UNIVERSIDADE ESTADUAL DE FEIRA DE SANTANA
DEPARTAMENTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BOTÂNICA

IARA OLIVEIRA COSTA

# ALGAS CORALINÁCEAS INCRUSTANTES DA ORDEM CORALLINALES (CORALLINOPHYCIDAE, RHODOPHYTA) NO ATLÂNTICO SUL: ASPECTOS MORFOLÓGICOS E MOLECULARES

FEIRA DE SANTANA – BAHIA
2017

**UNIVERSIDADE ESTADUAL DE FEIRA DE SANTANA**
**DEPARTAMENTO DE CIÊNCIAS BIOLÓGICAS**
**PROGRAMA DE PÓS-GRADUAÇÃO EM BOTÂNICA**

# ALGAS CORALINÁCEAS INCRUSTANTES DA ORDEM CORALLINALES (CORALLINOPHYCIDAE, RHODOPHYTA) NO ATLÂNTICO SUL: ASPECTOS MORFOLÓGICOS E MOLECULARES

**IARA OLIVEIRA COSTA**

Tese apresentada ao Programa de Pós-Graduação em Botânica da Universidade Estadual de Feira de Santana como parte dos requisitos para a obtenção do título de Doutor em Botânica.

ORIENTADOR: PROF. DR. JOSÉ MARCOS DE CASTRO NUNES (UFBA)
COORIENTADOR: PROF. DR. PAULO ANTUNES HORTA (UFSC)

FEIRA DE SANTANA – BAHIA
2017

**Ficha Catalográfica - Biblioteca Central Julieta Carteado**

C872a Costa, Iara Oliveira
Algas coralináceas incrustantes da ordem Corallinales (Corallinophycidae, Rhodophyta) no Atlântico Sul: aspectos morfológicos e moleculares / Iara Oliveira Costa. - 2017.
178 f.: il.

Orientador: José Marcos de Castro Nunes.
Coorientador: Paulo Antunes Horta.
Tese (doutorado) - Universidade Estadual de Feira de Santana, Programa de Pós-Graduação em Botânica, 2017.

1. Algas coralináceas incrustantes – Taxonomia. 2. Corallinaceae. I. Nunes, José Marcos de Castro, orient. II. Horta, Paulo Antunes, coorient. III. Universidade Estadual de Feira de Santana. IV. Título.

CDU: 582.273

**A**

**Domingos Costa (*In memorian*), meu pai.**

"*Hoje eu sei que quem me deu a ideia de uma nova consciência e juventude, está em casa guardado por Deus*..." (Belchior).

## AGRADECIMENTOS

A Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela concessão da bolsa de Doutorado através do Programa de Pós Graduação em Botânica da Universidade Estadual de Feira de Santana (PPGBot-UEFS). Agradeço também aos professores do programa por contribuírem com minha formação acadêmica e à secretaria por instrução e viabilização dos trâmites burocráticos.

Ao Conselho Nacional de Pesquisa e Desenvolvimento Tecnológico – CNPq (BrBOL 564945/2010-2 e UNIVERSAL 477614/2013-2) e à Fundação de Amparo à Pesquisa do Estado da Bahia - FAPESB (PRONEM T.O. PNE. 0020/2011 e REDES T.O. RED006/2012) pelo apoio financeiro concedidos aos projetos de pesquisa que viabilizaram o desenvolvimento deste trabalho.

Ao meu orientador, Prof. Dr. José Marcos de Castro Nunes, por me conceder a oportunidade de realizar este trabalho, por todo aprendizado desde a minha iniciação científica.

Ao meu coorientador, Prof. Dr. Paulo Antunes Horta, por toda atenção, disponibilidade, orientação e ensinamentos. Agradeço pela paciência, discussões elucidativas, e também pelo acolhimento e hospitalidade.

A equipe do Laboratório de Algas Marinhas da UFSC pelo acolhimento e auxílio durante minha estadia. Ao Laboratório Central de Microscopia Eletrônica (LCME) da UFSC por disponibilizar a infraestrutura para o processamento das imagens de microscopia eletrônica de varredura. E à Vanessa Andrade pela supervisão durante uso dos equipamentos do LCME.

Aos professores Dra. Kelly Regina Batista Leite e Dr. Lázaro Benedito da Silva por disponibilizarem a infraestrutura do LAVIM/UFBA e aos estagiários por toda atenção e auxílio quando necessário.

A professora Dra. Alessandra Selbach Schnadelbch por disponibilizar a infraestrutura do LAGEV/UFBA para as análises moleculares, por todas as orientações e incentivos. Ao professor Dr. Henrique Batalha por disponibilizar a infraestrutura do LEBI/UFBA para as análises moleculares, pela disponibilidade e auxílio quando necessário.

A professora Dra. Moema Cortizo Bellintani por disponibilizar a infraestrutura e área de convivência do LCTV/UFBA.

As professoras Dra. Mariana Cabral e Dra. Valéria Cassano por disponibilizarem a infraestrutura do LAM/USP. Agradeço também à Fábio Nauer, Beatriz Torrano, Janaína Pires e aos técnicos do LAM por todo auxílio.

Ao professor Dr. Rafael Riosmena-Rodriguez que, apesar do nosso pouco contato, me auxiliou em muitos pontos da pesquisa.

A Msc. Marina Sissini e Dra. Talita Vieira-Pinto pela disponibilidade, parceria e discussões acerca das algas coralináceas incrustantes.

As professoras Dra. Renata Reis e Dra. Beatriz de Barros Barreto agradeço pela receptividade, hospitalidade e auxílio nas coletas do Rio de Janeiro. Ao professor Dr. Alexandre Pedrini agradeço pelo envio de amostras. Ao Pedro Bastos agradeço pelo auxílio na coleta do Ceará. Agradeço também à receptividade e auxílio dos donos das pousadas ao longo das coletas: D. Rita do Espírito Santo, Vítor do Piauí, Luiza, Ricardo e Morgana do Paraná.

Aos estagiários que me acompanharam ao longo dessa jornada e sem os quais eu não teria conseguido concluir o trabalho. A Tiana pelo árduo trabalho de bancada tanto na parte anatômica, quanto na molecular. A Poline por sua paciência e eficiência nos cortes em micrótomo. A Valter que em tão pouco tempo trabalhou tanto e com uma dedicação exemplar.

Aos colegas do Laboratório de Algas Marinhas (LAMAR): Aaron, Adriele, Cibele, Cristina, Edilene, Gabriel, Larissa, Lorena, Patrícia e Viviane. Aos colegas de bancada do Laboratório de Genética e Evolução de Plantas (LAGEV) e do Laboratório de Cultura de Tecidos Vegetais (LCTV). Agradeço a todos pela companhia e pelos momentos de descontração.

A Nazaré Marchi e Zafira Gurgel agradeço pelas pausas para o café e conversas descontraídas.

Ao querido Neilton Andrade, que mesmo distante continua perto, por toda disponibilidade e boa vontade em resolver problemas de diversas ordens.

Ao Dr. Fábio Espírito Santo por toda amizade, hospitalidade, por ser meu procurador oficial nas matrículas e por todos os momentos de descontração.

A Dra. Goia de Mattos Lyra por todas as dicas e revisões de texto. A Dra. Priscila Barreto de Jesus por todas a discussões e direcionamentos nas análises moleculares.

As minhas companheiras de viagens, Taiara Aguiar Caires e Priscila Barreto de Jesus, agradeço por toda paciência e emoções que vivemos neste período. “Deus protege os inocentes” e como nos protegeu e livrou de várias ciladas! Agradeço também a Helen Affe, a mais recente integrante do grupo que veio para formar o “Quarteto”. Ah Helen, obrigada pelo mapa! Somos tão diferentes, mas juntas nos completamos! E por último, mas nem por isso menos importante, o nosso “quinto elemento” Diogo Rocha, sempre inteirado dos nossos assuntos. Foram tantas resenhas, desabafos, gargalhadas. Não teria conseguido sem vocês!

A minha família por todo apoio, incentivo e carinho. Principalmente à minha mãe e meu querido irmão por toda torcida, vibração com minhas vitórias e amor a mim dedicado. E agradeço pelo auxílio nas coletas também!

A Luiz Felipe, meu marido, por toda compreensão, paciência, estímulo, carinho e amor. Obrigada por entender meus muitos momentos de ausência e por me incentivar nos momentos de desânimo. Te amo!

Enfim, a todos que eu possa ter esquecido de mencionar, mas que sabem que contribuíram para a concretização deste trabalho: Muito Obrigada!!!

# SUMÁRIO

# INTRODUÇÃO

## *Caracterização geral*

As algas coralináceas pertencem ao filo Rhodophyta e apresentam como principal característica a presença de impregnação de carbonato de cálcio na forma de calcita nas paredes celulares, o que não é observado nos demais grupos de algas vermelhas, e isso lhes confere textura rígida (Chamberlain, 1983; Bailey & Chapman, 1998).

As algas coralináceas estão distribuídas dos trópicos aos pólos, desde a zona de entremarés até os limites inferiores da zona fótica, mais de 200 m de profundidade (Foster, 2001).

Morfologicamente as algas coralináceas podem ser divididas em dois grupos artificiais principais: as algas coralináceas geniculadas ou articuladas – consistem em segmentos não calcificados e calcificados alternados, chamados, respectivamente, de genículo e intergenículo; e as algas coralináceas incrustantes, também chamadas de não geniculadas ou não articuladas – são integralmente calcificadas, resultando em formas incrustantes do talo (Harvey et al., 2005).

As algas coralináceas incrustantes podem se desenvolver aderidas a um substrato contínuo ou desprendidas destes, formando estruturas denominadas de rodolitos que são nódulos calcários de vida livre (Harvey et al., 2005).

Por muito tempo a forma articulada foi separada da incrustante, sem levar em consideração as características anatômicas ou reprodutivas. Cabioch (1988) apresentou pela primeira vez a presença de conexões celulares secundárias (“pit-connections”Secundárias) e/ou de células fusionadas como características filogeneticamente mais relevantes do que a presença ou não de genículo, o que foi confirmado posteriormente com os estudos moleculares (Bailey & Chapman, 1998; Bailey, 1999).

As formas incrustantes desempenham papel fundamental na construção e na ocupação do substrato nos recifes coralíneos em todo o mundo, protegendo esse ecossistema contra a ação erosiva das ondas e possibilitando a manutenção e crescimento dos recifes (Steneck & Testa, 1997). Também podem formar bancos de rodolitos, que compõem um ecossistema único onde abriga grande diversidade de algas, invertebrados e vertebrados associados direta ou indiretamente a eles (Steller et al., 2003; Litter & Litter, 2008; Riul et al., 2008).

A maior extensão de bancos de rodolitos está na costa brasileira, onde estão distribuídos de forma descontínua desde a plataforma continental do Amapá até Santa Catarina, havendo uma concentração relevante na região do alargamento da plataforma continental que vai do sul da Bahia ao norte do Espírito Santo (Amado Filho et al., 2012a; Moura et al., 2016; Horta et al., 2016). Recentemente, foi descoberto o maior banco de rodolitos do mundo na região da Plataforma Continental de Abrolhos (Amado Filho et al., 2012a). Este banco ocupa uma área de mais de 20.000 km², e tem uma importância global na produção de carbonato de cálcio comparável aos recifes coralíneos do Caribe e à Grande Barreira de Corais da Austrália (Amado Filho et al., 2012a; Moura et al., 2013). Outra descoberta recente foi o recife carbonático de aproximadamente 10.000 km² na região entre a Guiana Francesa e o Brasil, que compreende a região da Foz do Rio Amazonas, sendo este o primeiro recife descoberto sob a pluma de um grande rio (Moura et al., 2016).

A taxonomia das algas coralináceas incrustantes tem sido considerada a mais complexa do filo Rhodophyta, devido à incrustação do carbonato de cálcio na parede celular destas algas, as diferentes formas de desenvolvimento e a similaridade dos morfotipos entre táxons filogeneticamente distintos (Chamberlain, 1983; Woelkerling, 1998; Harvey et al., 2003).

Inicialmente a taxonomia das algas coralináceas incrustantes esteve baseada em caracteres vegetativos (p. e. Cabioch, 1972) e posteriormente foram incorporados caracteres reprodutivos (Woelkerling, 1988). Todavia, a classificação destas algas tem estado sob constante mudança (Harvey et al., 2003). O valor de alguns dos caracteres originais utilizados para fins taxonômicos foi testado e, principalmente os vegetativos, foram descartados e substituídos por caracteres reprodutivos (Woelkerling, 1985; Harvey & Woelkerling, 2007).

No entanto, a alta plasticidade fenotípica e a sobreposição de muitos caracteres taxonômicos entre espécies e gêneros dificulta a delimitação dos táxons (Maneveldt & Keats, 2008; Vidal et al. 2003). A incorporação de novas técnicas de observação, como a microscopia eletrônica de varredura (MEV), tem proporcionado avanços na busca e na interpretação dos caracteres anatômicos dos talos (Woelkerling, 1988; Riosmena-Rodriguez et al., 1993).

Novas abordagens utilizando a análise molecular tem propiciado um crescente avanço no conhecimento da diversidade das algas coralináceas nas últimas décadas, muitas vezes confirmando antigas circunscrições baseadas em dados anatômicos e também

trazendo novidades em todos os níveis de classificação (Bailey & Chapman, 1996; Kato et al., 2011; 2013; Hind & Saunders, 2013). Este tipo de estudo viabiliza a identificação de espécies crípticas, espécies estas que possuem aspecto morfológico não diferenciado, mas que guardam em seu DNA uma variação genética importante para a manutenção e adaptação da biodiversidade de determinada população (Thorpe & Solé-Cava, 1994; Maggs et al., 2007).

***Classificação***

Inicialmente, Linnaeus (1767), classificou as algas coralináceas como corais hermatípicos, formadores de recifes, baseado na natureza calcária e na existência de pequenos poros. A família Corallinaceae foi proposta em 1812 por Lamouroux, mas ainda seguia a classificação proposta por Linnaeus. Em 1816, Lamouroux propôs uma família chamada Corallinés, que incluía algas não calcárias, coralináceas articuladas e coralináceas não articuladas. Kützing (1843) revisou as publicações de Lamouroux e separou as algas coralináceas das demais algas, e essas passaram a pertencer à duas famílias: Spongitae (algas não geniculadas) e Corallinae (algas geniculadas). Inicialmente a ordem Corallinales foi proposta por Funk (1927) e Hylander (1928), mas estes não apresentaram justificativas para a nova ordem. Então, Silva e Johansen (1986), com base em estudos bioquímicos, ultraestruturais, anatômicos e do desenvolvimento, estabelecerem definitivamente a ordem Corallinales. Na citada ordem foram incluídas algas de talo calcificado, que cresciam sobre diversos tipos de substratos; apresentando meristema intercalar recoberto por uma camada de células; e forma das conexões celulares primárias apresentando “plug” com a camada mais externa em forma de domo.

A primeira filogenia molecular de Corallinales foi publicada por Bailey e Chapman (1996, 1998), onde foi confirmado o cenário evolutivo hipotetizado por Cabioch (1988) de que as formas geniculadas teriam evoluído independentemente em linhagens distintas na citada da ordem. Desde então, as abordagens moleculares tem elucidado as relações filogenéticas das algas coralináceas (Bailey, 1999; Harvey et al., 2003; Broom et al., 2008; Le Gall & Saunders, 2007; Le Gall, 2010; Bittner et al., 2011; Kato et al., 2011; Hind & Saunders, 2013; Nelson et al., 2015; Rösler et al., 2016).

Até recentemente, as algas coralináceas foram classificadas em uma única ordem (Corallinales) constituída de três famílias: Corallinaceae, Hapalidiaceae e Sporolithaceae. Esta classificação se baseava em uma combinação de dados morfológicos, anatômicos, bioquímicos, ultraestruturais e moleculares (Harvey et al., 2003).

Le Gall e Saunders (2007) publicaram a filogenia supraordinal de Florideophyceae e propuseram a criação da subclasse Corallinophycideae para agrupar os membros das ordens Corallinales e Rhodogorgonales, formando um grupamento monofilético. A citada subclasse é caracterizada por precipitação de calcita na parede celular, entretanto, os membros de Rhodogorgonales precipitam este mineral apenas em células vegetativas específicas, enquanto que os membros de Corallinales o fazem por todo o talo (Le Gall & Saunders, 2007; Le Gall, 2010).

Le Gall et al. (2010), com base em estudos moleculares, propuseram a mudança de *status* da família Sporolithaceae, elevando-a a uma nova ordem: Sporolithales, que compreende uma única família com dois gêneros (*Sporolithon* Heydrich e *Heydrichia* R.A.Townsend, Y.M.Chamberlain & Keats). Foram utilizadas como características para separar a nova ordem, a presença de tetrasporângios cruciados produzidos em compartimentos calcificados que geralmente estão agrupados em soros e apresentam tampão apical.

A ordem Corallinales passou, então, a englobar duas famílias Corallinaceae e Hapalidiaceae que produzem tetrasporângios divididos zonadamente (Harvey & Woelkerling, 2007). A família Corallinaceae é caracterizada por possuir conceptáculos tetra/bisporangiais uniporados sem plug apical (Harvey et al., 2003) e é composta, hoje em dia, de sete subfamílias (Bittner et al., 2011; Kato et al., 2011). A família Hapalidiaceae engloba táxons que possuem conceptáculos tetra/bisporangiais multiporados com plug apical (Harvey et al., 2003) e possui três subfamílias (Harvey et al., 2003; Bittner et al., 2011).

Nelson et al. (2015), através de análises moleculares concatenadas com táxons da Nova Zelândia, revisaram a posição filogenética de Hapalidiaceae e, baseados nos mesmos critérios que Le Gall et al. (2010), propuseram a elevação desta família à ordem Hapalidiales.

A ordem Corallinales, atualmente, é composta apenas por uma família – Corallinaceae – e seis subfamílias: Corallinoideae, Hydrolithoideae, Lithophylloideae, Mastophoroideae, Metagoniolithoideae e Neogoniolithoideae (Rosler et al., 2016). No entanto, o status taxonômico de alguns táxons ainda é polêmico (Bailey & Champan, 1998; Kato et al., 2013; Nelson et al., 2015). A síntese da atual classificação das algas coralináceas é encontrada na tabela 1 e as principais características morfológicas e anatômicas utilizadas na classificação atual encontram-se na tabela 2 e Figuras 1 a 3.

**Tabela 1:** Classificação das algas coralináceas incrustantes até o nível de subfamília.

| | | | |
|---|---|---|---|
| Filo | Rhodophyta Wettstein 1901 | | |
| Subfilo | Eurodophytina Saunders & Hommersand 2004 | | |
| Classe | Florideophyceae Cronquist 1960 | | |
| Subclasse | Corallinophycidae Le Gall & Saunders 2007 | | |
| Ordem | Corallinales Silva & Johansen 1986 | Hapalidiales Nelson et al. 2015 | Sporolithales Le Gall et al. 2010 |
| Família | Corallinaceae Lamouroux 1812 | Hapalidiaceae Gray 1864 | Sporolithaceae Verheij 1993 |
| Subfamília | Corallinoideae Foslie 1908<br>Hydrolithoideae Kato et al. 2011<br>Lithophylloideae Setchell 1943<br>Mastophoroideae Setchell 1943<br>Metagoniolithoideae (Johansen) Rösler et al. 2016<br>Neogoniolithoideae (Kato et al.) Rösler et al. 2016 | Austrolithoideae Harvey & Woelkerling 1995<br>Choreonematoideae Woelkerling 1987<br>Melobesioideae Bizzozero 1885 | Não se aplica |

**Tabela 2:** Caracteres considerados diagnósticos para as algas coralináceas com base na classificação atual (Referências: Harvey et al., 2003; Harvey et al., 2005; Harvey & Woelkerling, 2007; Le Gall et al., 2010; Kato et al., 2011; Hind et al. 2016; Rösler et al. 2016).

| Ordem / Família | Estrutura de desenvolvimento de esporângios | Arranjo dos esporos | Plug apical | Subfamília | Presença de genículo | Tipo de conexão entre filamentos | Células do teto do conceptáculo | Filamentos espermatangiais |
|---|---|---|---|---|---|---|---|---|
| Corallinales / Corallinaceae | Conceptáculos uniporados | Zonados | Ausente | Corallinoideae | Sim/Não | Fusão celular | Células do poro paralelas à superfície do talo | Não ramificado, situados no teto e no piso do conceptáculo |
| | | | | Hydrolithoideae | Não | Fusão celular | Células do poro perpendiculares à superfície do talo | Não ramificado, situados no piso do conceptáculo |
| | | | | Lithophylloideae | Sim/Não | "Pit-connections"Secundárias | Células do poro perpendiculares à superfície do talo | Não ramificado, situados no piso do conceptáculo |
| | | | | Mastophoroideae | Não | Fusão celular | Células do poro paralelas à superfície do talo | Não ramificado, situados no piso do conceptáculo |
| | | | | Metagoniolithoideae | Sim/Não | Fusão celular | Células do poro perpendiculares à superfície do talo | Não ramificado, situados no piso do conceptáculo |
| | | | | Neogoniolithoideae | Não | Fusão celular | Células do poro paralelas à superfície do talo | Não ramificado, situados no teto e no piso do conceptáculo |
| Hapalidiales / Hapalidiaceae | Conceptáculos multiporados | Zonados | Presente | Austrolithoideae | Não | Sem conexão | Várias camadas | Ramificados, situados no teto e no piso do conceptáculo |
| | | | | Choreonematoideae | Não | Sem conexão | Única camada | Não ramificados |
| | | | | Melobesioideae | Não | Fusão celular | Várias camadas | Ramificados ou não ramificados, situados no piso do conceptáculo |
| Sporolithales / Sporolithaceae | Compartimentos calcificados | Cruciados | Presente | Não se aplica | Não | Fusão celular e "pit-connections"Secundárias | Não se aplica | Ramificados ou não ramificados, situados no teto e no piso do conceptáculo |

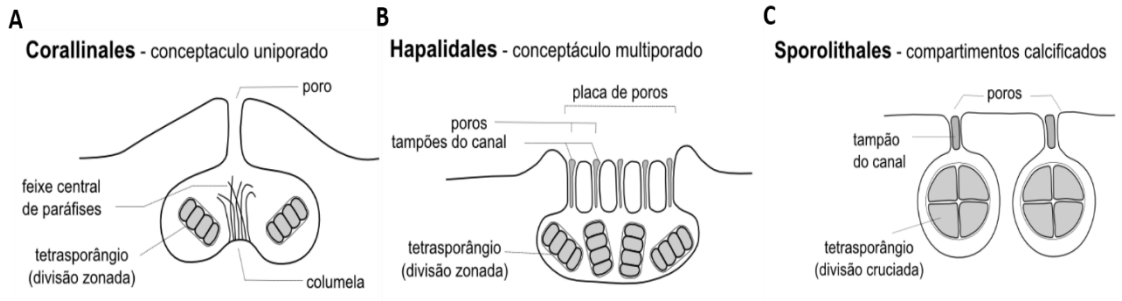


**Figura 1.** Caracterização esquemática das estruturas reprodutivas das algas coralináceas. **A.** Conceptáculo tetrasporangial de Corallinales. **B.** Conceptáculo tetrasporangial de Hapalidiales. **C.** Compartimentos tetrasporangiais calcificados de Sporolithales. Adaptado de Farr et al. (2009).

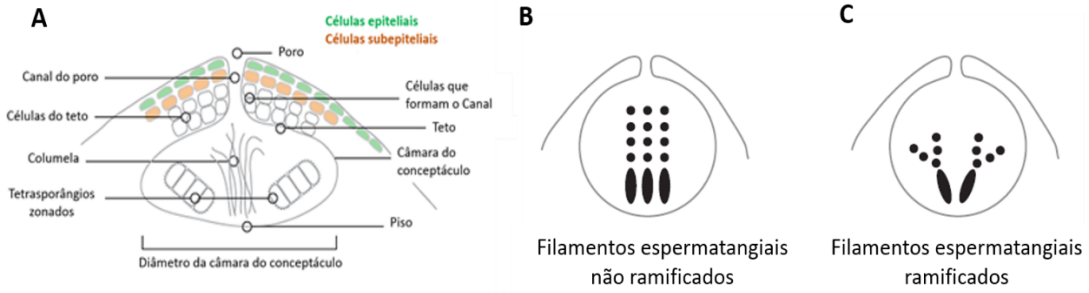


**Figura 2.** Caracterização esquemática das estruturas reprodutivas das algas coralináceas. **A.** Conceptáculo tetrasporangial uniporado, evidenciando estruturas utilizadas para a identificação dos táxons. **B.** Conceptáculo espermatangial com filamentos espermatangiais não ramificados. **C.** Conceptáculo espermatangial com filamentos espermatangiais ramificados. Adaptado de Farr et al. (2009).

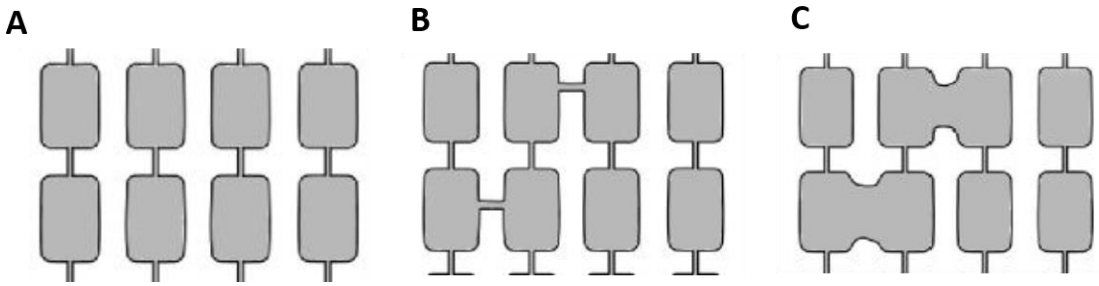


**Figura 3.** Tipos de ligação celular encontradas nas algas coralináceas. **A.** Somente ligações primárias (Pit-connections) entre células de um mesmo filamento. **B.** Pit-connections secundárias entre células de filamentos adjacentes. **C.** Fusão celular entre células de filamentos adjacentes. Adaptado de Farr et al. (2009).

***Estudos no Brasil***

Até a década de 70 os estudos no Brasil sobre algas coralináceas incrustantes se limitaram a algumas citações de espécies (Martens, 1870; Taylor, 1931; Oliveira Filho et al., 1979). Yamaguishi-Tomita (1976) realizou o primeiro estudo taxonômico no Brasil, entretanto, este estudo foi restrito ao gênero *Sporolithon*, onde foram descritas oito espécies para o litoral brasileiro. Após mais de duas décadas sem que fossem realizados trabalhos taxonômicos com algas coralináceas incrustantes no litoral brasileiro, Horta (2000) ao estudar as algas marinhas do infralitoral das Regiões Sul e Sudeste do Brasil, descreveu oito espécies de coralináceas incrustantes. Horta (2002) apresenta um levantamento histórico sobre os estudos taxonômicos em coralináceas incrustantes realizados na costa brasileira e as bases para identificação deste grupo.

No Brasil, somente na última década as algas coralináceas incrustantes ganharam destaque com o aumento do número de estudos publicados, apresentando enfoque tanto ecológico quanto taxonômico (Horta et al., 2016). Até a presente data 26 espécies de algas coralináceas incrustantes pertencentes a ordem Corallinales foram reconhecidas na flora brasileira (Tabela 3).

O aumento no número de estudos neste campo tem possibilitado a descoberta de novas espécies da ordem Corallinales, como: *Lithophyllum depressum* Villas-Boas, Figueiredo & Riosmena-Rodriguez no estado do Espírito Santo (Villas-Boas et al., 2009); *Lithophyllum atlanticum* T. Vieira-Pinto, M. C. Oliveira & P. A. Horta no litoral Sul do Brasil (Vieira-Pinto et al., 2014); e *Neogoniolithon atlanticum* Tâmega, Riosmena-Rodríguez, Mariath & M. Figueiredo no Arquipélago de Abrolhos, Bahia (Tâmega et al., 2014).

