THE CORALLINE GENERA *SPOROLITHON* AND *HEYDRICHA* (SPOROLITHALES, RHODOPHYTA) CLARIFIED BY SEQUENCING TYPE MATERIAL OF THEIR GENERITYPES AND OTHER SPECIES

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Interspecific systematics in the red algal order Sporolithales remains problematic. To re-evaluate its species, DNA analyses were performed on historical type material and recently collected specimens assigned to the two genera *Sporolithon* and *Heydrichia*. Partial *rbcL* sequences from the lectotype specimens of *Sporolithon ptychoides* (the generitype species) and *Sporolithon molle*, both from El Tor, Egypt, are exact matches to field-collected topotype specimens. *Sporolithon crassum* and *Sporolithon erythraeum* also have the same type locality; material of the former appears to no longer exist, and we were unable to PCR amplify DNA from the latter. A new species, *Sporolithon eltorensis*, is described from the same type locality. We have not found any morpho-anatomical characters that distinguish these three species. No sequenced specimens reported as *S. ptychoides* from other parts of the world represent this species, and likely reports of *S. ptychoides* and *S. molle* based on morpho-anatomy are incorrect. DNA sequences from topotype material of *Heydrichia woelkerlingii*, the generitype species, and isotype material of *Heydrichia cerasina* confirm that these are distinct species; the taxon reported to be *H. woelkerlingii* from New Zealand is likely an undescribed species. Type specimens of all other *Sporolithon* and *Heydrichia* species need to be sequenced to confirm that they are distinct species; morpho-anatomical studies have proved inadequate for this task.

**Key index words:** COI; Egypt; Gulf of Suez; LSU; new species; phylogenetics; *psbA*; *rbcL*; Red Sea; UPA

**Abbreviations:** bp, base pairs; BS, bootstrap value; ML, maximum likelihood; NWGMx, Northwest Gulf of Mexico; SEGMx, Southeast Gulf of Mexico

To determine if any genus is monophyletic and which species belong in the genus, one must begin by characterizing the generitype species. Historically, this characterization was done for coralline algae by morpho-anatomical comparisons using gross morphology at first, but soon thereafter augmented by anatomical characters through decalcification, sectioning and light microscopy, and finally in the late 20th century aided by scanning electron microscopy (Johansen 1981, Woelkerling 1988). Recently, this approach has been further augmented by DNA sequence data that has, thus far, supported the monophyly of only one genus, *Bossiella* (Hind et al. 2014a, 2015), but revealed that many others were polyphyletic, e.g., *Calliarthron* (Gabrielson et al. 2011), *Clathromorphum* (Adey et al. 2015), *Corallina* (Hind and Saunders 2013), *Hydrolithon*, *Porolithon*, and *Spongites* (Rössler et al. 2016). Furthermore, the
generitype species of three additional genera, *Pachyarthron*, *Serraticardia* and *Yamadaia* were all shown to belong in *Corallina*, and these genera were placed in synonymy by Hind et al. (2014b), Hind and Saunders (2013) and Martone et al. (2012), respectively. Many other coralline genera have yet to be assessed using this methodology.

DNA sequencing of the type specimens of generitype species has been critical to correctly applying these names. For example, *Bossiella plumosa*, the generitype species and *B. fongsifera* both share the same type locality, and sequencing recently collected topotype material did not allow names to be correctly applied until the holotype specimen of each was sequenced (Hind et al. 2015). In the case of the generitype species of *Lithophyllum*, *L. incrustans*, that name had been misapplied to specimens for 60 years (Hernández-Kantún et al. 2015). Western European field-collected specimens with *rbcL* sequences identical to the lectotype specimen were subtidal rhodoliths, or less commonly, subtidal, epilithic, epizoic, or epiphytic crusts, not a common, epilithic intertidal crust as widely reported (Cabioc and Boudouresque 1992, Chamberlain and Irvine 1994).

Four currently recognized *Sporolithon* species, *S. ptychoides*, the generitype, *S. erythraeum*, *S. crassum*, and *S. molle*, all have their type locality at El Tor, Egypt in the Gulf of Suez, Red Sea, and all have intermingled taxonomic histories. *Sporolithon erythraeum* was originally described as *Lithothamnion erythraeum* (Rothpletz 1893) based on material found on the beach. In the protologue of *S. ptychoides*, two forms were described, *S. ptychoides* f. *dura* (Heydrich 1897a, pl. III, figs. 20–23) and *S. ptychoides* f. *mollis* (Heydrich 1897a, pl. III, figs. 15–19). Heydrich (1897b) subsequently split the *S. ptychoides* f. *mollis* material and recognized two species, *S. molle* (Heydrich 1897a, pl. III, figs. 16, 18, 19) and *S. crassum* (Heydrich 1897a, pl. III, fig. 15), leaving *S. ptychoides*, based on *S. ptychoides* f. *dura* as the generitype species. Woelkerling and Townsend (in Woelkerling 1988: 207) noted that Heydrich’s original material of *S. ptychoides*, *S. molle*, and *S. crassum* was destroyed, but that syntype material of the first two was present in the Foslie herbarium (TRH). They designated this syntype material of *S. ptychoides* in TRH as the lectotype specimen. Much earlier, Foslie (1900c) (as *Archaeolithamnium erythraeum*) had considered *S. ptychoides* to be a later, heterotypic synonym of *S. erythraeum*, and this was confirmed by Woelkerling and Townsend (in Woelkerling 1988: 207) based on a morpho-anatomical comparison of the two type specimens, both in TRH. However, Verheij (1993) examined the arrangement of tetrasmusporangia in the type specimens of both *S. ptychoides* and *S. molle* and, based on the presence or absence of a distinct layer of elongated cells among the tetrasmusporangia, determined that they were distinct species, in addition to *S. erythraeum*. He also noted that the lectotype specimen of *S. molle*, which had been considered a later heterotypic synonym of *S. ptychoides* since the early 20th century, was more similar to *S. erythraeum*, leaving only two equivocal characters to distinguish the three species: (i) size of tetrasmusporangia (partially overlapping in length and width), and (ii) whether or not old, buried terasmusporangia were sometimes infilled (Verheij 1993, table 2). To date, no original material of *S. crassum* has been located in other herbaria. Thus, at present, all four species are recognized, and *S. erythraeum*, *S. molle*, and *S. ptychoides* are reported to be widely distributed based on morpho-anatomy (Guiry and Guiry 2017, accessed January 20, 2017).

