

Article



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Sporolithon sinusmexicanum sp. nov. (Sporolithales, Rhodophyta): a new rhodolith-forming species from deepwater rhodolith beds in the Gulf of Mexico

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Abstract

Sporolithon is a non-geniculate, marine taxon that occurs in tropical to subtropical and warm temperate habitats worldwide. On the basis of specimens collected from offshore Louisiana and Florida in the Gulf of Mexico, Sporolithon sinusmexicanum sp. nov. is described. rbcL and psbA sequence analyses showed that S. sinusmexicanum is closely related to Sporolithon yoneshigueae (type locality: Bahia, Brazil). These two species can be distinguished by the number of rosette cells (10–12 in S. sinusmexicanum vs. 19–24 in S. yoneshigueae) and by the habit of the tetrasporangial structures after spore release (sloughed off in S. sinusmexicanum vs. overgrown and buried in Sporolithon yoneshigueae).

Keywords: biodiversity, coralline algae, Hapalidiales, marine algae, molecular phylogenetics, new species, seaweeds

Abbreviations: GB = GenBank; GMx = Gulf of Mexico; NWGMx = northwestern Gulf of Mexico; SEM = Scanning Electron Microscope; SEGMx = southeastern Gulf of Mexico; WTA = Western Tropical Atlantic

Introduction

Recent studies have revealed a great diversity of non-geniculate coralline red algae (Corallinales, Hapalidiales, and Sporolithales) from deepwater rhodolith beds at 42–80 m depth in the northwestern Gulf of Mexico (NWGMx) and from the surrounding offshore areas of the Gulf of Mexico (GMx) (Richards *et al.* 2014, Krayesky-Self *et al.* 2016, Richards *et al.* 2016, Richards *et al.* 2017). These studies stemmed largely from specimens collected during seven biodiversity assessment expeditions following the 2010 Deepwater Horizon oil spill (Fredericq *et al.* 2014, Felder *et al.* 2014).

Sporolithon Heydrich is among the incompletely described taxa of coralline algae from the Gulf of Mexico. Minnery (1990) reported one species of *Sporolithon* (as *Archaeolithothamnium* Rothpletz ex Foslie) from offshore Texas in the Flower Garden Banks National Marine Sanctuary at depths ranging from 21–90 m, but no specific epithet was applied. Other previous accounts of coralline algal diversity from the Gulf of Mexico did not report *Sporolithon* (Dawes & Van Breedveld 1969, Dawes 1974, Fredericq *et al.* 2009).

Richards *et al.* (2017) showed that a species of *Sporolithon* from the NWGMx and the SEGMx has unique DNA sequences that are distinct from *Sporolithon episporum* (M.Howe) E.Y.Dawson and *Sporolithon dimotum* (Foslie & M.Howe) Yamaguishi-Tomita ex M.J.Wynne, species with type localities in the Caribbean, and *Sporolithon yoneshigueae* Bahia, Amado-Filho, Maneveldt & W.H.Adey and *Sporolithon tenue* Bahia, Amado-Filho, Maneveldt & W.H.Adey, species with type localities in Brazil. Herein, we investigate the morpho-anatomy of this new *Sporolithon* species using SEM and provide a taxonomic account and description of this new species. Phylogenetic analyses were conducted to compare the plastid *psbA* (photosystem II reaction center protein D1 gene) and *rbcL* (gene which encodes the large subunit of the enzyme ribulose-1, 5-bisphosphate carboxylase/oxygenase [RuBisCO]) sequences of the new species to *Sporolithon indopacificum* Maneveldt & P.W.Gabrielson, recently described by Maneveldt *et al.* (2017).

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Material and methods

Subtidal specimens were collected from offshore Louisiana and Florida in the Gulf of Mexico as described in Richards *et al.* (2014, 2016). DNA extraction, amplification, and sequencing methods followed the protocols of the above-mentioned studies.

