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Asia Pacific *Sporolithon* (Corallinophycidae, Rhodophyta) species revised based on DNA sequencing of type specimens and including *S. crypticum sp. nov.*, *S. immotum sp. nov.* and *S. nodosum sp. nov.*

Paul W. Gabrielson [1], Jeffery R. Hughey [2], Viviana Peña [3,4], Joseph L. Richards [5], Gary W. Saunders [6], Brenton Twist [7,8], Tracy Farr [8] and Wendy A. Nelson [8,9]

[1]*Biology Department and Herbarium, Coker Hall CB 3280, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, 27599-3280, USA*
[2]*Division of Mathematics, Science, and Engineering, Hartnell College, 411 Central Ave., Salinas, California 93901, USA*
[3]*BioCost Research Group Universidade da Coruña, Facultad de Ciencias, campus de A Zapateira S/N, 15071, A Coruña, Spain*
[4]*Institut de Systématique, Évolution, Biodiversite (ISYEB), Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, 57 rue Cuvier, CP 39, 75005, Paris, France*
[5]*Department of Biology, University of Louisiana at Lafayette, Lafayette, LA 70504-3602 USA*
[6]*Department of Biology, Centre for Environmental & Molecular Algal Research, University of New Brunswick, Fredericton, New Brunswick E3B5A3, Canada*
[7]*Department of Botany and Beaty Biodiversity Research Centre, University of British Columbia, 3200-6270 University Boulevard, Vancouver, British Columbia, V6T 1Z4, Canada*
[8]*National Institute of Water and Atmospheric Research, Private Bag 14901, Wellington 6241, New Zealand*
[9]*School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand*

ABSTRACT

To address the taxonomic uncertainty of *Sporolithon* species named in the early to mid-20th century, targeted PCR sequencing was performed on eight historical type specimens and on recently collected specimens. Six type specimens amplified for the *rbc*L gene and were Sanger sequenced yielding sequences ranging in length from 118 to 280 base pairs (bp). One, *S. australasicum*, failed to amplify and another, *S. howei*, was amplified for the *psb*A gene yielding a sequence 544 bp in length. The 118 bp long *rbc*L sequence of the lectotype of *S. crassiramosum* showed that it is a later, heterotypic synonym of *S. molle*. The *rbc*L sequences of type specimens of *S. episoredion, S. schmidtii, S. sibogae* and *S. timorense* ranged from 118 to 228 bp, and each is a distinct species. The 544 bp long *psb*A sequence of *S. howei* is also unique. The 280 bp long *rbc*L sequence of the lectotype of *S. durum* did not match any sequence with that name in any public repository, including the previously published complete plastome and mitogenome sequences. However, it was identical in sequence to a specimen in GenBank from the southern coast of Western Australia as well as several other sequences generated from field-collected specimens from the states of South Australia and Western Australia. The rhodolith specimens from New Zealand previously called *S. durum* are *S. nodosum sp. nov*. The species is endemic to New Zealand. The epilithic specimens from New Zealand previously called *S. durum* are *S. immotum sp. nov*., which is also found along the southeastern coast of Australia. *Sporolithon crypticum sp. nov*. is described from the southern coast of Western Australia. RAxML and Bayesian phylogenetic analyses of *Sporolithon psb*A and *rbc*L sequences are congruent between the two plastid encoded genes. DNA sequencing of type specimens of species of corallines is demonstrated to be the only reliable method to correctly apply names.

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INTRODUCTION

Sporolithales is a distinct order of Corallinophycidae based on both phylogenetic analyses of DNA sequences, including single marker and concatenated analyses of LSU, *psb*A, *rbc*L and SSU (Le Gall *et al.* 2010; Nelson *et al.* 2015; Richards *et al.* 2017, 2022; Richards & Fredericq 2018; Peña *et al.* 2020), and on two morpho-anatomical features: production of tetrasporangia singly within calcified sporangial compartments and cruciate cleavage of each tetrasporangium (Le Gall *et al.* 2010). Based on phylogenetic analyses of *psb*A and *rbc*L sequences, three genera are currently recognized, namely *Heydrichia* R.A. Townsend, Y.M. Camberlain & Keats, *Roseapetra* W.A. Nelson, Twist & K.F. Neill and *Sporolithon* Heydrich, along with several morpho-anatomical characters that distinguish both *Heydrichia* and *Roseapetra* from *Sporolithon*, but none that distinguish *Heydrichia* from *Roseapetra* (Nelson *et al.* 2021). At the generic rank, diversity is greatest in the southern hemisphere where all three genera occur; only *Sporolithon* has been reported from the northern hemisphere. Species in this order occur as rhodoliths and/or are epilithic or are only epizoic; none are epiphytic. All are marine, occurring from the intertidal to the deep subtidal, and are found in waters ranging from cool temperate

CONTACT Paul W. Gabrielson drseaweed@hotmail.com
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to tropical, but are absent from high latitude cold waters (Guiry & Guiry 2022).

Roseapetra is monotypic and reported only from New Zealand (Nelson *et al.* 2021). *Heydrichia* is comprised of only four extant and one fossil species. Three of the extant species, *H. cerasina* Maneveldt & E. van der Merwe, *H. groeneri* Keats & Y.M. Chamberlain and the generitype, *H. woelkerlingii* R.A. Townsend, Y.M. Chamberlain & Keats, occur in South Africa, and one, *H. homalopasta* R.A. Townsend & Borowitzka, has its type locality in Australia and was also reported from New Zealand. *Sporolithon* is the most speciose with 29 extant species and 13 fossil species (Guiry & Guiry 2022). Of the 29 extant species, 13 have had their type specimens sequenced. Richards *et al.* (2017) demonstrated that only by sequencing type specimens can species names be correctly applied in this genus. Based on DNA sequencing of type specimens, Richards *et al.* (2017) found that the name *S. ptychoides* Heydrich, the generitype species (type locality: El Tor, Egypt), was misapplied in Brazil (Bahia *et al.* 2011), Hawai'i, USA (Sherwood *et al.* 2010) and New Caledonia (Bittner *et al.* 2011), and further that the name *S. molle* (Heydrich) Heydrich (type locality: El Tor, Egypt, Gulf of Suez) was misapplied in Brazil (Bahia *et al.* 2014), and that *S. dimotum* (Foslie & M. Howe) Yamaguishi-Tomita *ex* M.J. Wynne (type locality: Lemon Bay near Guánica, Puerto Rico) was incorrectly placed in synonymy by Bahia *et al.* (2011) under *S. ptychoides*. The first report of *Sporolithon* from New Zealand (Harvey *et al.* 2005) noted the presence of both a free-living rhodolith form and an immobile epilithic form growing attached to fixed rock substrate. This was consistent with *S. durum sensu* Harvey *et al.* (2002) from south-eastern Australia. However, DNA sequencing consistently supported the two ecological forms from New Zealand as separate taxa (Broom *et al.* 2008; Farr *et al.* 2009; Twist *et al.* 2019). The application of names based on perceived morpho-anatomical differences has been an abject failure. Based on these earlier results, we have sought to sequence all remaining type specimens of *Sporolithon* species.

Herein, we report our successes and failures attempting to sequence the following type specimens of *Sporolithon* species: *S. autralasicum* (Foslie) Yamaguishi-Tomita *ex* M. J. Wynne (type locality: Cape Jaffa, South Australia, Australia), *S. crassiramosum* (Pilger) P.C. Silva (type locality: Juan de Nova Island, Mozambique Channel), *S. durum* (Foslie) R.A. Townsend & Woelkerling (type locality: Cape Jaffa, South Australia, Australia); *S. episoredion* (W.H. Adey, R.A. Townsend & Boykins) Verheij (type locality: St Rogatien Bank, Northwest Hawai'ian Islands, USA), *S. howei* (Me. Lemoine) Yamaguishi-Tomita *ex* M.J. Wynne [type locality: Isla Coiba, Gulf of Panama, Panama (Pacific Ocean)], *S. schmidtii* (Foslie) G.D. Gordon, T. Masaki & Akioka (type locality: Ko Kahdat, Ko Chang Archipelago, Thailand), *S. sibogae* (Weber Bosse & Foslie) P.C. Silva (type locality: Pearl Bank, Sulu Archipelago, Indonesia) and *S. timorense* (Foslie) P.C. Silva [type locality: Pulau Sailus-besar, Paternoster Islands (Kepulauan Tengah), Indonesia]. Any type specimen of an extant species of *Sporolithon* not in this list has already been sequenced or, to our knowledge, sequencing has not yet been attempted. We also name and describe three new species, two of them previously assigned to *S. durum*.