The generitype species of *Heydrichia*, *H. woelkerlingii*, is based on specimens collected from Oudekraal, Western Cape Province, South Africa (Townsend et al. 1994). Sequencing of the holotype, isotype, and other specimens examined in the protologue is likely not possible due to formalin and glutaraldehyde preservation. The first published SSU sequence of *H. woelkerlingii* was from a specimen collected from Betty’s Bay near the type locality (Bailey and Chapman 1998). Subsequently, Farr et al. (2009) provided *psbA* sequences of specimens identified as *H. woelkerlingii* from New Zealand. More recently, Mateo-Cid et al. (2014) published a *psbA* sequence of a specimen collected at the type locality in South Africa. Bahia et al. (2015a) showed that the *psbA* sequence of a specimen identified as *H. woelkerlingii* from New Zealand did not form a clade with the *psbA* sequence of the specimen from South Africa.

Herein, we apply DNA sequencing methodology to the generitype species, *Sporolithon ptychoides* and *Heydrichia woelkerlingii*. For the former, we obtained an *rbcL* sequence from the lectotype specimen; for the latter, an *rbcL* sequence from topotype material. We also sequenced the lectotype of *S. molle* and the holotypes of *Sporolithon* dimotum (type locality: Lemon Bay, near Guánica, Puerto Rico), currently considered a synonym of *S. ptychoides*, and of *S. episporum* (type locality: Point Toro, near Cólon, Panama). Based on the results of the DNA sequence analyses and SEM observations of morpho-anatomical characters, we assess the identity of species of *Sporolithon* and *Heydrichia* and describe a new coralline alga, *S. eltorensis* sp. nov.

**MATERIAL AND METHODS**

*Specimen collection.* Collections of *Sporolithon* spp. specimens were made in the shallow subtidal zone (1–1.5 m deep) near El Tor, Egypt as well as Dahab, Egypt, along fringing reefs and reef flats during a field expedition focused on assessing algal biodiversity in the northern Red Sea (Table 1). Specimens of non-geniculate coralline algae were collected by hand while snorkeling and preserved in silica gel; vouchers were deposited at NCU or LAF (herbarium codes follow Thiers 2017). Specimens identified as *S. episporum* and *Sporolithon* sp. were collected using SCUBA from the subtidal zone in the Caribbean (3 m) and Pacific Panama (6–7 m),
Table 1. List of taxa with collection data and GenBank numbers for newly generated sequences. Sequences included in the concatenated analyses shown in italics.

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<th>Taxa</th>
<th>ID no./reason for inclusion</th>
<th>Locality</th>
<th>Collector/date habitat</th>
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<td><strong>Antithamnion sp.</strong></td>
<td>LAF 4355 phylogeny</td>
<td>Ewing Bank, NWGMx 28°06.066' N; 91°02.146' W</td>
<td>S. Fredericq, J. Richards, 29.viii.2011 (collected from site), 25.i.2012 (collected from microcosm), epilithic, 58–91 m depth</td>
<td>KY980440 KY994115 – KY994130 –</td>
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<td><strong>Heydrichia cerasina</strong></td>
<td>NCU 617165 (UWC 10/144)</td>
<td>l’Agulhas, Cape Agulhas, Western Cape Province, South Africa 34°49'6.69&quot; S; 20°01'7.83&quot; E</td>
<td>G. W. Maneveldt 16.vi.2010, on pebbles in sand, low intertidal</td>
<td>KY980439 KY994114 MF034551 KY994128 –</td>
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<td><strong>Mesophyllum lichenoides</strong></td>
<td>NCU 590286 Phylogeny</td>
<td>Wembury, South Devon, England 50°19' N; 4°04' 59.88&quot; W</td>
<td>J. Brodie, 22.vii.2009, epilithic in tidepool</td>
<td>– – MF034552 KY994129 –</td>
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<td><strong>Sporolithon dimotum</strong></td>
<td>NY 900045 (Howe 2667) Holotype of Archaeolithothamnium dimotum</td>
<td>Lemon Bay, near Guanica, Puerto Rico</td>
<td>M. A. Howe, 27.vi.1903</td>
<td>– – – KY994131 –</td>
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<td><strong>Sporolithon episporum</strong></td>
<td>NY 900041 (Howe 6832) Holotype of Archaeolithothamnium episporum</td>
<td>Point Toro, near Colon, Panama, Caribbean Sea</td>
<td>M. A. Howe, 7.i.1910, low water mark to several meters depth</td>
<td>– – – KY994125 –</td>
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<td><strong>Sporolithon molle</strong></td>
<td>C A92529 Lectotype of Sporolithon ptychoides f. mollis</td>
<td>El Tor, Egypt, Gulf of Suez</td>
<td>A. Kaiser, no date, no habitat data</td>
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<td>El Tor, Egypt, Gulf of Suez</td>
<td>A. Kaiser, no date, no habitat data</td>
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<td>Sporolithon ptychoides</td>
<td>NCU 606663 (LAF 5846)</td>
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<td>T. Sauvage, W. E. Schmidt, D. Gabriel, 8.x.2012, 1–2 m depth</td>
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<td>Sporolithon sp.</td>
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<td>LAF 6970B</td>
<td>Dry Tortugas Vicinity, SEGmX</td>
<td>J. Richards &amp; S. Fredericq, 10.x.2014 (collected from site), 15.xi.2014 (collected from microcosm), 69 m depth</td>
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<td>Sporolithon yoneshigueae</td>
<td>RB 600559</td>
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<td>R.G. Bahia 27.x.2013, 28 m depth</td>
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**THE CORALLINE GENERA SPOROLITHON AND HEYDRICHA**

