & L.E. Mateo-Cid	20.48783 N 86.96806 W	1069	MT470903	MT470851	MT465397
<i>S. polyceratium</i>	Chankanab, Cozumel Quintana Roo. July 2011, D. González Nieto & L.E. Mateo-Cid	20.43784 LN 86.99889 LW	1045	MT470879	MT470838	MT465399
<i>S. polyceratium</i>	Playa Azul, Cozumel, Quintana Roo. July 2011, D. González Nieto & L.E. Mateo-Cid	20.54535 N 86.9325 W	1075	MT470878	MT470840	MT465402
<i>S. polyceratium</i> var. <i>ovatum</i>	Playa Garrafón, Isla Mujeres, Quintana Roo. March 2012, D. González Nieto & A. Senties	21.20985 N 86.72255 W	1046	MT470883	MT470837	MT465404
<i>S. polyceratium</i> var. <i>ovatum</i>	Punta Morena, Cozumel, Quintana Roo. May 2013, D. González Nieto & L.E. Mateo-Cid	20.40861 N 86.85333 W	1047	MT470895	-	MT465401
<i>S. polyceratium</i> var. <i>ovatum</i>	Punta Chiquero, Cozumel, Quintana Roo. May 2013, D. González Nieto & L.E. Mateo-Cid	20.50788 N 86.96038 W	1059	MT470889	-	MT465396
<i>S. ramifolium</i>	Punta Morena, Cozumel, Quintana Roo. May 2013, D. González Nieto & L.E. Mateo-Cid	20.40861 N 86.85333 W	1062	MT470876	-	MT465394
<i>S. vulgare</i>	Playa Hermosa, Veracruz. Sep. 2011, D. González Nieto	18.66447 N 95.13006 W	1030	MT470907	MT470865	MT465411
<i>S. vulgare</i>	Playa El Morro (La Mancha), Veracruz. Sep. 2011, D. González Nieto	19.60047 N 96.37217 W	1026	MT470897	MT470859	MT465421
<i>S. vulgare</i>	Playa Escondida, Veracruz. Sep. 2011, D. González Nieto	18.59302 N 95.05012 W	1031	MT470900	MT470855	MT465423
<i>S. vulgare</i>	Punta Delgada, Veracruz. Sep. 2011, D. González Nieto	19.85894 N 96.45902 W	1039	MT470901	MT470861	MT465416
<i>S. vulgare</i>	Villa Rica, Veracruz. Sep. 2011, D. González Nieto	19.67583 N 96.39222 W	1028	MT470896	MT470858	MT465412
<i>S. vulgare</i>	Villa Rica, Veracruz. Sep. 2011, D. González Nieto	19.67583 N 96.39222 W	1029	MT470898	MT470864	MT465410

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TABLE S1 (Continued)

Taxa name	Locality. Date, collector	GPS coordinates	Voucher number UAMIZ	GeneBank accession numbers		
				COI-5P	<i>rbcL_S</i>	ITS2
<i>S. vulgare</i>	Playa Caracol, Quintana Roo. March 2012, D. González Nieto & A. Senties	20.28472 N 86.96751 W	1057	MT470893	MT470843	
<i>S. vulgare</i>	Punta Chiquero, Cozumel, Quintana Roo. May 2013, D. González Nieto & L.E. Mateo-Cid	20.50788 N 86.96038 W	1072	MT470890	MT470848	MT465395
<i>S. xochitlae</i>	Lado Oriente de Isla Mujeres, Quintana Roo. March 2012, D. González Nieto	21.1316 N 86.4320 W	1056	MT470873	MT470853	MT465424
<i>S. xochitlae</i>	Lado Oriente de Isla Mujeres, Quintana Roo. March 2012, D. González Nieto	21.2250 N 86.7033 W	1055	MT470874	MT470854	MT465425
<i>T. tricostrata</i>	Akumal, Quintana Roo. July 2011, D. González Nieto & L.E. Mateo- Cid	20.39667 N 87.31417 W	2514	MT470871	-	-
<i>T. tricostrata</i>	Punta Morena, Cozumel, Quintana Roo. July 2011, D. González Nieto & L.E. Mateo-Cid	20.50788 N 86.96038 W	1078	MT470872	MT470868	-
<i>T. tricostrata</i>	Punta Morena, Cozumel, Quintana Roo. July 2011, D. González Nieto & L.E. Mateo-Cid	20.40861 N 86.85333 W	2513	MT470870	MT470869	-

TABLE S2. Data of the molecular sequences of *Sargassum* and *Turbinaria* obtained from GenBank used in this study.