**Tabela 3**: Espécies de algas coralináceas incrustantes descritas para o litoral brasileiro, com sua distribuição e referências. Código das localidades: AL= Alagoas; AM = Foz do Rio Amazonas; AR = Atol das Rocasl; BA= Bahia; CE= Ceará; ES= Espírito Santo; FN = Fernando de Noronha; IT = Ilha de Trindade; PE= Pernambuco; RJ= Rio de Janeiro; RN= Rio Grande do Norte; SC= Santa Catarina; SP= São Paulo; SPSP = Arquipélago de São Pedro e São Paulo. *Estudos que incluíram análise molecular.

| Táxons | Distribuição | Referência |
|---|---|---|
| **ORDEM CORALLINALES** | | |
| Família Coralinaceae | | |
| *Hydrolithon boergesenii* (Foslie) Foslie | BA | Figueiredo & Steneck (2000), Jesionek et al. (2016)* |
| *Hydrolithon breviclavium* (Foslie) Foslie | IT | Henriques et al. (2014b) |
| *Hydrolithon farinosum* (J.V. Lamouroux) D. Penrose & Y.M. Chamberlain | RJ, SP | Taylor (1960), Oliveira Filho (1977), Horta (2000) |
| *Harveylithon rupestre* (Foslie) A.Rösler, Perfectti, V.Peña & J.C.Braga | AR, BA, ES, FN, IT, SPSP | Villas-Boas (2008), Amado-Filho et al. (2012 a,b), Pereira-Filho et a. 2012, Crespo et al. (2014), Bahia (2014), Villas-Boas et al. (2015), Amado Filho et al. (2016), Moura et al. (2016) |
| *Harveylithon samoënse* (Foslie) A.Rösler, Perfectti, V.Peña & J.C.Braga | RJ | Tâmega & Figueiredo (2005) |
| *Lithophyllum atlanticum* Vieira-Pinto, Oliveira & Horta | AL, RJ, RS, SC, SP | Vieira-Pinto et al. (2014)*, Torrano-Silva (2015)*, Henriques (2016)* |
| *Lithophyllum corallinae* (P.L. Crouan & H.M. Crouan) Heydrich | AL, BA, ES, FN, RJ | Villas-Boas (2008), Villas-Boas et al. (2009), Amado-filho et al. (2012b), Bahia (2014), Henriques et al. (2014b), Torrano-Silva (2015)* |
| *Lithophyllum depressum* Villas-Boas, Figueiredo & Riosmena-Rodriguez | ES, BA, IT | Villas-Boas et al. (2009), Torrano-Silva (2015)* |
| *Lithophyllum johansenii* Woelkerling & Campbell | ES | Villas-Boas et al. (2009) |
| *Lithophyllum kaiseri* (Heydrich) Heydrich | BA | Figueiredo & Steneck (2000), Jesionek et al. (2016)* |
| *Lithophyllum margaritae* (Hariot) Heydrich | AL, ES, RJ, SC, SP | Horta (2000), Pascelli et al. (2013), Vieira-Pinto et al. (2014)*, Torrano-Silva (2015)*, Henriques (2016)* |
| *Lithophyllum rugosum* (Foslie) Me.Lemoine | SC | Pascelli et al. (2013) |
| *Lithophyllum stictaeforme* (Areschoug) Hauck | BA, SC, RJ, ES, PE | Nunes et al. (2008), Villas-Boas et al. (2009), Burgos (2011), Amado-Filho et al. (2012a), Pascelli et al. (2013), Bahia (2014), Costa et al. (2014b), Henriques et al. (2014b) |
| *Neogoniolithon accretum* (Foslie & Howe) Setch & Mason | BA | Figueiredo & Steneck (2000) |
| *Neogoniolithon atlanticum* Tâmega, Riosmena-Rodriguez, Mariath & Figueiredo | BA | Tâmega et al. (2014) |
| *Neogoniolithon brassica-florida* (Harvey) Setchell & Mason | BA, ES | Villas-Boas (2008), Amado-Filho et al. (2012a), Villas-Boas et al. (2015) |

Continuação da tabela 3.

| Táxons | Distribuição | Referência |
|---|---|---|
| **ORDEM CORALLINALES** | | |
| Família Coralinaceae | | |
| *Neogoniolithon fosliei* (Heydrich) Setchell & Mason | BA, SP | Taylor (1960), Oliveira Filho (1977), Bahia (2014) |
| *Pneophyllum conicum* (Dawson) Keats, Chamberlain & Baba | BA | Mariath et al. (2012), Jesionek et al. (2016)* |
| *Pneophyllum fragile* Kützing | BA, AR, ES, FN, RJ, SP | Taylor (1960), Oliveira Filho (1977), Bahia (2014) |
| *Porolithon improcerum* (Foslie & Howe) Lemoine | IT | Bahia et al. (2014c) |
| *Porolithon onkodes* (Heydrich) Foslie | BA, FN, IT | Taylor (1960), Oliveira Filho (1977), Henriques et al. (2014b), Jesionek et al. (2016)* |
| *Spongites fruticulosa* Kützing | ES, IT, SPSP | Crespo et al. (2014), Bahia (2014), Henriques et al. (2014b) |
| *Spongites yendoi* (Foslie) Chamberlain | BA, ES | Henriques et al. (2011), Costa et al. (2014a) |
| *Titanoderma prototypum* (Foslie) Woelkerling, Y.M. Chamberlain & P.C. Silva | BA, IT | Pereira Filho et al. (2012), Bahia (2014), Torrano-Silva (2015)*, Jesionek et al. (2016)* |
| *Titanoderma pustulatum* (J.V. Lamouroux) Nägeli | BA, ES, FN, SP, SPSP | Taylor (1960), Oliveira Filho (1977), Horta (2000), Crespo et al. (2014), Bahia (2014), Henriques et al. (2014b) Torrano-Silva (2015)* |

## JUSTIFICATIVA

As algas coralináceas incrustantes são consideradas construtoras de ecossistemas, produzindo importantes depósitos sedimentares, promovendo mudanças no ambiente, aumentando a heterogeneidade do habitat e disponibilidade de nichos, o que resulta em maior diversidade de espécies. O Atlântico Sul é notável por ter extensas áreas de recifes coralíneos. Apesar da grande importância deste grupo de algas para a resiliência da biodiversidade e considerando que muitas espécies de algas coralináceas incrustantes conhecidas para o Atlântico Sul ainda não foram devidamente estudadas segundo os caracteres taxonômicos atuais, faz-se necessária a realização de um grande esforço amostral com análise concatenada entre os dados morfológicos, anatômicos e moleculares para que as lacunas existentes na taxonomia do grupo sejam resolvidas. Desta forma, o presente estudo visa ampliar o conhecimento das algas coralináceas incrustantes pertencentes à ordem Corallinales que ocorrem na costa brasileira.

## OBJETIVO GERAL

O presente estudo tem por objetivo contribuir para o conhecimento da taxonomia das espécies de algas coralináceas incrustantes, pertencentes à ordem Corallinales, no Atlântico Sul, baseado em estudos morfoanatômicos e moleculares.

## OBJETIVOS ESPECÍFICOS

- Investigar a diversidade de espécies incrustantes pertencentes à ordem Corallinales no litoral brasileiro, através da combinação de dados morfoanatômicos e moleculares.
- Estabelecer critérios taxonômicos para melhor delimitação das espécies estudadas através da seleção de características consistentes para a taxonomia do grupo à luz de dados morfoanatômicos.
- Avaliar a aplicação de distintos marcadores moleculares (COI-5P, *psb*A, *rbc*L-3P, SSU rDNA, no reconhecimento de espécies de algas coralináceas incrustantes.

## METODOLOGIA GERAL

### *Coleta e Preservação das Amostras*

Foram realizadas 25 saídas de campo, entre os anos de 2011 e 2016, que abrangeram 75 localidades da costa brasileira, estendendo-se desde o Maranhão até o Rio Grande do Sul. A amostragem incluiu desde as porções do mediolitoral durante as marés baixas de sizígia, até o infralitoral (2-25m de profundidade) em marés de quadratura através de mergulho SCUBA. As estações de coleta foram georeferenciadas com o auxílio do GPS de campo (Garmin eTrex Legend® HCx) e estão dispostas na figura 4 e tabela 4, respectivamente.

As amostras foram limpas e secas ao ar livre durante no mínimo 24 horas e armazenadas em sílica-gel. Na identificação de cada amostra foram incluídos, além de informações gerais sobre a coleta, dados específicos como a cor, textura e tipo de substrato.

Em laboratório, as amostras foram examinadas utilizando estereomicroscópio (Leica® Zoom 2000) para a separação dos fragmentos para análise molecular e morfoanatômica. O material utilizado na análise molecular foi mantido em sílica-gel e os fragmentos utilizados na análise morfoanatômica foram preservados em formaldeído 4%.

### *Análise morfoanatômica*

Para a identificação morfoanatômica foram retirados fragmentos das porções vegetativas e reprodutivas (quando presentes). As técnicas histológicas para a microscopia óptica foram utilizadas seguindo método adaptado de Horta (2002):

- Descalcificação: as amostras foram submergidas em ácido nítrico ($HNO_3$) a 0,6 M por um período máximo de 24 horas; a comprovação de descalcificação do material foi realizada através da perfuração com agulha hipodérmica visando verificar se a rigidez do talo diminuiu; as amostras foram lavadas em água corrente para a retirada do agente descalcificante.

- Desidratação: utilização de série gradual de etanol - $C_2H_5OH$ (30, 50, 70, 90 e 100%), mantendo-se a amostra durante 20 minutos em cada concentração, em temperatura ambiente.
- Pré-infiltração: inserção da amostra na mistura que contém a solução de infiltração (50 ml de 2-hidroxietil metacrilato acrescida de 0,5g de benzoilo peróxido) e etanol 100%, mantendo-se o frasco em geladeira, por um período de 1-2 horas ou até o material afundar.
- Infiltração: colocação da amostra na solução de infiltração pura (sem etanol) e manutenção em geladeira por cerca de 12 horas.
- Inclusão: as amostras foram colocadas em moldes de polietileno, levando-se em consideração a orientação desejada, adicionando-se sobre esta a mistura de inclusão (15 ml de solução de infiltração + 1 ml de dimetilsulfóxido - endurecedor) até cobri-la.
- Polimerização: inserção do molde contendo a amostra imersa em resina em estufa à 40° C (cerca de 1-2 horas) ou em temperatura ambiente (cerca de 5-6 horas).
- Após a polimerização o material foi retirado do molde e fixado no suporte de madeira (cubo de cerca de 1,5 $cm^3$) com cola de secagem rápida, levando em consideração a orientação desejada para o seu seccionamento.
- Cortes histológicos: os blocos foram seccionados em 4-10 µm de espessura no micrótomo rotativo Thermo Cientific (modelo HM 325). Utilizou-se a navalha de aço da marca Leica perfil D para o corte.
- Os cortes foram distendidos, um a um, com pincel e pinça em lâminas histológicas contendo um filme de água destilada com detergente (30 ml de água com 3 gotas de detergente dermatológico) para quebrar tensão superficial desta.
- As lâminas foram colocadas em placa aquecedora à 42-44° C para adesão dos cortes (cerca de 5 minutos) e levadas à estufa à 35-40° C (1-2 horas) até secagem completa;
- A coloração das lâminas foi realizada empregando-se azul de toluidina acidificado com ácido bórico (0,16 g de toluidina e 1,6 g ácido bórico diluído em 100 ml de água). O tempo da coloração foi de cerca de 1 – 3 minutos à temperatura ambiente.
- Após a coloração as lâminas foram lavadas com água destilada para a retirada do excesso do corante e secas ao ar livre.
- As lâminas foram mantidas em dessecador para melhor visualização dos cortes histológicos.

A observação das lâminas com cortes histológicos foi realizada através de microscópio binocular óptico de captura (Olympus – CX 31) equipado com câmera acoplada (Q Imaging - Go Series) na qual a captura de imagens foi realizada via software (Q Imaging - QCapture PRO 7). As medidas foram realizadas utilizando-se o software AxioVision Rel. 4.8, onde a altura das células foi considerada como a distância entre as conexões celulares primárias e o diâmetro a distância perpendicular das mesmas. Para a altura dos conceptáculos, foi considerada a distância entre o assoalho e o teto da câmara do conceptáculo e o diâmetro a distância entre as paredes internas laterais da câmara do conceptáculo (Chamberlain, 1983).

Para a utilização da técnica de microscopia eletrônica de varredura (MEV) o material armazenado em formol a 4%, foi transferido para uma solução de álcool 70% e seco em estufa a aproximadamente 50°C por 48 horas (Horta, 2002). Com a utilização de estereomicroscópio de rotina (Leica – Zoom 2000) foram selecionados espécimes que apresentavam margens e/ou conceptáculos. Ainda sob o referido estereomicroscópio, utilizando-se alicates de tamanhos variados, o respectivo material foi fraturado, devidamente posicionado em suporte (*stubs*) com o auxílio de fitas adesivas dupla face e metalizado em ouro, com atmosfera estabilizada em 50 mTor de Argônio (Denton Vacuum - Desk IV), com tempo de exposição de cinco minutos, a uma corrente de 50 mA, com a voltagem de 12-25 kV, para se obter uma maior resolução. O microscópio eletrônico de varredura utilizado para captura de imagens foi o modelo JEOL - JSM 6390 LV.

**Identificação Taxonômica**

Foram observadas as seguintes características para as algas coralináceas incrustantes:

**Características vegetativas (morfologia externa e interna):**

- Coloração do talo na alga viva;
- Forma de crescimento;
- Conexões citoplasmáticas secundárias (tipos “pit” ou fusão);
- Células epitaliais (forma e número de camadas);
- Células subepitaliais (tamanho);
- Organização do talo (dímero ou monômero);
- Tricocitos (presença e localização).

**Características reprodutivas:**

- Conceptáculos tetra / bisporangiais (uni ou multiporado);
- Posição do conceptáculo no talo (elevados, nivelados ou afundados na superfície);
- Forma do conceptáculo (dimensões das cavidades, presença de anéis e columela).
- Características do teto dos conceptáculos tetrasporangiais (número e formato das células).

**Herborização**

As amostras identificadas foram acondicionadas tanto em lâminas definitivas devidamente etiquetadas com número de herbário, em sílica gel e acondicionadas em potes plásticos foscos etiquetados com os dados pertinentes.

Todo o material acima citado foi depositado no Herbário Alexandre Leal Costa (ALCB) da Universidade Federal da Bahia e Herbário da Universidade de Feira de Santana (HUEFS).

***Extração e Amplificação de DNA***

O DNA total foi extraído a partir de fragmentos oriundos de amostras secas acondicionadas em sílica-gel, utilizando-se o protocolo de extração com resina Chelex (Goff & Moon, 1993) ou método CTAB (*Cetyl Trimethyl Ammonium Bromide*) de Doyle e Doyle (1987) modificado. O DNA foi extraído dos mesmos espécimes utilizados na análise morfológica (Tabela 3).

Quatro marcadores moleculares (SSU rDNA, *psb*A, *rbc*L-3P e COI-5P) foram amplificados por PCR nas seguintes condições: 1x tampão de PCR, MgCl2 1,5 mM, dNTP 0,2 mM cada, *primer* de 0,2 μM, 5 ng de DNA e 1,25 U de Taq DNA polimerase (Invitrogen), num volume total de 25 μL. Para as reações de amplificação e sequenciamento de cada marcador, foram utilizados pares de iniciadores específicos: para o mitocondrial COI-5P, GazF1 e GazR1 (Saunders, 2005); para o plastidial *rbc*L-3P, F1150cor (Sissini et al., 2014) e R1460cor (Hernández-Kantún et al. 2016) ou e RrbcS (Freshwater et al., 1994); para o plastidial *psb*A, psbAF1 e psbAR2 (Yoon et al., 2002); e para o nuclear SSU rDNA, 18S5 'e 1055R, e 1055F e 18S3' (Milstein e Oliveira 2005).

A PCR para COI-5P foi conduzida com uma desnaturação inicial a 94°C seguida por 40 ciclos a 94°C durante 1 min. (desnaturação), 45°C durante 1 min. (anelamento) e 72°C (extensão) durante 1 min. seguido por uma extensão final a 72°C durante 5 min.

A PCR para *rbc*L-3P foi realizada com uma desnaturação inicial de 94°C durante 4 min. seguida de 2 ciclos a 94°C durante 1 min. (desnaturação), 40°C durante 1 min. (anelamento) e 72°C (extensão) para 2 min. depois 40 ciclos a 94°C durante 1 min. (desnaturação), 42°C durante 1 min. (anelamento) e 72°C (extensão) durante 2 min. seguido de uma extensão final a 72°C durante 5 min.

A PCR para *psb*A foi realizada uma desnaturação inicial a 94°C durante 2 min. seguido de 35 ciclos a 94°C durante 30 segundos (desnaturação), 47°C durante 1 min. (anelamento) e 72°C durante 2 min. (extensão) seguido de uma extensão final a 72°C durante 7 min.

A PCR para SSU rDNA foi realizada com uma desnaturação inicial de 94°C durante 4 min. seguida de 35 ciclos a 94°C durante 30 segundos (desnaturação), 60°C durante 1 min. (recozimento de iniciador) e 72°C (extensão) para 2 min. seguido por uma extensão final a 72°C durante 7 min. As reações foram realizadas no termociclador Vertic® 96-Well Thermal Cycler (Applied Biosystems, Foster City, Califórnia, EUA). As purificações e reações de sequenciamento dos produtos de PCR foram realizadas pela empresa GeneWiz (Cambridge, Massachusetts, EUA, http://www.genewiz.com/).

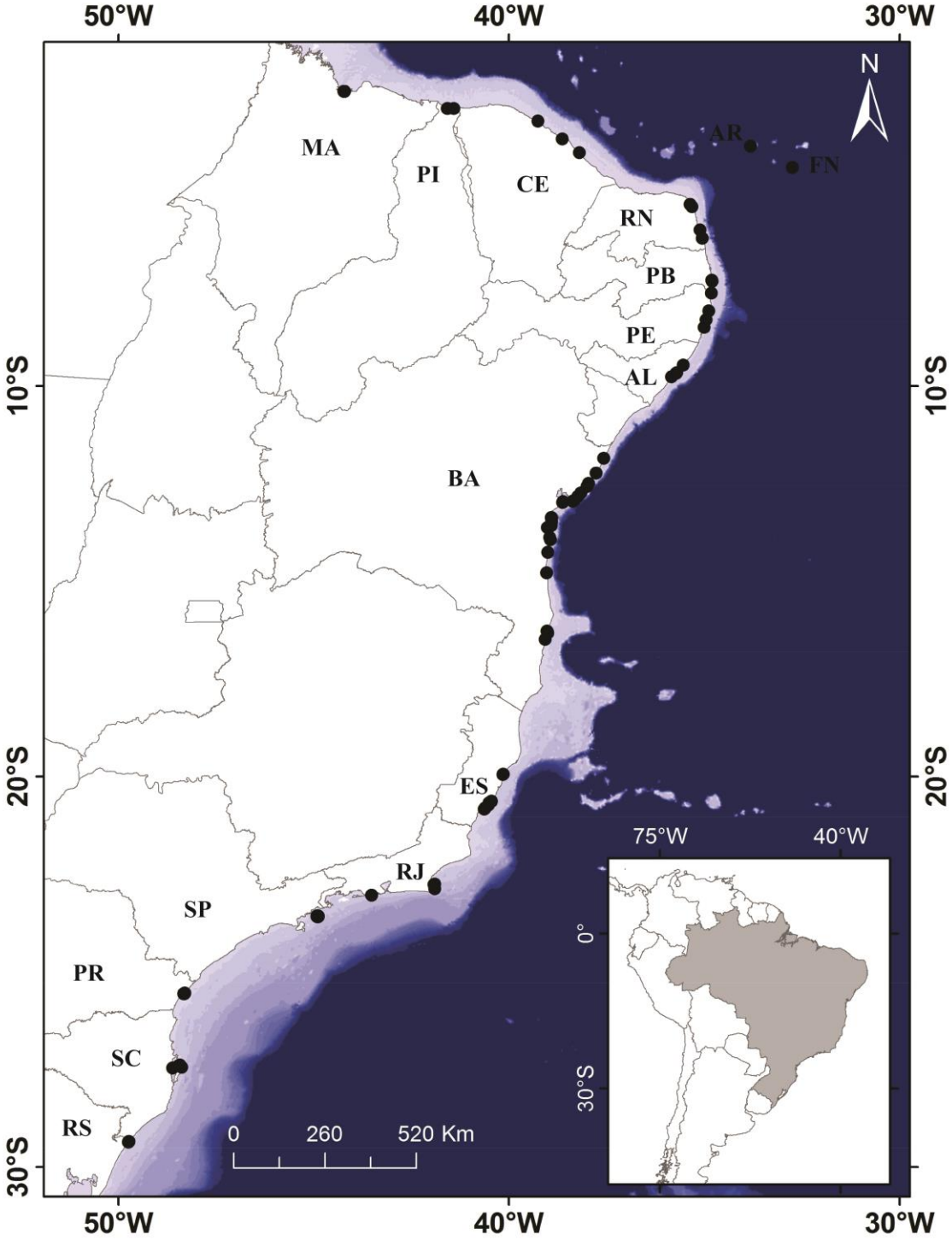


**Figura 4:** Mapa do litoral do Brasil mostrando os pontos de coleta e os respectivos estados amostrados: MA – Maranhão, PI – Piauí, CE – Ceará, RN – Rio Grande do Norte, AR – Atol das Rocas, FN – Fernando de Noronha, PB – Paraíba, PE – Pernambuco, AL – Alagoas, BA – Bahia, ES – Espírito Santo, RJ – Rio de Janeiro, SP – São Paulo, PR – Paraná, SC – Santa Catarina, RS – Rio Grande do Sul.

**Tabela 4:** Pontos de coleta com respectivas coordenadas geográficas.

| Estado | Município | Pontos de Coleta | Coordenadas |
|---|---|---|---|
| Maranhão | São Luís | Olhos D'Água | 2°28'16.83"S x 44°13'40.49"W |
| | São José do Ribamar | Araçagi | 2°28'00.75"S x 44°12'17.19"W |
| Piauí | Luís Correia | Praia do Coqueiro | 2°54'19.64"S x 41°34'31.25"W |
| | Luís Correia | Praia do Farol | 2°54'8.75"S x 41°33'49.78"W |
| | Cajueiro da Praia | Barra Grande | 2°54'22.70"S x 41°24'25.16"W |
| | Cajueiro da Praia | Cajueiro da Praia | 2°55'33.10"S x 41°20'10.42"W |
| Ceará | Caucaia | Pacheco | 3°41'9.18"S x 38°37'57.19"W |
| | Trairi | Flecheiras | 3°13'25.99"S x 39°15'00.63"W |
| | Cascavel | Caponga | 4°2'17.88"S x 38°11'35.68"W |
| Rio Grande do Norte | | Atol das Rocas | 3°52'26.8"S x 33°48'13.5"W |
| | Nísia Floresta | Pirambúzios | 6°00'44.62"S x 35°6'26.48"W |
| Paraíba | João Pessoa | Ponta do Seixas | 7°8'47.05"S x 34°47'52.01"W |
| | Conde | Carapibus | 7°17'59.56"S x 34°47'55.34"W |
| | Conde | Coqueirinho | 7°19'40.62"S x 34°47'43.42"W |
| Pernambuco | | Fernando de Noronha | 3°85'9.89"S x 32°44'18.85"W |
| | Goiana | Ponta das Pedras | 7°37'47.9"S x 34°48'31.2"W |
| | Cabo de Santo Agostinho | Enseada dos Corais | 8°18'50.3"S x 34°56'47.8"W |
| | Cabo de Santo Agostinho | Gaibú | 8°18'49.5"S x 34°56'48.4"W |
| | Recife | Pina | 8°05'25.52"S x 34°52'46.59"W |
| Alagoas | Maceió | Ponta Verde | 9°39'47.86"S x 35°41'42.84"W |
| | Maceió | Pajuçara | 9°40'50.49"S x 35°43'14.79"W |
| | Marechal Deodoro | Praia do Francês | 9°46'13.62"S x 35°50'19.52"W |
| | Paripueira | Praia Brava | 9°28'01.64"S x 35°32'10.88"W |
| Bahia | Entre Rios | Sítio do Conde | 11°51'11"S x 37°33'49"W |
| | Entre Rios | Subaúma | 12°14'10"S x 37°46'05"W |
| | Mata de São João | Imbassaí | 12°30'11"S x 37°57'36"W |
| | Mata de São João | Praia do Forte | 12°34'42"S x 38°00'06"W |
| | Camaçari | Arembepe | 12°44'25"S x 38°08'58"W |
| | Camaçari | Jauá | 12°49'38"S x 38°13'22"W |
| | Salvador | Stella Maris | 12°56'22"S x 38°19'41"W |
| | Salvador | Pedra do Sal | 12°57'06"S x 38°20'42"W |
| | Salvador | Itapuã | 12°57'22"S x 38°21'31"W |
| | Salvador | Pituba | 13°00'24.2"S x 38°27'17.5"W |
| | Salvador | Barra | 13°00'11"S x 38°32'01"W |
| | Vera Cruz (Ilha de Itaparica) | Mar Grande | 12°58'00"S x 38°36'30"W |
| | Vera Cruz (Ilha de Itaparica) | Penha | 12°59'08"S x 38°37'02"W |
| | Cairú | Tassimirim (Boipeba) | 13°34'49.01"S x 38°54'50.84"W |
| | Cairú | Garapuá | 13°29'13.14"S x 38°54'25.5"W |
| | Cairú | Morro de São Paulo | 13°22' 44"S x 38°54' 46"W |
| | Cairú | 2ª Praia Morro de S. Paulo | 13°22'44"S x 38°54'46"W |
| | Cairú | Ilha do Caitá | 13°22'53.31"S x 38°54'14.22"W |
| | Maraú | Taipu de Fora | 13°56'18.1"S x 38°55'40.7"W |
| | Maraú | Ponta do Mutá | 13°52'45.61"S x 38°56'50.79"W |
| | Maraú | Três Coqueiros | 13°52'55.16"S x 38°56'32.35"W |
| | Maraú | Praia do Francês | 13°56'18.1"S x 38°55'40.7"W |
| | Itacaré | Havaizinho | 14°15'45"S x 38°59'56"W |
| Espírito Santo | Guarapari | Praia das Castanheiras | 20°40'19.64"S x 40°29'46.01"W |
| | Guarapari | Pontal de Guaibura | 20°43'40.86"S x 40°31'19.32"W |
| | Guarapari | Praia de Setiba | 20°38'05.08"S x 40°26'24.5"W |
| | Anchieta | Praia dos Castelhanos | 20°50'05.08"S x 40°37'24.84"W |
| | Anchieta | Praia de Ubú | 20°48'49.36"S x 40°36'42.45"W |
| | Anchieta | Praia de Parati | 20°48'19.28"S x 40°36'07.45"W |
| | Serra | Praia da Curva da Baleia | 20°38'06.1"S x 40°26'24.7"W |

| Estado | Município | Pontos de Coleta | Coordenadas |
|---|---|---|---|
| Espírito Santo | Aracruz | Praia de Santa Cruz | 19°57'13.1"S x 40°09'08.44"W |
| | Aracruz | Praia do Coqueiral | 19°56'34.7"S x 40°08'30.6"W |
| Rio de Janeiro | Búzios | Praia Rasa | 23°02'34.4"S x 43°30'29.1"W |
| | Búzios | Manguinhos | 22°46'07.8"S x 41°54'44.5"W |
| | Cabo Frio | Praia das Conchas | 22°52'16.8"S x 41°53'51.2"W |
| | Cabo Frio | Ilha do Japonês | 22°45'47.8"S x 41°53'51.2"W |
| | Rio de Janeiro | Recreio dos Bandeirantes | 23°02'32.48"S x 43°30'27.3"W |
| | Rio de Janeiro | Praia do Pontal | 23°01'57.92"S x 43°28'51.35"W |
| São Paulo | Ubatuba | Ilha Rapada | 23°51'76.79"S x 45°04'10.72"W |
| Paraná | Paranaguá (Ilha do Mel) | Ponta de Nhá Pina | 25°33'59.0"S x 48°17'59.0"W |
| | Paranaguá (Ilha do Mel) | Praia do Miguel | 25°33'43.8"S x 48°18'05.4"W |
| | Paranaguá (Ilha do Mel) | Para da Pontinha | 25°33'43.58"S x 48°19'08.84"W |
| | Paranaguá (Ilha do Mel) | Praia Grande | 25°33'18.8"S x 48°17'56.8"W |
| | Paranaguá (Ilha do Mel) | Ponta do Joaquim | 25°32'46.9"S x 48°17'33.7"W |
| Santa Catarina | Florianópolis | Praia dos Ingleses | 27°26'33.88"S x 48°22'11.24"W |
| | Florianópolis | Ponta das Canas | 27°23'39.59"S x 48°26'08.08"W |
| | Bombinhas | Ilha Deserta | 27°27'54.22"S x 48°35'91.44"W |
| Rio Grande do Sul | Torres | Praia Grande | 29°20'27.1"S x 49°43'24.7"W |
| | Torres | Prainha | 29°20'41.5"S x 49°43'35.6"W |
| | Torres | Praia do Cal | 29°20'41.5"S x 49°43'35.8"W |
| | Torres | Paria da Guarita | 29°21'31.1"S x 49°44'05.3"W |

Continuação tabela 4.

Esta tese está estruturada em três capítulos, onde cada capítulo constitui um artigo, sendo os dois primeiros já submetidos para publicação e o último a ser submetido. No Capítulo 1, tratamos da diversidade específica do gênero *Harveylithon* no Atlântico Sul Tropical e Temperado Quente, incluindo a descrição de três novas espécies a partir da combinação de análise morfoanatômica, evidências filogenéticas e métodos de delimitação específica; no Capítulo 2, trazemos o primeiro estudo de espécies de *Neogoniolithon* para o Atlântico Sudoeste, baseado em caracteres moleculares e morfoanatômicos, onde três espécies foram identificadas, incluindo uma nova espécie e uma nova ocorrência para o Atlântico Sul; e no Capítulo 3, tratamos das relações filogenéticas entre as espécies brasileiras de algas coralinaceas incrustantes da ordem Corallinales.

## REFERÊNCIAS

AMADO-FILHO, G.M; MANEVELDT, G.; MARINS, B.V.; MANSO, R.C.C. PACHECO, M. R.; GUIMARÃES, S.P.B. 2007. Structure of rhodolith beds from a depth gradient of 4 to 55 meters at the south of Espírito Santo coast, Brazil. *Cienc. Mar.*, 32: 34-65.