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respectively. Dredged specimens from the NWGMx and SEGMx were collected according to the protocol of Richards et al. (2014, 2016; Table 1) and were either preserved directly in the field or subsequently collected from microcosms established with field-collected material. Two specimens of Sporolithon yoneshigueae originating from Bahia State, Brazil, were obtained from the Smithsonian Institution in Washington, D.C. and a specimen of Hydrichia cerasina was collected from Western Cape Province, South Africa.

**DNA sequencing of newly collected specimens.** Markers chosen for PCR included the chloroplast-encoded genes *rbcL* (encodes the large subunit of the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase), *psbA* (encodes the photosystem II reaction center protein D1 gene), and “Universal Plastid Amplicon” (UPA, partial 23S rDNA), as well as the mitochondrial-encoded gene “cytochrome oxidase subunit I” (COI), and the nuclear-encoded LSU (partial 28S rDNA). Total DNA was extracted from specimens at the University of North Carolina at Chapel Hill following the protocol established by Hughey et al. (2001) and modified by Gabrielson et al. (2011). Amplification of *rbcL* and *psbA* (in part) markers was performed at the University of North Carolina at Chapel Hill following, respectively, Gabrielson et al. (2011) and Sissini et al. (2014). Resulting PCR products were sent to the DNA Analysis Core Facility at the University of North Carolina at Wilmington for sequencing as in Adey et al. (2015). Aliquots of extracted DNA were sent to the University of Louisiana at Lafayette where PCR and sequencing for COI, LSU, and UPA, and *rbcL* and *psbA* (in part), were performed as in Richards et al. (2014, 2016).

**DNA sequencing of type specimens.** Specimens were received on loan from the following herbaria: C, NY, and TRH. Specimens that consisted of multiple fragments were compared to the protologue to identify the most representative fragment to choose for DNA extraction. Specimens were examined with a dissecting microscope and fragments were selected from the thallus portions with no epiphyte overgrowth or if epiphytes were present, they were removed. Total DNA was extracted from all type specimens in an independent laboratory at Hartnell College following the precautionary guidelines for historical specimens proposed by Hughey and Gabrielson (2012); extraction and primers for amplification of a short portion of *rbcL* followed Hernández-Kantún et al. (2016).

**Alignment and phylogenetic analyses.** COI (661 bp), LSU (550 bp), *psbA* (863 bp), UPA (369 bp), and *rbcL* alignments were constructed as in Richards et al. (2014, 2016). Three alignments of varying sequence length were generated for *rbcL*, including an alignment of short sequences (263–296 bp), an alignment of medium and long sequences (644–1,387 bp) and an alignment of mixed short, medium, and long sequences (263–1,387 bp). Publically available sequences of *Sporolithon* spp. and *Hydrichia* spp. were downloaded from GenBank (last accessed 20 January 2017) and included in the alignments, as well as those for several members of the Hapalidiales, Corallinales, and Rhodogorgonales (Table S1 in the Supporting Information) for phylogenetic context. Members of the Ceramiales that were downloaded from GenBank or were newly generated (Table 1, Table S1) served as the outgroup in all analyses. A concatenated alignment of LSU, COI, *psbA*, and *rbcL* (3,454 bp) was constructed using Sequence Matrix 1.7.8 (Vaidya et al. 2011). PartitionFinder (Landear et al. 2012) was used to determine the best partition scheme and model(s) of evolution as implemented by RAxML. For the concatenated data set, 10 data blocks were used by PartitionFinder to find the best partitioning scheme using ‘search=all’; nine blocks consisted of a data block for each codon position of each protein coding gene and a single data block for LSU, respectively. ML analyses were performed with the RAxML-HPC2 program as implemented on the online server “The CIPRES Science Gateway V. 3.3” (Miller et al. 2010) with a GTR+I+G model of evolution and the partition scheme found by PartitionFinder, 1,000 topological searches from random restarts, and 1,000 bootstrap replicates to assess branch support.

**Evaluation of pair wise distance distribution for five markers.** The distribution of raw pairwise distances (i.e., divergence) was computed for the five markers utilized in the present study in order to evaluate their phylogenetic informativeness within the Sporolithales ingroup. Raw pairwise distances (number of bp differences) were calculated in R with package “Ape” (Paradis et al. 2004; R Core Team 2014) and divided by the alignment length. Prior to doing so, alignments were cropped at their 5’ and 3’ ends when missing data were present, and some short sequences were removed as not to overinflate computed pair wise distances because of missing data. Short (296 bp) and medium length (578 bp) alignments of *rbcL* were each analyzed separately.