MATERIAL AND METHODS

Recent field-collected specimens were obtained intertidally or subtidally by snorkeling or SCUBA. Specimens were removed by hand or with a hammer and chisel. Historical type collections were examined on site or received on loan from L, PC, TRH and US; herbarium acronyms follow Thiers (2023). Vouchers of recently collected specimens were deposited at NCU, UNB or WELT. Type material was extracted and amplified at two different institutions: the Muséum National d'Histoire Naturelle, Paris (MNHN) and Hartnell College (HC). Field-collected material was extracted and amplified at the National Institute of Water and Atmospheric Research (NIWA), the University of New Brunswick (UNB), and the University of North Carolina, Chapel Hill (UNC). Extractions and amplifications of types and historical collections were performed separate from recent collections, and they were accompanied by negative controls in every step. At the MNHN, DNA of one type specimen was extracted using QIAamp®DNA Micro Kit (Qiagen S.A.S., Les Ulis, France) following the manufacturer's protocol for tissues. Samples collected from around the New Zealand coastline were treated as detailed in Twist *et al.* (2019). Subsamples dried in silica gel were extracted using the Qiagen DNeasy Blood and Tissue DNA Extraction Kit (Qiagen GmbH, Hilden) following slight modifications to manufacturer's protocols. At UNC recent collections were extracted following Gabrielson *et al.* (2011); at HC type material was extracted according to Hernández-Kantún *et al.* (2016) following the guidelines proposed by Hughey & Gabrielson (2012); at UNB recent collections were extracted following Saunders & McDevit (2012). Two genes (*rbc*L and *psb*A) were amplified in this study. For type specimens and historical collections, *rbc*L sequences were obtained at HC with two primer combinations, F1150Cor-R1460 or F1150Cor-R1308 yielding a fragment trimmed to a maximum of 263 bp (1172–1434) or 118 bp (1172–1290), respectively; for recent collections at NIWA, DNA was amplified with psbA-F1 and psbA-R1/psbA-R2 for *psb*A (Yoon *et al.* 2002) and for *rbc*L in two parts using F57 paired with R1150, and F753 with RrbcL-Start (Freshwater & Rueness 1994); at UNC, *rbc*L sequences of 1383 bp were obtained with two overlapping primer combinations F57-R1150 and F753-RrbcS and at UNC *rbc*L sequences of 691 bp with primer combination F753/RrbcS-Start trimmed to 691 bp (772–1464); at UNB, *rbc*L sequencing followed Saunders & Moore (2013); at UNC, DNA was amplified using the primer pair psbA-F1/psbA-R2 following Adey *et al.* (2015); at MNHN, using the primer pairs psbA-F1/psbA-R2 and psbA-F1/psbA-600R (Yoon *et al.* 2002), following Peña *et al.* (2015). At the MNHN, PCR products were purified and sequenced by Eurofins (Eurofins Scientific, Nantes, France); at NIWA, PCR products were purified according to Twist *et al.* (2019) and sequenced by Macrogen Inc. (Seoul, Korea); at UNC, PCR products were purified according to Hughey *et al.* (2001) and

sequenced at the DNA Analysis Core Facility, Center for Marine Sciences, University of North Carolina, Wilmington; at HC, PCR products were purified and sequenced by Functional Biosciences, Inc. (Madison, Wisconsin, USA); at UNB following Saunders & Moore (2013). Sequences were assembled and aligned with the assistance of CodonCode Aligner® (CodonCode Corporation, USA) or with Sequencher (Gene Codes Corp., Ann Arbor, Michigan, USA) and adjusted manually using MUSCLE as implemented in Geneious Prime (2020.2.4, Biomatters, Auckland, New Zealand); they were submitted to GenBank (accession numbers listed in Table S1).

The *rbc*L and *psb*A datasets were constructed using previously published sequences from GenBank and new sequences generated in this study (Table S1). Sequences in the *rbc*L dataset ranged from 118 to 1387 bp in length; *psb*A sequences from 509 to 850 bp. For the *rbc*L dataset, two taxa from the order Rhodogorgonales were used as outgroups, *Rhodogorgon ramosissima* J.N. Norris & Bucher and *Renouxia marerubra* D. Gabriel, J.N. Norris & Fredericq; for the *psb*A dataset only *R. ramosissima* was used. The dataset was compiled and aligned using MUSCLE (Edgar 2004) as implemented in Geneious Prime (2020.2.4, Biomatters, Auckland, New Zealand).

Phylogenetic reconstructions with maximum likelihood (ML) and Bayesian inference (BI) were carried out using the RAxML (Stamatakis 2014) and Mr. Bayes (Huelsenbeck & Ronquist 2001) Geneious Prime plugins, respectively. The RAxML analyses were performed using the GTR + CAT + I model and Rapid hill-climbing algorithm for 20 random trees to determine the best starting tree for determining node confidence. Node confidence was assessed by 1,000 bootstrap replications and search for best-scoring ML tree. Bayesian analyses were performed using the GTR + gamma + I sites model with four heated Monte-Carlo Markov Chains for 1,000,000 generations, sampling every 750 generations and with a burn-in length value of 250,000 generations.

Specimens for WELT were prepared for anatomical examination following methods described by Harvey *et al.* (2005). Small samples were decalcified in 0.6 M HNO_3, stained with 5% aqueous $KMnO_4$, dehydrated through step changes to 100% ethanol, then embedded in 'LR White' resin (London Resin Co., Reading, Berkshire, England). Permanent slides for light microscopy were prepared with 10-µm sections cut with a slide microtome (American Optical, Buffalo, New York), cleared with HistoClear II (National Diagnostics, Atlanta, Georgia, USA) and mounted with Eukitt (Kindler, Germany). Permanent slides were examined by light microscopy using a Zeiss Axiovert 200 microscope (Zeiss, Jena, Germany) and photomicrographs taken with a Zeiss Axiocam HR digital camera. All slides were deposited with voucher collections held at WELT.

At UL Lafayette, thallus habit images were obtained with a Canon PowerShot A3300 camera. Surface views of conceptacles were observed using a Zeiss Stemi 2000-C dissecting microscope and images were captured with a Canon Rebel Eos T2-I mounted on the microscope. For scanning electron microscopy, material was removed from desiccated specimens using a single edge razor blade or wire cutter tool, and vertical fractures were performed using a new single edge razor blade for each fracture. Fractured pieces were mounted with forceps onto aluminium stubs using conductive adhesive tape and liquid graphite, then coated with 18 nm of gold to prevent charging. Specimens were viewed with a Scios 2 Dual Beam Focused Ion Beam scanning electron microscope (FIB-SEM) at an accelerating voltage of 15 kV.

For cell measurements, lengths denote the distance between primary pit connections, and diameter the maximum width of the cell lumen at right angles (Irvine & Chamberlain 1994). Conceptacle measurements followed Adey & Adey (1973). The determination and presentation (i.e. ranges and not averages with standard deviations/errors) of all measurements followed Maneveldt *et al.* (2017). Thallus anatomical terminology followed Irvine & Chamberlain (1994). Morphological (growth forms) terminology followed Woelkerling *et al.* (1993).

RESULTS

Phylogenetic analyses

Separate *rbc*L (Fig. 1) and *psb*A (Fig. 2) phylograms were inferred, as six additional *Sporolithon* species are present in the *rbc*L phylogram, namely *S. crassiramosum, S. dimotum, S. episoredion, S. schmidtii, S. sibogae* and *S. timorense*, although one, *S. crassiramosum*, is a heterotypic synonym of *S. molle*. No *psb*A sequences are available for these taxa, and indeed all are known only based on unique *rbc*L sequences from their type specimens. A different species, *S. howei*, is only present in the *psb*A phylogram and is also known only from its type specimen sequence. Nevertheless, there is strong congruence in the phylogenetic relationships among the taxa that are present in both phylograms (Figs 1, 2). In both phylograms, there is full support in the Bayesian analyses and strong support in RAxML [92% in *rbc*L phylogram (Fig. 1); 90% in *psb*A phylogram (Fig. 2)] for a clade consisting of *S. crypticum sp. nov.* (south coast of Western Australia), *S. eltorense* J.L. Richards & P.W. Gabrielson (Gulf of Suez) and *S. franciscanum* L.A.S. Leão & Bahia (Brazil) that in *rbc*L also includes *S. dimotum* (Puerto Rico) and *S. sibogae* (Indonesia); full (*psb*A) to strong (*rbc*L) support for a clade of *S. amadoi* J.L. Richards & Bahia (Brazil), *S. molle* (Gulf of Suez and Mozambique Channel) and *S. ptychoides* (Gulf of Suez) that in the *rbc*L phylogram also includes *S. episoredion* (Hawai'i), *S. schmidtii* (Thailand) and *S. timorense* (Timor Islands); a fully supported clade in both phylograms of *S. episporum* (M. Howe) E.Y. Dawson, *S.* '*erythraeum*' (Taiwan) and *S. indopacificum* Maneveldt & P.W. Gabrielson; a fully supported clade in both phylograms of *S. durum* (south coast of Australia) and *S. nodosum sp. nov.* (New Zealand), which in *rbc*L also includes an undescribed *Sporolithon* species from Jervis Bay, New South Wales, Australia, that previously was incorrectly named *S. durum* (Harvey *et al.* 2002); and in both phylograms a fully supported clade that contains the same species, *S. gracile* J.L. Richards, Kittle & Fredericq (Gulf of Mexico), *S. mesophoticum* J.L. Richards, P.W. Gabrielson & C.W. Schneider (Bermuda), *S. sinusmexicanum* J.L. Richards & Fredericq (Gulf of Mexico) and *S. yoneshigueae* Bahia, Amado-Filho, Maneveldt & W.H. Adey (Brazil). The sister taxon relationships in both phylograms of *S. immotum sp. nov.* (New Zealand