Phylogenetic analysis. Alignment and ML analyses of plastid rbcL and psbA were performed according to the protocol of Richards et al. (2017). The rbcL and psbA alignments were 1387 bp and 863 bp in length, respectively. The rbcL alignment included 21 Sporolithales sequences in the ingroup; the psbA alignment included 42 Sporolithales sequences (see Table S1 for complete list of sequences analyzed). Preliminary BLAST searches of an rbcL sequence (KP142787) of Sporolithon cf. ptychoides from Brazil indicated it is a chimeric sequence of two taxa; the 5' end of the sequence was related to members of the Peyssonneliales whereas the 3' end was related to members of the Sporolithales. Therefore, the 5' end of sequence KP142787 was cropped and only the 3' end of the sequence (691 bp) was used in these analyses.

SEM and light microscopy. Sample preparation and SEM were conducted according to the protocol of Richards *et al.* (2016). Light microscopy of tetrasporangial sori was performed with a Zeiss Stemi 2000-C dissecting microscope and images were captured with a Cannon Rebel Eos T2-I. Cell dimensions were measured from SEM micrographs following the protocols of Irvine and Chamberlain (1994) and Adey *et al.* (2005). Terminology follows Woelkerling (1988) and Adey *et al.* (2015).

Results

Phylogenetic analysis. Results of the rbcL (Fig. 1) and psbA (Fig. 2) analyses showed the new species is sister to S. yoneshigueae. The new species did not form a clade with the other species that have type localities in the Caribbean, S. episporum and S. dimotum, and did not form a clade with the recently described S. indopacificum from the western Indian and Pacific Oceans. Based on the diagnostic DNA sequences of the holotype and additional specimen examined and the morpho-anatomical results presented in this study, we herein describe a new species of Sporolithon from the Gulf of Mexico.

Sporolithon sinusmexicanum J.Richards & Fredericq sp. nov. (Figs. 3–23)

Holotype: LAF 6956A (Figs. 3–17), Sackett Bank, NWGMx (28° 38.0' N; 89° 33.028' W), 65–68 m deep, *leg.* J. L. Richards & S. Fredericq, 7.ix.2014.

Additional material examined: LAF 6970B (Figs. 18–23), Dry Tortugas Vicinity, SEGMx (24° 31.494'N; 83° 19.793'W), 69 m deep, *leg.* J. L. Richards & S. Fredericq, 10.ix.2014 (collected from site in field), 15.xii.2014 (collected from microcosm).

Etymology: The specific epithet refers to the Gulf of Mexico, the locality of the holotype and Florida specimen.

Description

DNA sequences: DNA sequences from the holotype: rbcL (GB accession = KY994126), psbA (GB accession = MF034549), LSU (GB accession = KY980437), and UPA (GB accession = KY980429); and from the Florida specimen: LSU (GB accession = KY980438), rbcL (GB accession = KY994127), and psbA (GB accession = MF034550).

Morphology and Habit: Thallus non-geniculate, forming biogenic rhodoliths that are smooth to warty (Fig. 3) or with numerous protuberances (Fig. 18). Found growing in benthic rhodolith beds at a depth of 65–69 m.

Vegetative Anatomy: It was not determined with certainty if thallus construction is dimerous or monomerous, though some areas of the thallus appeared putatively monomerous. New vegetative layers (Fig. 4) formed by a secondary hypothallium with one to two layers of basal filaments (Fig. 5). Hypothallial cells rectangular in shape, $11.5-20~\mu m \log x 3-7 \mu m$ wide. Perithallium with abundant cell fusions (Figs. 6, 20); secondary pit connections not observed. Perithallial cells $6.6-19~\mu m \log x 7-10.6~\mu m$ wide. Meristematic cells $4.2-12~\mu m \log x 7.8-15.3~\mu m$ wide (Figs. 7, 8, 20, 21). Epithallium (Figs. 7, 8, 20, 21) a single layer of armored epithallial cells that are $2-3~\mu m \log x 4-7.8~\mu m$ wide, with thick, heavily calcified cell walls and a trapezoidal-shaped lumen.