**Scanning electron microscopy.** Portions of the thallus from silica gel-dried specimens were removed using a single-edged razor blade and forceps. Vertical fractures were performed on crustose portions, whereas protuberances were sectioned longitudinally using a new razor blade for each fracture. Sections were mounted using liquid graphite and coated with 10–16.5 nm of gold. Specimens were viewed using a Hitachi S-3000N SEM at a voltage of 15 or 20 kV, housed in the Microscopy Center at UL Lafayette. Cell dimensions were measured from SEM micrographs following the protocols of Irvine and Chamberlain (1994) and Adey et al. (2005). Ten cells of each vegetative cell type (hypothallial, perithallial, epithallial, and meristematic cells) and 10 reproductive structures were measured for each specimen, except where otherwise noted. Terminology follows Woelkerling (1988) and Adey et al. (2015).

**Results**

**Historical type specimens and toptotypes.** Unique *rbcL* sequences were obtained from the type specimens of four *Sporolithon* species, *S. ptychoideas*, *S. molle*, *S. dimotum*, and *S. episporum* (Fig. 1). The *rbcL* sequences obtained from the lectotype specimens of *S. ptychoideas f. dura* (263 bp) and *S. ptychoideas f. mollis* (296 bp) were each an exact match to this *rbcL* segment of longer sequences newly generated from our collection of these morphotypes from the type locality at El Tor, Egypt. The *rbcL* sequence of the holotype specimen of *Archaeolithothamnion dimotum* (263 bp) was within the *Sporolithon* clade, but did not match any existing or newly generated *Sporolithon* sequences. The *rbcL* sequence (296 bp) obtained from the holotype specimen of *Archaeolithothamnion episporum* was an exact match over its entire length to our recently collected specimen of *S. episporum* from Caribbean Panama. Unfortunately, PCR amplification of the type specimen of *Archeolithothamnion erythraeum* was unsuccessful.

**Phylogenetics.** The results of the ML analyses with concatenated (Fig. 2) or single genes (Fig. 1, Figs. S1–S6 in the Supporting Information) showed some variation with regard to the monophyly of *Sporolithon* caused by the variable positioning of sister clade species assigned to this genus, namely
S. yoneshigueae and newly sequenced specimens from the Gulf of Mexico. The clade formed by these two taxa branches at the base of other Sporolithon spp. in the concatenated analyses had good support (87 BS). However, in single gene analyses, except with LSU (Fig. S4), the positioning of this clade received no support (psbA, COI, UPA; Figs. S3, S5, S6) and was paraphyletic with respect to the other Sporolithon species in rbcL phylogenies (Fig. 1, Figs. S1 and S2). Depending on the markers and concatenation, the support within Heydrichia was generally low to moderate, and most of its taxa lie on long branches. The order Sporolithales was monophyletic in concatenation and when inferred from single gene psbA and COI analyses, but paraphyletic with rbcL and LSU analyses. Divergence analyses show that LSU is the most conserved marker, followed by UPA, psbA, COI, and rbcL (Fig. S7 in the Supporting Information; Tables S2–S7 in the Supporting Information). As indicated from pair wise distances (see Tables S2–S6), sister clade species of Sporolithon (S. yoneshigueae and S. sp. [LAF 6956A, LAF 6970B]) were as divergent from the remaining Sporolithon spp. as they were from Heydrichia spp.

Sporolithon and Heydrichia names. ML analyses of the single gene alignments (Fig. 1, Figs. S1–S6) and concatenated gene alignment (rbcL, psbA, COI, LSU; Fig. 2) show that none of the sequences deposited in GenBank of specimens identified as Sporolithon ptychoides formed a monophyletic clade with sequences of the authentic S. ptychoides specimens (NCU 606660, NCU 606663), which were exact matches with the lectotype specimen. Sequences of specimens identified as S. ptychoides from Hawaii (represented by COI, LSU and UPA) and Brazil (represented by rbcL and psbA) could not be compared to each other due to a lack of corresponding markers. The rbcL and psbA sequences of toptype material of H. woelkerlingii from South Africa were distinct from the rbcL or psbA sequences of the New Zealand specimen identified as H. woelkerlingii in the single (Fig. 1, Figs. S1–S3) and concatenated gene analyses (Fig. 2). Heydrichia homalopasta formed a strongly supported clade with the South African H. woelkerlingii (Fig. 2, Fig. S3), whereas H. cerasina comprised a clade with lower support (Figs. 1 and 2, Figs. S1–S3; BS = 56–68).

Based on the rbcL, psbA, UPA, COI, and LSU sequence data, as well as the morpho-anatomical characters, we herein emend the descriptions of S. ptychoides and S. molle, resurrect the name S. dimotum, and formally describe S. eltorensis sp. nov.

Sporolithon Heydrich 1897a: 66.

To the generic description provided by Verheij (1993), we add the postfertilization developmental features of Bahia et al. (2015b), including fertilized carpogonia that each produce short, 1–2 celled gonimoblasts (a central fusion cell is absent) each
bearing terminal, oblong carposporangia, which are found along the floor and walls of carposporangial conceptacles.

**Sporolithon ptychoides** Heydrich 1897a: 67–69, figs. 2–3; pl. III.

**Basionym:** *S. ptychoides f. dura* Heydrich 1897a: 67–69, figs. 2–3, pl. III.


**DNA sequences:** A 263 bp sequence of *rbcL* was obtained from the lectotype specimen of *S. ptychoides*. This sequence was an exact match over its entire length to two 1,387 bp *rbcL* sequences, each generated from specimens collected at the type locality in the shallow subtidal (1–1.5 m deep) of the Gulf of Suez at El Tor, Egypt. Other diagnostic sequences from these topotype specimens included *psbA*, *UPA*, *COI*, *LSU* (Table 1), and *tufA* (GenBank accession = KU362143).