AMADO FILHO G.M.; PEREIRA-FILHO, G.H.; BAHIA, R.G.; ABRANTES, D.P.; VERAS, P.C.; MATHEUS, Z. 2012a. Occurrence and distribution of rhodolith beds on the Fernando de Noronha Archipelago of Brazil. *Aquat. Bot.*, 101: 41-45.

AMADO-FILHO, G.M.; MOURA, R.L.; BASTOS, A.C.; SALGADO, L.T.; SUMIDA, P.Y.; GUTH, A.Z.; FRANCINI-FILHO, R.B.; PEREIRA-FILHO, G.H.; ABRANTES, D.P.; BRASILEIRO, P.S.; BAHIA, R.G.; LEAL, R.N.; KAUFMAN, L.; KLEYPAS, J.A.; FARINA,M.; THOMPSON, F.L. 2012b. Rhodolith Beds Are Major CaCO3 Bio-Factories in the Tropical South West Atlantic. *PLoS ONE*, 7: e35171 [online].

AMADO-FILHO, G.M.; MOURA, R.L.; BASTOS, A.C.; FRANCINI-FILHO, R.B.; PEREIRA-FILHO, G.H.; BAHIA, R.G.; MORAES, F.C.; MOTTA, F.S. 2016. Mesophotic ecosystems of the unique South Atlantic atoll are composed by rhodolith beds and scattered consolidated reefs. *Mar. Biodivers.*, 10.1007/s12526-015-0441-6 [online].

BAHIA, R. G.; ABRANTES, D. P.; BRASILEIRO, P. S. 2010. Rhodolith bed structure along a depth gradient on the northern coast of Bahia, Brazil. *Braz. J. Oceanogr.*, 58: 323-337.

BAHIA, R.G.; RIOSMENA-RODRIGUEZ, R.; MANEVELDT, G.W.; AMADO-FILHO, G.M. 2011. First report of *Sporolithon ptychoides* (Sporolithales, Corallinophycidae, Rhodophyta) for the Atlantic Ocean. *Phycol. Res.*, 59:64–69.

BAHIA, R.G.; AMADO-FILHO, G.M.; MANEVELDT, G.W.; ADEY, W.H.; JOHNSON, G.; MARINS, B.V.; LONGO, L.L. 2013. *Sporolithon tenue* sp. nov. (Sporolithales, Corallinophycidae, Rhodophyta): a new rhodolith-forming species from the tropical southwestern Atlantic. *Phycol. Res.*, 62: 44-54.

BAHIA, R.G. 2014. Algas coralináceas formadoras de rodolitos da plataforma continental tropical e ilhas oceânicas do Brasil: levantamento florístico e taxonomia. Instituto de Pesquisas Jardim Botânico do Rio de Janeiro. Tese de Doutorado, 220 pp.

BAHIA, R.G.; AMADO-FILHO, G.M.; MANEVELDT, G.W. 2014a. *Sporolithon molle* (Heydrich) Heydrich (Sporolithales, Corallinophycidae, Rhodophyta): an addition to the Atlantic flora found on a remote oceanic island. *Cryptogamie Algologie*, 35 (1): 7-14.

BAHIA, R.G.; AMADO-FILHO, G.M.; AZEVEDO, J.; MANEVELDT, G.W. 2014b. *Porolithon improcerum* (Porolithoideae, Corallinaceae) and *Mesophyllum macroblastum* (Melobesioideae, Hapalidiaceae): new records of crustose coralline red algae for the Southwest Atlantic Ocean. *Phytotaxa,* 190: 38-44.

BAHIA, R.G.; AMADO-FILHO, G.M.; MANNEVELT, G.W.; ADEY, W.H.; JOHNSON, G.; JESIONEK, J.B.; LONDON, L.L. 2015. *Sporolithon yoneshigueae* sp. nov. (Sporolithales, Corallinophycidae, Rhodophyta), a new rhodolith-forming coralline alga from the southwest Atlantic. *Phytotaxa*, 224(2): 140-158.

BAILEY, J.C.; CHAPMAN, R.L. 1996. *Evolutionary relationships among coralline red algae (Corallinaceae, Rhodophyta) determined by 18S rRNA gene sequence analysis*. In: Chaudhary, B.R. & Agrawal, S.B. (Eds.) Cytology, Genetics and Molecular Biology of Algae. SPB Academic Publishing, Amsterdam, p. 363-376.

BAILEY, J.C.; CHAPMAN, R.L. 1998. A phylogenetic study of the Corallinales (Rhodophyta) based on nuclear small-subunit rRNA gene sequences. *J. Phycol.*, 34: 692-705.

BAILEY, J.C. 1999. Phylogenetic positions of *Lithophyllum incrustans* and *Titanoderma pustulatum* (Corallinaceae, Rhodophyta) based on 18S rRNA gene sequence analyses, with a revised classification of the Lithophylloideae. *Phycologia*, 38 (3): 208-216.

BITTNER, L.; PAYRI, C.E.; MANEVELDT, G.W.; COULOUX, A.; CRUAUD, C.; REVIERS, B.; LE GALL, L. 2011. Evolutionary history of the Corallinales (Corallinophycidae, Rhodophyta) inferred from nuclear, plastidial and mitochondrial genomes. *Mol. Phylogenet. Evol.*, 61: 697-713.

BORGES, V.P.; BASTOS, E.; BATISTA, M.B.; BOUZON, Z.; LHUILLIER, C.; SCHMIDT, E.C.; SISSINI, M.N.; HORTA, P.A. 2014. The genus *Melobesia* (Corallinales, Rhodophyta) from the subtropical South Atlantic, with the addition of *M. rosanoffii* (Foslie) Lemoine. *Phytotaxa*, 190(1): 268-276.

BROOM, J.E.S.; HART, D.R.; FARR, T.J.; NELSON, W.A.; NEILL, K.F.; HARVEY, A.S.; WOELKERLING, W.J. 2008. Utility of psbA and SSU rDNA for phylogenetic

reconstruction in the Corallinales based on New Zealand taxa. *Mol. Phylogenet. Evol.,* 46: 958-973.

CABIOCH, J. 1972. Etude sur les Corallinacées. II. La morphogénese: consequences systématiques et phylogénétiques. *Cah. Biol. Mar.;* 13: 137–287.

CABIOCH, J. 1988. Morphogenesis and generic concepts in coralline algae - a reappraison. *Helgolander. Meeresunters.,* 42: 493-509.

CHAMBERLAIN, Y. M. 1983. Studies in the Corallinaceae with special reference to *Fosliella* and *Pneophyllum* in the British Isles. *Bulletin of the British Museum (Natural History) Botany,* 11: 291-463.

COSTA, I.O.; HORTA P.A.; NUNES, J.M.C. 2014a. *Spongites yendoi* (Foslie) Chamberlain (Corallinales, Rhodophyta) on the coast of Bahia, Brazil. *Braz. J. Bot.* 37(4): 637-641.

COSTA, I. O.; HORTA, P. A.; BERGSTROM, E. R.; NUNES, J. M. C. 2014b. Taxonomic study of crustose coralline algae off the northeastern Brazilian coast. *Phytotaxa,* 190(1): 130-161.

CRESPO, T. M.; BAHIA, R. G.; MANEVELDT, G. W.; AMADO FILHO, G. M. 2014. Floristic composition of crustose coralline algae from the St. Peter and St. Paul Archipelago, a summit of the Mid-Atlantic Ridge. *Phytotaxa,* 190(1): 17-37.

FARIAS, J.; RIOSMENA-RODRÍGUEZ, R.; BOUZON, Z.; OLIVEIRA, E.C.; HORTA, P.A. 2010. *Lithothamnion superpositum* (Corallinales; Rhodophyta): First description for the Western Atlantic or rediscovery of a species? *Phycol. Res.,* 58: 210-216.

FARR, T.; BROOM, J.; HART, D.; NEILL, K.; NELSON, W. 2009. Common coralline algae of northern New Zealand: an identification guide. *NIWA Information Series,* 70: 125p.

FIGUEIREDO, M.A.O. 1997. Colonization and growth of crustose coralline algae in Abrolhos, Brazil. *Proceedings of the 8$^{th}$ International Coral Reef Symposium,* 1: 689-693.

FIGUEIREDO, M.A.O.; STENECK, R.S. 2000. Floristic and ecological studies of crustose coralline algae on Brazil's Abrolhos reefs. *9th International Coral Reef Symposium, Bali,* 1: 493-497.

FOSTER, M.S. 2001. Rhodoliths: between rocks and soft places - Minireview. *J. Phycol.,* 37: 659-667.

HALL, T.A. 1999. BioEdit: a user-friendly biological alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.* 41: 95-98.

HARVEY, A.S.; BROADWATER, S.T.; WOELKERLING, W.J.; MITROVSKI, P.J. 2003. *Choreonema* (Corallinales, Rhodophyta): 18S rDNA Phylogeny and resurrection of the Hapalidaceae for the subfamilies Choreonematoideae, Austrolithoideae, and Melobesioideae. *J. Phycol.*, 39: 988-998.

HARVEY, A.; WOELKERLING, W.; FARR, T.; NEILL, K.; NELSON, W. 2005. Coralline algae of central New Zealand, an identification guide to commom 'crustose' species. *NIWA Information series,* 57: 145.

HARVEY, A.; WOELKERLING, W. 2007. A guide to non geniculate coralline red algal (Corallinales, Rhodophyta) rhodolith identification. *Cienc. Mar.,* 33: 411-426.

HENRIQUES, M.C.; VILLAS-BOAS, A.; RIOSMENA RODRIGUEZ, R.; FIGUEIREDO, M.A.O. 2011. New records of rhodolith-forming species (Corallinales, Rhodophyta) from deep water in Espírito Santo, Brazil. *Helgol Mar Res.,* 66: 219-231.

HENRIQUES, M. C.; RIOSMENA-RODRIGUEZ, R.; COUTINHO, L. M.; FIGUEIREDO, M.A. 2014a. Lithophylloideae and Mastophoroideae (Corallinales, Rhodophyta) from the Brazilian continental shelf. *Phytotaxa*, 190(1): 112-129.

HENRIQUES, M. C.; COUTINHO, L. M.; RIOSMENA-RODRIGUEZ, R.; BARROS-BARRETO, M. B.; KHADER, S.; FIGUEIREDO, M. A. 2014b. Three deep water species of *Sporolithon* (Sporolithales, Rhodophyta) from the Brazilian continental shelf, with the description of Sporolithon elevatum sp. nov. *Phytotaxa*, 190(1): 320-330.

HENRIQUES, M.C. 2016. Taxonomia e filogenia molecular de algas calcárias incrustantes da Bacia de Campos e importância da gestão da sua biodiversidade no Brasil. Universidade Federal do Rio de Janeiro. Tese de Doutorado, 222 pp.

HERNÁNDEZ-KANTÚN, J.J.; GABRIELSON, P.; HUGHEY, J.R.; PEZZOLESI, L.; RINDI, F.; ROBINSON,N.M.; PEÑA, V.; RIOSMENA-RODRIGUEZ, R.; LE GALL, L.; ADEY, W. 2016. Reassessment of branched *Lithophyllum* spp. (Corallinales, Rhodophyta) in the Caribbean Sea with global implications. *Phycologia*, 55 (6): 619-639.

HIND K.R.; SAUNDERS G.W. 2013. A Molecular Phylogenetic Study of the Tribe Corallineae (Corallinales, Rhodophyta) with an Assessment of Genus-Level Taxonomic Features and Descriptions of Novel Genera. *Phycologia*, 49: 103-114.

HIND, K.R.; GABRIELSON, P.W.; LINDSTROM, S.C.; MARTONE, P.T. 2014. Misleading morphologies and the importance of sequencing type specimens for resolving coralline taxonomy (Corallinales, Rhodophyta): *Pachyarthron cretaceum* is *Corallina officinalis*. *J. Phycol.*, 50: 760-4.

HORTA, P.A. 2000. *Macroalgas do infralitoral do sul e sudeste do Brasil: Taxonomia e Biogeografia.* Tese de Doutorado. Universidade de São Paulo, São Paulo. 301p.

HORTA, P.A. 2002. Bases para a identificação das coralináceas não articuladas do litoral brasileiro – uma síntese do conhecimento. *Biotemas,* 15: 7-44.

HORTA, P.A; AMÂNCIO, E.; COIMBRA, C.S.; OLIVEIRA, E.C. 2001. Considerações sobre a distribuição e origem da flora de macroalgas brasileiras. *Hoehnea,* 28: 243-265.

HORTA, P.A.; SCHERNER, F.; BOUZON, Z.L.; RIOSMENA-RODRIGUES, R.; OLIVEIRA, E.C. 2011. Morphology and reproduction of *Mesophyllum erubescens* (Foslie) Me. Lemoine (Corallinales, Rhodophyta) from Southern Brazil. *Revista Brasileira de Botânica,* 34: 125-134.

HORTA, P. A., RIUL, P., AMADO FILHO, G. M., GURGEL, C.F.D. et al. 2016. Rhodoliths in Brazil: Current knowledge and potential impacts of climate change. *Braz. J. Oceanogr*. 64:117-36.

KATO, A.; BABA, M.; SUDA, S. 2011. Revision of the Mastophoroideae (Corallinales, Rhodophyta) and polyphyly in nongeniculate species widely distributed on Pacific coral reefs. *J. Phycol.,* 47(3): 662-672.

LAMOUROUX, J. V. 1816. *Histoire des polypiers coralligenes flexibles*.

LAMOUROUX, J. V. F. 1812. Extrait d'un mémoire sur la classification des Polypiers coralligènes non entièrement pierreux. *Nouveau Bulletin des Sciences, par la Société Philomatique de Paris,* 3(63): 181-188.

LE GALL, L.; PAYRI, C.E.; BITTNER, C.E.; SAUNDERS, G.W. 2009. Multigene phylogenetic analyses support recognition of the Sporolithales ord. nov. *Mol. Phylog. Evol.;* 54: 302-305.

LINNAEUS, C. 1767. *Mantissa Plantarum. Generum Editionis vi et Specierum Editionis ii. Laurentius Salvius, Stockholm*.

LITTLER, M.M.; LITTLER, D.S. 2008. Coralline algal rhodoliths form extensive benthic communities in the Gulf of Chiriqui, Pacific Panama. *Coral Reefs*, 27: 553-553.

MAGGS, C. A.; VERBRUGGEN, H.; CLERCK, O. 2007. Molecular systematic of red algae: building future structures on firm foundations. In BRODIE, J.; LEWIS, J.

(Eds). Unravelling the algae: the past, present and future. *Taylor; Francis group*, 1: 103-121.

MANEVELDT, G.; KEATS, D. 2008. Effects of herbivore grazing on the physiognomy of the coralline alga *Spongites yendoi* and on associated competitive interactions. *Afr. J. Mar. Sci.*, 30: 581-593.

MARIATH, R.; RIOSMENA-RODRIGUEZ R.; FIGUEIREDO, M. 2012. *Lithothamnion steneckii* sp. nov. and *Pneophyllum conicum*: new coralline red algae (Corallinales, Rhodophyta) for coral reefs of Brazil. *Algae*, 27: 249-258.

MILSTEIN, D.; OLIVEIRA, M.C. 2005. Molecular phylogeny of Bangiales (Rhodophyta) based on small subunit rDNA sequencing: emphasis on Brazilian Porphyra species. *Phycologia*, 44:212-221.

MOURA, C.W.N.; KRAUS, J.E.; CORDEIRO-MARINO, M. 1997. Metodologia para obtenção de cortes histológicos com historresina e coloração com azul de toluidina O para algas coralináceas (Rhodophyta, Corallinales). *Hoehnea*, 24: 17-27.

MOURA, R.L.; SECCHIN, N.A.; AMADO-FILHO, G.M.; FRANCINI-FILHO, R.B.; FREITAS, M.O.; MINTE-VERA, C.V.; TEIXEIRA, J.B.; THOMPSON, F.L.; DUTRA, G.F.; SUMIDA, P.Y.G.; GUTH, A.Z.; LOPES, R.M.; BASTOS, A.C. 2013. Spatial patterns of benthic megahabitats and conservation planning in the Abrolhos Bank. *Cont. Shelf. Res.*, 70: 109-117.

MOURA, R.L.; AMADO-FILHO, G.M.; MORAES, F.C.; et al. 2016. An extensive reef system at the Amazon River mouth. *Science Advances*, 2: e1501252-e1501252.

NELSON W.A.; SUTHERLAND J.E.; FARR T.J.; HART D.R.; NEILL K.F.; KIM H.J.; YOON H.S. 2015. Milti-gene phylogenetic analyses of New Zealand coralline algae: *Corallinapetra novaezelandiae* gen. et sp. nov. and recognition of the Hapalidiales ord. nov. *J. Phycol.*, 51: 545-468.

NUNES, J.M.C.; GUIMARÃES, S.M.P.B.; DONNANGELO, A.; FARIAS, J.; HORTA, P.A. 2008. Aspectos taxonômicos de três espécies de coralináceas não geniculadas do litoral do Estado da Bahia, Brasil. *Rodriguesia* 59(1): 75-86.

OLIVEIRA FILHO, E.C. 1977. *Algas Marinhas Bentônicas do Brasil.* Tese de Livre Docência. Universidade de São Paulo, São Paulo, 407p.

PASCELLI, C., RIUL, P., RIOSMENA-RODRÍGUEZ, R., SCHERNERA, F, NUNES, M., HALL-SPENCER, J.M., OLIVEIRA, E.C. & HORTA, P. 2013. Seasonal and depth-driven changes in rhodolith bed structure and associated macroalgae off Arvoredo island (southeastern Brazil). *Aquat. Bot.* 111: 62–65.

RIOSMENA-RODRIGUEZ, R. 1993. Una Propuesta de tecnica histológica para el estudio de algas coralinas (Corallinales: Rhodophyta). *Revista Investigación Científica,* 4: 65-73.

RIUL, P.; LACOUTH, P.; PAGLIOSA, P.R.; CHRISTOFFERSEN, M.L.; HORTA, P.A. 2009. Rhodolith beds at the easternmost extreme of South America: Community structure of an endangered environment. *Aquat. Bot.,* 90: 315–320.

RONQUIST, F.; HUELSENBECK, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.

RÖSLER, A.; PERFECTTI, F.; PEÑA, V.; BRAGA, J.C. 2016. Phylogenetic relationships of corallinaceae (Corallinales, Rhodophyta): taxonomic implications for reef-building corallines. *J. Phycol.,* 52(3):412-431.

SISSINI, M. N.; OLIVEIRA, M. C.; GABRIELSON, P. W.; ROBINSON, N. M.; OKOLODKOV, Y.B.; RIOSMENA-RODRÍGUEZ, R.;; HORTA, P.A. 2014. *Mesophyllum erubescens* (Corallinales, Rhodophyta)—so many species in one epithet. *Phytotaxa,* 190(1): 299-319.

STELLER, D.L.; RIOSMENA-RODRÝGUEZ, R.; FOSTER, M.S.; ROBERTS, C. 2003. Rhodolith bed diversity in the Gulf of California: the importance of rhodolith structure and consequences of anthropogenic disturbances. *Aquat. Conserv.,* 13: 5–20.

STENECK, R.S.; TESTA, V. 1997. Are coralline algae important to reefs today on in the past? Symposium summary. *Proc. 8th Int. Coral Reef Symposium,* 1: 685-688.

TÂMEGA, F.T.S.; FIGUEIREDO, M.A.O. 2005. Distribuição das algas calcáreas incrustantes (Corallinales, Rhodophyta) em diferentes habitats na Praia do Forno, Armação dos Búzios, Rio de Janeiro. *Rodriguésia,* 56: 123-132.

TÂMEGA, F.T.S.; RIOSMENA-RODRIGUES, R.; MARIATH, R.; FIGUEIREDO, M.A.O. 2014. Nongeniculate coralline red algae (Rhodophyta: Corallinales) in coral reefs from Northeastern Brazil and a description of *Neogoniolithon atlanticum* sp. nov. *Phytotaxa,* 190(1): 277-298.

TAYLOR, W.R. 1960. Marine algae of the eastern tropical and subtropical coasts of the Americas. University of Michigan Press, Michigan.

THORPE, J. P.; SOLÉ-CAVA, A. M. 1994. The use of allozyme electrophoresis in invertebrate systematics. *Zool. Scr.;* 23:3–18.

TORRANO-SILVA, B.N.; RIOSMENA-RODRÍGUEZ, R.; DE OLIVEIRA, M.C. 2014. Systematic position of *Paulsilvella* in the Lithophylloideae (Corallinaceae, Rhodophyta) confirmed by molecular data. *Phytotaxa*, 190(1): 94-111.

TORRANO-SILVA, B.N. 2015. *Taxonomia e Filogenia de Lithophylloideae no Brasil.* Universidade de São Paulo. Tese de doutorado. 250pp.

VIEIRA-PINTO, T.; OLIVEIRA, M. C.; BOUZON, J.; SISSINI, M.; RICHARDS, J. L.; RIOSMENA-RODRÍGUEZ, R.; HORTA, P. A. 2014. *Lithophyllum* species from Brazilian coast: range extension of *Lithophyllum margaritae* and description of *Lithophyllum atlanticum* sp. nov. (Corallinales, Corallinophycidae, Rhodophyta). *Phytotaxa*, 190(1): 355-369.

VIEIRA-PINTO, T. 2016. *Diversidade das algas calcárias crostosas do Brasil baseada em marcadores moleculares e morfologia.* Universidade de São Paulo. Tese de doutorado. 312pp.

VILLAS-BOAS, A.B.; RIOSMENA-RODRIGUEZ, R.; AMADO-FILHO, G.M.; MANEVELDT, G.W.; FIGUEIREDO, M.A.O. 2009. Rhodolith-forming species of Lithophyllum (Corallinales; Rhodophyta) from Espírito Santo, Brazil, including the description of L. depressum sp. nov. *Phycologia*, 48: 237-248.

WOELKERLING, W. J. 1988. *The Coralline red algae: An analysis of the genera and subfamilies of nongeniculate corallinaceae.* Oxford University Press, Oxford. 268p.

WOELKERLING, W. J. 1985. A taxonomic reassessment of *Spongites* (Corallinaceae, Rhodophyta) based on studies of Kützing's original collections. *Br. Phycol.*, 20: 123-153.

YOON, H.S.; HACKETT, J.D.; BHATTACHARYA, D. 2002. A single origin of the peridinin- and fucoxanthin- containing plastids in dinoflagellates through tertiary endossymbiosis. *Proc. Nat. Acad. Sci. Unit. St. Am.*, 99(18): 11724-11729.

# CAPÍTULO 1

## REEF-BUILDING CORALLINE ALGAE FROM THE SOUTH ATLANTIC: FILLING GAPS WITH THE RECOGNITION OF *HARVEYLITHON* (CORALLINACEAE, RHODOPHYTA) ON THE BRAZILIAN COAST*

IARA OLIVEIRA COSTA, PRISCILA BARRETO DE JESUS, TIANA DA SILVA DE JESUS, POLINE DOS SANTOS SOUZA, PAULO ANTUNES HORTA, JOSÉ MARCOS DE CASTRO NUNES

***Manuscrito submetido ao periódico Journal of Phycology**

# REEF-BUILDING CORALLINE ALGAE FROM THE SOUTH ATLANTIC: FILLING GAPS WITH THE RECOGNITION OF *HARVEYLITHON* (CORALLINACEAE, RHODOPHYTA) ON THE BRAZILIAN COAST[1]

Iara Oliveira Costa [2]; Programa de Pós-Graduação em Botânica, Universidade Estadual de Feira de Santana, Av. Transnordestina, s/n, Feira de Santana, BA, 44031-460, Brazil

Priscila Barreto de Jesus; Programa de Pós-Graduação em Botânica, Universidade Estadual de Feira de Santana, Av. Transnordestina, s/n, Feira de Santana, BA, 44031-460, Brazil

Tiana da Silva de Jesus; Laboratório de Algas Marinhas, Instituto de Biologia, Universidade Federal da Bahia, Rua Barão de Jeremoabo, s/n, Salvador, BA, 40.170-115, Brazil

Poline dos Santos Souza; Laboratório de Algas Marinhas, Instituto de Biologia, Universidade Federal da Bahia, Rua Barão de Jeremoabo, s/n, Salvador, BA, 40.170-115, Brazil

Paulo Antunes Horta; Departamento de Botânica, Universidade Federal de Santa Catarina, Caixa Postal 476, 88010-970 Florianópolis, SC, Brazil

José Marcos de Castro Nunes; Laboratório de Algas Marinhas, Instituto de Biologia, Universidade Federal da Bahia, Rua Barão de Jeremoabo, s/n, Salvador, BA, 40.170-115, Brazil

[2]Author for correspondence and present address: Laboratório de Algas Marinhas, Instituto de Biologia, Universidade Federal da Bahia, Rua Barão de Jeremoabo, s/n, Salvador, BA, 40.170-115, Brazil. Email: iaraoc@hotmail.com; phone: 55(71) 32836598; Fax: 55(71)32836511.

Condensed title: *HARVEYLITHON* IN THE SOUTH ATLANTIC

ABSTRACT

The South Atlantic is notable for having extensive reef areas cemented by coralline red algae. The current study constitutes an advancement in the knowledge of the specific diversity of *Harveylithon* in Tropical and Warm Temperate South Atlantic, one of the most important reef builders in tropical and temperate environments. The combination of morphological analysis, phylogenetic evidence and specific delimitation methods (mBGD, ABGD, SPN and PTP), the latter using four molecular markers, resulted in the circumscription of three new species for the scientific community: *H. catarinense* sp. nov., *H. maris-bahiensis* sp. nov. and *H. riosmenum* sp. nov. In addition, SSU rDNA, COI-5P, *psb*A and *rbc*L-3P regions are discussed as promising markers to be used in specific delimitation studies of coralline algal reef builders.

**Key index words:** Crustose coralline algae; Corallinales; Metagoniolithoideae; Single-Marker Delimitation Methods; Taxonomy.

**Abbreviations:** ABGD, Automated Barcode Gap Discovery; BP, Bootstrap percentage; CCA, crustose coralline algae; COI-5P, the standard DNA barcode region of the mitochondrial cytochrome c oxidase 1 gene (*cox*1); dNTP, triphosphate deoxyribonucleotide; mBGD, Manual Barcode Gap Discovery; MCMC, Markov Chain Monte Carlo; ML, maximum likelihood; PP, Bayesian Posterior probability; PSH, Primary Species Hypothesis; *psb*A, gene encoding the D1 protein of photosystem II; PTP, Poisson Tree Processes; *rbc*L, gene encoding the large subunit of RuBisCo; SPN, Statistical Parsimony Network; SSH, Secondary Species Hypothesis; UPA, Universal Plastid Amplicon 23S rRNA gene.

# INTRODUCTION

The South Atlantic is notable for having extensive reef areas. Such areas have been studied with focus on different algal groups; the subject of the studies were the origin of biodiversity, evolutionary relationships, and connectivity (e.g. Costa et al. 2014a, b, Sissini et al. 2014, Jesus et al. 2015, 2016, Horta et al. 2016, Lyra et al. 2016). Many of these reefs are mostly made of crustose coralline algae (CCA); an example is Atol das Rocas, the only atoll in the South Atlantic (Gherardi & Bonsence 2001). Even coastal areas have their coral and rocky reefs cemented and covered by CCA which mitigate erosion and elevate both substrate stability and niche complexity. Among these CCA, the most frequent and abundant genera of Corallinaceae are: *Hydrolithon*, *Mastophora*, *Metamastophora*, *Neogoniolithon*, *Porolithon*, *Pneophyllum*, and *Spongites*. All these taxa are formerly known as Mastophoroideae, and are now circumscribed by the family Corallinaceae (Kato et al. 2011).

Rösler et al. (2016) analyzed the phylogenetic relationships of these genera and proposed the genus *Harveylithon* as the most suitable placement for species of the genus *Hydrolithon* sensu Penrose (1996). These authors re-circumscribed the subfamily Metagoniolithoideae in order to include three genera: *Metagoniolithon*, *Porolithon* and *Harveylithon*. Metagoniolithoideae was then characterized as having geniculate thalli with conspicuous mucilaginous caps; genicula, when present, have many-celled untiered filaments (Johansen 1969), or non-geniculate thalli with monomerous organization and lateral cell fusions present; secondary connections absent; trichocytes occurring singly or in horizontal fields; tetrasporangial uniporate conceptacle roofs are formed by filaments peripheral to the fertile area and interspersed among tetrasporangial initials; and spermatangia developed on the floor of the male conceptacle (Rösler et al. 2016).

*Harveylithon* presents monomerous thallus organization and a plumose ventral core; trichocytes usually occur singly on the surface or buried in the thallus; and cells lining the pore canal of tetrasporangial conceptacle are oriented perpendicular to the thallus surface. This genus is typified by *H. rupestre* and is further represented by *H. munitum*; *H. samoënse*; and *H. canariense*. The first three species had previously been described as *Hydrolithon*, while the latter was initially described as *Goniolithon accretum* f. *canariense* by Foslie (1906). The form *canariense* was raised to the category of species by Rösler et al. (2016), standing out, until now, as an endemic species of Canary Islands.