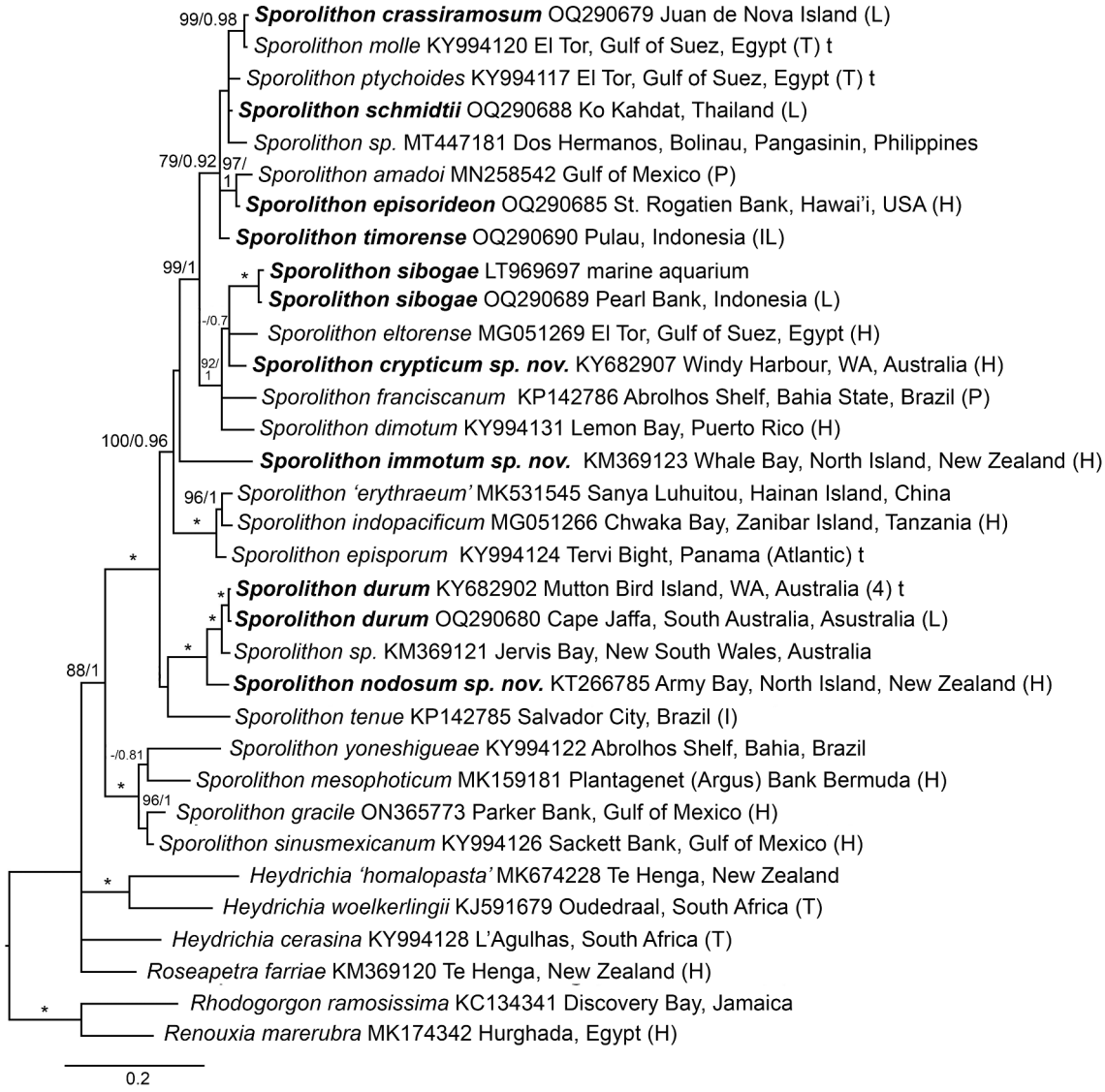


Fig. 1. Phylogram of Sporolithales inferred by RAxML and Bayesian analyses of *rbc*L sequences; outgroups *Rhodogorgon* and *Renouxia* species (Rhodogorgonales). New species and newly sequenced historical type specimens are in bold. GenBank accession number and locality provided. Capital letters in parentheses are: H, holotype; I, isotype; IL, isolectotype; L, lectotype; P, paratype; T, topotype (specimen collected at type locality). Sequences linked to type specimen are marked 't'. Numbers in parentheses indicate the total number of sequences for new species and *Sporolithon durum*. Bootstrap percentages (1,000 replications) and Bayesian posterior probability values are shown at nodes when >75% and >0.7, respectively; nodes with maximal support are marked with *.

and Australia) and *S. tenue* Bahia, Amado-Filho, Maneveldt & W. H. Adey (Brazil) is unsupported.

Historical type specimens

Sporolithon australasicum (Foslie) Yamaguishi-Tomita*ex* M.J. Wynne (1986, p. 2258)

BASIONYM: *Archaeolithothamnion australasicum* Foslie (1907, p. 12).

LECTOTYPE: (designated by Adey in Adey & Lebednik 1967, p. 84): TRH C19-3370, collected in 1900 by A. Engelhart at Cape Jaffa, South Australia, Australia (no habitat data).

COMMENT: Attempts to obtain *rbc*L sequences from the lectotype specimen failed. Townsend *et al.* (1995) noted that this lectotype specimen is a rhodolith and lacked organic remains, the latter perhaps explaining our failure to obtain any DNA sequence. Based on the morpho-anatomy completed by Townsend *et al.* (1995), the specimen appears to belong to *Sporolithon*, but could not be assigned to any species. Perhaps future workers will be able to obtain a DNA sequence that will allow this name to be correctly applied. Meanwhile, it should not be used as its correct application is unknowable.

Sporolithon durum (Foslie) R.A. Townsend & Woelkerling in Townsend *et al.* (1995, p. 86, figs 1–17)

BASIONYM: *Archaeolithothamnion durum* Foslie (1907, p. 11).

LECTOTYPE: (designated by Adey in Adey & Lebednik 1967, p. 84): TRH C19-3381, collected in 1899 by A. Engelhart at Cape Jaffa, South Australia, Australia (no habitat data). [Specimen is a rhodolith (Townsend *et al.* 1995)].

COMMENT: The 280 bp *rbc*L sequence (GenBank accession number: OQ290680) from the lectotype specimen of *S. durum* is identical over its length to five specimens from South Australia and Western Australia, all occurring along the southern Australian coast (Table S1). The *rbc*L sequence from the lectotype of *S. durum* differs from three

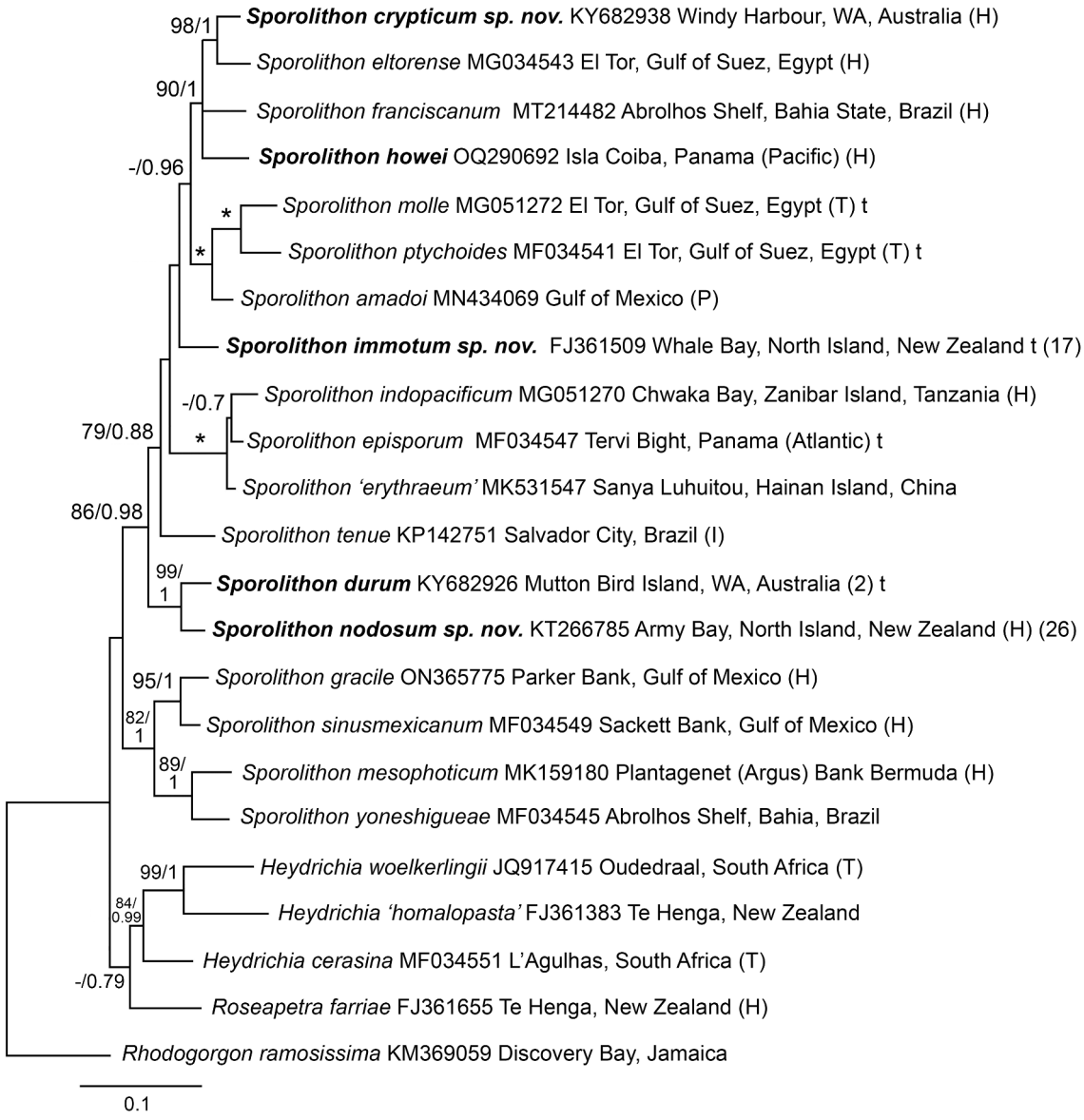


Fig. 2. Phylogram of Sporolithales inferred by RAxML and Bayesian analyses of *psb*A sequences; outgroup *Rhodogorgon ramosissima* (Rhodogorgonales). New species and newly sequenced historical type specimens are in bold. GenBank accession number and locality provided. Capital letters in parentheses are: H, holotype; I, isotype; P, paratype; T, topotype (specimen collected at type locality). Sequences linked to type specimen are marked 't'. Numbers in parentheses indicate the total number of sequences for new species and *Sporolithon durum*. Bootstrap percentages (1,000 replications) and Bayesian posterior probability values are shown at nodes when >75% and >0.7, respectively; nodes with maximal support are marked with*.

sequences deposited in GenBank that were identified previously based on morpho-anatomy as *S. durum*. From LTB 21140 from Jervis Bay, New South Wales (currently an undescribed species), the lectotype sequence differs over its length by 6 bp (2.1% sequence divergence), from *S. nodosum sp. nov.* (see below) from New Zealand by 13 bp (4.6% sequence divergence), and from *S. immotum sp. nov.* (see below) from Australia and New Zealand by 28 bp (10% sequence divergence).