Reproduction: Tetrasporangial sori are sloughed off after spore release. Sori were observed in the process of sloughing off the surface of the rhodoliths (Figs. 9–13, 22). Pores with rosette cells that remained intact (n=5) showed 10–12 rosette cells surrounding each tetrasporangial compartment pore (Fig. 14). Sections showed tetrasporangial compartments at the protuberance tip (Figs. 15–17) and no buried tetrasporangial structures embedded in the perithallium (Figs. 15, 19). Intact tetrasporangial compartment measured 83 μm long x 59 μm wide (n=1), subtended by a stalk cell 14 μm long x 28 μm wide (n=1) (Fig. 16). Tetrasporangial compartments surrounded by paraphyses with non-elongated cells at the base of tetrasporangial compartments (Figs. 16, 17). Female and male structures were not observed.

Distribution: Sackett Bank, NWGMx, and the vicinity of the Dry Tortugas, SEGMx.

Comments: Unidentified spherical inclusions were observed in the perithallium of specimen LAF 6970B, which may be unidentified life history stages of microalgal organisms (Fig. 23), as reported in Krayesky-Self *et al.* (2017).

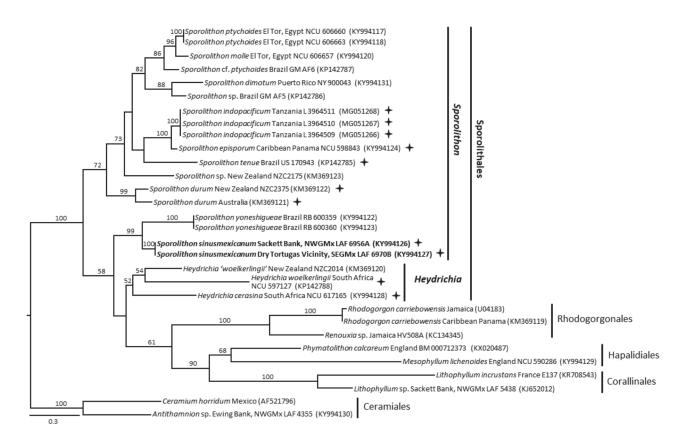


FIGURE 1. Phylogeny based on ML analyses of *rbc*L sequences. Branch numbers indicate bootstrap values out of 1,000 replicates. *Sporolithon sinusmexicanum* sp. nov. shown in boldface. Stars indicate species that slough off their tetrasporangial sori after spore release.

Discussion

Sporolithon sinusmexicanum did not form a clade with the other Sporolithon species that have type localities in the Caribbean, namely S. episporum and S. dimotum (Figs. 1, 2). The results of the rbcL and psbA analyses in this study (Figs. 1, 2), as well as the analyses of concatenated plastid rbcL and psbA, mitochondrial COI (cytochrome oxidase subunit I gene), and a portion of LSU (nuclear-encoded 28S rDNA gene) sequences performed in Richards et al. (2017), showed that S. sinusmexicanum is closely related to S. yoneshigueae (type locality: Bahia, Brazil). Sequence divergence values indicate S. yoneshigueae and S. sinusmexicanum are different species. For example, the rbcL and psbA divergence values between these two taxa are 9.75 and 6.8%, respectively (Richards et al. 2017), which is greater than the rbcL and psbA divergence values between Sporolithon ptychoides and Sporolithon molle (6.5% and 3.5%, respectively), and between S. episporum and S. indopacificum (2.7% and 3.1%, respectively) (Maneveldt et al. 2017).