Rosler et al. (2016) sequenced only the type material of *Goniolithon accretum* f. *canariensis*. *Harveylithon munitum*, *H. rupestre*, *H. samoënse* had their circumscription based on materials from the Indo-Pacific, which were analyzed by morpho-anatomical and molecular characterization. The representatives of the genus listed for the South Atlantic had their determination based only on morpho-anatomical characters, lacking molecular basis: *H. rupestre* (Amado Filho et al. 2012a, b, Crespo et al. 2014, Villas-Boas et al. 2015), *H. samöense* (Tâmega & Figueiredo 2005), and *H. munitum* (Woelkerling 1998). Such a lack is relevant considering the distinct macro-ecological conditions of the taxa areas of occurrence (Spalding et al. 2007) and their potential biogeographic isolation because distance or ecophysiological barriers (Sanches et al. 2016). Additionally, it should be highlighted that important examples of cryptic species can be found among the representatives of CCA in the South Atlantic (Sissini et al. 2014, Vieira-Pinto et al. 2014).

The insufficiency of anatomical characters to segregate taxa has been revealed for different groups of coralline algae considering different molecular markers (Hernández-Kantún et al. 2004, Kato et al. 2013, Peña et al. 2014, Sissini et al. 2014, Hind et al. 2015). Thus, new approaches using molecular analysis have been providing a better understanding of the phylogenetic relationships of coralline algae. Occasionally, such analyses confirm ancient circumscriptions based on anatomical data and bring novelty at all classification levels (Bailey & Chapman 1996, Kato et al. 2011, 2013, Hind & Saunders 2013, Hind et al. 2016).

A large part of these studies has used the nuclear marker SSU rDNA to analyze relationships at the levels of orders, families and subfamilies (Bailey & Chapman 1996, Bailey 1999, Harvey et al. 2003, Broom et al. 2008, Vidal et al. 2008, LeGall et al. 2010, Nelson et al. 2015, Hind et al. 2016). For analysis at the generic and specific level, most molecular studies have used less conserved markers, such as the plastidial *psb*A (Sissini et al. 2014, Torrano-Silva et al. 2014, Hind et al. 2015, Maneveldt et al. 2015, Hernandez-Kantun et al. 2016, Hind et al. 2016), the *rbc*L (Sissini et al. 2014, Torrano-Silva et al. 2014, Hind et al. 2015, Hernandez-Kantun et al. 2016, Hind et al. 2016), and the mitochondrial COI-5P (Vieira-Pinto et al. 2014, Hind et al. 2015, Hind et al. 2016).

The only existing nucleotide sequences for the species of the genus *Harveylithon* were produced by Rösler et al. (2016) for the LSU rDNA, SSU rDNA, *psb*A and UPA markers; with the exception of the SSU rDNA, sequence attributed to *H. samöense* (as *Hydrolithon samöense*) from South Australia by Bailey et al. (2004).

The present study aims to characterize morphological and molecularly the representatives of Metagoniolithoideae in the Tropical and Warm Temperate South Atlantic. Additionally, we will discuss the taxonomic and biogeographic implications related to the presence of *Harveylithon* on this region. For this, in addition to the morpho-anatomical characters, four molecular markers were evaluated: the nuclear SSU rDNA, the plastidial *psb*A and *rbc*L-3P, and the mitochondrial COI-5P. This work contributes to filling an important gap in the distribution of the genus *Harveylithon*, and complementing the knowledge of its diversity with three new species (*H. catarinense* sp. nov., *H. maris-bahiensis* sp. nov. and *H. riosmenum* sp. nov.). Finally, this study provides a careful analysis of the advantages and disadvantages of the use of each marker in taxonomic studies on CCA.

## MATERIALS AND METHODS

### *Sampling, morphological and anatomical observations*

Twenty samples were collected at nine localities on the Brazilian coast (Table 1). Sampling took place at intertidal region during syzygy low tides and at infralittoral (2-5m deep) in quadrature tides by scuba diving. Samples were cleaned and dried outdoors for at least 24 hours and then stored on silica gel. All samples were examined using a stereomicroscope (Leica® Zoom 2000, Wetzlar, Germany) for the separation of the fragments for molecular and morpho-anatomical analysis. Materials for morpho-anatomical observations were subsequently preserved in formaldehyde 4%.

For optical microscopy analysis, a fragment was decalcified with 0.6M HNO3 for 24 hours, followed by dehydration in an ethanol series (30, 50, 70, 90 and 100%) and by infiltration and inclusion in “Historesin embedding Kit” (Leica, Heidelberg, Germany) according to the instructions provided by the manufacturer. The sectioning (4-10 μm thickness) was performed with a Thermo Scientific microtome (model HM 325, Walldorf, Germany), stained with toluidine blue acidified with borax (Riosmena-Rodriguez 1993, Moura et al. 1997). Identifications and photomicrographs were performed using a photomicroscope (Olympus trinocular CX31RTS5®, Tokyo, Japan) coupled with digital camera (QImaging GO-3). The specimens were deposited in the Herbarium Alexandre Leal Costa (ALCB) of the Universidade Federal da Bahia (UFBA/Brazil).

### *DNA extraction, PCR and sequencing*

The total DNA was extracted from fragments of dry samples using the Chelex resin extraction protocol (Goff & Moon 1993) or the CTAB method (Cetyl Trimethyl Ammonium Bromide), modified from Doyle and Doyle (1987). The DNA was extracted from the same specimens used in the morphological analysis. Fifteen samples were sequenced for the four markers used in this study (SSU rDNA, *psb*A, *rbcL*-3P and COI-5P) (Table 1).

Molecular markers were PCR-amplified in a final volume of 25 µL: 1x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.2 µM primer, 5 ng DNA, and 1.25 U Taq DNA polymerase (Invitrogen, São Paulo, SP, Brazil). For the amplification and sequencing reactions of each marker, specific primer pairs were used. For the plastidial *psb*A and the mitochondrial COI-5P, the PCR were conducted following Sissini et al. (2014) and Jesus et al. (2016), respectively. For the plastidial *rbc*L-3P, the PCR was performed with an initial denaturation at 94° C for 4 min, 2 cycles at 94° C for 1 min (denaturation), 40° C for 1 min (annealing), and 72° C (extension) for 2 min; then, 40 cycles at 94° C for 1 min (denaturation), 42°C for 1 min (annealing), and 72°C (extension) for 2 min; and a final extension at 72° C for 5 min, using the primers F1150cor (Sissini et al. 2014) and R1460cor (Hernández-Kantún et al. 2016) or RrbcS (Freshwater et al. 1994). For the nuclear SSU rDNA, the primers 18S5' and 1055R, and 1055F and 18S3' (Milstein & Oliveira 2005) were used. The PCR was performed with an initial denaturation at 94°C for 4 min, 35 cycles at 94°C for 30 sec. (denaturation), 60°C for 1 min (primer annealing), and 72° C (extension) for 2 min; and a final extension at 72° C for 7 min. Reactions were performed on the Vertic® 96-Well Thermal Cycler (Applied Biosystems, Foster City, California, USA). The purifications and sequencing reactions of the PCR products were carried out by the company GeneWiz (Cambridge, Massachusetts, USA, http://www.genewiz.com/).

*Molecular analyses*

Electropherograms were manually assembled using the Staden package (Staden et al. 2003) and edited in BioEdit 5.0.6 (Hall 1999). For each marker, a multiple alignment was generated with the ClustalW tool (Thompson et al. 1994), available in BioEdit 5.0.6 (sequences of the PCR primers were excluded from alignment). In addition to the sequences generated in this study, sequences of other species of Corallinaceae available from GenBank were used and included in the analyses (www.ncbi.nlm.nih.gov/genbank/,

searched in January 2017 - Supplementary Table S1). Unalignable regions of the SSU rDNA gene were removed before analysis.

We analyzed each marker individually for species delimitation and phylogenetic analyses. The concatenated data were used for better presentacion of the delimitacion results. To concatenated alignment, 43 partial sequences of the markers were obtained (12 SSU rDNA, 12 *psb*A, 11 *rbc*L-3 P, and 8 COI-5 P) and combined with 30 sequences available at GenBank (11 SS U rDNA, 7 *psb*A, 6 *rbc*L, and 6 COI-5 P). The final SSU rDNA alignment included 23 sequences of 1,544 bp. For *psb*A, the final alignment counted on 19 sequences of 760 bp. For the *rbc*L-3P, the final alignment had 17 sequences of 247 bp. For COI-5P, the final alignment included 14 sequences of 534 bp. For the individual analysis of the SSU r DNA marker, we added 88 sequences from the GenBank, in order to observe the relationship between our species studied and other species of Corallinaceae.

*Methods for species delimitation*

Four different specific delimitation methods were used for each marker (SSU rDNA, *psb*A, rbcL and COI-5P): (1) manual Barcode Gap Discovery (mBGD), described by Freshwater et al. (2010); (2) Automated Barcode Gap Discovery (ABGD), by Puillandre et al. (2012); (3) Poisson Tree Process model (PTP) by Zhang et al. (2013), with the implementation of Maximum Likelihood; and (4) the analysis of Statistical Parsimony Network (SPN) developed by Templeton et al. (1992).

To assess the level of variation in the data set, estimates of divergence values within and between species were calculated using MEGA 6.0 (Tamura et al. 2013). The mBGD estimates how many times the minimum divergence of species diversity is greater than the maximum value found in internal divergence for one species (Freshwater et al. 2010). The *barcode gap* is present when the calculated value is greater than 1, when the values are equal to or less than 1 it indicates overlapping of genetic divergence (Tables 2 to 5). The ABGD infers the number of groups present (as preliminary hypotheses of species) by verifying the *barcoding gap* of the peer-to-peer differences for a given data set (Puillandre et al. 2012). The estimate was made through the available online platform: http://wwwabi.snv.jussieu.fr/public/abgd; where a fasta-like alignment was used as input file, with the standard parameters from ABGD (pmin = 0.001, pmax = 0.1, number of compartments = 20, X (relative width of the range) = 1.5). The Genetic distances JC69 (Jukes-Cantor 1969) and K2P (Kimura 1980) were tested. The PTP takes into account the length of the branches (the number of substitutions) for estimating the boundary between

species (Zang et al. 2013). For the implementation of this analysis, Max Likelihood tree files were used (obtained in the same way as for phylogenetic analyses). The analysis was done on the online platform: http://species.h-its.org/ptp. An analysis of the Statistical Parsimony Network (SPN) was performed using the TCS software version 1.21 (Clement et al. 2000). The TCS calculates the minimum number of mutational steps by which sequences of the same species can be associated with> 95% confidence. In this analysis, different species are presented as unconnected sequences or networks because of their greater evolutionary distinction measured by mutations.

The results of the specific delimitation of each marker were compared with the results of the detailed phylogenetic and morpho-anatomical analyses of the studied specimens, including a large bibliographical survey. This approach allowed to transform preliminary hypotheses of species (PSHs) into secondary concluding hypotheses (SSHs) and to extract taxonomic conclusions.

*Phylogenetic Analyses*

The analyses of Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI) were performed to infer phylogenetic relationships. The analyses were based on the data set of each of the markers (COI-5P, *psb*A, *rbc*L-3P and SSU rDNA), and on the dataset of all four markers (concatenated). The outgroup included were *Sporolithon ptychoides* (*psb*A: KC870927 / COI-5P: HQ422711 / SSU rDNA: DQ629014) and *Sporolithon durum* (*rbc*L: KM369122). The MP trees were constructed on PAUP* 4.0b.10 (Swofford 2002), using a heuristic search algorithm with the following definitions: initial tree(s) obtained by stepwise addition; tree-bisection-reconnection (TBR) as *branch-swapping* algorithm and 10 trees retained by replication. All characters were given equal weights, their states were considered unordered, and the character-optimization criterion was ACCTRAN (*Accelerated Transformation*). Branches with a maximum length of zero collapsed to produce polytomies. The strict consensus analysis was calculated and the bootstrap values were accessed using 1.000 replicates with random addition of terminals. The consistency index (CI), retention index (RI), and rescaling consistency index (CR) resulting from MP analysis were calculated (Supplementary Table S2).

The most suitable evolutionary model was selected using the software MrModeltest 2.3 (Nylander 2008), using the Akaike Information Criterion (AIC), as recommended by Posada and Buckley (2004). ML analysis was conducted using RAxML (*Randomized Axelerated Maximum Likelihood*, version 7.0.4; Stamatakis 2006) with the GTR+I+G

model. The best ML tree score and 500 bootstrap trees were obtained using a fast scale algorithm (Stamatakis et al. 2008).

The Bayesian analysis was carried out using the software Mr. Bayes 3.1 (Ronquist & Huelsenbeck 2003) using the Marcov - Monte Carlo (MCMC) method with the selected model for each data set: GTR+I+G (COI-5P, *psb*A, *rbc*L-3P, SSU rDNA, and concatenated). Five million generations in two independent races with four chains each were carried out, saving one tree every 100 generations. The *burn-in* was identified graphically to determine the period in which the likelihood values had reached a plateau. The trees sampled after stabilization were used to infer the posterior Bayesian probability.

## RESULTS

### *Specific Delimitation Methods*

The final SSU rDNA alignment had variable sites at 92 positions (6%) and 171 positions (11%) were parsimoniously informative. The final *psb*A alignment had variable sites at 61 positions (8%) and 140 positions (18%) were parsimoniously informative. The final *rbc*L-3P alignment had with variable sites at 23 positions (9%) and 58 positions (23%) were parsimoniously informative. The final COI-5P alignment had variable sites at 80 positions (15%) and 112 positions (21%) were parsimoniously informative. Intra- and interspecific genetic variations, and *barcode gaps* for the markers used in this study are shown in Tables 2 to 5.

The results of the species delimitation methods (mBGD, ABGD, PTP and SPN) are shown in Figure 1, together with the tree topology of maximum likelihood from all four concatenated markers (SSU rDNA, *psb*A, *rbc*L-3P and COI-5P). Most of the methods presented similar results for all markers, separating the taxa *Harveylithon catarinense*, *H. maris-bahiensis*, and *H. riosmenum* as distinct entities from other already-described species of *Harveylithon*.

The *psb*A and COI-5P markers were highly congruent in all used methods (mBGD, ABGD, PTP and SPN) and recovered the taxa *Harveylithon catarinense*, *H. maris-bahiensis*, and *H. riosmenum* (with the exception of the taxon *H. catarinense* for the COI-5P marker, for which it was not possible to generate sequences). The SPN analysis also recovered *H. riosmenum* as a distinct species, but evidenced the existence of different haplotypes depending on the marker (Table 6).

The marker *rbc*L-3P recovered the three taxa - here proposed as taxonomic novelty - only for the SPN analysis. This analysis revealed high diversity for the taxon *Harveylithon riosmenum*, which was evidenced by the existence of five haplotypes (Table 6). The PTP method, joined *H. riosmenum* and *H. maris-bahiensis* as a single taxon. Additionally, the same method considered *H. catarinense* a distinct entity, although with low support (0.21). The mBGD and ABGD methods, however, recovered *H. riosmenum* as three distinct and biogeographically separated entities (group 1: Santa Catarina - south of Brazil; group 2: Pernambuco - northeastern Brazil; and group 3: Rio de Janeiro and Espírito Santo - southeast of Brazil) (see ranges of divergences in Table 4).

The SSU rDNA marker presented inconsistent results among the different delimitation methods used in the present study. The PTP method considered the taxa. *H. catarinense*, *H. maris-bahiensis*, and *Harveylithon riosmenum* sp. nov as a single PSH, although with low support (0.55). The mBGD and SPN methods recovered the taxa as three distinct entities, the SPN having identified five haplotypes within the taxon *H. riosmenum* (Table 6). The ABGD method, however, recovered the taxa *H. riosmenum* and *H. catarinense* as distinct entities. The same method grouped *H. maris-bahiensis* and *H. samöense* as a single taxon. The latter two presented divergence rates of 0.53-0.66% (8-10 bp) (Table 2).

*Phylogenetic Analysis*

The phylogenetic tree of SSU rDNA reinforced the robustness of main taxa of Corallinaceae (Fig. 2) and clusters the species of the genus *Harveylithon* as a distinct clade, presenting high support only in Bayesian Inference (PP: 0.99) within the subfamily Metagoniolithoideae (PP: 0.93 and MP: 85%). Our Southern Tropical and Warm Temperate species have grouped with the Indo-Pacific species, including the generitype *H. rupestre*. *H. samöense* differs from *H. maris-bahienis* by 0.53-0.66% and *H. riosmenum* by 0.73-0.99%. On the other hand, *H. rupestre* differs from *H. catarinense* from 1.39% (Table 2).

All phylogenetic analyses revealed the clades *Harveylithon catarinense*, *H. maris-bahiensis*, and *H. riosmenum* as distinct and well-supported species. The different phylogenetic analyses for the SSU rDNA marker showed the *H. samöense* branch with low-resolution. The divergence analyses (Table 2) showed that this taxon is closely related to *H. maris-bahiensis*, diverging by only 0.53-0.66%. The topologies of ML, MP and BI for concatenated data and SSU rDNA identified three distinct groups, with moderate to

high support and strictly related to the other species of the genus *Harveylithon* (Fig 1 and 2).

*Morphological and Anatomical Analysis*

CORALLINALES Silva and Johansen (1986: 250)

Corallinaceae Lamouroux (1812: 185)

Metagoniolithoideae (H.W. Johansen) emend. A. Rösler, Perfectti, V. Peña & J.C. Braga (2016)

*Harveylithon* A. Rösler, Perfectti, V. Peña & J.C. Braga

DESCRIPTION: Thallus nongeniculate, monomerous organization and a plumose ventral core (Fig. 3A). Trichocytes usually occur singly and/or buried within the thallus (Fig. 3B and 3C). Spermatangial conceptacles small, with spermatangia restricted to the conceptacle chamber floor (Fig. 3D). Carposporangial conceptacles with carposporangia supported for gonimoblast filaments that arise peripherally to surface of the continuous fusion cell (Fig. 3E). Cells lining the pore canal of tetrasporangial conceptacles are oriented perpendicular to the thallus surface (Fig. 3F).

*Harveylithon catarinense* I.O. Costa, P.A. Horta & J.M.C. Nunes sp. nov. (Fig. 4 and 5)

HOLOTYPE: Brazil, Santa Catarina State, Bombinhas, Ilha Deserta, 27°16'30"S, 48°19'83"W; coll. P.A. Horta s.n. 02 Mar 2016; ALCB 125627; GenBank accessions: *rbc*L-3P – MF966182 / *psb*A – MF966195.

ISOTYPE: Brazil, Santa Catarina State, Bombinhas, Ilha Deserta, 27°16'30"S, 48°19'83"W; coll. P.A. Horta s.n. 02 Mar 2016; ALCB 125628; GenBank accessions: *rbc*L-3P – MF966181 / *psb*A – MF966194 / SSU rDNA – MF611675.

ETYMOLOGY: The epithet refers to the place of occurrence of the species, until then restricted to the coast of Santa Catarina, Brazil.

DISTRIBUTION: Brazil: Santa Catarina State.

DIAGNOSIS: With the characteristics of *Harveylithon*, differing from other species of the genus in having trichocytes common, single at the surface and buried in the thallus. Tetrasporangial conceptacles elliptical, measure 82–174 µm long and 127–250 µm in diameter; conceptacle roof 4–8 cells thick, with similar-shapped. *rbc*L-3P, *psb*A and SSU rDNA sequences unique.

Figures: 4A-C and 5A-F.

DESCRIPTION: Thallus encrusting and smooth; pink color (Fig. 4A and 4B). Monomerous and medullary region consists of a central plumose core (Fig. 5A). Epithallial cells single, squat to elliptical to spherical, 2.7–4.7 µm long and 4.3–7.7 µm in diameter (Fig. 5B). Subepithallial initials (3.3–7.9 µm long and 3.4–8 µm in diameter) as long or longer than the cells immediately subtending them (Fig. 5B). Cells of the perithallial filaments, 5.9–18.6 µm long and 4.5–7.1 µm in diameter. Cells of the hypothallial filaments, 4.9–20 µm long and 5–10.8 µm in diameter. Trichocytes rectangular to bottle-shaped to elongate, single at the surface and buried in the thallus, and measure 8.5–18.3 µm long and 8–12.3 µm in diameter (Fig. 5B and 5C). Cells of the adjacent filaments joined by cell fusions (Fig. 5C). Secondary pit-connections not observed. Gametangial thalli dioecious. Carposporangial conceptacle, measure 94.8 µm long and 237.5 µm in diameter (Fig. 5D). Carposporangia measure 21.4–25.6 µm long and 30–31.2 µm in diameter. Uniporate tetrasporangial conceptacles flush in the thallus surface, chambers elliptical, measure 82–174 µm long and 127–250 µm in diameter (Fig. 5E). Central columella absent. Conceptacle roofs 4-8 cells thick, with similar-shapped cells. A ring of enlarged, domed cells lines the base of the pore canal and is oriented more-or-less perpendicular (vertically orientated) to the roof surface; these cells do not project into the pore canal (Fig. 5E and 5F). Zonately divided tetrasporangia, 25.5–71.7 µm long and 18–26 µm in diameter (Fig. 5F). Spermatangial thallus not observed.

MATERIAL EXAMINED: BRAZIL. SANTA CATARINA: Bombinhas, Ilha Deserta, 27°16'30"S, 48°19'83"W, coll. P.A. Horta s.n. 02 Mar 2016, (ALCB 125627 and 125628♀).

*Harveylithon maris-bahiensis* I.O. Costa, P.A. Horta & J.M.C. Nunes sp. nov. (Fig. 6)

HOLOTYPE: Brazil, Bahia State, Cairú, Morro de São Paulo, 13°23'16.8"S, 38°54'18.6"W; coll. I.O. Costa et al. s.n., 06 Oct 2013; ALCB 125620; GenBank accessions: COI-5P – MF966174 / *rbc*L-3P – MF966185 / *psb*A – MF966197 / SSU rDNA – MF611672.

ISOTYPE: Brazil, Bahia State, Cairú, Morro de São Paulo, 13°23'16.8"S, 38°54'18.6"W; coll. I.O. Costa et al. s.n., 06 Oct 2013; ALCB 125622; GenBank accessions: *rbc*L-3P – MF966183 / *psb*A – MF966196 / SSU rDNA – MF611674.

ETYMOLOGY: The epithet refers to the place of occurrence of the species, until then restricted to the coast of Bahia, Brazil.

DISTRIBUTION: Brazil: Bahia State.

DIAGNOSIS: With the characteristics of *Harveylithon*, differing from other species of the genus in having trichocytes common, single at the surface of the thallus. Tetrasporangial conceptacles spherical; conceptacle roof 2–3 cells thick, comprising a single squat to elliptical to spherical epithallial cell, a single elliptical meristematic cell that is 1.5 times the length of the epithallial cell, and a small inner cell. COI-5P, *rbc*L-3P, *psb*A and SSU rDNA sequences unique.

Figure: 6A-G.

DESCRIPTION: Thallus encrusting and smooth; pink color (Fig. 6A and 6B). Monomerous and medullary region consists of a central plumose core (Fig. 6C). Epithallial cells single, squat to elliptical to spherical, 4.3–7.5 µm long and 4.8–8.5 µm in diameter (Fig. 6D). Subepithallial initials (4.3–9.4 µm long and 4.2–7.9 µm in diameter) as long or longer than the cells immediately subtending them (Fig. 6D). Cells of the perithallial filaments, 4.6–10 µm long and 4.4–8.6 µm in diameter. Cells of the hypothallial filaments, 4–12 µm long and 8–10 µm in diameter. Trichocytes rectangular to bottle-shaped to elongate, single at the surface of the thallus, and measure 10–15.4 µm long and 7–11.6 µm in diameter (Fig. 6D). Cells of the adjacent filaments joined by cell fusions (Fig. 6E). Secondary pit-connections not observed. Uniporate tetrasporangial conceptacles flush in the thallus surface, chambers spherical, measure 50–71.6 µm long and 70–111.1 µm in diameter (Fig. 6F). Central columella absent. Conceptacle roofs 2-3 cells thick, comprising a single squat to elliptical to spherical epithallial cell, a single elongated meristematic cell (of elliptical shape) that is 1.5 times the length of the epithallial cell, and a small inner cell. A ring of enlarged cells lines the base of the pore canal and is oriented more-or-less perpendicular (vertically orientated) to the roof surface, these cells do not project into the pore canal (Fig. 6F). Zonately divided tetrasporangia, 30–69.3 µm long and 12–27.6 µm in diameter (Fig. 6G). Gametangial thalli not observed.

MATERIAL EXAMINED: BRAZIL. BAHIA: Cairú, Morro de São Paulo, 13°23'16.8"S, 38°54'18.6"W, coll. I.O. Costa et al. s.n., 06 Oct 2013, (ALCB 125620, 125621 and 125622).

*Harveylithon riosmenum* I.O. Costa, P.A. Horta & J.M.C. Nunes sp. nov. (Fig. 7 and 8)

HOLOTYPE: Brazil, Espírito Santo State, Anchieta, Praia de Ubú, $20^{0}$48'49,36”S x $40^{0}$36'42,45”W; coll. I.O. Costa et al. s.n., 21 Aug 2013; ALCB 125617; GenBank

accessions: COI-5P – MF966170 / *rbc*L-3P – MF966177 / *psb*A – MF966189 / SSU rDNA – MF611668.

PARATYPE: Brazil, Pernambuco State, Cabo de Santo Agostinho, Enseada dos Corais, 08°18'50.3"S, 34°56'47.8"W, coll. I.O. Costa et al. s.n., 26 April 2014; ALCB 125624; GenBank accessions: *rbc*L-3P – MF966176 / *psb*A – MF966187 / SSU rDNA – MF611670.

ETYMOLOGY: The epithet is a tribute to the phycologist Rafael Riosmena-Rodriguez, a specialist in coralline algae and the first to suggest that the taxon could be a new species for science.

DISTRIBUTION: Brazil: States of Pernambuco, Espírito Santo, Rio de Janeiro and Santa Catarina.

DIAGNOSIS: With the characteristics of *Harveylithon*, differing from other species of the genus in having trichocytes common, single and in small vertical rows at the surface or buried in the thallus. Gametangial thalli monoecious. Tetrasporangial conceptacle roof 2–4 cells thick, comprising a single squat to elliptical to spherical epithallial cell, a single collunar meristematic cell that is 2–4 times the length of the epithallial cell, and a small inner cell. COI-5P, *rbc*L-3P, *psb*A and SSU rDNA sequences unique.

Figures: 7A-F and 8A-H.

DESCRIPTION: Thallus encrusting and smooth; pink color (Fig. 7). Monomerous and medullary region consists of a central plumose core (Fig. 7E). Epithallial cells single, squat to elliptical to spherical, 2.6–6.5 µm long and 7.5–9 µm in diameter. Subepithallial initials (3.2–8 µm long and 3.3–7 µm in diameter) as long or longer than the cells immediately subtending them (Fig. 7F). Cells of the perithallial filaments, 2.4–14.5 µm long and 3.5–8.5 µm in diameter. Cells of the hypothallial filaments, 4.1–12.8 µm long and 8–11 µm in diameter. Trichocytes rectangular to bottle-shaped to elongate, single and in small vertical rows at the surface or buried in the thallus, and measure 9–20.7 µm long and 5.2–18 µm in diameter (Fig. 8A and 8B). Cells of the adjacent filaments joined by cell fusions (Fig. 7F). Secondary pit-connections not observed. Gametangial thalli monoecious (Fig. 8C). Carposporangial conceptacles with 48–66.1 µm long and 90–125 µm in diameter. Carposporangial conceptacle roofs 2-4 cells thick, comprising a single squat to elliptical to spherical epithallial cell, a single collunar meristematic cell that is 2–4 times the length of the epithallial cell, and a small inner cell. Carposporangia with 22–33 µm long and 24–32.5 µm in diameter, supported for gonimoblast filaments that arise peripherally to surface

of the continuous fusion cell (Fig. 8D). Spermatangial conceptacles small and inconspicuous, chambers measure 24.4–26 µm long and 46–54 µm in diameter. Simple spermatangial systems restricted to the conceptacle chamber floor (Fig. 8E). Uniporate tetrasporangial conceptacles flush in the thallus surface, chambers elliptical to spherical, measure 43–126 µm long and 72–164.8 µm in diameter (Fig. 8F). Central columella absent. Conceptacle roofs 2-4 cells thick, comprising a single squat to elliptical to spherical epithallial cell, a single collunar meristematic cell that is 2–4 times the length of the epithallial cell, and a small inner cell (Fig. 8H). A ring of enlarged cells lines the base of the pore canal and is oriented more-or-less perpendicular (vertically orientated) to the roof surface, these cells do not project into the pore canal. Zonately divided tetrasporangia, 11.5–89 µm long and 8–34 µm in diameter. Old tetrasporangial conceptacles discarded on the surface of the thallus (Fig. 8G).

MATERIAL EXAMINED: BRAZIL. PERNAMBUCO: Cabo de Santo Agostinho, Enseada dos Corais, 08°18'50.3"S, 34°56'47.8"W, coll. I.O. Costa et al. s.n., 26 April 2014, (ALCB 125624); Recife, Praia de Boa Viagem, 08°05'25.52"S, 34°53'46.59"W, coll. I.O. Costa et al. s.n., 29 April 2014, (ALCB 125625 and 125626); ESPÍRITO SANTO: Guarapari, Pontal de Guaibura, 20°43'40.86"S, 40°31'19.32"W, coll. I.O. Costa et al. s.n., 20 Aug 2013, (ALCB 125612, 125613, 125614 and 125615♀); Anchieta, Praia de Ubú, 20°48'49.36"S, 40°36'42.45"W, coll. I.O. Costa et al. s.n., 21 Aug 2013, (ALCB 125616 and 125617); RIO DE JANEIRO: Rio de Janeiro, Recreio dos Bandeirantes, 23°02'32.48"S, 43°30'27.3"W, coll. I.O. Costa et al. s.n., 24 May 2013, (ALCB 125623); Búzios, Praia Rasa, 23°02'34.4"S, 43°30'29.1"W, coll. I.O. Costa et al. s.n., 25 May 2013, (ALCB 25618 and 125619); Cabo Frio, Praia das Conchas, 22°52'16.8"S, 41°53'51.2"W, coll. I.O. Costa et al. s.n., 26 May 2013, (ALCB 123210 and 125611♂♀); SANTA CATARINA, Bombinhas, Ilha Deserta, 27°27'54.22"S, 48°35'91.44"W, coll. P.A. Horta s.n., 02 Mar 2016, (ALCB 123166).