Taxa	Collection data (country: site; collector; collection date)	COI-5P	<i>rbcL_S</i>	ITS-2
<i>S. agardhianum</i>	USA: Catalina; S.Murry.	-	AY256964	-
<i>S. aquifolium</i>	<i>rbcL_S</i> : -. ITS-2: Fiji.	-	AF076689	EU833432
<i>S. aquifolium</i>	<i>rbcL_S</i> : Sri Lanka: Hikkaduwa, Chaaya Tranz. ITS-2: France: New Caledonia.	-	KX620746	FJ170435
<i>S. aquifolium</i>	<i>rbcL_S</i> : Malaysia. ITS-2: French Polynesia.	-	KF437917	EU100795
<i>S. buxifolium</i>	Mexico: Veracruz, Muñecos; A.Ávila	-	LN850358	-
<i>S. carpophyllum</i>	COI-5P: New Caledonia. ITS-2: New Caledonia.	HQ416036	KY935452	EU100799
<i>S. cymosum</i>	<i>rbcL_S</i> : South Africa. ITS-2: Reunion.	-	KP720538	KF413691
<i>S. cymosum</i>	South Africa	-	KP720533	-
<i>S. cymosum</i>	Colombia: Magdalena, Granate (TNNP); O. Camacho; Apr 2009.	-	KF437953	-
<i>S. cymosum</i>	Colombia: Magdalena, Concha (TNNP); O.Camacho; Apr 2009.	-	KF437956	-
<i>S. cymosum</i>	Colombia: Magdalena, Concha (TNNP); O.Camacho; Apr 2009.	-	KF437957	-
<i>S. cymosum</i>	Colombia: Magdalena, Neguanje (TNNP); O.Camacho; May 2009.	-	KF437960	-
<i>S. fallax</i>	<i>rbcL_S</i> : Australia. ITS-2: Australia; R.R.M.Dixon and K.R.Dixon; 15-Nov-2009.	-	AF244333	JN243808
<i>S. filipendula</i>	<i>rbcL_S</i> : Colombia. ITS-2: Panama: Flat Rock Beach, Bocas del Toro; S.Fredericq; Aug-2010.	-	KF437955	KM461672
<i>S. filipendula</i>	<i>rbcL_S</i> : USA: Masonboro, North Carolina; O.Camacho; May-2011. ITS-2: USA: Masonboro, North Carolina; O.Camacho; May-2011.	-	KP064349	KM461673
<i>S. filipendula</i>	Panama: Flat Rock Beach, Bocas del Toro; S.Fredericq; Aug-2010.	-	KP064348	-
<i>S. fluitans</i>	COI-5P: 26-May-2012. <i>RbcL_S</i> : 26-May-2012. ITS-2: USA: Fort Fisher, North Carolina; O.Camacho; Aug-2011.	KY084910	KY206067	KM461674
<i>S. fluitans</i>	<i>rbcL_S</i> : USA: Holly Beach, Gulf of Mexico; O.Camacho; Jun-2014. ITS-2: USA: Holly Beach, Gulf of Mexico; O.Camacho; Jun-2014.	-	KP064351	KM461675
<i>S. fusiforme</i>	COI-5P: China: Haiyang Island, Liaoning; J.D.Liu. <i>RbcL_S</i> : China.	JX999787	KY432486	FJ712732
<i>S. hemiphylum</i>	ITS-2: Japan.	JF718398	KY432489	GU586230
<i>S. herporhizum</i>	Mexico: Gulf of California?	-	-	JX560130
<i>S. horneri</i>	<i>rbcL_S</i> : South Korea: Seosang, Namhaedo.	-	AY372981	AY149999
<i>S. horridum</i>	Mexico: Gulf of California?	JX560110	-	-
<i>S. hystrix</i>	USA: Geyer Bank, Gulf of Mexico; E.Hickerson; Sep-2011.	-	KP064353	-
<i>S. hystrix</i>	USA: Geyer Bank, Gulf of Mexico; O.Camacho; Sep-2011.	-	KP064352	-
<i>S. ilicifolium</i>	COI-5P: New Caledonia. ITS-2: Tanzania.	HQ416042	KY935455	HQ416060
<i>S. johnstonii</i>	<i>rbcL_S</i> and ITS-2: Mexico: Gulf of California?	JX560103	-	JX560129
<i>S. lapazeanum</i>	COI-5P: Mexico: Gulf of California?. <i>RbcL_S</i> : Mexico: Baja California; R. Scrosati. ITS-2: Mexico: Gulf of California?.	JX560104	AY256965	JX560125
<i>S. muticum</i>	<i>rbcL_S</i> : Japan: Miyagi, Nagatsu-ura. ITS-2: Japan: Miyagi, Nagatsu-ura.	EU681423	AF292068	AB043503
<i>S. natans</i>	T15:00Z; 04-Jan-2016.	-	KY206504	-
<i>S. natans</i>	T13:23Z; 03-Dec-2014.	-	KY206358	-
<i>S. natans</i>	T15:00Z; 27-May-2012.	-	KY206675	-
<i>S. nozhouense</i>	-	-	JN687999	-