DISTRIBUTION: Based on DNA sequenced specimens along the southern coast of Australia from Cape Jaffa, South Australia in the east to the mainland across from Shelter Island, Western Australia in the west (Fig. 3).

Sporolithon episoredion (W.H. Adey, R.A. Townsend & Boykins) Verheij (1992, p. 501)

BASIONYM: *Archaeolithothamnion episoredion* W.H. Adey, R.A. Townsend & Boykins (1982, p. 51, fig. 35).

HOLOTYPE: US 190919, collected in August 1971 by D. Child at St. Rogatien Bank, Northwest Hawai'i Islands, USA; rhodolith, 70–95 m depth.

COMMENT: The 228 bp *rbc*L sequence of the holotype of *S. episoredion* (GenBank accession number: OQ290685) differs unambiguously over its length by 5 bp (2.2% sequence divergence) from *S. amadoi*, its closest relative (Fig. 1). Only the holotype specimen was sequenced; paratype specimens are reported from other localities in the Hawai'ian Islands and need to be sequenced. The observation of zonately divided tetrasporangia in one of the paratype specimens also needs to be re-investigated (Adey *et al.* 1982). It is possible that this is a mixed collection, but the holotype specimen clearly belongs in *Sporolithon*.

DISTRIBUTION: Known only from the type locality in the Hawai'ian Islands (Fig. 3).

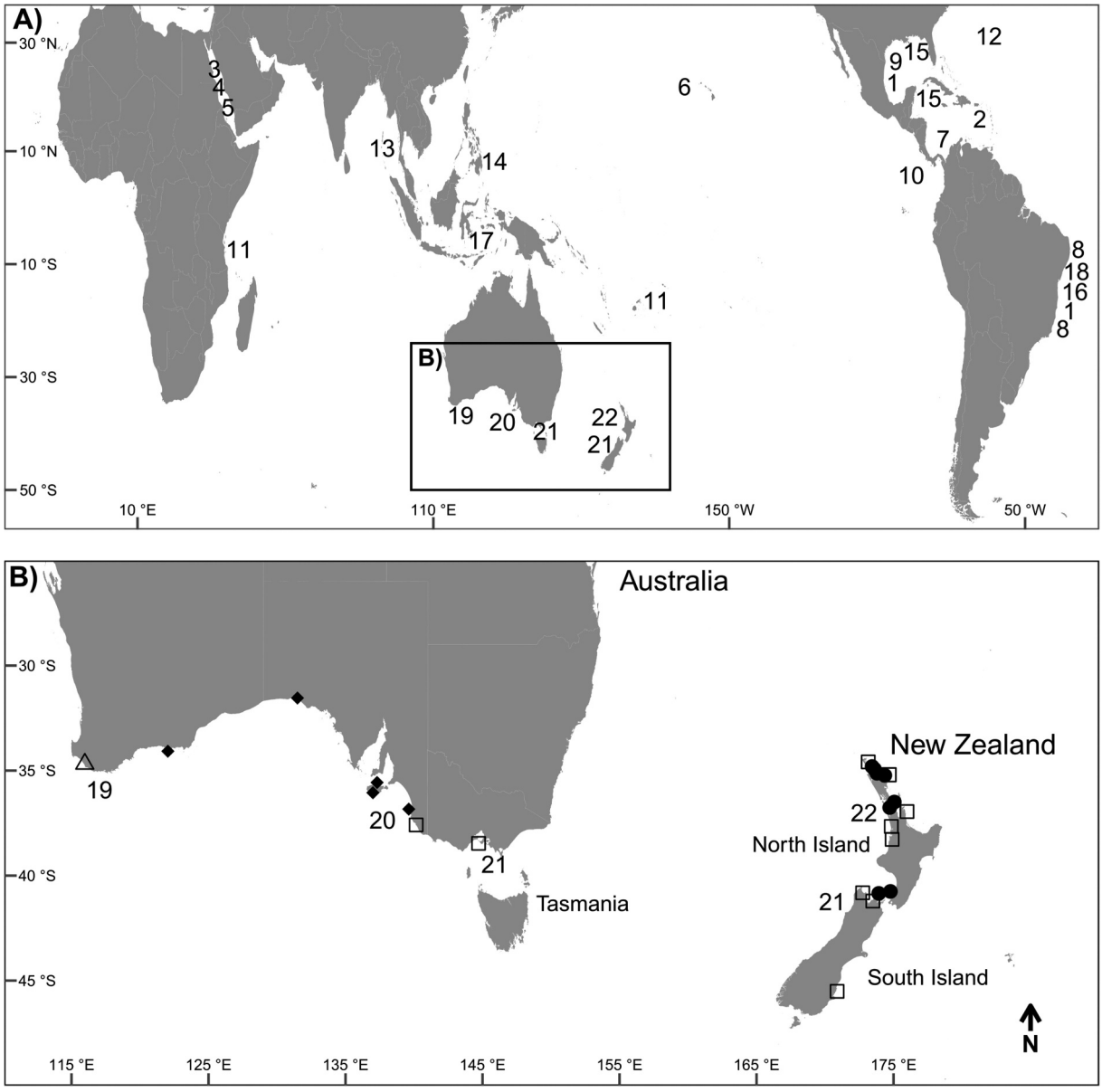


Fig. 3. Map of *Sporolithon* species whose type specimens have been sequenced: A) globally, and B) within the NZ and Australia region. Numbers and symbols correspond to the following species: 1, *S. amadoi*; 2, *S. dimotum*; 3, *S. eltorense*; 4, *S. molle*; 5, *S. ptychoides*; 6, *S. episoredion*; 7, *S. episporum*; 8, *S. franciscanum*; 9, *S. gracile*; 10, *S. howei*; 11, *S. indopacificum*; 12, *S. mesophoticum*; 13, *S. schmidtii*; 14, *S. sibogae*; 15, *S. sinusmexicanum*; 16, *S. tenue*; 17, *S. timorense*; 18, *S. yoneshigueae*; 19 and Δ, *S. crypticum*; 20 and ♦, *S. durum*; 21 and □, *S. immotum*; 22 and ●, *S. nodosum*.

Sporolithon howei (Me. Lemoine) Yonishigue-Tomita*ex* M. J. Wynne (1986, p. 2258)

BASIONYM: *Archaeolithothamnion howei* Me. Lemoine (1929, p. 40, figs 1, 2; pl. I, fig. 4).

HOLOTYPE: PC 0731949, undated, collected by C. Crossland at Isla Coiba, Gulf of Panama (Pacific Panama); attached to the substratum, dredged 9 m depth.

COMMENT: The 544 bp *psb*A sequence from the holotype of *S. howei* (GenBank accession number: OQ290692) differs from its most closely related species, *S. franciscanum* by 38 bp (7.5% sequence divergence) where these sequences overlap each other (506 bp). Aside from the original description by Lemoine (1929) the only other reports of this species are in checklists or keys from the eastern Pacific (Dawson 1961), from Pacific Central America (Dawson 1962; Fernández-García *et al.* 2011), or from Panama (Earle 1972).

DISTRIBUTION: Based on the DNA sequencing, known only from the type locality (Fig. 3).

Sporolithon molle (Heydrich) Heydrich (1897b, p. 416)

BASIONYM: *Sporolithon ptychoides* f. *molle* Heydrich (1897a, p. 67, pl. III, figs 16, 18, 19; as '*mollis*').

HOMOTYPIC SYNONYM: *Archaeolithothamnion erythraeum* f. *molle* (Heydrich) Foslie (1904, p. 38; as '*mollis*').

HETEROTYPIC SYNONYM: *Sporolithon crassiramosum* (Pilger) P.C. Silva in Silva *et al.* (1996, p. 276) – basionym: *Archaeolithothamnion crassiramosum* Pilger (1908, p. 39, pl. 5, figs 1–3).

LECTOTYPE (DESIGNATED HEREIN): TRH C19-3376, collected in 1894 by Voeltzkow at Juan de Nova Island (Mozambique Channel); no habitat data.

COMMENT: Woelkerling (1993, p. 66) and Woelkerling *et al.* (2005, p. 476) indicated that a type specimen had not been designated for

S. crassiramosum and that a syntype specimen was located in TRH. It is this specimen, TRH C19-3376, that was sequenced and that we designate as the lectotype. Woelkerling (1993, p. 66) noted that the fragment was 9 mm long at its largest dimension. Two fragments were present in 2022, both about 3 mm^3 (Fig. S1). One of these was cut in half to obtain the extracted DNA. Pilger's illustrations (1908, pl. 5, figs 1, 2) of tetrasporangial compartments clearly indicate that this species belongs in the Sporolithales, and his illustration of cell fusions (Pilger 1908, pl. 5, fig. 3) is not at odds with the ordinal circumscription. The 118 bp *rbc*L sequence (GenBank accession number: OQ290679) is identical over its length to the 296 bp sequence (GenBank accession number: KY994121) of the lectotype (C A92529) and a topotype specimen of *S. molle* (Richards *et al.* 2017). While the sequence is short, all other Sporolithales species with *rbc*L sequences differ by a minimum of 7 bp (5.9% sequence divergence) over this variable 118 bp region of *rbc*L.

DISTRIBUTION: Based on DNA sequences, confirmed from two localities, El Tor, Egypt, Gulf of Suez, and Juan de Nova Island, Mozambique Channel (Fig. 3).