## DISCUSSION

Phylogenetic (Fig. 1 and 2) and morpho-anatomical analyses (Fig. 3 to 8) corroborate the circumscription of *Harveylithon* (Rösler et al. 2016) and attest its occurrence in tropical and warm temperate regions of the South Atlantic's west coast. This integrated approach has been favoured in the scientific community because it makes concomitant use of the broadest range of independent data types, improving taxonomic

robustness (Puillandre et al. 2012, Hind et al. 2015, 2016, Jesus et al. 2016, Lyra et al. 2016).

*Comparison of methods of species delimitation*

The concatenated analysis, considering the congregation of all four markers (SSU rDNA, *psb*A, *rbc*L-3P and COI-5P), with all the main representatives of the subfamily, showed well supported clades for *Harveylithon catarinense*, *H. maris-bahiensis* and *H. riosmenum.* These observations corroborated the results of the different methods of specific delimitation and of the anatomical analyses.

Among the four delimitation methods used, the mBGD and SPN methods recovered the same PSHs for the markers SSU rDNA, *psb*A and COI-5P, corroborating circumscription of *Harveylithon maris-bahiensis* and *H. riosmenum.* The methods mBGD, ABGD and SPN recovered *H. catarinense* as a distinct entity for SSU rDNA, *psb*A and *rbc*L-3P.

The SSU rDNA is the only marker for which there is a published sequence of the taxon *Harveylithon samöense*. Despite the morphological similarity between *H. samöense*, *H. maris-bahiensis* and *H. riosmenum* (Table 2), the values are within the range considered as interspecific (0.53-2.90%) for SSU rDNA in other groups of CCA (Bailey 1999, Bailey et al. 2004).

For the *rbc*L-3P marker, the mBGD and ABGD methods evidenced the biogeographic separation between the specimens of *Harveylithon riosmenum.* Both methods are based on differences between intra and interspecific divergences; therefore, such separation was due to the high genetic divergence (Table 4). This high divergence, in addition to being explained by the geographic distance between the collected specimens (Table 4).In the current study, the PTP method incorporated more than one morphological / phylogenetic group into a single PSH for SSU rDNA and *rbc*L-3P, although poorly supported. These results are in agreement with those found by Kozak et al. (2015) and Jesus et al. (2016), while Zhang et al. (2013) and Modica et al. (2014) considered that the PTP can overestimate the number of species when the taxa sampling is uneven among the species.

The mBGD, ABGD and SPN methods were more efficient for species delimitation in our data set, and showed that the results may vary according to the marker that is used. According to Muangmai et al. (2014), the incongruent results obtained between the

delimitation methods for different markers can be influenced by different evolutionary markers rates.

*Phylogenetic Analysis*

In our broader phylogenetic analyses, based on SSU rDNA, Metagoniolithoideae and Lithophylloideae were resolved as sister subfamilies (PP: 1.0, ML: 90%, and MP: 93%). *Hydrolithon* and *Spongites* clade were resolved as sisters with high support only in the ML (91%). Our result was in agreement with that found by Bailey et al. (2004), Broom et al. (2008), and Hind et al. (2016). In the study by Rosler et al. (2016) Lithophylloideae was sister of the *Spongites* group, with moderate support (see Rosler et al. 2016) what now was presented in a much more clear perspective.

The phylogenetic tree of SSU rDNA, the *Harveylithon* genus had strong support only in Bayesian Inference (Fig 2). The species of this genus, however, had moderate to strong support in all phylogenetic analyses. The SSU rDNA has been successful in the specific and generic delimitation of coralline algae (Harvey et al. 2002, Vidal et al. 2008, Peña et al. 2011, Vieira-Pinto et al. 2014, Hernández-Kantún et al. 2015). However, some studies (e.g., Torrano-Silva et al. 2014, Hind et al. 2016) reported that this marker was unsuccessful to delimit species, because it is considered very conserved (Bailey & Chapman 1996, Bailey 1999, Vidal et al. 2003, Bailey et al. 2004, Le Gall et al. 2010, Bittner et al. 2011, Nelson et al. 2015).

In our *psb*A tree, *Harveylithon catarinense*, *H. maris-bahiensis* and *H. riosmenum* were very strong supported as distinct species (PP: 1.0, ML and MP: 100%) (Fig. S1). Our values of interspecific divergence, for *psb*A, (Table 2) were in accordance with that found by Mateo-Cid et al. (2014) and Van der Merwe et al. (2015). The *psb*A marker has been also widely used in phylogenetic analyses for coralline algae, as well as the SSU rDNA. *psb*A presents better resolution at the genus and species levels, identifying taxa that have diverged more recently, which couldn't be identified with more conserved markers (Broom et al. 2008, Peña et al. 2011, Torrano-Silva et al. 2014, Hernández-Kantún et al. 2015, 2016).

Phylogenetic analyses for the COI-5P marker showed high support for the taxa *Harveylithon riosmenum* and *H. maris-bahiensis* (Fig. S2). Unfortunately, we did not succeed in sequencing *H. catarinense* specimens for this marker. COI-5P has been widely used for species separation in several groups in Rhodophyta and, more recently, for

coralline algae, presenting greater variation than SSU rDNA and *psb*A (Bittner et al. 2011, Hind & Saunders 2013, Carro et al., 2014, Torrano et al. 2014, Vieira-Pinto et al. 2014).

Phylogenetic analyses for the marker *rbc*L-3P showed high support for the taxa *Harveylithon maris-bahiensis* and *H. catarinense*. However, the sequence of *Porolithon onkodes* (as *Hydrolithon onkodes* - Nelson et al. 2015) was positioned within the clade of the genus *Harveylithon*, as a sister of *H. maris-bahiensis.* The cited sequence of a specimen collected in New Zealand needs revision, as it is probably an erroneously identified species of *Harveylithon*.

The taxon *Harveylithon riosmenum* was shown as paraphyletic for the marker *rbc*L-3P (Fig S3), with high rates of genetic divergence between the specimens of different geographical locations (Table 4). According to Leliaert et al. (2014), lineages of recent divergence go through stages where individuals of one species are paraphyletic before becoming monophyletic, which is caused by incomplete lineage separation. Freshwater et al. (2010) considered any value > 2% of divergence for *rbc*L as an interspecific divergence. However, they used the complete *rbc*L gene (1,356 bp) while in the current study we used only a variable fragment (247 bp) corresponding to 18% of the complete gene, which may explain the high values of divergence we found.

The *rbc*L gene is widely used for phylogenetic inference in Florideophyceae (Freshwater et al. 1994, Gurgel & Fredericq 2004, Jesus et al. 2016, Lyra et al. 2016). Saunders and Moore (2013) proposed the possibility of using the 3' portion of this gene (*rbc*L-3P) in the *DNA barcoding* technique (using a small DNA sequence from a designated region of the genome as a diagnostic tool for species-level identification). Our results corroborate previous studies (Sissini et al. 2014, Adey et al. 2015, Hernandez Kantun et al. 2016, Hind et al. 2016) where *rbc*L-3P was successful for analyses of species delimitation, in our study it even recovered biogeographic groups.

The morphological similarity between *Harveylithon riosmenum*, *H. maris-bahiensis*, and *H. samoënse*, and the low genetic divergence between them, reveals the existence of cryptic species under the epithet of *H. samoënse*. It is necessary to analyze in depth the specimens from different localities that are attributed to *H. samoënse*, as well as their synonyms so that we can understand the real distribution and diversity of taxa existing under this epithet. Putative species, discovered through delimitation methods, are the first step in arriving at the calculation of true species diversity (Muangmai et al. 2014).

*Harveylithon riosmenum* presented a wide distribution along the Brazilian coast, occurring from the northeast to the southern region of the country. Nevertheless, the

species *H. maris-bahiensis* and *H. catarinense* presented restricted distribution to the type localities.

There is a scarcity of molecular data available in the databases for the species of the genus *Harveylithon.* Additionally, there is a limited number of analyzed specimens, which represent a real problem in the phylogenetic reconstruction of this group. *H. rupestre* has only one sequence for each of the two different markers (SSU rDNA and *psb*A - as *Hydrolithon rupestre*), *H. samoënse* has one sequence for SSU rDNA (as *Hydrolithon samoënse*), while *H. munitum* (as *Hydrolithon munitum*) and *H. canariense* (as *Neogoniolithon accretum*) have only one sequence each for *psb*A (https://www.ncbi.nlm.nih.gov/). More molecular data for specimens morphologically attributed to this genus are essential to better understand the evolutionary history of the group and to solve systematic problems.

The record of occurrence of *Harveylithon* in the South Atlantic is rare and limited to morpho-anatomic analyses (as *Hydrolithon* - Tâmega and Figueiredo 2005, Amado Filho et al. 2012 a, b, Crespo et al. 2014, Villas-Boas et al. 2015). Despite the fact that this group of algae belong to the main reef-building in shallow waters (Steneck 1986), there is a scarcity in efforts to identify them. The current study constitutes an important advance in the knowledge of the specific diversity of this genus in a tropical and still underestimated region (Nunes & Guimarães 2008, Nunes et al. 2011, 2014, Jesus et al 2015). However, it is still necessary to revisit the sites where the specimens identified as *H. samoënse* and *H. rupestre* were collected so that the taxonomic status of these entities is confirmed by molecular analyses; as well as a re-evaluation of other taxa identified as *Hydrolithon* in the South Atlantic.

*Anatomical delimitation of the species*

The genus *Harveylithon* is characterized by presenting: 1) monomerous thallus organization; 2) plumose ventral core; 3) trichocytes usually occurring single on the surface or buried in the thallus; 4) tetrasporangial conceptacle pore canal cells perpendicularly oriented to the surface of the thallus (Rösler et al. 2016). The species studied here are in accordance with the diagnostic characters of this genus, and we added the description of characteristics of the male and carposporangial conceptacle (Fig. 3).

Initially, the taxa *Harveylithon riosmenum* was characterized as *H. samöense* because they presented anatomical characteristics very similar to those described for the latter species (Table 7). The morphological differences between two species often only

appear after a long time since the lineage divergence and, therefore, the recently divergent species will probably remain undetected morphologically (Leliaert et al. 2014), thus dealing with cryptic species. Phylogenetic molecular data have revealed numerous cases of closely related species that are morphologically indistinct (Kato et al. 2013, Sissini et al. 2014, Jesus et al. 2011, Lyra et al. 2016).

Maneveldt et al. (2015) performed a taxonomic review on *Harveylithon samöense* (as *Hydrolithon samöense*) based on morpho-anatomical analysis of the type material and on comparison with other closely related specimens. In this study, the taxa *Neogoniolithon caribeum*, *N. erosum*, and *N. rugulosum* were synonymized with *Harveylithon samöense*, in addition to being evidenced that this taxon presents dioecious gametangial thalli. The authors comment on the need for molecular analysis to support these synonyms, but emphasize that while this is not possible, efforts should be made to revise the diagnostic characteristics of the type material for the advancement of taxonomic knowledge.

*Harveylithon riosmenum* differs from *H. samöense* only because it presents monoecious gametangial thalli (Fig. 8C). On the other hand, *H. maris-bahiensis* differs from *H. riosmenum* and *H. samöense* by the size and shape of the meristematic cells of the roof of the tetrasporangial conceptacle (Table 7) and by presenting trichocytes only on the surface of the thallus (Fig. 6E). *H. catarinense* consists of a more divergent species, differentiating from the previously mentioned taxa because it does not presents the roof of the tetrasporangial conceptacle with elongated meristematic cells, having 4-8 cells in the roof of the tetrasporangial conceptacle with similar shape between them (Table 7); besides, presenting isolated trichocytes buried in the thallus (Fig. 5C). *H. catarinense* differs still from *H. rupestre* and *H. canariense* because it presents differences in the diameter of the tetrasporangial conceptacle (Table 7) and presence of isolated trichocytes buried in the thallus; and of *H. munitum* by the presence of the central columella in the latter and absence in *H. catarinense*.

The morphological and anatomical characters used for the delimitation of the new species were: location of the trichocytes; size of tetrasporangial conceptacles; shape and quantity of the cells that make up the roof of the tetrasporangial conceptacle (Table 7).

## CONCLUSION

The combination of morphological analysis, phylogenetic evidence and specific delimitation methods with molecular data confirmed the occurrence of *Harveylithon* in both Tropical and Warm Temperate South Atlantic. Consequentially, it resulted in the

circumscription of three new species for the scientific community: *H. catarinense*, *H. maris-bahiensis* and *H. riosmenum*. Despite the limitation of some markers, the combination of multiple methods support delimitation among reef builder species. Our findings evidenced the need for a worldwide effort to produce new sequences of different *Harveylithon* species based on multiple standard molecular markers for the taxonomy and systematics of Metagoniolithoideae. Our study is in agreement with the previous researches, where COI-5P, *psb*A and *rbc*L-3P markers were successful for the delimitation of species, while ratifying that the use of SSU rDNA at deeper levels (phylogenetic studies) seems to be the most appropriate, and should be used with caution for the delimitation of genera and species.

## ACKNOWLEDGEMENTS

This research has been funded by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, BrBOL 564945/2010-2 and UNIVERSAL 477614/2013-2) and the Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB, PRONEM T.O. PNE. 0020/2011 and REDES T.O. RED006/2012). IOC and PBJ acknowledge scholarships from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and CNPq, respectively. JMCN acknowledge CNPq (307368/2015-7). PAH acknowledge CNPq (PROTAX 440575/2015-0), Fundação de Apoio à Pesquisa Científica e Tecnológica do Estado de Santa Catarina (FAPESC, 2015TR1702), CAPES, Rede Clima, FAPESP, Fundação BOTICÁRIO.

## REFERENCES

Adey, W.H., Hernandez-Kantun, J.J., Johnson, G. & Gabrielson, P.W. 2015. DNA sequencing, anatomy, and calcification patterns support a monophyletic, subarctic, carbonate reef-forming *Clathromorphum* (Hapalidiaceae, Corallinales, Rhodophyta). *J. Phycol*. 51:189-203.

Amado-Filho, G.M., Moura, R.L., Bastos, A.C., Salgado, L.T., Sumida, P.Y., et al. 2012a. Rhodolith Beds Are Major CaCO3 Bio-Factories in the Tropical South West Atlantic. *PLoS ONE* 7:e35171 [online].

Amado Filho G.M., Pereira-Filho, G.H., Bahia, R.G., Abrantes, D.P., Veras, P.C., Matheus, Z. 2012b. Occurrence and distribution of rhodolith beds on the Fernando de Noronha Archipelago of Brazil. Aquat. Bot. 101: 41-45.

Bailey, J.C. 1999. Phylogenetic positions of *Lithophyllum incrustans* and *Titanoderma pustulatum* (Corallinaceae, Rhodophyta) based on 18S rRNA gene sequence analyses, with a revised classification of the Lithophylloideae. *Phycologia* 38:208–16.

Bailey, J.C. & Chapman, R.L. 1998. A phylogenetic study of the Corallinales (Rhodophyta) based on nuclear small-subunit rRNA gene sequences. *J. Phycol*. 34:692–705.

Bailey, J.C. & Chapman, R.L. 1996. Evolutionary relationships among coralline red algae (Corallinaceae, Rhodophyta) determined by 18S rRNA gene sequence analysis. In: Chaudhary, B.R. & Agrawal, S.B. (Eds.) Cytology, Genetics and Molecular Biology of Algae. SPB Academic Publishing, Amsterdam. 363–76.

Bailey, J.C., Gabel, J.E. & Freshwater, D.W. 2004. Nuclear 18S rRNA gene sequence analyses indicate that the Mastophoroideae (Corallinaceae, Rhodophyta) is a polyphyletic taxon. *Phycologia* 43:3–12.

Bittner, L., Payri, C.E., Maneveldt, G.W., Couloux, A., Cruaud, C., de Reviers, B. and Le Gall, L. 2011. Evolutionary history of the Corallinales (Corallinophycidae, Rhodophyta) inferred from nuclear, plastidial and mitochondrial genomes. *Mol. Phylogenet. Evol.* 61:697–713.

Broom, J. E. S., Hart, D. R., Farr, T. J., Nelson, W. A., Neil, K. F., Harvey, A. S. & Woelkerling, W. J. 2008. Utility of psbA and SSU rDNA for phylogenetic reconstruction in the Corallinales based on New Zealand taxa. *Mol. Phylogenet. Evol.* 46:958-73.

Carro, B., Lopez, L., Peña, V., Bárbara, I., & Barreiro, R. 2014. DNA barcoding allows the accurate assessment of European maerl diversity: a Proof-of-Concept study. *Phytotaxa* 190:176-89.

Clement, M., Posada, D. & Crandall, K. A. 2000. TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* 9:1657–59.

Costa, I.O., Horta, P.A., Bergstrom, E.R. & Nunes, J.M.C. 2014. Taxonomic study of crustose coralline algae off the northeastern Brazilian coast. Phytotaxa 190:130-61.

Crespo, T. M., Da Gama Bahia, R., Maneveldt, G. W. & Amado Filho, G. M. 2014. Floristic composition of crustose coralline algae from the St. Peter and St. Paul Archipelago, a summit of the Mid-Atlantic Ridge. *Phytotaxa* 190:17-37.

Doyle, J. J. & Doyle, J. L. 1987. A rapid DNA isolation method for small quantities of fresh tissues. *Phytochem. Bull.* 19:11–15.

Farr, T., Broom, J., Hart, D., Neill, K. & Nelson, W. 2009. Common coralline algae of northern of New Zeland. An identification guide. *NIWA Information*. Ser. No. 70. Wellington, New Zealand. 125p.

Foslie, M. 1906. Algologiske notiser. II. Det Kongelige Norske Videnskabers Selskabs Skrifter 1906:1–28.

Freshwater, D. W., Tudor, K., O'Shaughnessy, K. & Wysor, B., 2010. DNA barcoding in the red algal order Gelidiales: comparison of COI with *rbcL* and verification of the barcoding gap. *Cryptogam. Algol*. 31:435–49.

Freshwater, D.W., Fredericq, S., Butler, B.S., Hommersand, M. H. & Chase, M.W. 1994. A gene phylogeny of the red algae (Rhodophyta) based on plastid *rbcL*. *Proc. Natl. Acad. Sci. U.S.A.* 91:7281–85.

Gabrielson P.W. 2008. Molecular sequencing of Northeast Pacific type material reveals two earlier names for *Prionitis lyallii*, *Prionitis sternbergii* and *Prionitis jubata* (Halymeniaceae, Rhodophyta). *Phycologia* 47:89–97.

Gabrielson, P.W., Miller, K.A. & Martone, P.T. 2011. Morphometric and molecular analyses confirm two distinct species of *Calliarthron* (Corallinales, Rhodophyta), a genus endemic to the northeast pacific. *Phycologia* 50:298-316.

Gherardi, D.F.M & Bosence, D.W.J. 2001. Composition and community structure of the coralline algal reefs from Atol das Rocas, South Atlantic, Brazil. *Coral Reefs* 19:205-19.

Guiry, M.D. & Guiry, G.M. 2017. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Available at: http://www.algaebase.org (last accessed 30 January 2017).

Gurgel, C.F.D. & Fredericq, S. 2004. Systematics of the Gracilariaceae (Gracilariales, Rhodophyta): a critical assessment based on rbcL sequence analysis. *J. Phycol*. 40:138-59.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser*. 41:95–98.

Harvey, A. S.; Woelkerling, W. J. & Millar, A. J. K. 2002. The Sporolithaceae (Corallinales, Rhodophyta) in southeastern Australia: taxonomy and 18S rRNA phylogeny. *Phycologia* 41:207-27.

Harvey, A. S., Broadwater, S. T., Woelkerling, W. J. & Mitrovski, P. J. 2003. *Choreonema* (Corallinales, Rhodophyta): 18S rDNA phylogeny and resurrection of the Hapalidiaceae for the subfamilies Choreonematoideae, Australithoideae, and Melobesioideae. *J. Phycol.* 39:988–98.

Harvey, A. S., Phillips, L. E., Woelkerling, W. J. & Millar, A. J. K. 2006. The Corallinaceae, subfamily Mastophoroideae (Corallinales, Rhodophyta) in south-eastern Australia. *Aust. Syst. Bot.* 19:387–429.

Hernandez-Kantun, J. J., Rindi, F., Adey, W. H., Heesch, S., Peña, V., Le Gall, L., And Gabrielson, P. W. 2015. Sequencing type material resolves the identity and distribution of the generitype *Lithophyllum incrustans*, and related European species *L. hibernicum* and *L. bathyporum* (Corallinales, Rhodophyta). *J. Phycol*. 51: 791-807.

Hernández-Kantún, J. J., Riosmena-Rodriguez, R., Adey, W. H., & Rindi, F. 2014. Analysis of the cox 2-3 spacer region for population diversity and taxonomic implications in rhodolith-forming species (Rhodophyta: Corallinales). *Phytotaxa* 190:331-54.

Hernandez-Kantun, J. J., Rindi, F., Adey, W. H., Heesch, S., Peña, V., Le Gall, L., & Gabrielson, P.W. 2015. Sequencing type material resolves the identity and distribution of the generitype *Lithophyllum incrustans*, and related European species *L. hibernicum* and *L. bathyporum* (Corallinales, Rhodophyta). *J. Phycol.* 51:791-807.

Hernández-Kantún, J.J., Gabrielson, P., Hughey, J.R., Pezzolesi, L., Rindi, F., Robinson, N.M., Peña, V., Riosmena-Rodriguez, R., Le Gall, L., & Adey, W. 2016. Reassessment of branched *Lithophyllum* spp. (Corallinales, Rhodophyta) in the Caribbean Sea with global implications. *Phycologia* 55:619-39.

Hind, K. R., & Saunders, G. W. 2013. A Molecular Phylogenetic Study of the Tribe Corallineae (Corallinales, Rhodophyta) with an Assessment of Genus-Level Taxonomic Features and Descriptions of Novel Genera. *J. Phycol*. 49:103-14.

Hind, K., Miller, K.A., Young, M., Jensen, C., Gabrielson, P.W. & Martone, P.T. 2015. Resolving cryptic species of *Bossiella* (Corallinales, Rhodophyta) using contemporary and historical DNA. *Am. J. Bot.* 102:1-19.

Hind, K. R., Gabrielson, P. W., P Jensen, C., & Martone, P. T. 2016. *Crusticorallina* gen. nov., a nongeniculate genus in the subfamily Corallinoideae (Corallinales, Rhodophyta). *J. Phycol.* 52:929-41.

Horta, P. A., Riul, P., Amado Filho, G. M., Gurgel, C.F.D. et al. 2016. Rhodoliths in Brazil: Current knowledge and potential impacts of climate change. *Braz. J. Oceanogr*. 64:117-36.

Jesus, P.B., Silva, M. S., Lyra, G. M., Nunes, J. M. C. & Schnadelbach, A. S. 2015. Extension of the distribution range of *Hypnea stellulifera* (Cystocloniaceae, Rhodophyta) to the South Atlantic: morphological and molecular evidence. *Aquat. Bot.* 123:26–36.

Jesus, P.B., Nauer, F., Lyra, G.M., Cassano, V., Oliveira, M.C., Nunes, J.M.C. & Schnadelbach, A.S. 2016. Species delimitation and phylogenetic analyses of some cosmopolitan species of *Hypnea* (Rhodophyta) reveal synonyms and misapplied names to *H. cervicornis*, including a new species from Brazil. *J. Phycol.* 52:774-92.

Johansen, H.W. 1969. Morphology and systematics of coralline algae with special reference to *Calliarthron. University of California Publications in Botany* 49:1-98.

Jukes, T.H. & Cantor, C. R. 1969. Evolution of protein molecules. *In* Munro, H. N. [Ed.] *Mammalian Protein Metabolism*, Academic Press, New York, pp. 21–132.

Kato, A., Baba, M. & Suda, S. 2011. Revision of the Mastophoroideae (Corallinales, Rhodophyta) and polyphyly in nongeniculate species widely distributed on Pacific coral reefs. *J. Phycol*. 3:662-72.

Kato, A., Baba, M. & Suda, S. 2013. Taxonomic circumscription of heterogeneous species *Neogoniolithon brassica-florida* (Corallinales, Rhodophyta) in Japan. *Phycol. Res.* 61:15–26.

Keats, D.W. & Chamberlain, Y.M. 1994. Three species of *Hydrolithon* (Rhodophyta, Corallinaceae): *Hydrolithon onkodes* (Heydrich) Penrose and Woelkerling, *Hydrolithon superficiale*, and *H. samoense* (Foslie) comb. nov. from South Africa. *South Afr. J. of Bot.* 60:8–21.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16:111–20.

Kozak, K. M., Wahlberg, N., Neild, A. F. E., Dasmahapatra, K. K., Mallet, J. & Jiggins, C. D. 2015. Multilocus species trees show the recent adaptive radiation of the mimetic *Heliconius* butterflies. *Syst. Biol*. 64:505–24.

Le Gall, L., Payri, C. E., Bittner, L. & Saunders, G. W. 2010. Multigene phylogenetic analyses support recognition of the Sporolithales ord. nov. *Mol. Phylogenet. Evol*. 54:302-5.

Leliaert, F., Verbruggen, H., Vanormelingen, P., Steen, F., Lópezbautista, J. M., Zuccarello, G. C., & De Clerck, O. 2014. DNA-based species delimitation in algae. *Eur. J. Phycol*. 49:179-96.

Lyra, G.M., Gurgel, F.D., Costa, E.S., Jesus, P.B., Oliveira, M.C., Oliveira, E.C., Davis, C.C., Nunes, J.M.C. 2016. Delimitating cryptic species in the *Gracilaria domingensis* complex (Gracilariaceae, Rhodophyta) using molecular and morphological data. *J. Phycol*. 52:997-1017.

Maneveldt, G.W., van der Merwe, E. & Keats, D.W. 2015. Taxonomic review of *Hydrolithon samoënse* (Corallinaceae, Corallinales, Rhodophyta) and other taxa found to be conspecific. *Phytotaxa* 192:230–53.

Milstein, D. & Oliveira, M.C. 2005. Molecular phylogeny of Bangiales (Rhodophyta) based on small subunit rDNA sequencing: emphasis on Brazilian *Porphyra* species. *Phycologia* 44:212-21.

Modica, M. V., Puillandre, N., Castelin, M. Zhang, Y. & Holford, M. 2014. A good compromise: rapid and robust species proxies for inventorying biodiversity hotspots using the Terebridae (Gastropoda: Conoidea). *PLoS One* 9:1–8.

Moura, C.W.N., Kraus, J.E & Cordeiro-Marino, M. 1997. Metodologia para obtenção de cortes histológicos com historresina e coloração com azul de toluidina O para algas coralináceas (Rhodophyta, Corallinales). *Hoehnea* 24:17-27.

Muangmai, N., West, J. A. & Zuccarello, G. C. 2014. Evolution of four Southern Hemisphere *Bostrychia* (Rhodomelaceae, Rhodophyta) species: phylogeny, species delimitation and divergence times. *Phycologia* 53:593–601.

Nelson, W. A., Sutherland, J. E., Farr, T. J., Hart, D. R., Neill, K. F., Kim, H. J., & Yoon, H. S. 2015. Multi-gene phylogenetic analyses of New Zealand coralline algae: *Corallinapetra novaezelandiae* gen. et and recognition of the Hapalidiales ord. nov. *J. Phycol.* 51:454–68.

Nunes, J.M.C. & Guimarães, S.M.P.B. 2008. Novas referências de rodofíceas marinhas para o litoral brasileiro. *Biota Neotrop.* 8:89–100.

Nunes, J.M.C., Guimarães, S.M.P.B., Bouzon, Z.L. & Horta, P.A. 2011. *Dotyophycus pacificum* I. A. Abbott (Liagoraceae Rhodophyta): a new record for the Atlantic Ocean. *Acta Bot. Bras.* 25:241–48.

Nunes, J.M.C., Jesus, P.B., Caires, T.A. & Guimarães, S.M.P.B. 2014. Occurrence of the genus *Tolypiocladia* F. Schmitz (Rhodomelaceae, Rhodophyta) in the Atlantic Ocean. *Bot. Mar.* 57:147–51.

N'Yeurt, A. D. R. & Payri, C. E. 2010. Marine algal flora of French Polynesia III. Rhodophyta, with additions to the Phaeophyceae and Chlorophyta. *Cryptogamie Algol.* 31:3–205.

Peña, V., Rousseau, F., De Reviers, B., & Le Gall, L. 2014. First assessment of the diversity of coralline species forming maerl and rhodoliths in Guadeloupe, Caribbean using an integrative systematic approach. *Phytotaxa* 190:190-215.

Penrose, D. 1996. Subfamily Mastophoroideae. In: Womersley HBSW (ed) The marine benthic flora of Southern Australia. Part IIIB, Gracilariales, Rhodymeniales, Corallinales and Bonnemaisoniales. Australian Biological Resources Study, Canberra. 237–83.