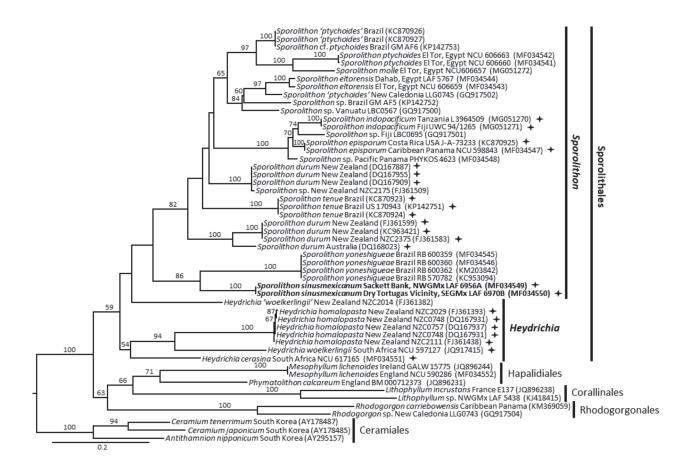


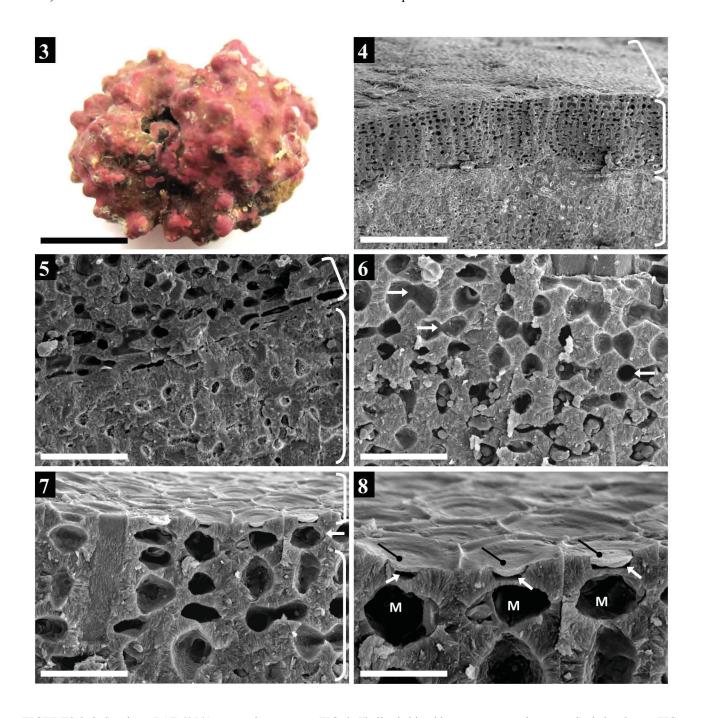
FIGURE 2. Phylogeny based on ML analyses of *psb*A sequences. Branch numbers indicate bootstrap values out of 1,000 replicates. *Sporolithon sinusmexicanum* sp. nov. shown in boldface. Stars indicate species that slough off their tetrasporangial sori after spore release.

The morpho-anatomical results presented in this study show *S. yoneshigueae* and *S. sinusmexicanum* are distinct from each other. *S. yoneshigueae* possesses tetrasporangial sori that are overgrown and buried after spore release (Bahia *et al.* 2015), whereas the tetrasporangial sori of *S. sinusmexicanum* are sloughed off after spore release. These taxa also differ in the number of rosette cells surrounding each tetrasporangial compartment pore (19–24 in *S. yoneshigueae* vs. 10–12 in *S. sinusmexicanum*). *S. sinusmexicanum* also differs from *S. episporum* and *S. indopacificum* with respect to connections between adjacent filaments. *S. sinusmexicanum* possesses abundant cell fusions and no secondary pit connections, whereas *S. episporum* has both cell fusions and secondary pit connections and *S. indopacificum* has primarily secondary pit connections with cell fusions rarely observed (table 2 in Maneveldt *et al.* [2017]).