Sporolithon schmidtii (Foslie) G.D. Gordon, T. Masaki & Akioka (1976, p. 250, pl. 1, figs 1–4)

BASIONYM: *Archaeolithothmnion schmidtii* Foslie (1901a, p. 16).

HOLOTYPE: TRH C19-3421, collected 15 February 1900 by Johs. Schmidt at Ko Kahdat, Ko Chang Archipelago, Thailand; on dead coral tines, 9 m depth.

COMMENT: Foslie (1904, pl. VIII, figs 15–17) illustrated for the first time the holotype of *Archeolithothamnion schmidtii*. It was from the specimen photographed in figure 15 in Foslie (1904, pl. VIII) that the 118 bp *rbc*L sequence was obtained (GenBank accession number: OQ290688). The sequence differs from the most closely related species, *S. ptychoides*, by 3 bp (2.5% sequence divergence) over its short length. If these were the same species, it would be expected that the sequences in this variable region would be identical. Foslie (1903) recognized a new variety, *Archaeolithothamnion schmidtii* f. *dissitum* Foslie, based on a specimen collected by Stanley Gardiner from 66 m deep from South Nilandu Atoll in the Maldives. We have not included this name as a heterotypic synonym of *S. schmidtii* as we do not know its identity based on DNA sequences, and morpho-anatomy cannot be used to reliably identify coralline specimens to species.

DISTRIBUTION: Based on a DNA sequence of the holotype known only from the type locality (Fig. 3).

Sporolithon sibogae (Weber Bosse & Foslie) P.C. Silva in Silva *et al.* (1987, p. 39)

BASIONYM: *Archaeolithothamnion sibogae* Weber Bosse & Foslie in Foslie (1901b, p. 3).

LECTOTYPE: TRH C19-3426, Weber van Bosse expedition, collection 297, station 96, collected 9 May 1899 by A. Weber van Bosse at Pearl Bank (southeast side), Sulu Archipelago, Philippine Islands; rhodolith, 15 m depth.

COMMENT: Adey (1970) designated the material cited in Foslie (1901b) as the 'holotype', which Verheij & Woelkerling (1992) corrected to lectotype. It was from this lectotype specimen (Fig. S2) in TRH (C19-3426) that we obtained a 118 bp *rbc*L sequence (GenBank accession number: OQ290689) that is distinct from all other *Sporolithon rbc*L sequences by a minimum of 7 bp (5.9% divergence) over the length of the sequence, with *S. crypticum sp. nov.* being most similar. There is a sequenced specimen in GenBank (LT969679) that matches *S. sibogae* (Fig. 1), but it is from a specimen in a marine aquarium with an apparently unknown provenance. Verheij (1993) reported four *Sporolithon* species from the Spermonde Archipelago, Indonesia, based on morpho-anatomy, namely *S. episoredion, S. episporum, S. molle* and *S. ptychoides*. However, this locality is over 1,200 km south of the type locality of *S. sibogae*, and in the absence of DNA sequences we do not know if any of these names are correctly applied.

DISTRIBUTION: Based on a DNA sequence of the lectotype, known only from the type locality (Fig. 3). Based on morpho-anatomy, a single report from Madagascar (Pichon 1978) that requires DNA confirmation.

Sporolithon timorense (Foslie) P.C. Silva in Silva *et al.* (1987, p. 39)

BASIONYM: *Archaeolithothamnion timorense* Foslie (1904, p. 42, pl. VIII, figs 1–14).

LECTOTYPE: (designated by Verheij & Woelkerling 1992): L 935.20713 (Siboga Expedition collection #443), collected 17-18 February 1900 by A. Weber van Bosse at Paternoster Islands (Kepulauan Tengah), Pulau Sailus-besar, Indonesia.

COMMENT: Verheij and Woelkerling (1992) proposed a new lectotype (L 935.207-13, Siboga Expedition collection #443) for *A. timorense*, arguing that the specimen in TRH selected by Adey (in Adey & Lebednik 1967, p. 85) was sterile, and therefore in serious conflict with the protologue where Foslie (1904) described sporangia. This single specimen in L was too small to be sent for DNA analysis, so one of 17 isolectotype specimens was sent for DNA sequencing. From this isolectotype specimen, L 935.207-13 Siboga Expedition collection #446 (Fig. S3), a 118 bp length *rbc*L sequence (GenBank accession number: OQ290690) was obtained that differs across its length by 3 bp (2.5% sequence divergence) from *S. schmidtii*, 5 bp (4.2% sequence divergence) from *S. ptychoides* and 6 bp (5% sequence divergence) from *S. amadoi*, its closest relatives. We do not know if the lectotype or other isolectotype specimens are this same species.

DISTRIBUTION: Based on DNA sequences, known only from the type locality (Fig. 3); based on morpho-anatomy a single report from the Philippine Islands (Velasquez *et al.* 1975).

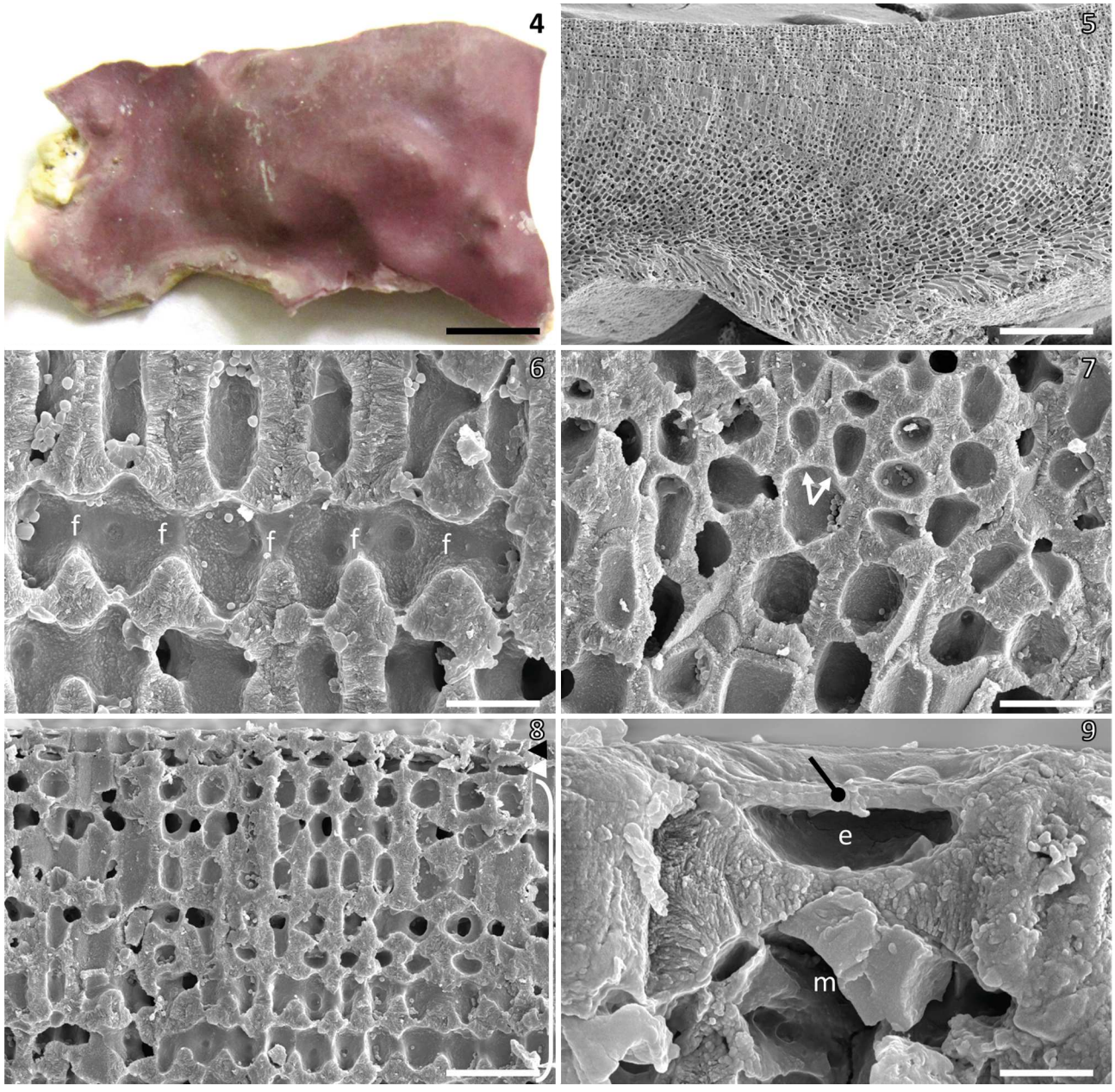
New Species

Sporolithon crypticum G.W. Saunders, J.L. Richards & P.W. Gabrielson *sp. nov.*

Figs 4–15

DESCRIPTION: Thallus habit encrusting, epilithic, lumpy to relatively smooth (Fig. 4). Thallus construction monomerous, with a well-developed plumose hypothallus comprised of 8–12 layers of filaments with rectangular hypothallial cells that grow perpendicular to the substratum; some hypothallial cell files curving downward toward substratum (Fig. 5). Perithallus with abundant cell fusions, often laterally joining multiple adjacent perithallial cell files, cells 6.0–16.5 µm long × 5–10 µm wide (Fig. 6). Pseudodichotomous branching observed in perithallus (Fig. 7). Intercalary meristematic cells shorter than wide or approximately isodiametric, 4–8 µm long × 7–8 µm wide (Figs 8, 9). Epithallus comprised of a single layer of epithallial cells that are 2–3 µm long × 5.5–7.0 µm wide, flared and armoured with highly calcified lateral cell walls, and a thin epithallial cell roof (Figs 8, 9). Uniporate gametangial conceptacles raised above thallus surface (Figs 10, 11). Some uniporate conceptacles observed nearby other uniporate conceptacles, with conceptacle chambers and pores observed directly next to each other in surface views and section views (Figs 11–13), with conceptacle chambers 200–275 µm in diameter. Other, larger conceptacles (chambers up to 600 um diameter), developed further away from other conceptacles (Figs 11, 14, 15). DNA sequences deposited in GenBank, holotype: COI, KY682899; CYTB, KY682920; nLSU, KY682891; *psb*A, KY682938; *rbc*L, KY682907; nSSU, KY682883. Sequences also deposited in BOLD: COI, OZSEA082-10; isotype COI, OZSEA083-10.