Puillandre, N., Lambert, A., Brouillet, S. & Achaz, G. 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Mol. Ecol.* 21:1864–877.

Riosmena-Rodriguez, R. 1993. Una propuesta de tecnica histological para el studio de algas coralinas (Corallinales: Rhodophyta). *Rev. Inv. Cient.* 4:65-73.

Ronquist, F. & Huelsenbeck, J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:572–1574.

Rösler, A., Perfectti, F., Peña, V. & Braga, J. C. 2016. Phylogenetic relationships of corallinaceae (Corallinales, Rhodophyta): taxonomic implications for reef-building corallines. *J. Phycol*. 52:412–31.

Sanches, P. F., Pellizzari, F. & Horta, P. A. 2016. Multivariate analyses of Antarctic and sub-Antarctic seaweed distribution patterns: An evaluation of the role of the Antarctic Circumpolar Current. *J. Sea Res.* 110:29-38.

Saunders, G. W. & Moore T. E. 2013. Refinements for the amplification and sequencing of red algal DNA barcode and RedToL phylogenetic markers: a summary of current primers, profiles and strategies. *Algae*. 28:31-43.

Saunders, G. W. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Phil. Trans. R. Soc. B* 360:1879–88.

Sissini, M. N., Oliveira, M. C., Gabrielson, P. W., Robinson, N. M., Okolodkov, Y. B., Riosmena-Rodríguez, R., & Horta, P. A. 2014. *Mesophyllum erubescens* (Corallinales, Rhodophyta)—so many species in one epithet. *Phytotaxa* 190:299-319.

Spalding M.D., Fox H.E., Allen, G.R, Davidson, N., Ferdaña, Z.A., Finlayson, M., Halpern, B.S, Jorge, M.A., Lombana, A., Lourie, S.A., Martin, K.D., Mcmanus, E., Molnar, J., Recchia, C.A., & Robertson J. 2007. Marine ecoregions of the world: A bioregionalization of coastal and shelf areas. *Bioscience* 57:573–583.

Staden, R., Judge, D. P. & Bonfield, J. K. 2003. Analysing sequences using the Staden Package and EMBOSS. *In* Krawetz, S. A. & Womble, D. D. [Eds.] *Introduction to Bioinformatics. A Theoretical and Practical Approach.* Human Press Inc, pp. 393-410.

Stamatakis, A., Hoover, P. & Rougemont, J. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Syst. Biol*. 57: 758–771.

Steneck, R.S. 1986. The ecology of coralline algal crusts: convergent patterns and adaptive strategies. *Ann. Rev. Ecol. Syst.* 17:299-319.

Swofford, D. L. 2002. *PAUP*: Phylogenetic Analysis Using Parsimony (*and Many Other Methods). Version 4.0b10.* Sinauer Associates, Sunderland, Massachusetts.

Tâmega, F. T. S. & Figueiredo, M. A. O. 2005. Distribuição das algas calcárias incrustantes (Corallinales, Rhodophyta) em diferentes habitats na Praia do Forno, Armação dos Búzios, Rio de Janeiro. *Rodriguésia* 56:123-32.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol.* 30:2725–29.

Templeton, A. R., Crandall, K. A. & Sing, C. F. 1992. A cladistic analysis of phenotypic association with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* 132:619–33.

Torrano-Silva, B. N., Riosmena-Rodríguez, R., & De Oliveira, M. C. 2014. Systematic position of Paulsilvella in the Lithophylloideae (Corallinaceae, Rhodophyta) confirmed by molecular data. *Phytotaxa* 190:94-111.

Van der Merwe, E., Miklasz, K., Channing, A., Maneveldt, G.W. & Gabrielson, P.W. 2015. DNA sequencing resolves species of Spongites (Corallinales, Rhodophyta) in the Northeast Pacific and South Africa, including *S. agulhensis Phycologia* 54:471–90.

Vidal, R., Meneses, I. & Smith, M. 2008. Phylogeography of the genus *Spongites* (Corallinales, Rhodophyta) from Chile. *J. Phycol*. 44:173–82.

Vieira-Pinto, T., Oliveira, M. C., Bouzon, J., Sissini, M., Richards, J. L., Riosmena-Rodríguez, R., & Horta, P. A. 2014. *Lithophyllum* species from Brazilian coast: range extension of *Lithophyllum margaritae* and description of *Lithophyllum atlanticum* (Corallinales, Corallinophycidae, Rhodophyta). *Phytotaxa* 190:355-69.

Villas-Bôas, A.B., Riosmena-Rodriguez, R., Tâmega, F.T.S., Amado-Filho, G.M., Maneveldt, G.W. & Figueredo, M.A.O. 2015. Rhodolith-forming species of the subfamilies Neogoniolithoideae and Hydrolithoideae (Rhodophyta, Corallinales) from Espírito Santo State, Brazil. *Phytotaxa* 222:169-84.

Woelkerling, W. J. 1998. Type collections of non-geniculate corallines housed at the Laboratoire de Cryptogamie (PC). In: Woelkerling, W.J. Eds. *Non-geniculate coralline red algae and the Paris Muséum: systematics and scientific history*. Paris: Muséum National d'Histoire naturelle / ADAC pp. 279-404.

Zhang, J., Kapli, P., Pavlidis, P. & Stamatakis, A. 2013. A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* 29:2869–76.

Yoon, H.S., Hackett, J.D. & Bhattacharya, D. 2002. A single origin of the peridinin and fucoxanthin containing plastids in dinoflagellates through tertiary endosymbiosis. *Proc. Natl. Acad. Sci.*99:11724–29.

Table 1. Sequences generated in this study, with voucher information, herbarium accession numbers and GenBank accession numbers for each DNA marker. (–) no molecular data was produced.

| Species | Voucher | Markers | | | | Locality | Coordinate | Collection date |
|---|---|---|---|---|---|---|---|---|
| | | 18S | *psb*A | COI | *rbc*L | | | |
| *Harveylithon riosmenum* | ALCB 123210 | MF611664 | MF966193 | MF966173 | MF966180 | Praia das Conchas, Cabo Frio, Rio de Janeiro, Brazil | 22$^0$52'16.8"S x 41$^0$53'51.2"W | 26/05/2013 |
| *Harveylithon riosmenum* | ALCB 125611 | - | - | - | - | Praia das Conchas, Cabo Frio, Rio de Janeiro, Brazil | 22$^0$52'16.8"S x 41$^0$53'51.2"W | 26/05/2013 |
| *Harveylithon riosmenum* | ALCB 125612 | MF611665 | MF966192 | - | - | Pontal de Guaibura, Guarapari, Espírito Santo, Brazil | 20$^0$43'40.86"S x 40$^0$31'19.32"W | 20/08/2013 |
| *Harveylithon riosmenum* | ALCB 125613 | - | - | - | - | Pontal de Guaibura, Guarapari, Espírito Santo, Brazil | 20$^0$43'40.86"S x 40$^0$31'19.32"W | 20/08/2013 |
| *Harveylithon riosmenum* | ALCB 125614 | MF611666 | MF966191 | MF966172 | MF966179 | Pontal de Guaibura, Guarapari, Espírito Santo, Brazil | 20$^0$43'40.86"S x 40$^0$31'19.32"W | 20/08/2013 |
| *Harveylithon riosmenum* | ALCB 125615 | MF611667 | MF966190 | MF966171 | MF966178 | Pontal de Guaibura, Guarapari, Espírito Santo, Brazil | 20$^0$43'40.86"S x 40$^0$31'19.32"W | 20/08/2013 |
| *Harveylithon riosmenum* | ALCB 125616 | - | - | - | - | Praia de Ubú, Anchieta, Espírito Santo, Brazil | 20$^0$48'49,36"S x 40$^0$36'42,45"W | 21/08/2013 |
| *Harveylithon riosmenum* | ALCB 125617 | MF611668 | MF966189 | MF966170 | MF966177 | Praia de Ubú, Anchieta, Espírito Santo, Brazil | 20$^0$48'49,36"S x 40$^0$36'42,45"W | 21/08/2013 |

| Species | Voucher | Markers | | | | Locality | Coordinate | Collection date |
|---|---|---|---|---|---|---|---|---|
| | | 18S | *psb*A | COI | *rbc*L | | | |
| *Harveylithon riosmenum* | ALCB 25618 | - | - | - | - | Praia Rasa, Búzios, Rio de Janeiro, Brazil | 23$^{0}$02'34.4”S x 43$^{0}$30'29.1”W | 25/05/2013 |
| *Harveylithon riosmenum* | ALCB 125619 | MF611669 | MF966188 | MF966169 | | Praia Rasa, Búzios, Rio de Janeiro, Brazil | 23$^{0}$02'34.4”S x 43$^{0}$30'29.1”W | 25/05/2013 |
| *Harveylithon riosmenum* | ALCB 125623 | - | - | - | - | Prainha do Recreio dos Bandeirantes, Rio de Janeiro, Brazil | 23$^{0}$02'32.48”S x 43$^{0}$30'27.3”W | 24/05/2013 |
| *Harveylithon riosmenum* | ALCB 125624 | MF611670 | MF966187 | - | MF966176 | Enseada dos Corais, Cabo de São Agostinho, Pernambuco, Brazil | 08°18'50.3”S x 34°56'47.8”W | 26/04/2014 |
| *Harveylithon riosmenum* | ALCB 125625 | - | - | - | - | Praia de Boa Viagem, Recife, Pernambuco, Brazil | 08°05'25.52"S x 34°53'46.59"W | 29/04/2014 |
| *Harveylithon riosmenum* | ALCB 125626 | - | - | MF966168 | - | Praia de Boa Viagem, Recife, Pernambuco, Brazil | 08°05'25.52"S x 34°53'46.59"W | 29/04/2014 |
| *Harveylithon riosmenum* | ALCB 123166 | MF611671 | MF966198 | MF966175 | MF966186 | Ilha Deserta, Bombinhas, Santa Catarina, Brazil | 27°27'54.22"S x 48°35'91.44"W | 28/03/2016 |
| *Harveylithon maris-bahiensis* | ALCB 125620 | MF611672 | MF966197 | MF966174 | MF966185 | Morro de São Paulo, Cairú, Bahia, Brazil | 13°23'16.8"S x 38°54'18.6"W | 06/10/2013 |

| Species | Voucher | Markers | | | | Locality | Coordinate | Collection date |
|---|---|---|---|---|---|---|---|---|
| | | 18S | *psb*A | COI | *rbc*L | | | |
| *Harveylithon maris-bahiensis* | ALCB 125621 | MF611673 | - | - | MF966184 | Morro de São Paulo, Cairú, Bahia, Brazil | 13°23'16.8"S x 38°54'18.6"W | 06/10/2013 |
| *Harveylithon maris-bahiensis* | ALCB 125622 | MF611674 | MF966196 | - | MF966183 | Morro de São Paulo, Cairú, Bahia, Brazil | 13°23'16.8"S x 38°54'18.6"W | 06/10/2013 |
| *Harveylithon catarinense* | ALCB 125627 | - | MF966195 | - | MF966182 | Ilha Deserta, Bombinhas, Santa Catarina, Brazil | 27°27'54.22"S x 48°35'91.44"W | 28/03/2016 |
| *Harveylithon catarinense* | ALCB 125628 | MF611675 | MF966194 | - | MF966181 | Ilha Deserta, Bombinhas, Santa Catarina, Brazil | 27°27'54.22"S x 48°35'91.44"W | 28/03/2016 |

Table 2. Levels of intraspecific genetic variation (diagonal in gray), interspecific variation (in percentage, under the diagonal) and DNA barcode gaps (over the diagonal) for SSU rDNA sequences, based on uncorrected p-distances between studied taxa. ( ), number of sequences/species analyzed; -, absent values.

| | *H. riosmenum* | *H. maris-bahiensis* | *H. catarinense* | *H. samöense* | *H. rupestre* |
|---|---|---|---|---|---|
| *H. riosmenum* (7) | 0-0.4 | 2.65x | 6.6x | 2x | 3.8x |
| *H. maris-bahiensis* (3) | 1.06-1.46 | 0.13-0.26 | 4.6x | 1.8x | 3.6x |
| *H. catarinense* (1) | 1.85-2.12 | 1.72-1.85 | - | - | - |
| *H. samöense* (1) | 0.73-0.99 | 0.53-0.66 | 1.59 | - | - |
| *H. rupestre* (1) | 1.52-1.79 | 0.93-1.06 | 1.39 | 0.99 | - |

Table 3. Levels of intraspecific genetic variation (diagonal in gray), interspecific variation (in percentage, under the diagonal) and DNA barcode gaps (over the diagonal) for *psb*A DNA sequences, based on uncorrected p-distances between studied taxa. ( ), number of sequences/species analyzed; -, absent values.

| | *H. riosmenum* | *H. maris-bahiensis* | *H. catarinense* | *H. rupestre* | *H. munitum* | *H. canariense* |
|---|---|---|---|---|---|---|
| *H. riosmenum* (8) | 0.0 - 0.39 | 15.5x | 21.5x | 19.5x | 23.2x | 21.2x |
| *H. maris-bahiensis* (2) | 6.05 - 6.45 | 0.79 | 9x | 8.2x | 9.65x | 10.2x |
| *H. catarinense* (2) | 8.42 - 8.55 | 7.11 - 7.63 | 0 | - | - | - |
| *H. rupestre* (1) | 7.63 - 7.89 | 6.45 - 6.97 | 4.47 | - | - | - |
| *H. munitum* (1) | 9.08 - 9.34 | 7.63 - 7.89 | 6.05 | 4.21 | - | - |
| *H. canariense* (1) | 8.29 - 8.42 | 8.03 - 8.29 | 2.5 | 4.61 | 6.45 | - |

Table 4. Levels of intraspecific genetic variation (diagonal in gray), interspecific variation (in percentage, under the diagonal) and DNA barcode gaps (over the diagonal) for *rbc*L-3P DNA sequences, based on uncorrected p-distances between studied taxa. ( ), number of sequences/species analyzed; -, absent values.

| | *H. riosmenum* SC | *H. riosmenum* PE | *H. riosmenum* PE | *H. maris-bahiensis* | *H. catarinense* | *P. onkodes* |
|---|---|---|---|---|---|---|
| *H. riosmenum* SC (1) | - | - | 6.1x | - | - | - |
| *H. riosmenum* PE (1) | 4.05 | - | 4x | - | - | - |
| *H. riosmenum* ES e RJ (4) | 2.43-2.83 | 1.62-2.02 | 0-0.4 | 14.2x | 23.3x | 12.1x |
| *H. maris-bahiensis* (3) | 6.07 | 6.07 | 5.67-6.07 | 0.0 | - | - |
| *H. catarinense*(2) | 8.91 | 9.72 | 9.31-9.72 | 10.12 | 0.0 | - |
| *P. onkodes* (1) | 6.48 | 4.86 | 4.86-5.26 | 4.45 | 9.31 | - |

Table 5. Levels of intraspecific genetic variation (diagonal in gray), interspecific variation (in percentage, under the diagonal) and DNA barcode gaps (over the diagonal) for COI-5P DNA sequences, based on uncorrected p-distances between studied taxa. ( ), number of sequences/species analyzed; -, absent values.

| | *H. riosmenum* | *H. maris-bahiensis* | *P. gardineri* |
|---|---|---|---|
| *H. riosmenum* (7) | 0.0 - 1.13 | 7.5x | 13.1x |
| *H. maris-bahiensis* (1) | 8.44 - 8.63 | - | - |
| *P. gardineri* | 14.82 | 13.13 | - |

Table 6. Number of haplotypes of the studied taxa for the different markers used, based on SPN analysis.

| | SSU rDNA | *psb*A | *rbc*L-3P | COI-5P |
|---|---|---|---|---|
| *H. riosmenum* | 5 | 3 | 5 | 2 |
| *H. maris-bahiensis* | 3 | 2 | 1 | 1 |
| *H. catarinense* | 1 | 1 | 1 | - |

Table 7: Morphological comparison between *Harveylithon* taxa (- = absent; ND = not disclosed; SS = singly surface; V = in small vertical rows; H = in horizontal rows; B = buried within the thallus).* Maneveldt et al. (2015)[1] – as *Hydrolithon samöense*; Penrose (1996)[2] – as *Hydrolithon rupestre* e *Hydrolithon munitum*; Foslie (1906)[3] – as *Goniolithon accretum* f. *canariensis*.

| Characters (measured in µm) | Lectotype[1] *H. samöense* | *H. samöense*[1] | *H. rupestre*[2] | *H. munitum*[2] | *H. canariense*[3] | *H. riosmenum* | *H. maris-bahiensis* | *H. catarinense* |
|---|---|---|---|---|---|---|---|---|
| Organization | Monomerous | Monomerous | Monomerous | Monomerous | Monomerous | Monomerous | Monomerous | Monomerous |
| Height of epithallial cells | 4 - 6 | 2-7 | 4 – 6 | 3-4 | ND | 2.6-6.5 | 4.3-7.5 | 2.7-4.7 |
| Diameter of epithallial cells | 5 - 7 | 3-10 | 5 – 10 | 3-6 | ND | 7.5-9 | 4.8-8.5 | 4.3-7.7 |
| Height of perithallial cells | 3 - 15 | 3-15 | 2-17 | 8-20 | ND | 2.4-14.5 | 4.6-10 | 5.9-18.6 |
| Diameter of perithallial cells | 3 - 9 | 3-13 | 2-6 | 3-6 | 7-9 | 3.5-8.5 | 4.4-8.6 | 4.5-7.1 |
| Height of hypothallial cells | 5 - 19 | 4-31 | 6 – 19 | ND | ND | 4.1-12.8 | 4-12 | 4.9-20 |
| Diameter of hypothallial cells | 3 - 17 | 3-17 | 5 – 11 | ND | ND | 8-11 | 8-10 | 5-10.8 |
| Trichocytes arrangement | SS, V, B | SS, V, B | SS, H | - | SS | SS, V, B | SS | SS, B |
| Height of trichocytes | 16 - 31 | 7-31 | 9 – 12 | ND | ND | 9-20.7 | 10-15.4 | 8.5-18.3 |
| Diameter of trichocytes | 7 - 10 | 5-16 | 6 – 8 | ND | ND | 5.2-18 | 7-11.6 | 8-12.3 |
| Height of tetrasporangial conceptacles | 31 - 60 | 30-80 | 77-82 | 117-164 | ND | 43-126 | 50-71.6 | 43-174 |
| Diameter of tetrasporangial conceptacles | 56 - 130 | 55-185 | 82-96 | 178-232 | 250 – 400 | 72-164.8 | 70-111 | 127-250 |
| Height of tetrasporangia | | 18-60 | 40-55 | 45-85 | - | 11.5-89 | 30-69.3 | 25.5-71.7 |

| Characters (measured in µm) | Lectotype[1] *H. samöense* | *H. samöense*[1] | *H. rupestre*[2] | *H. munitum*[2] | *H. canariense*[3] | *H. riosmenum* | *H. maris-bahiensis* | *H. catarinense* |
|---|---|---|---|---|---|---|---|---|
| Diameter of tetrasporangia | | 6-35 | 15-35 | 27-61 | - | 8-34 | 12-27.6 | 18-26 |
| Cells number in the roof of tetrasporangial conceptacle | 2 - 4 (3) | 2 - 4 (3) | 3-4 | 4-6 | 6-7 | 2 - 4 (3) | 2-3 | 4-8 |
| Typo of meristematic cells in the roof of tetrasporangial conceptacle (µm) and number of times it is greater than the epithallial cell | Columnar 2.5-5x | Columnar | Non columnar | Non columnar | Non columnar | Columnar (10.5-15) 2-4x | Non columnar (6.6-9.9) 1.5x | Non columnar |
| Gametangial thalli | Dioecious | Dioecious | - | - | - | Monoecious | - | Dioecious |
| Height of carposporangial conceptacles | 25-30 | 16-30 | - | - | - | 48-66.1 | - | 94.8 |
| Diameter of carposporangial conceptacles | 56-80 | 35-84 | - | - | - | 90-125 | - | 237.5 |
| Height of spermatangial conceptacles | 19-29 | 15-30 | - | - | - | 24.4-26 | - | 21.4-25.6 |
| Diameter of spermatangial conceptacles | 37-62 | 32-71 | - | - | - | 46-54 | - | 30-31.2 |

**Supplementary table S1.** List of taxa available in GenBank and included in our analyses.

| Species | Locality | GB access nº 18S | GB access nº psbA | GB access nº COI | GB access nº rbcL |
|---|---|---|---|---|---|
| *Amphiroa* sp. | Fiji | GQ917416 | - | - | - |
| *Amphiroa* sp. | Guadeloupe | GQ917380 | - | - | - |
| *Corallina officinalis* | Scotland | FM180104 | - | - | - |
| *Crusticorallina adhaerens* | Canada | KU983205 | - | - | - |
| *Crusticorallina muricata* | Canada | KU983206 | - | - | - |
| *Crusticorallina painei* | Canada | KX036713 | - | - | - |
| *Ellisolandia elongata* | South Africa | U60946 | - | - | - |
| *Harveylithon canariense* (as *Neogoniolithon accretum*) | Canary Island | - | KM407560 | - | - |
| *Harveylithon munitum* (as *Hydrolithon munitum*) | Australia | - | KM407531 | - | - |
| *Harveylithon rupestre* (as *Hydrolithon rupestre*) | Australia | KM073303 | KM407535 | - | - |
| *Harveylithon samoënse* (as *Hydrolithon samoënse*) | South Australia | AY234236 | - | - | - |
| *Hydrolithon reinboldii* | Hawaii | DQ628999 | - | - | - |
| *Hydrolithon reinboldii* | Japan | AB576017 | - | - | - |
| *Hydrolithon reinboldii* | Hawaii | DQ629003 | - | - | - |
| *Hydrolithon* cf. *reinboldii* | Hawaii | DQ629001 | - | - | - |
| *Hydrolithon* cf. *reinboldii* | Hawaii | DQ629000 | - | - | - |
| *Jania crassa* | South Africa | U62113 | - | - | - |
| *Jania rubens* | Brazil | KM044029 | - | - | - |

| | | | | | |
|---|---|---|---|---|---|
| *Lithophyllum atlanticum* | Brazil | KP192390 | - | - | - |
| *Lithophyllum* cf. *bamleri* | Fiji | GQ917406 | - | - | - |
| *Lithophyllum* cf. *bamleri* | Fiji | GQ917417 | - | - | - |
| *Lithophyllum dentatum* | Spain | KM073281 | - | - | - |
| *Lithophyllum incrustans* | Spain | KM073283 | - | - | - |
| *Lithophyllum incrustans* | Spain | KM073285 | - | - | - |
| *Lithophyllum incrustans* | Spain | KM073284 | - | - | - |
| *Lithophyllum incrustans* | UK | AF093410 | - | - | - |
| *Lithophyllum incrustans* | France | GQ917385 | - | - | - |
| *Lithophyllum insipidum* | Hawaii | - | - | HQ423075 | - |
| *Lithophyllum insipidum* | Japan | AB576007 | - | - | - |
| *Lithophyllum kotschyanum* | Hawaii | - | KM407548 | HQ423072 | - |
| *Lithophyllum kotschyanum* | Australia | KM073287 | - | - | - |
| *Lithophyllum kotschyanum* | Japan | AB576009 | - | - | - |
| *Lithophyllum kotschyanum* | Hawaii | DQ628974 | - | - | - |
| *Lithophyllum kotschyanum* | Japan | AB576008 | - | - | - |
| *Lithophyllum kotschyanum* | Japan | AB576010 | - | - | - |
| *Lithophyllum kotschyanum* | Fiji | U62117 | - | - | - |
| *Lithophyllum kotschyanum* | Japan | AB576011 | - | - | - |
| *Lithophyllum margaritae* | Brazil | KP192392 | JQ896253 | - | - |
| *Lithophyllum pygmaeum* | New Caledonia | GQ917403 | - | - | - |

| | | | | | |
|---|---|---|---|---|---|
| *Lithophyllum racemus* | Spain | KM073292 | - | - | - |
| *Lithophyllum* sp. | Fiji | GQ917413 | - | - | - |
| *Lithophyllum* sp. | Vanuatu | GQ917397 | - | - | - |
| *Lithophyllum stictaeforme* | Spain | KM073293 | - | - | - |
| *Mastophora pacifica* | Japan | AB576026 | - | - | - |
| *Mastophora pacifica* | Japan | AB576025 | - | - | - |
| *Mastophora pacifica* | French Polynesi | GQ917430 | - | - | - |
| *Mastophora rosea* | Japan | AB576024 | - | - | - |
| *Mesophyllum erubescens* | Brazil | - | - | - | KP050698 |
| *Mesophyllum lichenoides* | Spain | KP142819 | KP142728 | KJ592709 | KP142772 |
| *Mesophyllum lichenoides* | France | GQ917384 | - | - | - |
| *Metagoniolithon chara* | Australia | U60743 | - | - | - |
| *Metagoniolithon radiatum* | Australia | U61250 | - | - | - |
| *Metagoniolithon stelliferum* | Australia | U61251 | - | - | - |
| *Metamastophora flabellata* | South Australia | AY234239 | - | - | - |
| *Metamastophora flabellata* | South Australia | AY234240 | - | - | - |
| *Neogoniolithon brassica-florida* | Hawaii | DQ629008 | - | - | - |
| *Neogoniolithon brassica-florida* | Hawaii | DQ629007 | - | - | - |
| *Neogoniolithon brassica-florida* | Hawaii | DQ629009 | - | - | - |
| *Neogoniolithon fosliei* | Australia | KM073294 | - | - | - |
| *Neogoniolithon fosliei* | Moorea | KM073297 | - | - | - |

| | | | | | |
|---|---|---|---|---|---|
| *Neogoniolithon fosliei* | Japan | AB576020 | - | - | - |
| *Neogoniolithon fosliei* | Japan | AB713916 | - | - | - |
| *Neogoniolithon fosliei* | Japan | AB576019 | - | - | - |
| *Neogoniolithon fosliei* | Japan | AB713914 | - | - | - |
| *Neogoniolithon fosliei* | Japan | AB576018 | - | - | - |
| *Neogoniolithon megalocystum* | Japan | AB576021 | - | - | - |
| *Neogoniolithon* sp. | Spain | KM073298 | - | - | - |
| *Neogoniolithon* sp. | Spain | KM073300 | - | - | - |
| *Neogoniolithon* sp. | New Caledonia | GQ917424 | - | - | - |
| *Neogoniolithon* sp. | Vanuatu | GQ917396 | - | - | - |
| *Neogoniolithon* sp. | New Caledonia | GQ917425 | - | - | - |
| *Neogoniolithon* sp. | New Caledonia | GQ917426 | - | - | - |
| *Neogoniolithon* sp. | New Caledonia | GQ917434 | - | - | - |
| *Neogoniolithon* sp. | Fiji | GQ917410 | - | - | - |
| *Neogoniolithon* sp. | New Caledonia | GQ917402 | - | - | - |
| *Neogoniolithon* sp. | Vanuatu | GQ917387 | - | - | - |
| *Neogoniolithon spectabile* | Bahamas | AY234238 | - | - | - |
| *Pneophyllum confervicola* | Spain | KM073305 | - | - | - |
| *Pneophyllum* cf. *conicum* | Hawaii | DQ628989 | - | - | - |
| *Pneophyllum* cf. *conicum* | Hawaii | DQ628994 | - | - | - |
| *Pneophyllum conicum* | Hawaii | DQ628985 | - | - | - |

| | | | | | |
|---|---|---|---|---|---|
| *Pneophyllum conicum* | Hawaii | DQ628983 | - | - | - |
| *Pneophyllum conicum* | Japan | AB576022 | - | - | - |
| *Pneophyllum conicum* | Fiji | GQ917414 | - | - | - |
| *Pneophyllum conicum* | Fiji | GQ917408 | - | - | - |
| *Pneophyllum conicum* | Vanuatu | GQ917389 | - | - | - |
| *Porolithon gardineri* (as *Hydrolithon gardineri*) | Hawaii | - | - | HQ423069 | - |
| *Porolithon onkodes* (as *Hydrolithon* cf. *onkodes*) | Hawaii | DQ628996 | - | - | - |
| *Porolithon onkodes* (as *Hydrolithon onkodes*) | Indonesia | KM073275 | - | - | - |
| *Porolithon onkodes* (as *Hydrolithon onkodes*) | Japan | AB576013 | - | - | - |
| *Porolithon onkodes* (as *Hydrolithon onkodes*) | Australia | AY234237 | - | - | - |
| *Porolithon onkodes* (as *Hydrolithon onkodes*) | New Zealand | AY234237 | - | - | KM369154 |
| *Porolithon onkodes* (as *Hydrolithon onkodes*) | New Caledonia | GQ917372 | - | - | - |
| *Porolithon onkodes* (as *Hydrolithon onkodes*) | New Caledonia | GQ917373 | - | - | - |
| *Porolithon onkodes* (as *Hydrolithon onkodes*) | New Caledonia | GQ917371 | - | - | - |
| *Porolithon pachydermum* (as *Hydrolithon pachydermum*) | Japan | AY234235 | - | - | - |
| *Porolithon* sp. (as *Hydrolithon* sp.) | Hawaii | - | - | HQ422713 | - |
| *Spongites decipiens* | South Africa | - | - | - | KT184838 |
| *Spongites fruticulosa* | Spain | KM073306 | - | - | - |
| *Spongites hyperellus* | Australia | GQ917431 | - | - | - |
| *Spongites tumidum* | South Africa | - | - | - | KT184841 |

| | | | | | |
|---|---|---|---|---|---|
| *Spongites yendoi* | South Africa | U60948 | - | - | - |
| *Sporolithon durum* | New Zealand | - | - | - | KM369122 |
| *Sporolithon ptychoides* | Hawaii | DQ629014 | KC870927 | HQ422711 | HQ420971 |
| *Titanoderma prototypum* | Hawaii | DQ628981 | - | - | - |
| *Titanoderma pustulatum* | UK | AF093409 | - | - | - |
| *Titanoderma* sp. | Fiji | GQ917421 | - | - | - |