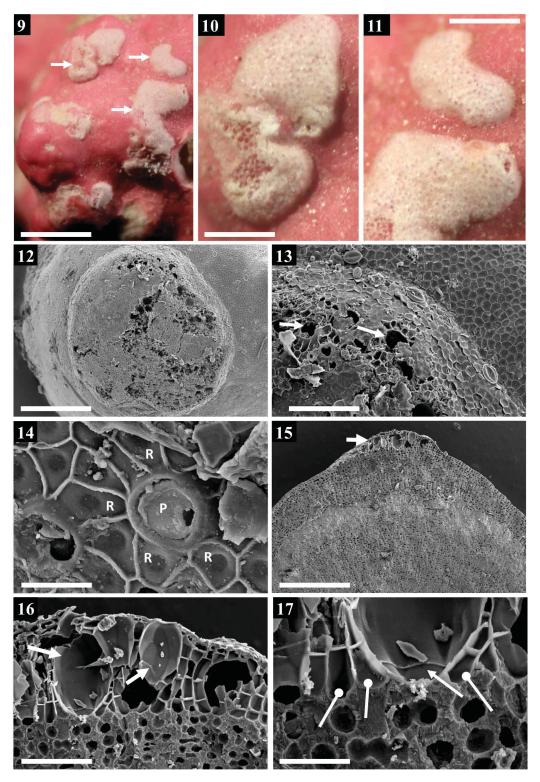
Considering sequence divergence values indicate the clade comprised of *S. sinusmexicanum* and *S. yoneshigueae* is as divergent from *Heydrichia* as it is from other species of *Sporolithon* (Richards *et al.* 2017), *S. sinusmexicanum* and *S. yoneshigueae* may represent a new genus separate from *Sporolithon* and *Heydrichia*. However, no characters were observed in *S. sinusmexicanum* that distinguish this taxon at the generic level from other members of *Sporolithon*. Characters considered diagnostic for species of *Heydrichia*, such as the presence of an involucre surrounding the tetrasporangia, multiple stalk cells, or multiple tetrasporangia within a single complex (Townsend *et al.* 1994, Maneveldt and van der Merwe 2012), were not observed in *S. sinusmexicanum*.

Sporolithon sinusmexicanum is morpho-anatomically similar to S. episporum, S. indopacificum and S. tenue, in regard to sharing the character of sloughing off tetrasporangial sori post spore release. However, the phylogenetic analyses of the DNA sequences presented herein (Figs. 1, 2) and in Richards et al. (2017) showed that S. sinusmexicanum is not closely related to any other Sporolithon species that slough off their tetrasporangial sori after spore release, and that this character has evolved independently in several clades. Sporolithon durum (Foslie) Townsend & Woelkerling

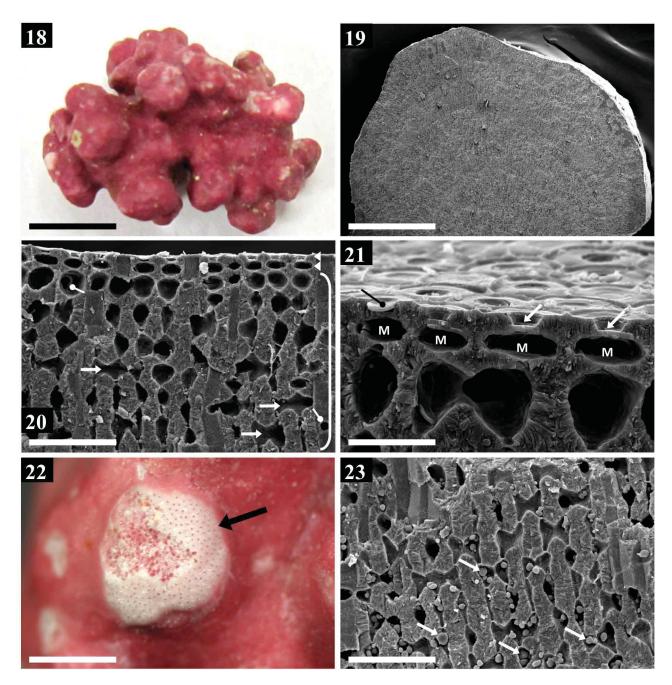
has also been reported to slough off its tetrasporangial sori; however, the lectotype collection of this species includes male gametangial specimens (Townsend *et al.* 1995) and multiple species are passing under this name (Richards *et al.* 2017). Further work needs to be done to confirm if this character is present in the true *S. durum*.