The following characterization of *S. ptychoides* is based only on our observations of field-collected specimens.

**Morphology and Habitat:** Thallus non-geniculate, with rounded protuberances; overgrowing coral on shallow fringing reefs and reef flats, 1–1.5 m deep (Fig. 3A).

**Anatomy:** Hypothallium monomericous with rectangular-shaped cells 18.75–52 µm long × 3.5–9 µm wide (Fig. 3, B and C). Perithallium with multiple fusions, secondary pit connections rarely observed (Fig. 3, D and E), with cells 7–11.5 µm long × 5–6.4 µm wide. Epithallium comprised of a single layer of armored epithallial cells 1–1.8 µm long × 3–6.7 µm wide (Fig. 3F). Meristematic cells subsisodiametric, or slightly longer or shorter than wide, 5–10.5 µm long × 4.8–10.2 µm wide (Fig. 3F).

**Reproduction:** Of the two specimens collected, one was tetrasporangial (NCU 606660), the other non-reproductive (NCU 606663). Tetrasporangial compartments borne among a basal layer of elongated cells, unshed and buried after spore release (Fig. 4, A–C). Tetrasporangia 45–52 µm long × 24–37 µm wide with triangular stalk cells 10–17 µm long × 10–20 µm wide (*N* = 8; Fig. 4D). Paraphyses 3–4 celled. Rosette cells not observed (tetrasporangial compartments were not present at the surface in the fragments examined).

**Distribution:** Confirmed only from the Gulf of Suez, at El Tor, Egypt.

**Sporolithon molle** (Heydrich) Heydrich 1897b: 416–417.

**Basionym:** *S. ptychoides f. mollis* Heydrich 1897a: 67–69, pl. III, figs. 16, 18, 19.


**Isolectotype:** TRH C19-3419, Heydrich #11, El Tor, Egypt.
**Fig. 3.** *Sporolithon ptychoides*, NCU 606665 (A, left, B–F), NCU 606660 (A, right). (A) Thallus habit. Scale bars 0.6 cm (left images), 0.3 cm (right images). (B) Vertical fracture of crustose portion of thallus; arrow indicates basal region, scale bar 380 μm. (C) Detail of area indicated by arrow in B showing monomorphous hypothallium (bracket) and upswept tiers of perithallial filaments, scale bar 85 μm. (D) Perithallium with multiple cell fusions (arrows), scale bar 19 μm. (E) Detail of cell fusion (arrow) neighboring a secondary pit connection (circle arrow), scale bar 20 μm. (F) Armored epithallial cells lacking epithallial cell roofs (arrows), meristematic cells (M), and recently divided meristematic cell (double arrowhead), scale bar 14 μm.

**Fig. 4.** *Sporolithon ptychoides* NCU 606660. (A) Longitudinal section of protuberance showing layers of unshed tetrasporangial compartments, scale bar 450 μm. (B) Layer of overgrown tetrasporangial compartments borne among layer of elongate cells, scale bar 80 μm. (C) Detail of paraphyses with elongate cells (arrows) growing among tetrasporangial instead of tetrasporangial compartments; stalk cell (circle arrow), scale bar 23 μm. (D) Detail of remnants of apical pore plug of tetrasporangial compartment, scale bar 25 μm.
DNA sequences: A 296 bp rbcL sequence was obtained from the lectotype specimen of *S. molle*. This sequence was an exact match over its entire length to a 694 bp sequence generated from a specimen (NCU 606657) collected at the type locality, El Tor, Egypt in the Gulf of Suez. Other diagnostic sequences from this topotype specimen included UPA, COI, LSU (Table 1), and tufA (GenBank accession = KU362144).

The following characterization of *S. molle* is based only on our observations of field-collected specimens.

Morphology and Habitat: Thallus non-geniculate, with warty to more slender protuberances; growing attached to the benthic substratum on shallow fringing reefs and reef flats, 1–1.5 m (Fig. 5A).

Anatomy: Hypothallium monomerous, with rectangular-shaped cells 16–30 μm long × 3–6.5 μm wide (Fig. 5, B and C). Perithallium with multiple fusions and secondary pit connections (Fig. 5, D and E). Perithallial cells 6.8–19.5 μm long × 5.5–10.2 μm wide. Epithallium comprised of a single layer of armored epithallial cells 1.5–2.5 μm long × 4.2–7.6 μm wide (Fig. 5F). Meristematic cells subsidiodometric to rectangular, 6.4–12 μm long × 5–6.8 μm wide (Fig. 5F).

Reproduction: One tetrasporangial specimen was observed. Tetrasporangial pores in surface view 6–10.8 μm diameter surrounded by 9–12 rosette cells (Fig. 6, A and B). Tetrasporangial compartments (Fig. 6, C–F) borne among a layer of elongate cells (Fig. 6D, Fig. S8 in the Supporting Information), unshed and buried after spore release (Fig. 6, D and F). Tetrasporangia 51–58.5 μm long × 21–41 μm wide (Fig. 6, C–F, Fig. S9 in the Supporting Information) with triangular stalk cells 10–18.75 μm long × 14–19.5 μm wide (Fig. 6, D–F). Paraphyses 3–4 celled.

Distribution: Confirmed only from the Gulf of Suez at El Tor, Egypt.

*Sporolithon dimotum* (Foslie & M. Howe) Yamagushi-Tomita ex Wynne 1986: 2258.
Isotype: TRH C19-3380.

DNA sequences: A 263 bp rbcL sequence (Table 1) was obtained from the holotype specimen of *S. dimotum*. This sequence is diagnostic for this species and was not identical to any sequences generated in...
this study or to any sequences in GenBank (last accessed 20 January 2017).