**Supplementary table S2.** Summary of parsimony analysis for four genes and concatenated data sets.

| | SSU | *psb*A | rbcL-3P | COI-5P | Concatenated |
|---|---|---|---|---|---|
| Number of taxa | 104 | 19 | 17 | 14 | 18 |
| Number of nucleotide (bp) included in analysis | 1,544 | 760 | 247 | 534 | 3,083 |
| Number of parsimony informative characters | 171 | 140 | 58 | 112 | 406 |
| Tree length | 1,584 | 389 | 143 | 363 | 1.091 |
| Consistency index (CI) | 0.4438 | 0.6170 | 0.7203 | 0.7355 | 0.7635 |
| Retention index (RI) | 0.8580 | 0.7140 | 0.7790 | 0.6811 | 0.7410 |
| Rescaled consistency index (RC) | 0.3808 | 0.4405 | 0.5611 | 0.5009 | 0.5657 |

FIGURES

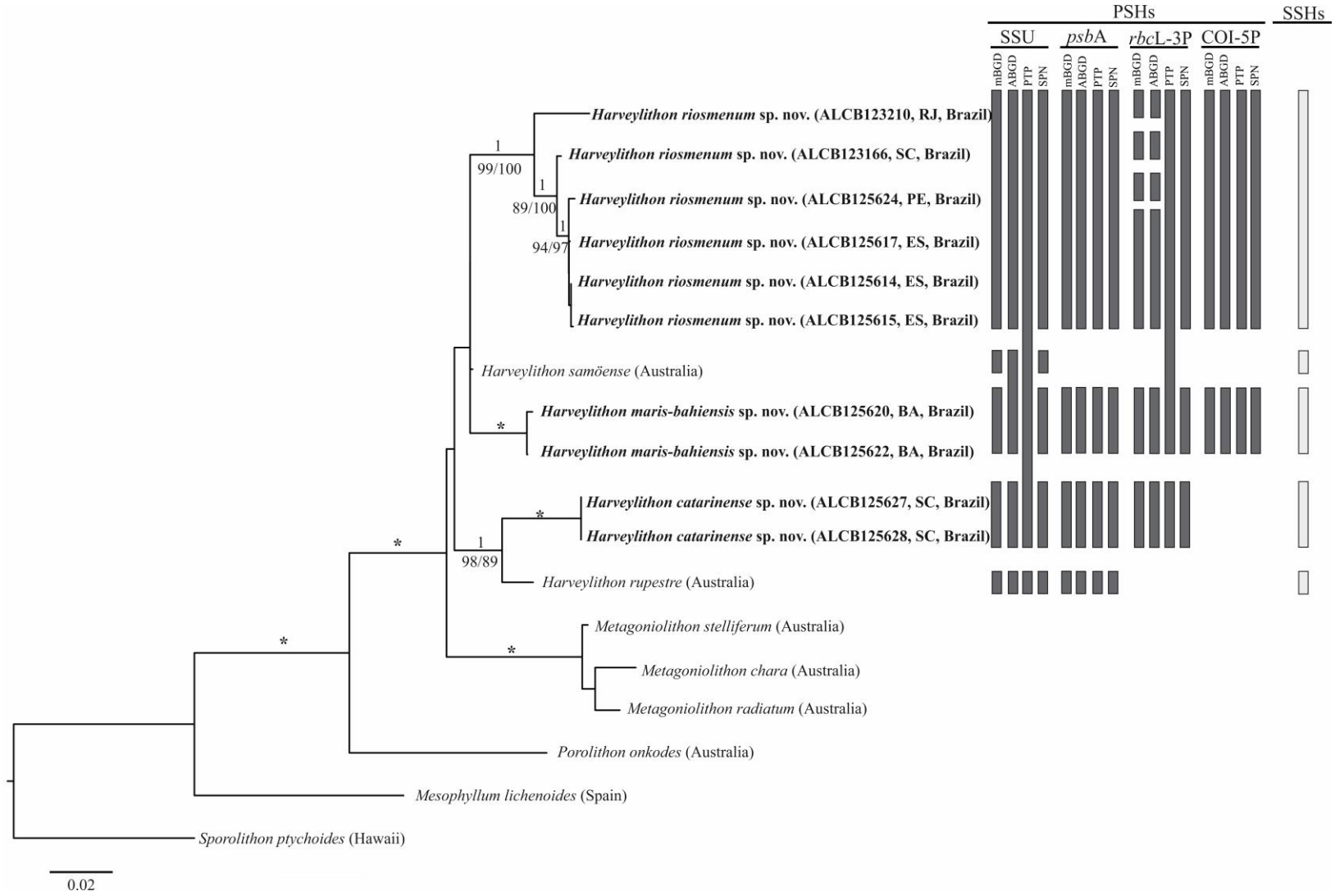
PSHs
SSHs
SSU
psbA
rbcL-3P
COI-5P
mBGD
ABGD
PTP
SPN
Harveylithon riosmenum sp. nov. (ALCB123210, RJ, Brazil)
Harveylithon riosmenum sp. nov. (ALCB123166, SC, Brazil)
Harveylithon riosmenum sp. nov. (ALCB125624, PE, Brazil)
Harveylithon riosmenum sp. nov. (ALCB125617, ES, Brazil)
Harveylithon riosmenum sp. nov. (ALCB125614, ES, Brazil)
Harveylithon riosmenum sp. nov. (ALCB125615, ES, Brazil)
Harveylithon samöense (Australia)
Harveylithon maris-bahiensis sp. nov. (ALCB125620, BA, Brazil)
Harveylithon maris-bahiensis sp. nov. (ALCB125622, BA, Brazil)
Harveylithon catarinense sp. nov. (ALCB125627, SC, Brazil)
Harveylithon catarinense sp. nov. (ALCB125628, SC, Brazil)
Harveylithon rupestre (Australia)
Metagoniolithon stelliferum (Australia)
Metagoniolithon chara (Australia)
Metagoniolithon radiatum (Australia)
Porolithon onkodes (Australia)
Mesophyllum lichenoides (Spain)
Sporolithon ptychoides (Hawaii)
1
99/100
1
89/100
1
94/97
*
*
*
1
98/89
*
0.02

FIGURE 1: Optimal maximum likelihood (ML) topology inferred from the concatenated data set of SSU rDNA, *psb*A, *rbc*L-3P and COI-5P, with results of single-marker species delimitation methods. Only bootstrap values in MP and ML ≥ 70% and Bayesian posterior probability (PP) ≥ 90% were plotted. New sequences produced in this study are in bold, others are from GenBank. Asterisks mark clades that are supported at 100% in all three analyses. Vertical thick lines indicate PSHs delineated with mBGD, ABGD, PTP and SPN methods based on SSU rDNA, *psb*A, *rbc*L-3P and COI-5P (dark gray). Conclusive SSHs obtained from congruent results of delimitation methods (light gray) are presented at the right edge of the tree.

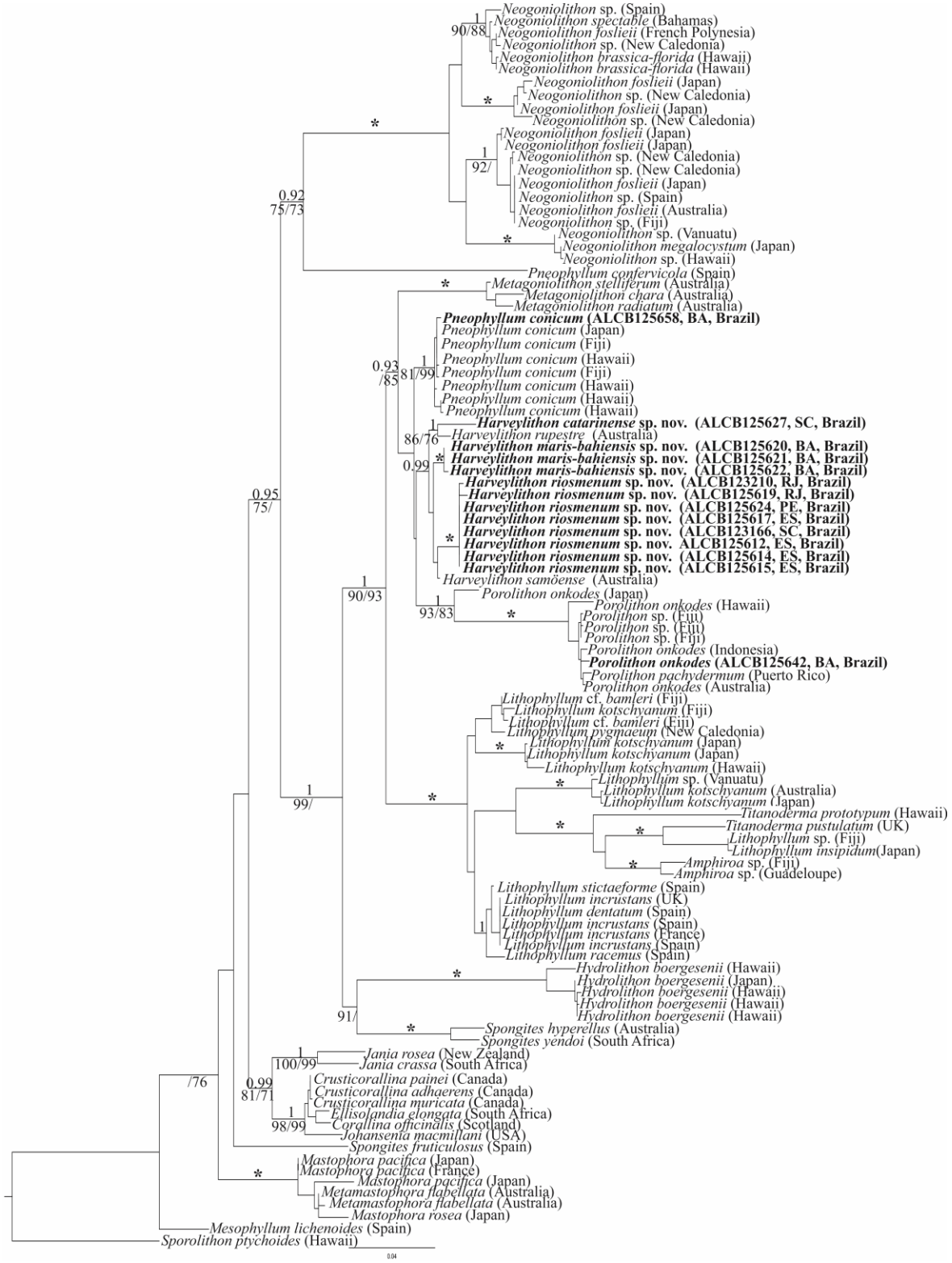


FIGURE 2: The optimal maximum likelihood (ML) topology based on the SSU rDNA data set. Values above each clade refer to Bayesian posterior probabilities, ML bootstrap values and MP (maximum parsimony), respectively. Only bootstrap values in MP and ML ≥ 70% and Bayesian posterior probability (PP) ≥ 90% were plotted. New sequences produced in

this study are in bold. Asterisks mark clades that are supported at 100% in all three analyses.

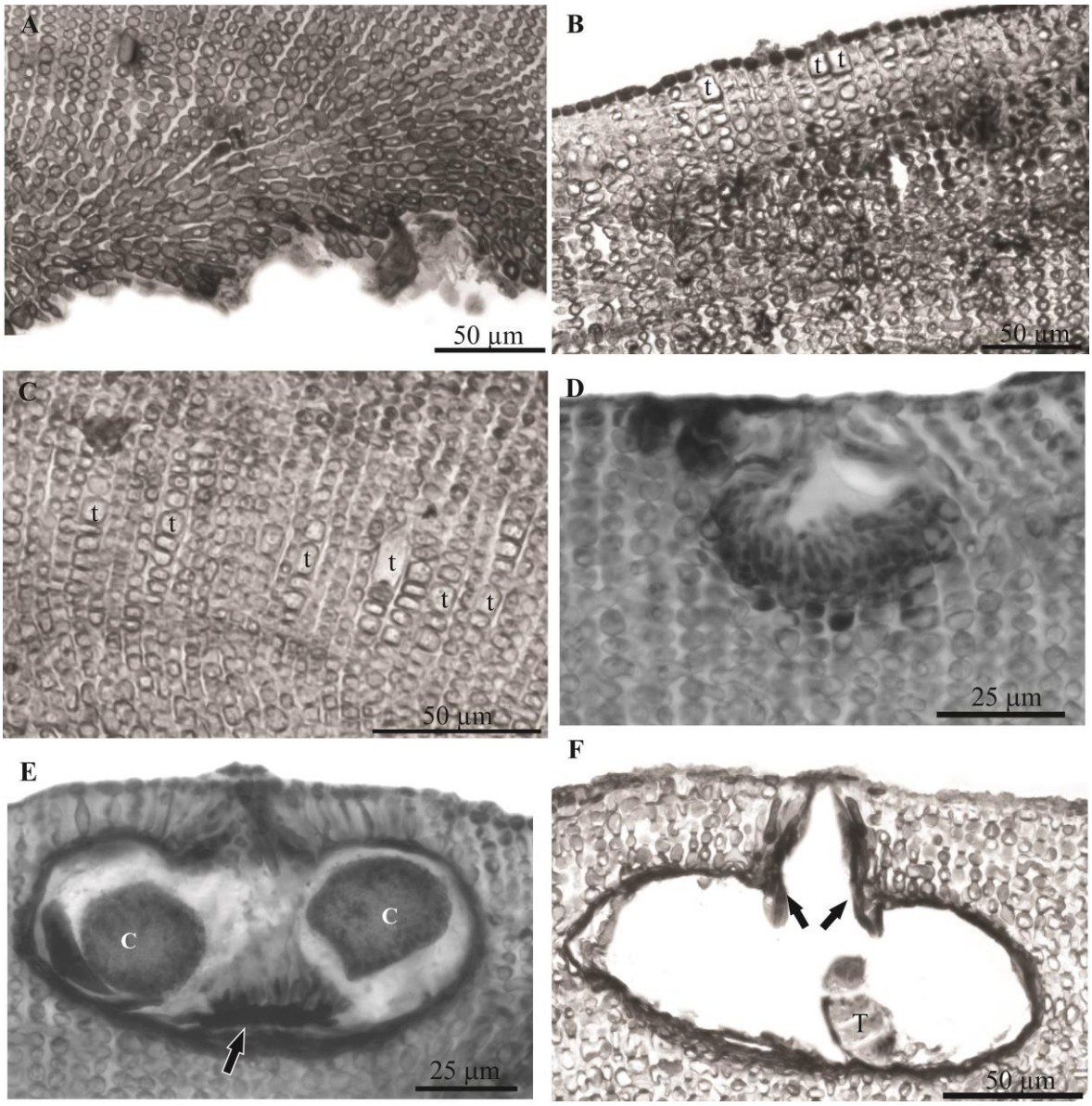


FIGURE 3 A-D: Diagnostic characters of *Harveylithon*. A. Longitudinal section through the thallus showing monomerous construction (ALCB 125613 – *H. riosmenum*). B. Longitudinal section through the thallus showing trichocytes (t) at the thallus surface (ALCB 125621 – *H. maris-bahiensis*). C. Longitudinal section through the thallus showing trichocytes (t) in small vertical rows (ALCB 125613 – *H. riosmenum*). D. Male conceptacle (S) showing unbranched spermatangial filaments across the chamber floor only (ALCB125611 – *H. riosmenum*). E. Carposporangial conceptacle showing central fusion cell (arrow) and peripheral gonimoblast filaments bearing terminal carposporangia (C) (ALCB125611 – *H. riosmenum*). F. Longitudinal section through tetrasporangial conceptacle showing pore canals lined by cells orientated perpendicularly to the thallus surface (arrows), and sporangium zonately divided (T) (ALCB 125628 – *H. catarinense*).

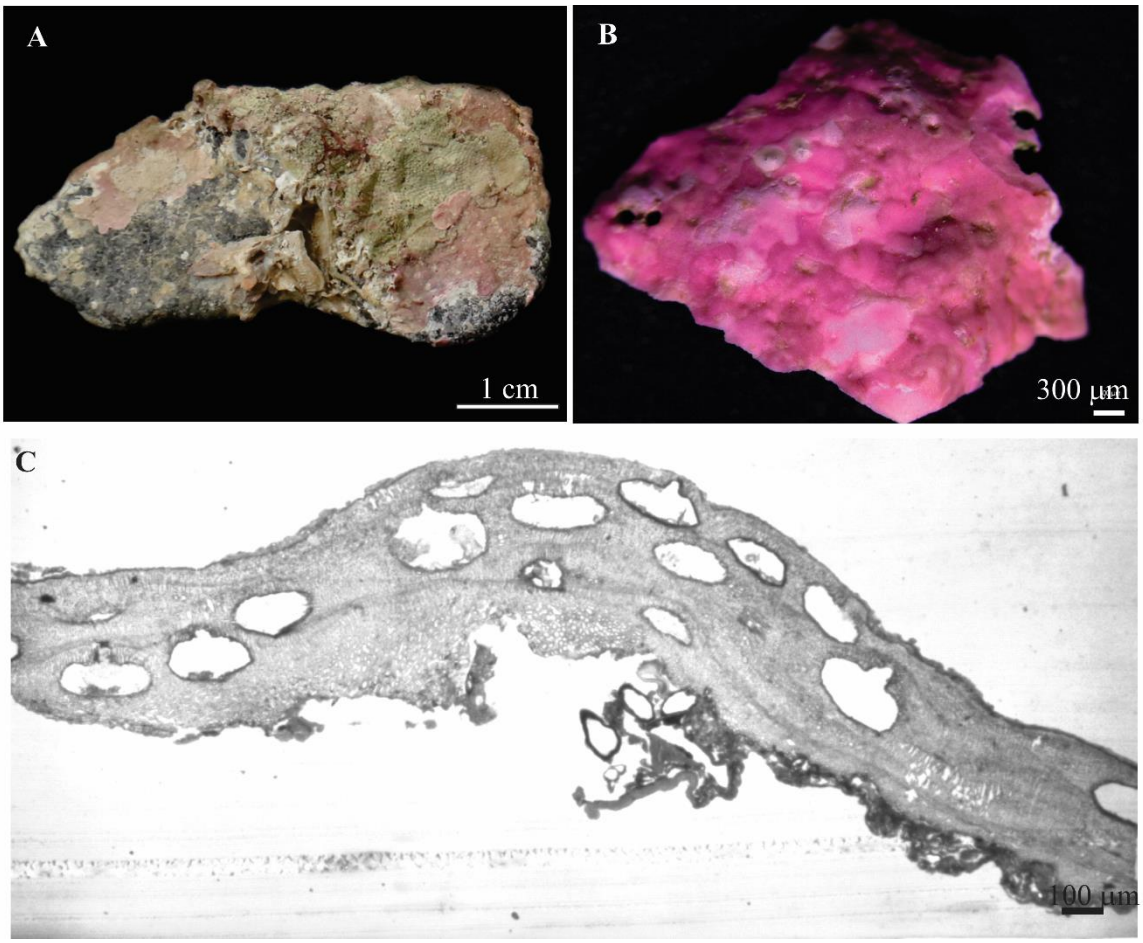


FIGURE 4 A-C: *Harveylithon catarinense* (ALCB125628). A. Encrusting growth-form on rock. B. Detail of uniporate conceptacle. C. Longitudinal section through the thallus showing old conceptacle buried in the thallus.

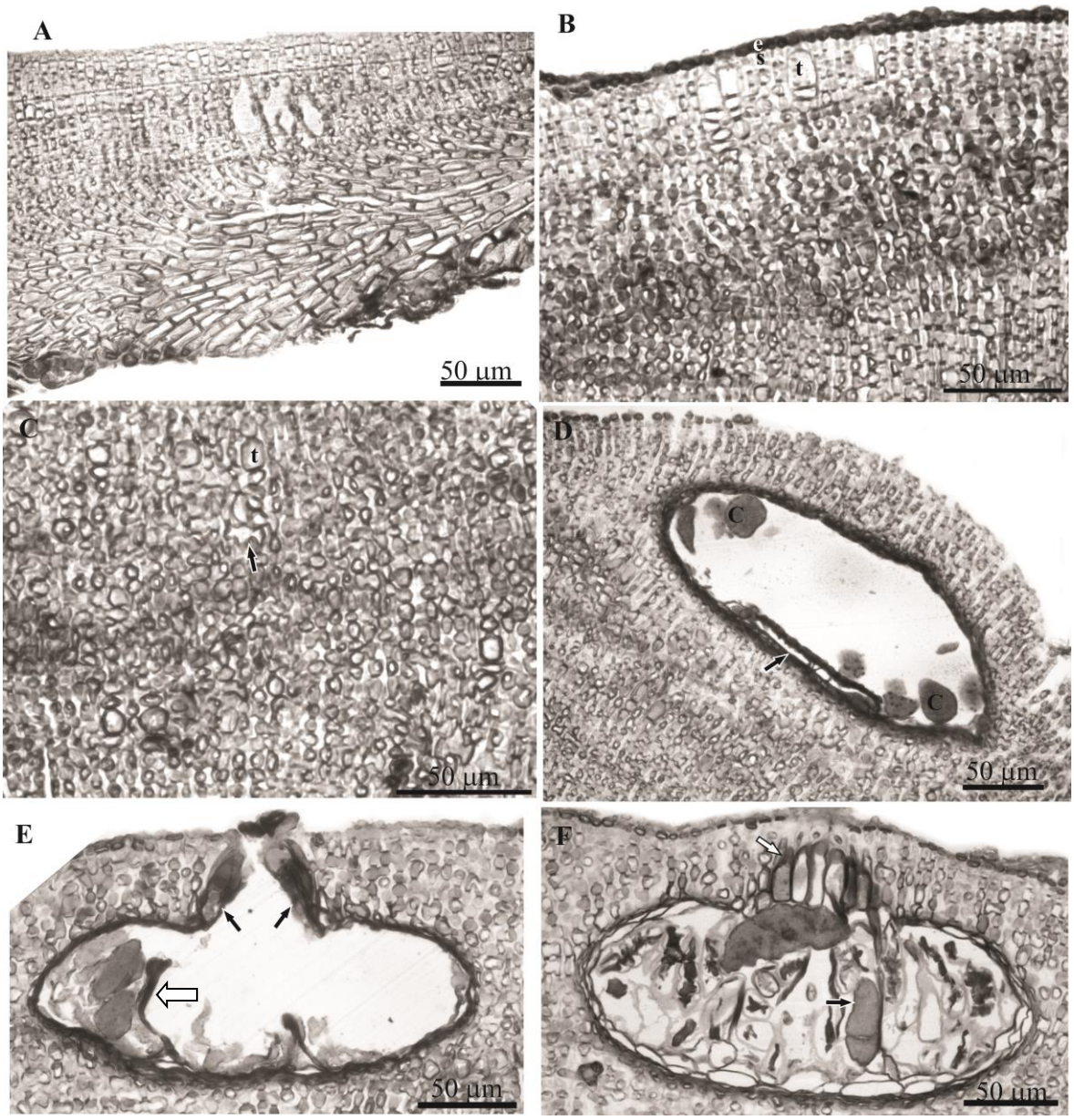


FIGURE 5 A-F: *Harveylithon catarinense* (ALCB125628). A. Longitudinal section through the thallus showing monomerous construction. B. Longitudinal section through the thallus showing epithallial cells (e) more or less elliptical, subepithallial initial cells (s) longer than subtending ones, and trichocytes (t) at the thallus surface. C. Longitudinal section through cells of adjacent filaments linked by lateral cell fusions (arrow) and trichocytes (t) in small vertical rows. D. Longitudinal section through carposporangial conceptacle showing central fusion cell (arrow) and peripheral gonimoblast filaments bearing terminal carposporangia (C). E. Longitudinal section through tetrasporangial conceptacle showing pore canals lined by cells orientated perpendicularly to the thallus surface (black arrows) and remaining of filaments surrounding and interspersed among sporangial initials (white arrow). F. Longitudinal section through tetrasporangial conceptacle showing elongate cells forming the pore canal (white arrow), and sporangium zonately divided (black arrow).

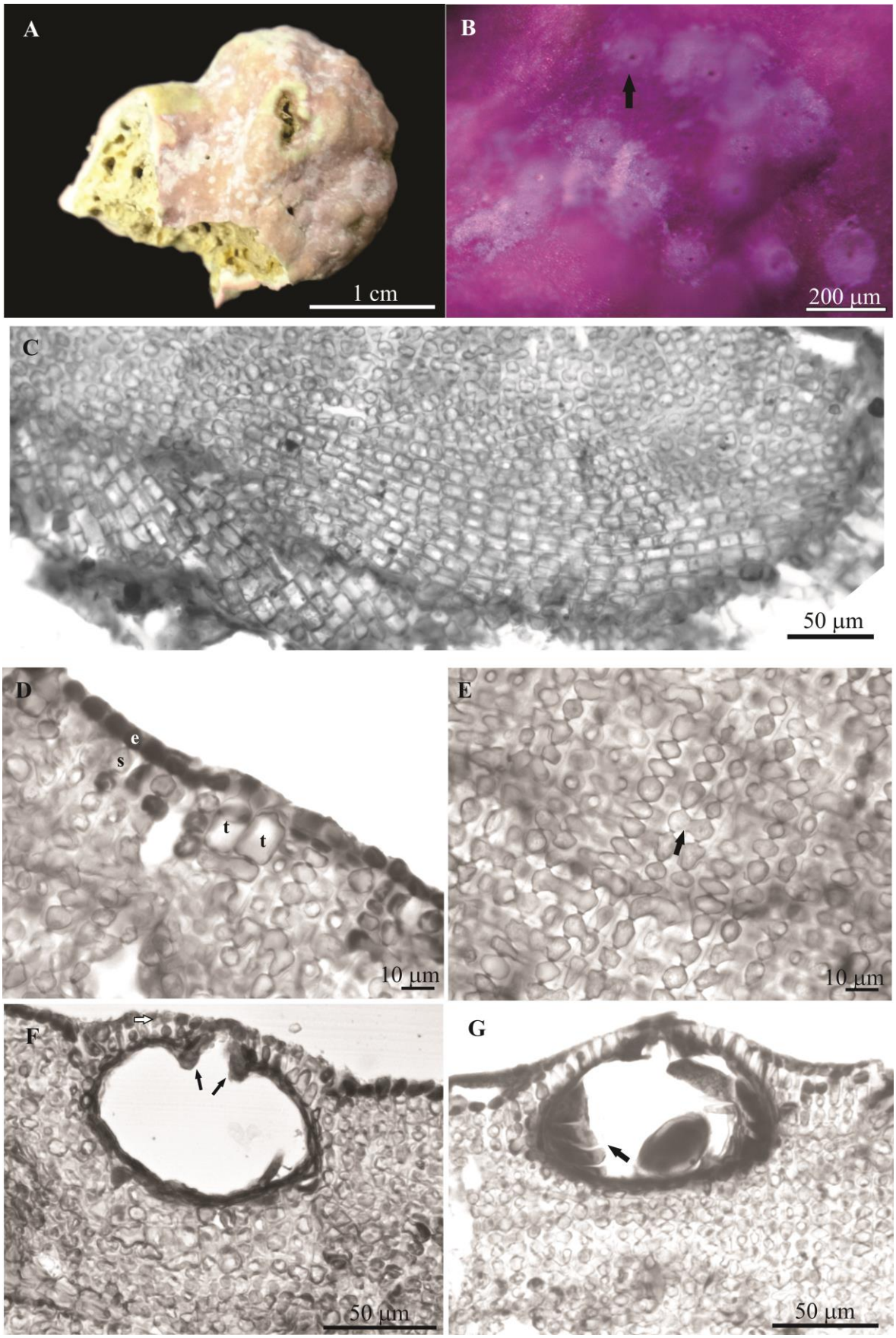


FIGURE 6 A-G: *Harveylithon maris-bahiensis* (ALCB125620). A. Encrusting growth-form on rock. B. Detail of uniporate conceptacle with distinct white aureole around

(arrow). C. Longitudinal section through the thallus showing monomerous construction. D. Longitudinal section through the thallus showing epithallial cells (e) more or less elliptical, subepithallial initial cells (s) longer than subtending ones, and trichocytes (t) at the thallus surface. E. Longitudinal section through cells of adjacent filaments linked by lateral cell fusions (arrow). F. Longitudinal section through tetrasporangial conceptacle showing pore canals lined by cells orientated perpendicularly to the thallus surface (black arrows), and conceptacle roof filaments comprising an epithallial cell, sustended by a characteristic elliptic cell and usually a smaller basal cell (white arrow). G. Longitudinal section through tetrasporangial conceptacle showing sporangium zonately divided (arrow).