FIGURES 3–8. Specimen LAF 6956A, vegetative anatomy. FIG. 3. Thallus habit with numerous protuberances. Scale bar 9 mm. FIG. 4. Surface view (upper bracket) and vertical fracture showing new vegetative layer (middle bracket) over older layer (lower bracket). Scale bar 176 μm. FIG. 5. Secondary hypothallium (upper bracket) over older vegetative layer (lower bracket). Scale bar 52 μm. FIG. 6. Perithallium showing cell fusions (arrows). Scale bar 34 μm. FIG. 7. Perithallium (lower bracket), epithallium (upper bracket) and intercalary meristem (arrow). Scale bar 24 μm. FIG. 8. Meristematic cells (M) and armored epithallial cells (arrows) with intact cell roofs (circle pointers). Scale bar 11 μm.



FIGURES 9–17. Specimen LAF 6956A, reproductive anatomy. FIG. 9. Dissecting microscope view of tetrasporangial sori (arrows). Scale bar 2 mm. FIGS. 10–11. Magnified views of sori. Scale bars 0.7 mm, 0.8 mm. FIG. 12. Surface view of tetrasporangial sorus undergoing sloughing off. Scale bar 400 μm. FIG. 13. Magnified view of sorus edge showing disintegrating pores (arrows) and rosette cells. Scale bar 110 μm. FIG. 14. Intact tetrasporangial pore (P) and rosette cells (R). Scale bar 24 μm. FIG. 15. Longitudinal section of protuberance showing tetrasporangial compartments at protuberance tip (arrow) and perithallium with no overgrown tetrasporangial compartments. Scale bar 400 μm. FIG. 16. Tetrasporangial compartments (arrows). Scale bar 70 μm. FIG. 17. Tetrasporangial stalk cell (arrow) and paraphyses surrounding tetrasporangial compartments with non-elongated cells at the base of the compartments (circle arrows). Scale bar 28.5 μm.



FIGURES 18–23. Specimen LAF 6970B. FIG. 18. Thallus habit showing numerous protuberances. Scale bar 6 mm. FIG. 19. Longitudinal section through protuberance showing perithallium with no overgrown tetrasporangial compartments. Scale bar 1.05 mm. FIG. 20. Epithallium (upper arrowhead), intercalary meristem (lower arrowhead) and perithallium (right bracket) showing cell fusions in the x-axis (arrows) and z-axis (circle pointers). Scale bar 4 μm. FIG. 21. Armored epithallial cells (arrows), one showing intact cell roof (circle pointer), and meristematic cells (M). Scale bar 14 μm. FIG. 22. Dissecting microscope view of thallus surface showing tetrasporangial sorus (arrow). Scale bar 1 mm. FIG. 23. Perithallium showing unidentified spherical inclusions (arrows), which may be unidentified life history stages of microalgal organisms. Scale bar 43 μm.

It is interesting that some species of *Sporolithon*, e.g. *S. sinusmexicanum*, *S. episporum*, and *S. indopacificum*, have the capacity to slough off their external cell layers (epithallial and upper perithallial layers) and tetrasporangial compartments, while this phenomenon has not been reported in other species of the genus (table 2 in Maneveldt *et al.* [2017]). Wegeberg and Pueschel (2002) documented that in other coralline algae, such as *Lithothamnion* Heydrich and *Phymatolithon* Foslie in the Hapalidiales, the sloughing off of epithallial cell layers can become replenished with a unique type of intercalary meristem. Since Krayesky-Self *et al.* (2017) documented with SEM, TEM and fluorescence microscopy previously unrecognized benthic life history stages of bloom-forming microalgae such as dinoflagellates (i.e. *Prorocentrum lima* (Ehrenberg) F.Stein) and haptophytes (i.e. *Ochrosphaera verrucosa* Schussnig) residing