HOLOTYPE: UNB GWS024693, collected 8 November 2010 by G.W. Saunders & K. Dixon at Windy Harbour (34°50.249′S, 116°0.855′E), Western Australia, Australia; epilithic, at 3 m depth.



Figs 4–9. Vegetative morpho-anatomy of *Sporolithon crypticum sp. nov*. holotype, UNB-GWS024693.
Fig. 4. Thallus habit. Scale bar = 3 mm.
Fig. 5. Vertical fracture showing monomerous thallus construction with a plumose hypothallus. Scale bar = 175 µm.
Fig. 6. Perithallus showing multiple cell fusions linking adjacent cell files (f). Scale bar = 10 µm.
Fig. 7. Perithallus showing pseudodichotomous branching (arrows). Scale bar = 14 µm.
Fig. 8. Vertical fracture showing epithallus (upper arrowhead), intercalary meristem (lower arrowhead) and perithallus (bracket). Scale bar = 24 µm.
Fig. 9. Epithallial cell showing cell lumen (e) and epithallial cell roof (circle pointer) and partial view of intercalary meristematic cell (m). Scale bar = 2.5 µm.

ISOTYPE: UNB GWS024694, epilithic, at 3 m depth (*leg.* G.W. Saunders & K. Dixon).

ETYMOLOGY: Specific epithet from Latin *crypticus, –a, –um*, referring to the identity of this species being hidden by the previous taxonomic confusion and misidentifications of *Sporolithon* spp. in the region.

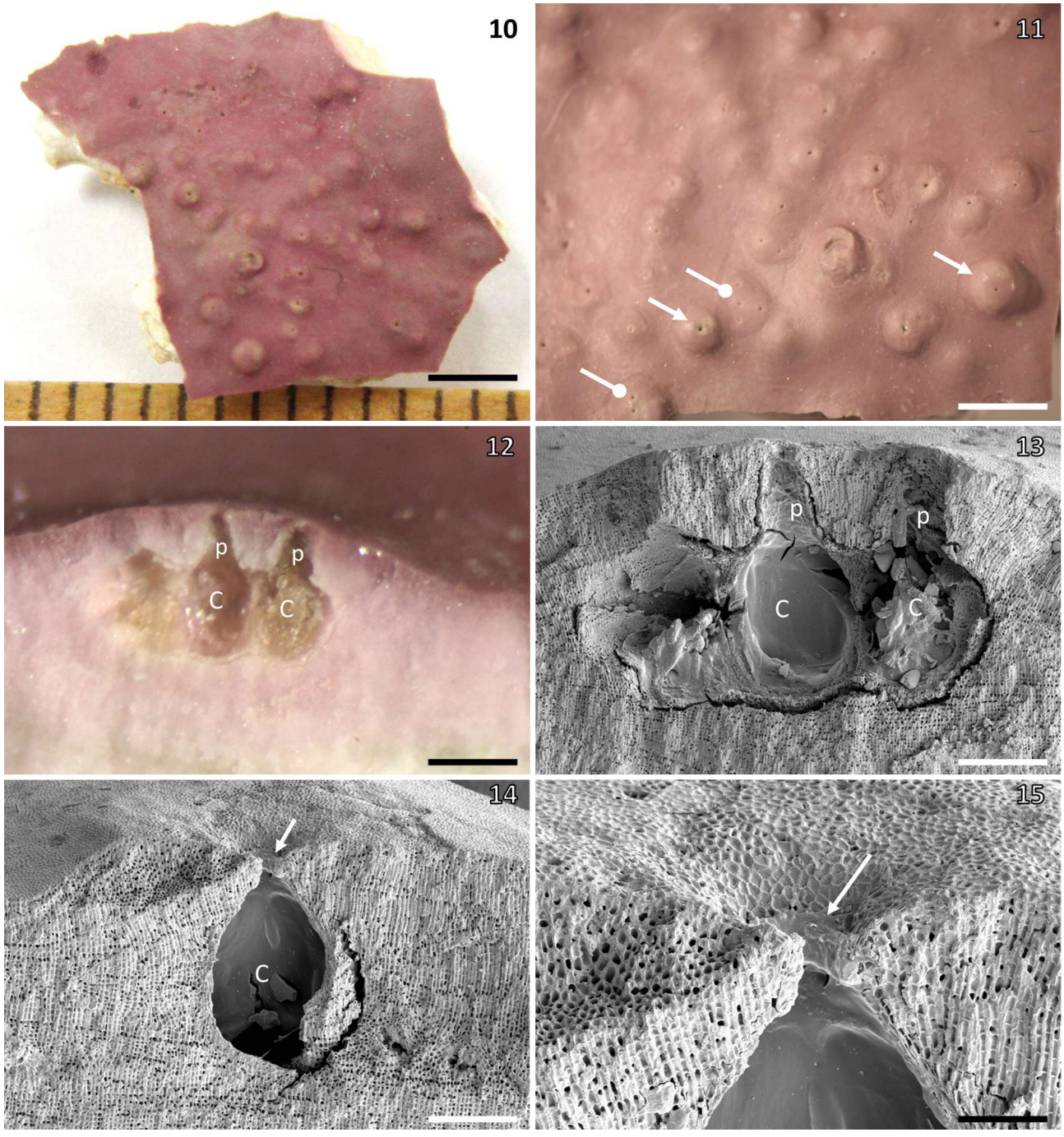
HABITAT: In shallow subtidal marine environment, at 3 m depth.

DISTRIBUTION: This species is currently only known from the type locality (Fig. 3).

Sporolithon immotum T.J. Farr & W.A. Nelson *sp. nov.*

Figs 16–19

DESCRIPTION: Encrusting and lumpy to warty, epilithic on rocky reef, stones and cobbles (Fig. 16); thallus up to 12 cm across; thallus thickness from 250 µm to 1 cm or more. Colour deep rose purple pink, bleaching to pale pink. Monomerous; flared epithallial cells; secondary pit connections predominate (Fig. 17); cell fusions, where present, occur in dark-staining bands (Fig. 17), often regularly spaced (every 3–4 to 6 rows). Tetrasporangia cruciately divided in calcified compartments, 80–105 µm high and 38–52 µm in diameter. Stalk cell conspicuous (Fig. 18);