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TABLE S2 (Continued)

Taxa	Collection data (country: site; collector; collection date)	COI-5P	<i>rbcL_S</i>	ITS-2
<i>S. obovatum</i>	Mauritius.	-	KF413681	-
<i>S. obtusifolium</i>	<i>rbcL_S</i> : USA: Kahala Beach Park Oahu; N. Phillips. ITS-2: French Polynesia.	-	AF244328	EU100787
<i>S. palmeri</i>	USA: San Clemente Island; S. Murray.	-	AF244339	-
<i>S. palmeri</i>	Colombia.	-	KF437959	-
<i>S. polyceratium</i>	Mexico: Quintana Roo, Playa Maya; A.Ávila	-	LN850359	-
<i>S. polyceratium</i>	Panama: East Rio Ca-averal, Bocas del Toro; O.Camacho; Aug-2010.	-	KP064356	-
<i>S. polycystum</i>	<i>rbcL_S</i> : Japan. ITS-2: Madagascar.	KJ872544	AF244326	KP720391
<i>S. polycystum</i>	ITS-2: Fiji.	-	KY935460	EU833422
<i>S. polyphyllum</i>	<i>rbcL_S</i> : USA: Kahala Beach Park, Oahu; N. Phillips. ITS-2: New Caledonia.	-	AF076690	EU833424
<i>S. polyporum</i>	Japan.	-	AF244327	-
<i>S. pteropleuron</i>	USA: Newfound Harbor Key, Florida; O.Camacho; Apr-2014.	-	-	KM461683
<i>S. schneiderii</i>	Colombia, Magdalena, Neguanje (TNNP); O. Camacho; Nov 2009.	-	-	KF437950
<i>S. sinclairii</i>	New Zealand: North Island.	-	-	FJ170459
<i>S. sinicola</i>	COI-5P and ITS-2: Mexico: Gulf of California?	JX560112	-	JX560124
<i>S. spinuligerum</i>	COI-5P: New Caledonia. <i>RbcL_S</i> : Australia; R.R.M.Dixon; 19-May-2009. ITS-2: France: New Caledonia.	HQ416051	JN243850	FJ170462
<i>S. swartzii</i>	France: New Caledonia.	-	-	EU882254
<i>S. thunbergii</i>	<i>rbcL_S</i> : Japan: Shirahama; T. Ajisaka. ITS-2: Japan; F.Mineur.	JF718399	AF244332	KF281927
<i>S. turbinarioides</i>	New Caledonia: Ile des Pins.	-	EU882265	-
<i>S. vachelianum</i>	-	-	JN935057	-
<i>S. vulgare</i>	<i>rbcL_S</i> : Panama: San Blas Archipelago; B.Wysor. ITS-2: Italy; 2012.	-	AY590502	KJ572483
<i>S. vulgare</i>	Italy; 2012.	-	-	KJ572486
<i>T. ornata</i>	-	-	JN688000	-
<i>T. turbinata</i>	Mexico: Quintana Roo, Playa Caletita; A.Ávila	-	LN850360	-