**Distribution:** Known only from the type locality.

*Sporolithon episporum* (M.Howe) E.Y.Dawson 1960: 40, figs. 11, 12.

**Basionym:** Archaeolithothamnium episporum Howe 1918: 2, pl. 1–6.

**Holotype:** NY 00900041 (Howe 6832), Point Toro, near Colon, Panama Canal Zone (Caribbean coast), 7.i.1910, leg. M. A. Howe.

**Paratypes:** In USNM, Cat. No. 35298. (Howe 6840).

**DNA sequences:** A 296 bp *rbcL* sequence was obtained from the holotype specimen of *S. episporum*, specifically the individual piece identified as the “technical type” by Howe (1918) in the prologue, and was an exact match over its entire length to a 1,362 bp *rbcL* sequence of a specimen collected from Caribbean Panama (Table 1). Other diagnostic sequences from this field-collected specimen included COI and *psbA* (Table 1), the latter was an exact match to a published sequence (Table S1).

**Distribution:** Caribbean Panama; Costa Rica (Bahia et al. 2014a,b).

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*Sporolithon eltorensis* sp. nov. J.Richards & P.W.Gabrielson

**Holotype:** NCU 606659, Gulf of Suez, El Tor, Egypt (28°14.080’ N; 33°36.174’ E), 8.v.2012, on sand and coral bottom, 1–2 m deep, leg. Will Schmidt, Daniela Gabriel & Thomas Sauvage.

**Isotype:** Specimen LAF 5767, Gulf of Aqaba, Dahab, Egypt (28°28.654’ N; 34°30.698’ E), 1–2 m deep, leg Will Schmidt, Daniela Gabriel & Thomas Sauvage.

**Etymology:** The specific epithet refers to the holotype locality, El Tor, Egypt.

**Diagnosis:** The diagnostic characters of this species are the following DNA sequences from the holotype: *psbA* and LSU; and from the isotype: *psbA*, UPA, COI, LSU (Table 1), and *tufA* (GenBank accession = KU362142). Thus far, no morpho-anatomical features distinguish this species from other *Sporolithon* species.

**Morphology and Habitat:** Thallus encrusting, smooth to warty growing over and consolidating underlying substratum of sand and coral (Fig. 7A); shallow subtidal zone, 2–6 m deep, along fringing reefs and reef flats.
**Vegetative Anatomy:** Thallus construction monomeric, with a non-coaxial hypothallium (Fig. 7, B and C). Hypothallial cells rectangular-shaped, 19–35 μm long × 3–5.5 μm wide (Fig. 7C). Perithallium with secondary pit connections and cell fusions in a ratio of 1:1. Perithalial cells 5.7–10.2 μm long × 4.2–6.2 μm wide. Epithallium a single layer of armored cells that are 1.8–2.6 μm long × 4.2–6 μm wide (Fig. 7E). Meristematic cells subsidodiometric or rectangular that are 3.4–6 μm long × 6.2–9 μm wide (Fig. 7E).

**Reproduction:** No reproductive structures were observed.

**Distribution:** Gulf of Suez, near El, Tor Egypt and Gulf of Aqaba, Dahab, Egypt.


**Holotype:** YMC 88/60, Oudekraal, Cape Peninsula, Western Cape, South Africa, 6.vi.1986, leg. L. Anderson.

**Isotype:** LTB.

**DNA sequences:** Topotype material sequenced: *psbA* (GenBank accession = JQ917415) and *rbCL* (GenBank accession = KP142788; Table S1).

**Distribution:** Confirmed only from Western Cape Province, South Africa.

**Heydrichia cerasina** Maneveldt & van der Merwe 2012: 11–21, figs. 1–27.

**Holotype:** L 0821464, Western Cape, South Africa, 16.vi.2010, leg. G.W. Maneveldt, E. van der Merwe, C. van Gass & O. van Gass.

**Isotypes:** L 0821455-63 and UWC 10/144.

**DNA sequences:** Isotype material sequenced: *psbA*, *rbCL*, COI, LSU (Table 1).

**Distribution:** Known only from a 10 km stretch of coast from Struisbaai to L’Agulhas, Western Cape Province, South Africa.

**DISCUSSION**

Sequencing type specimens of generitype species. Currently, the only method to unequivocally characterize the generitype species of coralline algae is by DNA sequencing of the type specimen; this is true for any coralline species, not just the generitype species. Relying on morpho-anatomical characterizations to identify many coralline taxa has resulted in frequent species misidentifications and, worse, polyphyletic genera (Gabrielson et al. 2011, Martone et al. 2012, Hind et al. 2014a,b, 2015, 2016, Sissini et al. 2014, Adey et al. 2015, Hernández-Kantún et al. 2015, 2016). Evidence for this...
also abounds with species-distinct sequences passing under single names in GenBank for numerous geniculate and non-geniculate species (e.g., *Amphi-roida beauvoisii, Spontiges yendoi, Sporolithon durium,* and as demonstrated herein for the generitype species of the only two genera in the order Sporolithales, *S. ptychoides* and *H. woelkerlingii.*