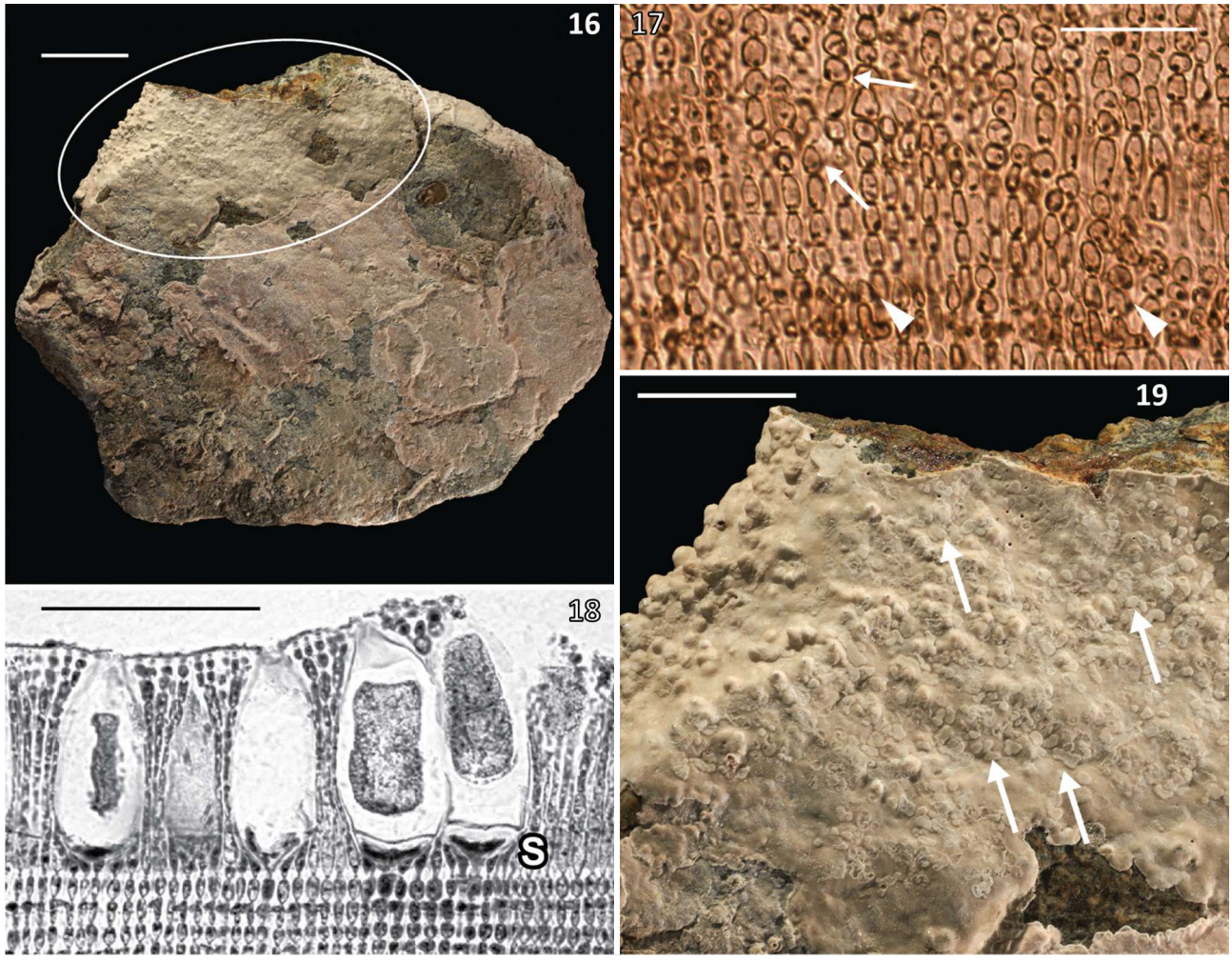


Figs 10–15. Reproductive morpho-anatomy of *Sporolithon crypticum sp. nov*. isotype, UNB-GWS024694.
Fig. 10. Thallus habit with conceptacles raised above the thallus surface. Scale bar = 2.5 mm.
Fig. 11. Magnified surface view of uniporate conceptacles (arrows), some neighbouring each other with pores closely next to each other (circle pointers). Scale bar = 2 mm.
Fig. 12. Dissecting scope view of vertical fracture showing neighbouring uniporate conceptacles, each with conceptacle chamber (c) and pore canal (p). Scale bar = 295 µm.
Fig. 13. Magnified SEM view of neighbouring uniporate conceptacles, each with conceptacle chamber (c) and pore canal (p). Scale bar = 175 µm.
Fig. 14. Partial surface view showing pore (arrow) and section view of conceptacle chamber (c). Scale bar = 140 µm.
Fig. 15. Surface view showing conceptacle pore (arrow) and partial section view. Scale bar = 55 µm.

pore plugs present. Calcified compartments arranged in irregularly shaped, raised sori 45–85(–105) µm above thallus surface (Fig. 19), pale to white. Cells in sori form paraphyses, 6–10 cells in height, but do not become colourless and fused; basal layer of elongate cells. Sori shed after spore release. Holotype DNA sequences deposited in GenBank: *psb*A, DQ167909; nSSU, EF628211.

HOLOTYPE: WELT A027045 (NZC0249), collected 20 March 2003 by N. Alcock & S. Brown at Cable Bay (41°9.346′S, 173°24.166′E), South Island, Nelson, New Zealand; epilithic, at 5–10 m depth.

ETYMOLOGY: Epithet from Latin *immotus, –a, –um* (immovable), in reference to the growth habit of this species attached to rock.



Figs 16–19. *Sporolithon immotum sp. nov.*
Fig. 16. Holotype WELT A027045 (CC-BY 4.0, Te Papa) showing *S. immotum* on rock (ellipse) substrate. Scale bar = 2 cm.
Fig. 17. Holotype WELT A027045, vertical section through thallus showing secondary pit connections (arrows) and cell fusions (stars). Scale bar = 50 μm.
Fig. 18. WELT A027043, vertical section of sorus with tetrasporangia and paraphyses and conspicuous stalk cells (S). Scale bar = 100 μm.
Fig. 19. Holotype WELT A027045 (CC-BY 4.0, Te Papa), showing sori (arrows). Scale bar = 1 cm.

HABITAT: Open coasts in moderate shelter, in embayments, from intertidal to upper subtidal and down to 18 m depth.

DISTRIBUTION: New Zealand: North Island, north and east coasts of the South Island extending to North Otago; Australia: Victoria (Fig. 3).

COMMENT: See Table S1 for other sequenced specimens of this species.

Sporolithon nodosum T.J. Farr & W.A. Nelson *sp. nov.*
Figs 20–23

DESCRIPTION: Free-living (rhodolith), rarely growing attached to rock; range in form and shape from spherical with short lumpy branches (Fig. 20) to more open to sparse twig-like branches, 2–4(–9) cm across; mostly lumpy to fruticose, occasionally warty to fruticose and encrusting. Colour glossy, deep rose purple pink, bleaching to pale pink. Monomerous construction; epithallial cells flared; cell fusions predominate (Fig. 21). Calcified compartments, containing tetrasporangia (Fig. 22), arranged in irregularly shaped, raised sori, 53–165 μm above thallus surface, pale to white (Fig. 23). Cells in sori form paraphyses, 4–6 cells in height, becoming 'fused', lacking pigmentation, with basal layer of elongated cells. Sori up to 1.3 mm across. Sori shed after spore release. Tetrasporangia cruciately divided in calcified compartments, 79.3–129 μm high and 42.4–55.8(–63) μm in diameter (Fig. 22). Stalk cells not apparent (Fig. 22); pore plugs present. Complete plastid genome sequence from holotype deposited in GenBank with accession number: KT266785.

HOLOTYPE: WELT A034583, collected 16 Nov 2012 by W.A. Nelson at Army Bay (36°36′S, 174°49′E), Whangaparaoa Peninsula, Hauraki Gulf, North Island, New Zealand; rhodolith, 0–1 m depth.

ETYMOLOGY: Epithet from Latin *nodosus, –a, –um*, in reference to the knobbly appearance of this rhodolith-forming species.

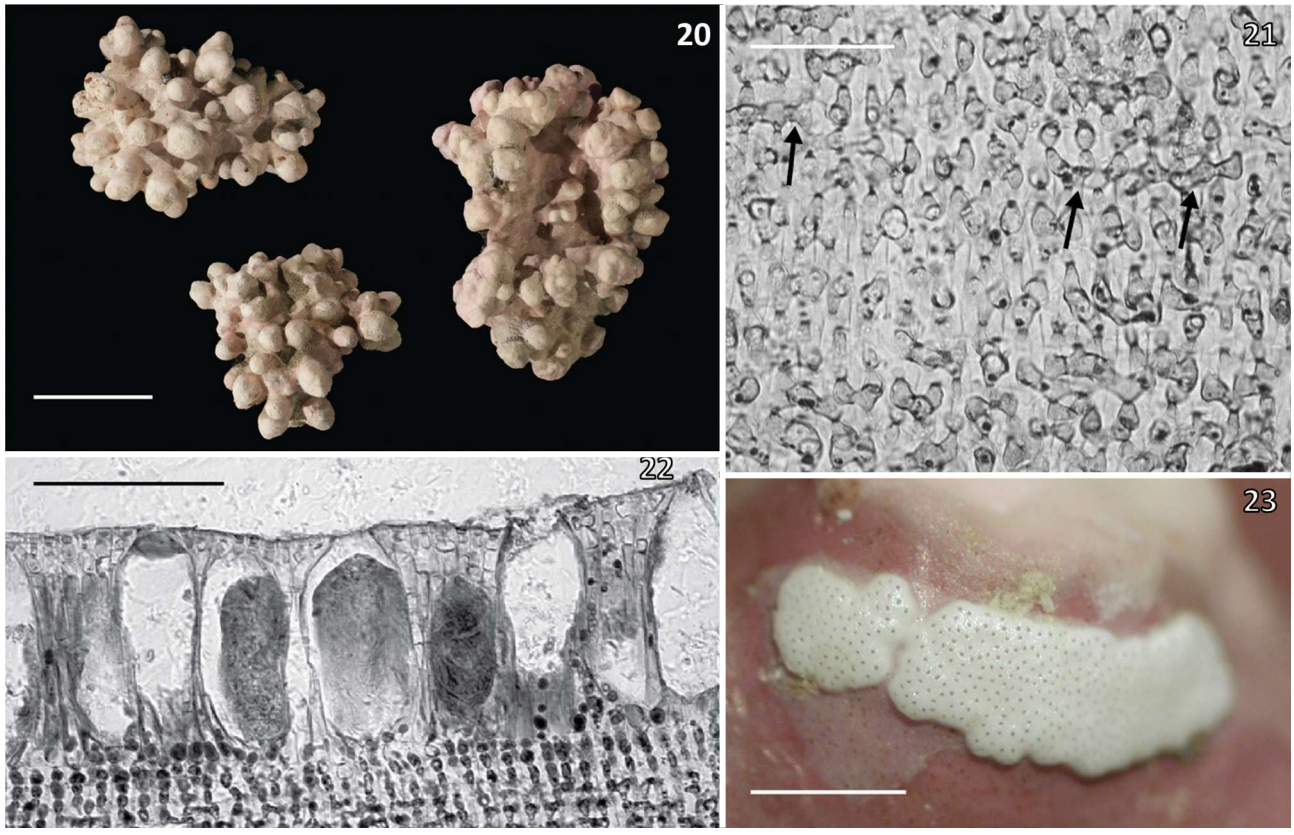
HABITAT: Open coasts in moderate shelter, in embayments; from upper subtidal down to 22 m depth.

DISTRIBUTION: New Zealand: North Island, and northern South Island (Fig. 3).

COMMENT: See Table S1 for other sequenced specimens of this species.