endolithically inside calcium carbonate-lined cell lumina of biogenic *Lithothamnion* rhodoliths, it is possible that the microalgal life history stages became passively surrounded by new coralline surface cell layer growth. Life history stages of a wide microbiotal diversity of photosynthetic eukaryotic algae including numerous red, green, and brown algae, ochrophytes and haptophytes inside *Lithothamnion* rhodoliths were also recovered from endolithic DNAs using plastid *tuf*A (elongation EF-*Tu*) (Sauvage *et al.* 2016) and 16S V4 rRNA (Sauvage *et al.* pers. comm.) metabarcoding (environmental amplicon sequencing). We speculate that *S. sinusmexicanum*, a species that sloughs off surface layers, could potentially harbor endolithic populations that are not permanent rhodolith residents but are instead transient life history stages (potentially resting stages). These stages may then form blooms once released in the water column from the rhodolith's interior following abrasion or sloughing off of its surface cell layers.

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TABLE S1. List of GenBank numbers for taxa included in phylogenetic analyses. N.A. = data not available. *sequence not analyzed in present study.

Taxa	Id. No.	Locality	Reference	GenBank Accession No.	
				psbA	rbcL
Antithamnion nipponicum	A28	South Korea	Yang & Boo 2004	AY295157	*AY295174
Antithamnion sp.	LAF 4355	Ewing Bank, NWGMx	Richards et al. 2017	-	KY994130
Ceramium horridum	N.A.	San Juan De La Costa, Baja California Sur, Mexico	Cho et al. 2008	-	AF521796
Ceramium japonicum	C135	South Korea	Seo et al. 2003	AY178485	-
Ceramium tenerrimum	C242	South Korea	Seo et al. 2003	AY178487	-
Heydrichia cerasina	NCU 617165	Western Cape Province, South Africa	Richards et al. 2017	MF034551	KY994128
Heydrichia homalopasta	NZC2015	New Zealand	Nelson et al. 2015	FJ361383	-
Heydrichia homalopasta	NZC2029	New Zealand	Farr et al. 2009	FJ361393	-
Heydrichia homalopasta	NZC2111	New Zealand	Farr et al. 2009	FJ361438	-
Heydrichia homalopasta	NZC0748	New Zealand	Broom et al. 2008	DQ167931	-
Heydrichia homalopasta	NZC0757	New Zealand	Unpublished	DQ167937	
Heydrichia woelkerlingii	NCU 597127	South Africa	Mateo-Cid et al. 2014, Adey et al. 2015	JQ917415	KP142788
Heydrichia woelkerlingii	NZC2014	New Zealand	Nelson et al. 2015	FJ361382	KM369120
Lithophyllum incrustans	GALW 15746 (E137)	France	Hernandez-Kantun <i>et al.</i> 2015	JQ896238	KR708543
Lithophyllum sp.	LAF 5438	NWGMx	Richards et al. 2014	KJ418415	KJ652012
Mesophyllum lichenoides	NCU 590286	South Devon, England	Richards et al. 2017	MF034552	KY994129
Mesophyllum lichenoides	LBC0031	France	Bittner et al. 2011	GQ917439	-
Phymatolithon calcareum	BM 000712373	Cornwall, England	Hernández-Kantún et al 2015, Hernandez- Kantun <i>et al</i> . 2016	JQ896231	KX020487
Phymatolithon calcareum	LBC0001	France	Bittner et al. 2011	GQ917436	-
Renouxia sp.	HV508A	Jamaica	West et al. 2016	-	KC134345
Rhodogorgon carriebowensis	WELT TBA	Panama, Caribbean Sea	Nelson et al. 2015	KM369059	KM369119
Rhodogorgon carriebowensis	N.A.	Jamaica	Freshwater et al. 1994	-	U04183
Rhodogorgon sp.	LLG0743	New Caledonia	Bittner et al. 2011	GQ917504	-
Sporolithon cf. ptychoides	GM AF6	Brazil	Adey et al. 2015	KP142753	KP142787
Sporolithon dimotum	NY 900043 (Howe 2667)	Lemon Bay, near Guanica, Puerto Rico	Richards et al. 2017	-	KY994131
Sporolithon durum	NZC2375	New Zealand	Nelson et al. 2015	FJ361583	KM369122
Sporolithon durum	Aus	Australia	Nelson et al. 2015	DQ168023	KM369121
Sporolithon eltorensis	NCU 606659 (LAF 5850)	El Tor, Egypt, Gulf of Suez	Richards et al. 2017	MF034543	-
Sporolithon eltorensis	LAF 5767 (NCU 649164)	Dahab, Egypt, Gulf of Aqaba	Richards et al. 2017	MF034544	-
Sporolithon episporum	NCU 598843 (PHYKOS 5467)	Bocas del Toro, Panama, Caribbean Sea	Richards et al. 2017	MF034547	KY994124
Sporolithon episporum	Steneck USA J-A-73233	Costa Rica	Bahia <i>et al</i> . 2014	KC870925	-
Sporolithon indopacificum	L 3964511	Tanzania	Maneveldt et al. 2017	-	MG051268
Sporolithon indopacificum	UWC 94/1265	Fiji	Maneveldt et al. 2017	MG051271	-