We obtained a diagnostic 296 bp *rbcL* sequence from the lectotype specimen of *Sporolithon ptychoides* that enabled us to unequivocally apply this name to field-collected material from the type locality, El Tor, Egypt. DNA sequences from the field-collected material allowed us to compare these with available GenBank sequences, including markers commonly used in phylogenetic analyses of corallines (*rbcL, psbA, UPA, COI, and LSU*). Specimens that were identified as *S. ptychoides* based on morpho-anatomy and then sequenced from Brazil (Bahia et al. 2014b), Hawaii (Sherwood et al. 2010), and New Caledonia (Bittner et al. 2011) did not match any sequence from the authentic *S. ptychoides* from El Tor, Egypt (Fig. 1 and 2, Figs. S3 and S4). Based on the several misapplications of the name *S. ptychoides* to sequenced specimens from other ocean basins, we hypothesize that other specimens attributed to *S. ptychoides* based on morpho-anatomy from the Mediterranean Sea (Alongi et al. 1996), Indonesia (Verheij 1993), South Africa (Keats and Chamberlain 1993), Gulf of Thailand and Andaman Sea (Kaewsuralikhit et al. 2012), and Brazil (Nunes et al. 2008, Bahia et al. 2011, Henriques et al. 2014) are also likely different species. Likewise, the taxon reported to be *H. woelkerlingii* from New Zealand (Nelson et al. 2015), far from its type locality of the Western Cape Province, South Africa, is not that species and likely represents an undescribed species. Material was identified as that species based on morpho-anatomy, but the DNA sequences for both *psbA* and *rbcL* indicate a different species (Figs. 1 and 2, Fig. S3).

Sporolithon species. There are four *Sporolithon* species whose type localities are El Tor, Egypt, *S. ptychoides, S. molle, S. erythraeum,* and *S. crassum.* Our ability to obtain diagnostic *rbcL* sequences from the lectotype specimens of *S. ptychoides* and *S. molle* to compare with field-collected toptype material at El Tor, Egypt enabled us to unequivocally apply these names. Unfortunately, we were unable to PCR amplify DNA from the lectotype specimen (TRH C19-3438) of *L. erythraeum* (Rothpletz 1893), the oldest epithet that could apply to *Sporolithon* species at this same type locality, El Tor, Egypt. Apparently, all original material of *S. crassum* was destroyed (Woelkerling 1988: 207), as also occurred for *S. ptychoides* and *S. molle,* except for specimens sent to other herbaria. We have been unable to locate any original material of *S. crassum.* In the future, original material of *S. crassum* may be found and type material of *S. erythraeum* may be sequenced with de novo technology, but until then, neither of these names should be applied anywhere in the world, as their use cannot be validated by comparison with type material. For this reason, we have provided a new name, *S. eltorensis,* for the third species of *Sporolithon* found at El Tor, Egypt (Fig. 2, Figs. S3–S6). We believe it is preferable to name a new species based on this newly collected material rather than designate that material as an epitype for either *S. erythraeum* or *S. crassum,* as that would merely be guessing at their identities.

*Sporolithon molle* had been distinguished morpho-anatomically from *Sporolithon ptychoides* by the absence of a layer of elongate cells at the base of tetrasporangial compartments (Verheij 1993, Bahia et al. 2011, 2014a). However, we demonstrated that both *S. molle* and *S. ptychoides* exhibit this character. During sectioning, tetrasporangial compartments that have a three-dimensional tapered base (where the stalk cell is cut-off from the subtending cell layers), may partially overlap and obscure the surrounding basal layer of cells. Furthermore, because the paraphyses may bend and curve as tetrasporangial compartments develop and enlarge (Townsend et al. 1995), and/or due to oblique section angles (Kaewsuralikhit et al. 2012, herein), it is inherently likely that incomplete views of basal cells will sometimes be observed in sections that may give the false impression that these cells are not elongate.

Much of our current information about *Sporolithon ptychoides* is based on specimens that we now know are not *S. ptychoides* from DNA sequence comparison. Other reports of specimens believed to be that species based on their uninformative morpho-anatomical characters cannot be corroborated currently. Notwithstanding our recent collections, we still are unable to adequately characterize *S. ptychoides* morpho-anatomically due to the absence of any reproductive material, except buried tetrasporangial compartments, and due to having only two field-collected specimens. The same is true for *S. molle* and *S. eltorensis,* for which we have only one and two field-collected specimens, respectively; however, having few specimens upon which to describe a species is true for many non-geniculate coralline species as pointed out by Hernández-Kantún et al. (2016). This problem is exacerbated when multiple species are passing under a single name, as is common with both geniculate (Hind and Saunders 2013, Hind et al. 2015, 2016) and non-geniculate (Sissini et al. 2014, Adey et al. 2015, Hernández-Kantún et al. 2015) species.

Despite the absence of any distinguishing morpho-anatomical characters and sharing the same type locality, *Sporolithon molle, S. ptychoides,* and *S. eltorensis* clearly are distinct species based on all markers that we sequenced (Figs. 1 and 2, Figs. S1–S5). Our clarification of their molecular identity will facilitate further taxonomic reassessment and eventual species description of specimens called “*S. ptychoides*” as well as other *Sporolithon* species at other locations. For example, reports of *S. molle* based only on morpho-anatomy, from the Spermonde...
Archipelago, Indonesia (Verheij 1993), the Persian Gulf (De Clerck and Coppejans 1996, John and Al-Thani 2014), and from Brazil (Bahia et al. 2014a) all need to be reassessed. Reports of *S. ptychoïdes* and other *Sporolithon* spp. fossils (Braga and Bassi 2007, Ghosh and Sarkar 2013), and the biogeographical implications of such reports, need to be re-evaluated considering the lack of diagnostic morpho-anatomical characters, especially considering the estimated age of the Sporolithales (136.4–130 million years B.P.; Aguirre et al. 2010, Yang et al. 2016).