DISCUSSION

Of the 33 recognized extant species of *Sporolithon* (Guiry & Guiry 2022 list 30, plus the three described herein), nine have



Figs 20–23. *Sporolithon nodosum sp. nov.*
Fig. 20. Holotype, WELT A034583. Scale bar = 2 cm.
Fig. 21. WELT A028375, vertical section with cell fusions (arrows). Scale bar = 50 µm WELT A029429.
Fig. 22. WELT A026998, vertical section through sorus with tetrasporangia, paraphyses with fused cells. Scale bar = 100 µm.
Fig. 23. Surface view of sorus with conspicuous pores. Scale bar = 0.5 mm.

been named and described in the DNA sequencing era and have diagnostic DNA sequences. Only for *S. elevatum* M.C. Henriques & Riosmena-Rodríguez, described in 2014, are there no DNA sequences (Henriques *et al.* 2014). Of the 23 *Sporolithon* species named before the DNA sequencing era, four historical type specimens were sequenced by Richards *et al.* (2017): *S. dimotum, S. episporum, S. molle* and the generitype, *S. ptychoides*, and herein we have added seven more. Two historical type specimens are missing: *S. crassum* Heydrich, presumed to have been lost in the bombing of the Berlin Herbarium in WWII, and *S. mediterraneum* Heydrich. For two other type specimens, *S. australasicum* and *S. erythraeum* (Rothpeltz) Kylin (type locality: El Tor, Egypt), we failed to amplify the extracted DNA. Richards *et al.* (2017) recommended that the name *S. erythraeum* not be used, but recently this name has been applied to DNA sequences from Taiwan, over 8,500 km from the type locality of *S. erythraeum*. It is our recommendation that neither *S. australasicum* nor *S. erythraeum* be used, as DNA sequences are lacking and these names cannot be correctly applied. To our knowledge, DNA sequencing has not been attempted for four historical *Sporolithon* type specimens: *S. indicum* V. Krishnamurthy & Jayagopal (type locality: Pamban, Tamil Nadu, India), *S. lemoineae* (Weber Bosse) Verheij (type locality: Kei Islands, Indonesia), *S. pacificum* E. Y. Dawson (type locality: Isla del Caño, Costa Rica) and *S. stefaninnii* (Raineri) P.C. Silva (type locality: Ras Hafun, Somalia). Sequencing of the type of *Sporolithon africanum* (Foslie) Afonso-Carrillo (type locality: Canary Islands) is currently in progress (V. Peña, personal information).

Three of the *Sporolithon* type specimen sequences reported herein (*S. schmidtii, S. sibogae* and *S. timorense*) are from southeast Asia, a region less well known phycologically. All of these names have been applied outside of their type localities based on specimens identified using morpho-anatomy, *S. schmidtii* from Guam (Gordon *et al.* 1976), *S. sibogae* from Madagascar (Pichon 1978) and *S. timorense* from the Philippine Islands (Velasquez *et al.* 1975). All of these reports need to be confirmed by DNA sequencing of the cited material or of field-collected material from the cited localities.

Verheij (1993), in a comprehensive study of *Sporolithon* species from the Spermonde Archipelago, Indonesia, based on morpho-anatomy and that included examining type specimens, reported five species from the region, namely *S. episoredion, S. episporum, S. lemoineae, S. molle* and *S. ptychoides*. He did not find specimens of *S. sibogae* and *S. timorense*, species with Indonesian type localities. All of Verheij's material was first preserved in 7% formalin-seawater before transfer to a 70% ethanol/2% glycerol solution. While it may be difficult to obtain DNA sequences from this material, it should be attempted to confirm the

application of names with type localities far removed from this region, i.e. *S. episoredion, S. episporum, S. molle* and *S. ptychoides*.

Another previously misapplied name has been *S. durum*. Harvey *et al.* (2002) were among the first to apply DNA sequencing to coralline algae, using 18S rDNA to sequence two species of the family Sporolithaceae found in southeast Australia, namely *Heydrichia homalopasta* and *S. durum*. For neither species was type material sequenced. The molecular concept of *S. durum* was based on a specimen LTB 21140 from Jervis Bay, New South Wales, Australia, which was later sequenced by Nelson *et al.* (2015) for *rbc*L. Over the same 280 bp sequence of the lectotype of *S. durum*, LTB 21140 differs by 6 bp, indicating that it is a different, undescribed species. The name *S. durum* had been incorrectly applied, based on morpho-anatomy, to all *Sporolithon* specimens from New Zealand, both rhodolith-forming and epilithic (Harvey *et al.* 2005). The complete mitogenome of a rhodolith from Whangaparaoa Peninsula, New Zealand (voucher: WELT A034583) identified as *S. durum* was assembled by Kim *et al.* (2013). Subsequently, three other rhodolith specimens from the same locality, cited as SKKU_SD01, SKKU_SD02 and SKKU_SD03, were sequenced for their complete plastid genome (Lee *et al.* 2016). This species, described herein as *S. nodosum sp. nov*., differs over a comparable length from the 280 bp lectotype *rbc*L sequence of *S. durum* by 14 bp, a sequence divergence of 5% that is very similar to 4.7% sequence divergence of the 1358 bp long sequences of field-collected material of the two species. All 26 sequenced specimens of *S. nodosum* from the North and South Islands of New Zealand occur as rhodoliths. The epilithic specimens from New Zealand, described herein as *S. immotum sp. nov*., differ over a comparable length from the 280 bp lectotype *rbc*L sequence of *S. durum* by 28 bp, a 10% divergence indicating that it is also a distinct species and not *S. durum*. This species has been collected on both the North and South Islands of New Zealand at 10 different localities. In addition, it was found epilithically at two localities along the south-eastern coast of Australia in the states of Victoria and South Australia (Table S1) and likely occurs at more localities in this region.

Based on DNA sequenced specimens that match the *rbc*L sequence of the lectotype specimen of *S. durum*, this species is endemic to Australia and occurs from its type locality, Cape Jaffa, South Australia, in the east to Shelter Island, Western Australia, in the west, essentially spanning the Great Australian Bight. All previous reports of *S. durum* based on morpho-anatomy from Australian localities outside this area, i.e. New South Wales, Victoria, and the west coast of Western Australia (Townsend *et al.* 1995), need to be confirmed by DNA sequencing. *Sporolithon durum* occurs as a rhodolith and attached to substratum from the low intertidal to 16 m depth.

Sporolithon crypticum sp. nov. is only known from its type locality, Windy Harbour, Western Australia, where it occurs attached to rock. A concerted collecting and sequencing effort of non-geniculate coralline algae is needed along the entire Australian coast to understand the diversity and distributions of these algae.

From the lectotype of *Sporolithon crassiramosum* only a 118 bp length *rbc*L sequence was obtained. While short, this sequence was an exact match to the *S. molle* holotype and to field-collected specimens from El Tor, Egypt, but differed by a minimum of 7 bp from all other *Sporolithon* species sequenced to date. *Sporolithon molle* is now known to occur at El Tor, Gulf of Suez, and at Juan de Nova Island in the Mozambique Channel – the two localities about 5,000 km apart (Fig. 3). DNA sequencing of corallines from the Red Sea and along the east coast of Africa is needed to determine if this species is present between these sites. Hernández-Kantún *et al.* (2016) discussed other markers that could be sequenced to help understand gene flow and patterns of connectivity in widely distributed non-geniculate coralline algae, e.g. *Lithophyllum kaiseri* (Heydrich) Heydrich (Hernández-Kantún *et al.* 2016), *Melyvonnea erubescens* (Foslie) Athanasiadis & D.J. Ballantine (Richards *et al.* 2020) and *Sporolithon indopacificum* (Maneveldt *et. al.* 2017).

Unfortunately, little can be said regarding the other successfully sequenced historical type specimens of *Sporolithon* that are known only from their type localities, namely *S. dimotum, S. episoredion, S. howei, S. schmidtii, S. sibogae* and *S. timorense*. However, we are confident that with additional sequencing, field-collected specimens will be found that match these type specimen sequences. It took five years to find sequences from field-collected specimens that matched the type sequence of the generitype species of *Lithophyllum, L. incrustans* Philippi, and three years to find field-collected specimens that matched the type specimen sequence of *L. intermedium* Foslie (Gabrielson, personal observations). It is also clear that after 11 years of sequencing coralline type specimens, this is the only method that enables names to be correctly applied to coralline species. DNA sequencing is essential to identify all coralline algal specimens to species.

An additional impediment, even when type specimens are sequenced, is that for many contemporary coralline species the *rbc*L gene is not being sequenced, and thus cannot be compared to the *rbc*L sequences generated for many type specimens to know if an historical name applies. It would be useful, both for correctly applying historical names and for phylogenetic analyses, for each coralline species to have *psb*A, *rbc*L and COI sequences generated from at least one specimen.

It is helpful that the *psb*A and *rbc*L gene trees for *Sporolithon* are congruent, despite the absence of numerous species from the *psb*A phylogram. As sequences from field-collected specimens are found that match the type specimen sequences, it will enable concatenated phylograms to be constructed that will be phylogenetically more informative. Only for one clade of *Sporolithon* species, namely *S. gracile, S. mesophoticum, S. sinusmexicanum* and *S. yoneshigueae*, is there a strong biogeographic pattern, all occurring in the western Atlantic Ocean, and that clade has full support in both the *psb*A and *rbc*L phylograms. No character or suite of characters, however, has been found that is diagnostic for this clade. And there are other western Atlantic species, namely *S. amadoi, S. episporum, S. franciscanum* and *S. tenue* that occur in clades with Pacific species. Encrusting (without protuberances) species of *Porolithon* (Foslie) Foslie, e.g. *P. imitatum* R. A. Townsend & Huisman, *P. onkodes* (Heydrich) Foslie, *P. penroseae* R.A. Townsend & Huisman and numerous

unnamed *Porolithon* species (Order Corallinales) that are found in warm temperate to tropical waters also have mixed clades of Atlantic and Pacific species (Gabrielson *et al.* 2018). These two examples, along with the aforementioned widely distributed non-geniculate coralline species, *Lithophyllum kaiseri, Melyvonnea erubescens* and *Sporolithon indopacificum*, illustrate our limited understanding of the processes that drive the evolution and biogeography of non-geniculate coralline algae.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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ORCID

Paul W. Gabrielson http://orcid.org/0000-0001-9416-1187
Jeffery R. Hughey http://orcid.org/0000-0003-4053-9150
Viviana Peña http://orcid.org/0000-0001-7003-3850
Joseph L. Richards http://orcid.org/0000-0002-7805-5453
Gary W. Saunders http://orcid.org/0000-0003-4813-6831
Brenton Twist http://orcid.org/0000-0002-4694-8628
Tracy Farr http://orcid.org/0009-0008-3665-8804
Wendy A. Nelson http://orcid.org/0000-0003-0014-5560

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