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TABLE S1. (Continued)

Taxa	Id. No.	Locality	Reference	GenBank Accession No.	
				psbA	<i>rbc</i> L
Sporolithon ptychoides	NCU 606663	El Tor, Egypt, Gulf of	Richards et al. 2017	MF034542	KY994118
	(LAF 5846)	Suez			
Sporolithon ptychoides	NCU 606660	El Tor, Egypt, Gulf of	Richards et al. 2017	MF034541	KY994117
	(LAF 5875)	Suez			
Sporolithon 'ptychoides'	US Amado-Filho	Brazil	Bahia et al. 2014	KC870926	-
	Brazil 8				
Sporolithon 'ptychoides'	US Amado-Filho	Brazil	Bahia et al. 2014	KC870927	-
	Brazil 7				
Sporolithon 'ptychoides'	LLG0745	New Caledonia	Bittner et al. 2011	GQ917502	-
Sporolithon sinusmexicanum	LAF 6956A	Sackett Bank,	Richards et al. 2017	MF034549	KY994126
		NWGMx			
Sporolithon sinusmexicanum	LAF 6970B	Vicinity of Dry Tortugas,	Richards et al. 2017	MF034550	KY994127
		SEGMx			
Sporolithon sp.	PHYKOS 4623	Gulf of Chiriquí, near	Richards et al. 2017	MF034548	-
		Mono Feliz, Panama,			
		Pacific Ocean			
Sporolithon sp.	LBC0567	Vanuatu	Bittner et al. 2011	GQ917500	-
Sporolithon sp.	LBC0695	Fiji	Bittner et al. 2011	GQ917501	-
Sporolithon sp. epilithic	NZC2175	New Zealand	Nelson et al. 2015	FJ361509	KM369123
Sporolithon sp.	GM AF5	Brazil	Adey et al. 2015	KP142752	KP142786
Sporolithon tenue	US 170943	Brazil	Adey et al. 2015	KP142751	KP142785
Sporolithon tenue	US Bahia Brazil	Brazil	Bahia et al. 2014	KC870923	-
	12512-4				
Sporolithon tenue	Bahia Brazil 33	Brazil	Bahia et al. 2014	KC870924	-
Sporolithon yoneshigueae	RB 570782	Brazil	Bahia et al. 2015	KC953094	-
Sporolithon yoneshigueae	RB 600359	Brazil	Richards et al. 2017	MF034545	KY994122
Sporolithon yoneshigueae	RB 600360	Brazil	Richards et al. 2017	MF034546	KY994123
Sporolithon yoneshigueae	RB 600362	Brazil	Bahia et al. 2015	KM203842	-