*Sporolithon dimotum* (type locality: Lemon Bay, Near Guánica, Puerto Rico) was placed in synonymy with *Sporolithon ptychoïdes* by Bahia et al. (2011) based on sharing the following morpho-anatomical features of tetrasporangial specimens: (i) tetrasporangia grouped in sori and raised above the thallus surface, (ii) presence of a basal layer of elongate cells in areas of developing tetrasporangia, (iii) tetrasporangial paraphyses comprised of 3–5 cells, (iv) deeply buried tetrasporangial compartments, and (v) size of tetrasporangia. Despite sharing all of these features, a 263 bp *rbcL* sequence from a holotype specimen shows *S. dimotum* is a distinct species more closely related to an undescribed species from Brazil than to *S. ptychoïdes* (Fig. 1, Fig. S1). We await new field collections to obtain longer, more phylogenetically informative DNA sequences of different markers for *S. dimotum* to confirm its evolutionary relationships.

*Sporolithon episporum* (type locality: Point Toro, near Colon, Panama Canal Zone, Caribbean coast) has been recognized as a distinct species since it was first described by Howe (1918). We confirm that the sequenced specimen named *S. episporum* from Atlantic Costa Rica (Bahia et al. 2014b) is correctly identified. The application of this name from the Natal and Western Cape Provinces of South Africa needs to be confirmed by DNA sequence data. *Sporolithon* sp. (PHYKOS 4623), collected from Gulf of Chiriquí, Pacific Panama, may correspond to *Sporolithon howei* (Me.Lemoine) N.Yamagushi-Tomita ex M.J.Wynne (type locality Coiba Island, Gulf of Chiriquí, Panama), or *S. pacificum* E.Y.Dawson (type locality Isla del Caño, Costa Rica). DNA sequencing needs to be performed on the holotype specimens of these species to determine the identity of PHYKOS 4623.

There are 15 additional names of extant species of *Sporolithon* (Guiry and Guiry 2017) not treated herein, and the type specimens of all of these need to be sequenced. We already know that there are multiple species passing under *S. durum* (Foslie) R.A.Townsend & Woelkerling based on DNA sequence comparisons (Figs. S2 and S3). We do not know which morpho-anatomical characters, if any, will be useful in segregating species of *Sporolithon*. To date, none of the characters previous considered to be diagnostic for these species has been supported by DNA sequence data. Thus far, no species reported to have a widespread distribution across different biogeographic provinces or ocean basins based on morpho-anatomical characters (e.g., *S. ptychoïdes*, *S. molle*, and *S. episporum*) has been confirmed by DNA sequence data. Based on the current evidence, there appears to have been much speculation in this genus with little concomitant morpho-anatomical change, despite the antiquity of Sporolithales.

**Sporolithales generic relationships.** Phylogenetic analyses of all of the data sets, particularly *rbcL* and *psbA*, for which we have more complete taxon sampling, suggest that *Sporolithon* is paraphyletic and the relationships among *Heydrichia* species are not well resolved. In particular the relationship of *S. yonishigae* from Brazil and an undescribed species from the Gulf of Mexico to other *Sporolithon* spp. needs to be investigated in greater detail. It is likely that DNA sequences from additional new world taxa will help to resolve these relationships.

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**Figure S1.** Phylogram based on ML analyses of short *rbcL* sequences (263–296 bp). Branch numbers indicate bootstrap values out of 1,000 replicates. Newly generated sequences shown in boldface. Stars indicate type specimens, diamonds indicate topotype specimens.

**Figure S2.** Phylogeny based on ML analysis of medium and long *rbcL* sequences (694–1,387 bp). Branch numbers indicate bootstrap values out of 1,000 replicates. Newly generated sequences shown in boldface. Stars indicate type specimens, diamonds indicate topotype specimens.

**Figure S3.** Phylogram based on ML analyses of *psbA* (863 bp). Branch numbers indicate bootstrap values out of 1,000 replicates. Newly generated sequences shown in boldface. Stars indicate type specimens, diamonds indicate topotype specimens.

**Figure S4.** Phylogram based on ML analyses of LSU (550 bp). Branch numbers indicate bootstrap values out of 1,000 replicates. Newly generated sequences shown in boldface. Stars indicate type specimens, diamonds indicate topotype specimens.

**Figure S5.** Phylogram based on ML analyses of COI (661 bp). Branch numbers indicate bootstrap values out of 1,000 replicates. Newly generated sequences shown in boldface. Stars indicate type specimens, diamonds indicate topotype specimens.

**Figure S6.** Phylogram based on ML analyses of UPA (369 bp). Branch numbers indicate bootstrap values out of 1,000 replicates. Newly generated sequences shown in boldface. Stars indicate type specimens, diamonds indicate topotype specimens.

**Figure S7.** Distribution of raw pair wise distances for each of the five markers analyzed in this study. Divergence values for *rbcL* presented for both short (296 bp) and medium length (578 bp) alignments.

**Figure S8.** *Sporolithon molle*, NCU 606657. Unshed tetraspergal compartments showing layer of neighboring elongate basal cells (arrows). Image shows the same tetraspergal compartments as Figure 6D in the mirroring section of the same fragment.

**Figure S9.** *Sporolithon molle*, NCU 606657. Layer of unshed tetraspergal compartments showing one with intact tetrasperrangium and apical pore plug (arrow).
Table S1. List of GenBank numbers for taxa included in analyses. Sequences included in the concatenated analyses shown in italics. N.A. = data not available. * = sequence not analyzed in present study.

Table S2. Divergence values (as %) for rbcL (296 bp).

Table S3. Divergence values (as %) for rbcL (578 bp).

Table S4. Divergence values (as %) for psbA (862 bp).

Table S5. Divergence values (as %) for LSU (446 bp).

Table S6. Divergence values (as %) for COI (661 bp).

Table S7. Divergence values (as %) for UPA (368 bp).