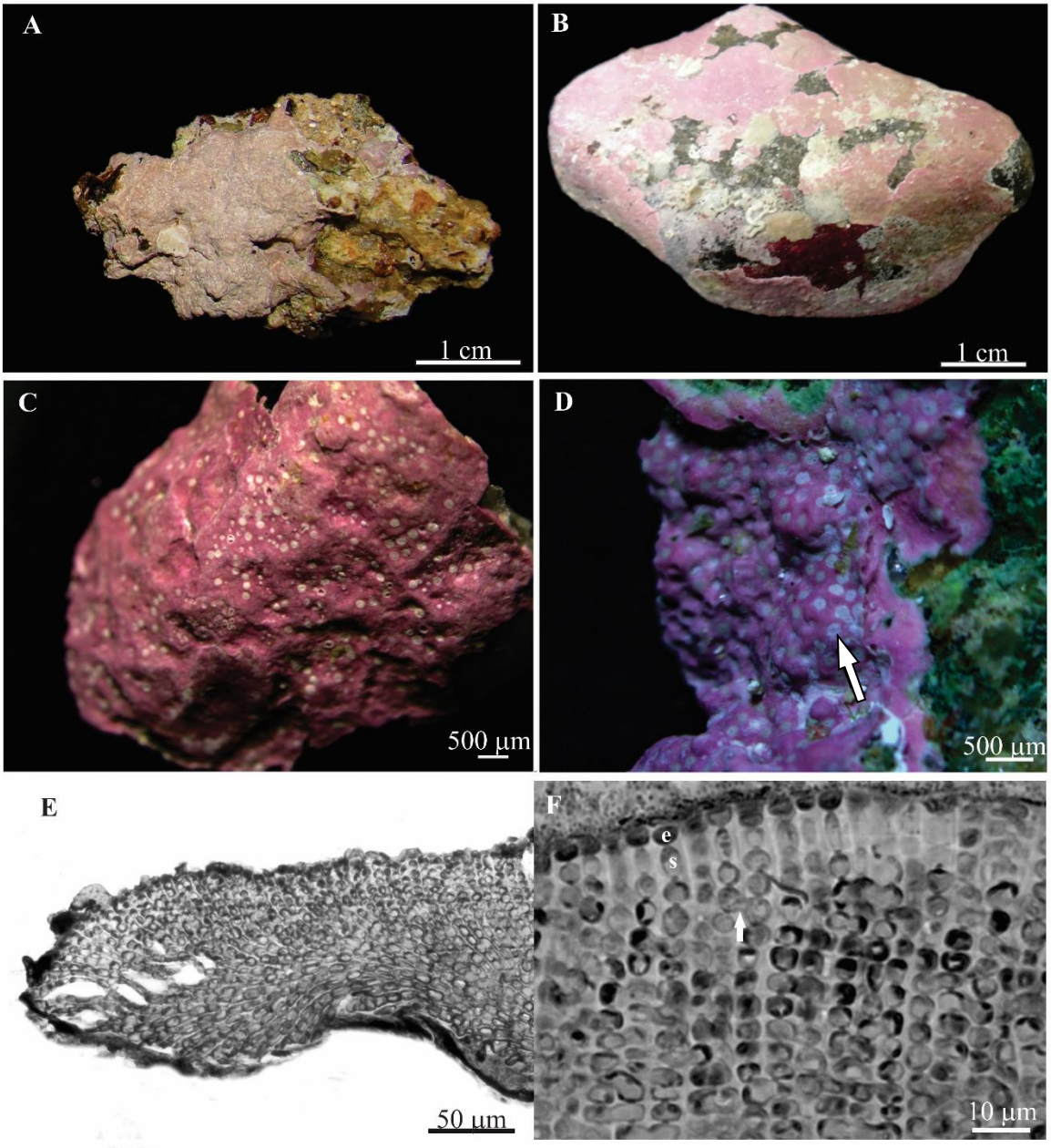


FIGURE 7 A-F: *Harveylithon riosmenum.* A. Encrusting growth-form on rock (ALCB125617). B. Very thin encrusting growth-form on rock (ALCB123166). C and D. Detail of uniporate conceptacle with distinct white roof (arrow) (ALCB125617). E. Longitudinal section through the thallus showing monomerous construction. F. Longitudinal section through the thallus showing epithallial cells (e) more or less elliptical, subepithallial initial cells (s) longer than subtending ones, and cells of adjacent filaments linked by lateral cell fusions (arrow).

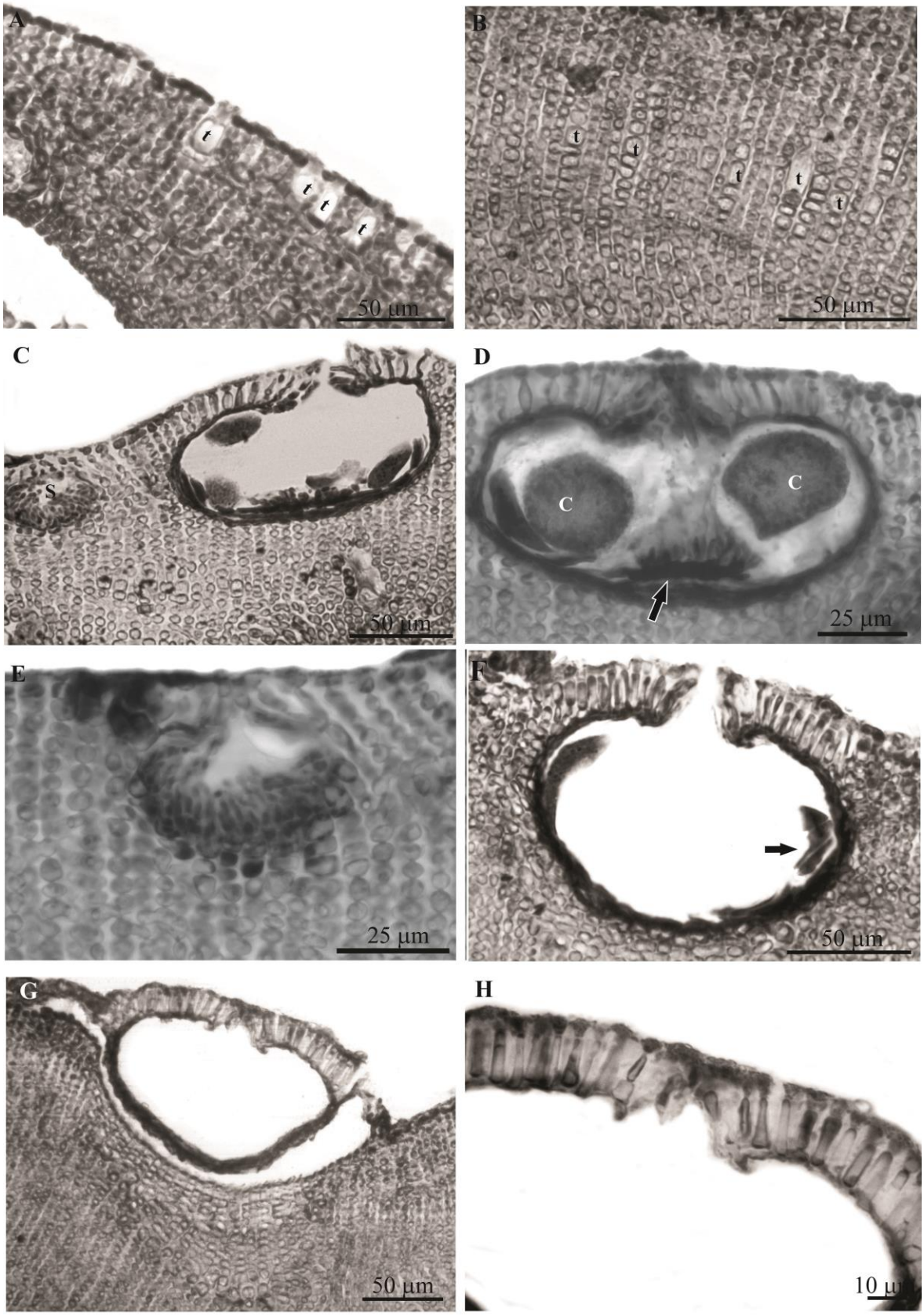


FIGURE 8 A-H: *Harveylithon riosmenum* (ALCB125617). A. Longitudinal section through the thallus showing trichocytes (t) at the thallus surface. B. Longitudinal section

through the thallus showing trichocytes (t) in small vertical rows. C. Longitudinal section through monoecius thallus with spermatangial and carposporangial conceptacles (ALCB125611). D. Carposporangial conceptacle showing central fusion cell (arrow) and peripheral gonimoblast filaments bearing terminal carposporangia (C). E. Male conceptacle (S) showing unbranched spermatangial filaments across the chamber floor only. F. Longitudinal section through tetrasporangial conceptacle showing pore canals lined by cells orientated perpendicularly to the thallus surface, and sporangium zonately divided (arrow) (ALCB125612). G. Old and empty conceptacles are outpours from the thallus. H. Detail of the conceptacle roof filaments comprising an epithallial cell, sustended by a characteristic columnar cell and usually a smaller basal cell.

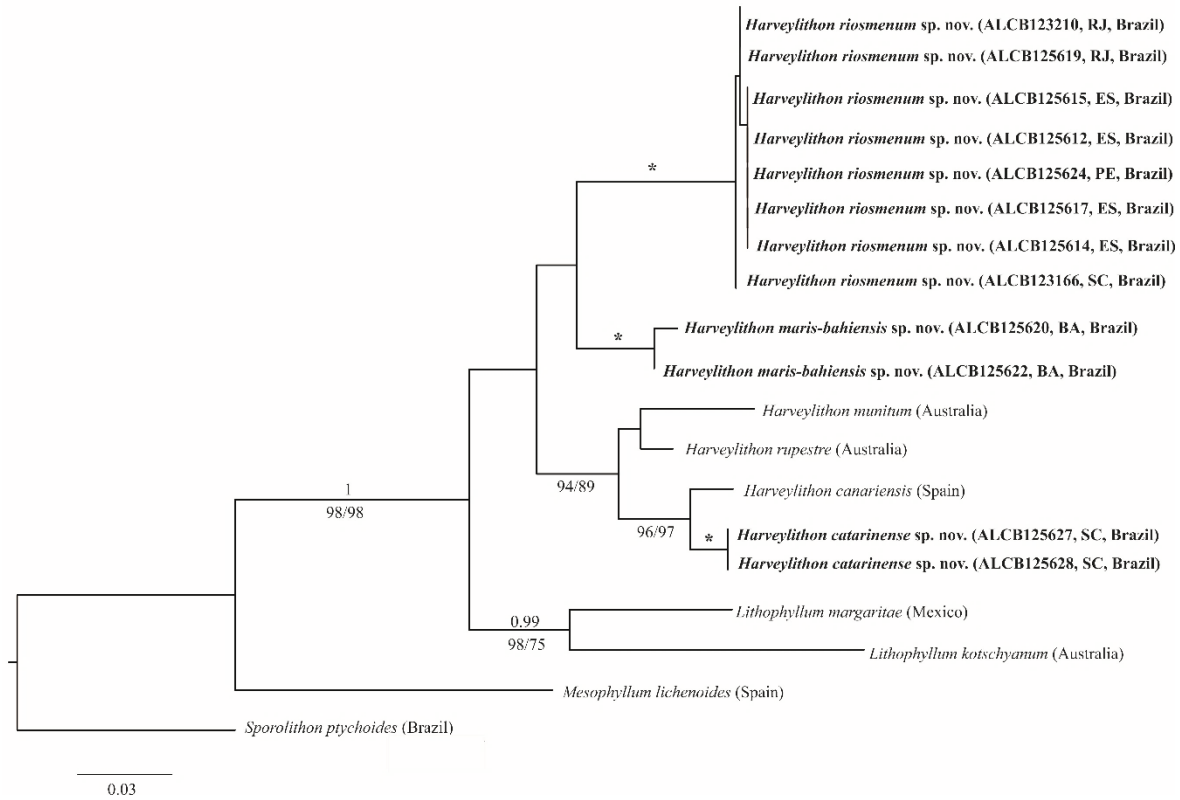


FIGURE S1: The optimal maximum likelihood (ML) topology based on the *psb*A data set. Values above each clade refer to Bayesian posterior probabilities, ML bootstrap values and MP (maximum parsimony), respectively. Only bootstrap values in MP and ML ≥ 70% and Bayesian posterior probability (PP) ≥ 90% were plotted. New sequences produced in this study are in bold. Asterisks mark clades that are supported at 100% in all three analyses.

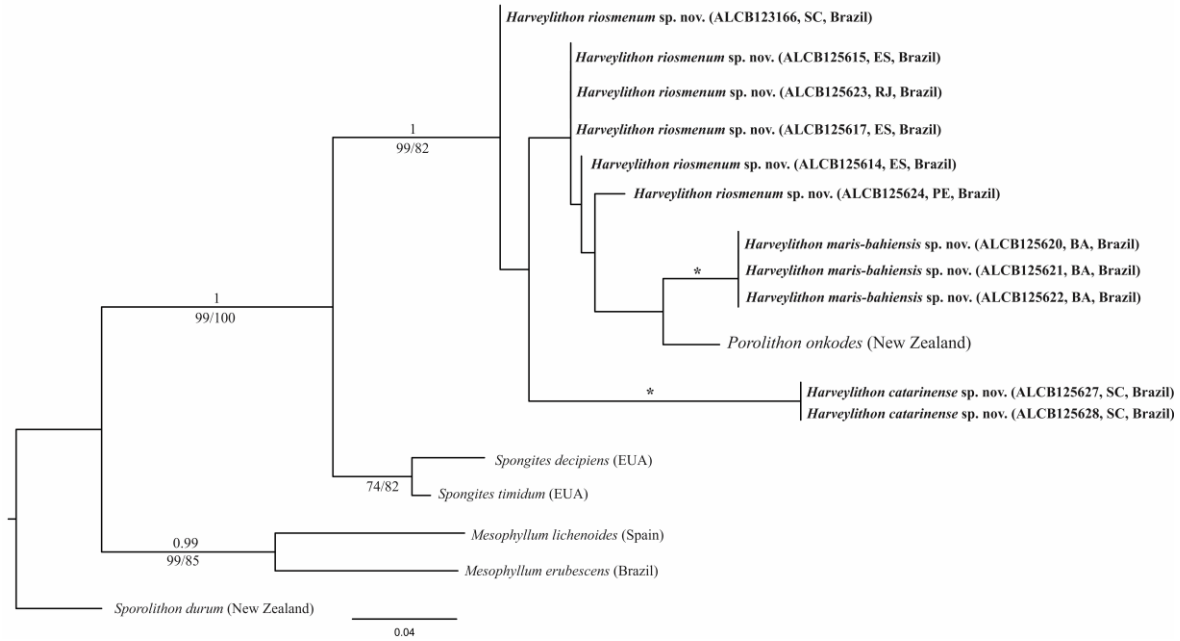


FIGURE S2: The optimal maximum likelihood (ML) topology based on the *rbc*L-3P data set. Values above each clade refer to Bayesian posterior probabilities, ML bootstrap values and MP (maximum parsimony), respectively. Only bootstrap values in MP and ML ≥ 70% and Bayesian posterior probability (PP) ≥ 90% were plotted. New sequences produced in this study are in bold. Asterisks mark clades that are supported at 100% in all three analyses.

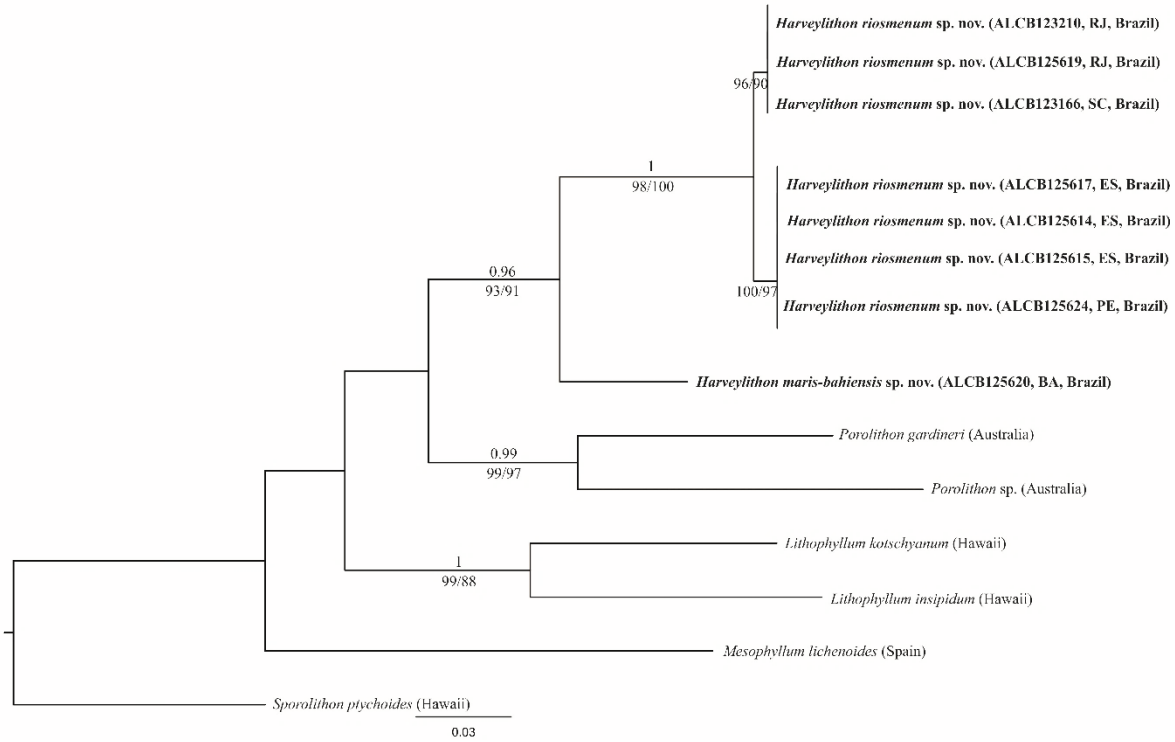


FIGURE S3: The optimal maximum likelihood (ML) topology based on the COI-5P data set. Values above each clade refer to Bayesian posterior probabilities, ML bootstrap values and MP (maximum parsimony), respectively. Only bootstrap values in MP and ML ≥ 70% and Bayesian posterior probability (PP) ≥ 90% were plotted. New sequences produced in this study are in bold. Asterisks mark clades that are supported at 100% in all three analyses.

# CAPÍTULO 2

## *NEOGONIOLITHON* FROM THE SOUTHWESTERN ATLANTIC: MOLECULAR AND MORPHO-ANATOMICAL ANALYSES REVEAL TAXONOMIC NOVELTY*

IARA OLIVEIRA COSTA, VALTER LOUREIRO DE ARAÚJO, PAULO ANTUNES HORTA, JOSÉ MARCOS DE CASTRO NUNES

***Manuscrito submetido ao periódico Journal of Phycology**

# *NEOGONIOLITHON* FROM THE SOUTHWESTERN ATLANTIC: MOLECULAR AND MORPHO-ANATOMICAL ANALYSES REVEAL TAXONOMIC NOVELTY [1]

Iara Oliveira Costa [2]; Programa de Pós-Graduação em Botânica, Universidade Estadual de Feira de Santana, Av. Transnordestina, s/n, Feira de Santana, BA, 44031-460, Brazil

Valter Loureiro de Araújo; Laboratório de Algas Marinhas, Instituto de Biologia, Universidade Federal da Bahia, Rua Barão de Jeremoabo, s/n, Salvador, BA, 40.170-115, Brazil

Paulo Antunes Horta; Departamento de Botânica, Universidade Federal de Santa Catarina, Caixa Postal 476, 88010-970 Florianópolis, SC, Brazil

José Marcos de Castro Nunes; Laboratório de Algas Marinhas, Instituto de Biologia, Universidade Federal da Bahia, Rua Barão de Jeremoabo, s/n, Salvador, BA, 40.170-115, Brazil

[2]Author for correspondence and present address: Laboratório de Algas Marinhas, Instituto de Biologia, Universidade Federal da Bahia, Rua Barão de Jeremoabo, s/n, Salvador, BA, 40.170-115, Brazil. Email: iaraoc@hotmail.com; phone: 55(71) 32836598; Fax: 55(71)32836511.

Condensed title: *NEOGONIOLITHON* IN THE SOUTHWESTERN ATLANTIC

ABSTRACT

This is the first study on *Neogoniolithon* species from the Southwestern Atlantic based on both molecular (COI-5P, *psb*A and SSU rDNA) and morpho-anatomical characters. We identified three species of this genus including one new species: *Neogoniolithon crypticum* sp. nov., which has molecular particularities and the conceptacle diameter as diagnostic character. We also described one new record for this region, *N. rhizophorae*. Ours results provided the first reference of carposporangial conceptacles in *N. rhizophorae*, and registered the occurrence of *N.* cf. *brassica-florida* on the South Atlantic. Our study contributes to the taxonomy of this challenging group and defines as important characters to distinguish between species: branched versus unbranched thalli, disposition of trichocytes, interior dimensions of carposporangial conceptacles, and number of cells comprising the conceptacle roof.

**Key words:** Crustose coralline algae; Corallinales; DNA sequences; Neogoniolithoideae; Taxonomy.

**Abbreviations:** BP, Bootstrap percentage; COI-5P, the standard DNA barcode region of the mitochondrial cytochrome c oxidase 1 gene (*cox*1); dNTP, triphosphate deoxyribonucleotide; MCMC, Markov Chain Monte Carlo; ML, maximum likelihood; PP, Bayesian Posterior probability; *psb*A, gene encoding the D1 protein of photosystem II.

## INTRODUCTION

Foslie (1898) described the genus *Goniolithon* by having monostromatic sterile frond structure and circumscribed *G. papillosum* as its type species. In 1900, Foslie rejected the original description and proposed an entirely new *Goniolithon* distinguished by the tetrasporangia distributed on the floor of the conceptacle and by the presence of heterocysts scattered through the perithallus (Setchell & Mason 1943). Setchell and Mason (1943) concluded that *Goniolithon* Foslie (1900) was the later homonym of *Goniolithon* Foslie (1898), thus replacing *Goniolithon* Foslie (1900) with *Neogoniolithon* and designating *N. fosliei* as the type species; originally based on specimens collected on the Gulf of Suez, Egypt.

Until recently, *Neogoniolithon* was formally assigned to the subfamily Mastophoroideae (Harvey et al. 2003), which includes 41 species (Guiry & Guiry 2017) of nongeniculate coralline algae with non-endophytic thallus; gonimoblast filaments derived from the entire dorsal surface of a fusion cell; spermatangia formed on both floor and roof of male conceptacle chambers; and tetrasporangia present across the floor chamber of the conceptacle (Penrose 1992, Iryu & Matsuda 1994).

In the previous molecular phylogeny for Mastophoroideae, Kato et al. (2011) proposed the segregation of *Neogoniolithon* to the new subfamily Neogoniolithoideae. Mateo-Cid et al. (2014) reviewed the taxonomy of *Neogoniolithon* on the Atlantic coast of Mexico based on both DNA sequences and morpho-anatomical characters. These authors identified nine species of the genus and described a new species *N. siankanensis* Mateo-Cid, Mendonza-Gonzaléz & P.W. Gabrielson, based on a distinctive branched thallus, apical tetrasporangial conceptacles and molecular particularities. Rösler et al. (2016) proposed the amendment of Neogoniolithoideae based on molecular phylogeny with five-markers, and included *Pneophyllum confervicola* (Kützing) Y.M.Chamberlain and *Spongites fruticulosus* Kützing in this subfamily.

All representatives of *Neogoniolithon* cited for the South Atlantic have been sampled in Brazil and were identified based only on morpho-anatomical characters: *N. accretum* (Foslie & Howe) Setch & Mason (Bahia state - Figueiredo & Steneck 2002); *N. brassica-florida* (Harvey) Setch & Mason (Bahia state - Amado-Filho et al. 2012); and the new specie *N. atlanticum* Tâmega, Riosmena-Rodriguez, Mariath & Figueiredo (Bahia state -

Tâmega et al. 2014). *N. accretum* and *N. brassica-florida* were only mentioned in a list of species, without any characterization being present.

Molecular studies including representatives of *Neogoniolithon* are still rare. Additionally, current studies revealed the diversity within this genus to be underestimated, especially due to a wide plasticity of morphological characters (Kato et al. 2011, 2013, Mateo-Cid et al. 2014, Rosler et al. 2016). Scarcity of morpho-anatomical diagnostic features can hide the presence of cryptic species produced by speciation processes related with physiological and oceanographical barriers (Sissini et al. 2014).

This paper is the first register of *Neogoniolithon* species in the Southwestern Atlantic, based on both DNA sequences (COI-5P, *psb*A and SSU rDNA) and morpho-anatomical characters. In this study, we described a new species: *Neogoniolithon crypticum* sp. nov.; we expanded the record of occurrence of *N. rhizophorae*, presenting for the first time carposporophytic specimens of *N. rhizophorae*.

## MATERIALS AND METHODS

### *Sampling, morphological and anatomical observations*

We collected twelve samples at nine localities along the Brazilian coast (Table 1); sampling took place on intertidal regions during syzygy low tides. After cleaned, samples were air dried for at least 24 hours and then stored on silica gel. Samples were examined using a stereomicroscope (Leica® Zoom 2000, Wetzlar, Germany) for the separation of the fragments for molecular and morpho-anatomical analysis. All samples were preserved in 4% formaldehyde.

For optical microscopy analysis, fragments were decalcified with 0.6M HNO3 for 24 hours, followed by dehydration in an ethanol series (30, 50, 70, 90 and 100%), and by infiltration and inclusion in "Historesin embedding Kit" (Leica, Heidelberg, Germany, following the instructions provided by the manufacturer). Sectioning (4-10 μm thickness) was performed with a Thermo Scientific microtome (model HM 325, Walldorf, Germany). Cuts were then stained with toluidine blue acidified with borax (Riosmena-Rodriguez 1993, Moura et al. 1997). Images were obtained using a microscope (Olympus trinocular CX31RTS5®, Tokyo, Japan) coupled with a digital camera (QImaging GO-3). For scanning electron microscopy (SEM), dry specimens were fractured to allow the search for diagnostic features and placed in stubs using carbon tapes, following the procedures

described by Chamberlain (1993). The specimens were deposited in the Herbarium Alexandre Leal Costa (ALCB) of Universidade Federal da Bahia (UFBA/Brazil).

*DNA extraction, PCR and sequencing*

Total DNA was extracted from fragments of dry samples (preserved on silica gel) using the Chelex resin extraction protocol (Goff & Moon 1993) or the CTAB method (Cetyl Trimethyl Ammonium Bromide) modified from Doyle and Doyle (1987). Ten samples were sequenced for COI-5P, *psb*A and SSU rDNA (Table 1).

Molecular markers were amplified by PCR under the following conditions: 1x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.2 µM primer, 5 ng DNA, and 1.25 U Taq DNA polymerase (Invitrogen, São Paulo, SP, Brazil), making a total volume of 25 µL. Specific primer pairs were used for amplification and sequencing reactions of each marker: the mitochondrial markers COI-5P, GazF1 and GazR1 (Saunders 2005); the plastidial markers *psb*A, *psb*AF1 and *psb*AR2 (Yoon et al. 2002); and the nuclear markers SSU rDNA, 18S5' and 1055R, and 1055F and 18S3' (Milstein & Oliveira 2005).

For the mitochondrial COI-5P and the plastidial *psb*A, the PCR was conducted following Jesus et al. (2016) and Sissini et al. (2014), respectively. For the nuclear SSU rDNA, the PCR was performed with an initial denaturation at 94°C for 4 min, 35 cycles at 94°C for 30 sec. (denaturation), 60°C for 1 min (primer annealing), 72° C (extension) for 2 min, and a final extension at 72° C for 7 min. Reactions were performed on the Vertic® 96-Well Thermal Cycler (Applied Biosystems, Foster City, California, USA). The company Genewiz (Cambridge, Massachusetts, USA, http://www.genewiz.com/) carried out both purification and sequencing reactions of PCR products.

*Molecular analyses*

Electropherograms were manually assembled using the Staden package (Staden et al. 2003) and edited in BioEdit 5.0.6 (Hall 1999). For each marker, a multiple alignment was generated with ClustalW tool (Thompson et al. 1994), available in BioEdit 5.0.6 (sequences of the PCR primers were excluded from alignment). In addition to the sequences generated in this study, sequences of other species of Corallinaceae available at GenBank were used and included in the analyses (www.ncbi.nlm.nih.gov/genbank/, searched in April 2017 - Supplementary Table S1).

*Phylogenetic Analyses*

Analyses of Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI) were performed to infer phylogenetic relationships among *Neogoniolithon* species and their relatives. The analyses were based on single-marker COI-5P, *psb*A and SSU rDNA datasets and included *Sporolithon ptychoides* Heydrich as outgroup (*psb*A: KC870927 / COI-5P: HQ422711 / SSU rDNA: DQ629014).

MP trees were constructed on PAUP* 4.0b.10 (Swofford 2002) using an heuristic search algorithm with the following definitions: initial tree(s) obtained by stepwise addition; tree-bisection-reconnection (TBR) as *branch-swapping* algorithm and 10 trees retained per replication. All characters were given equal weights, their states were considered unordered, and the ACCTRAN (*Accelerated Transformation*) character-optimization criterion was used. Branches with a maximum length of zero collapsed to produce polytomies. Strict consensus analysis was calculated and the bootstrap values were accessed using 1.000 replicates with random addition of terminals.

The most suitable evolutionary model was selected using the Akaike Information Criterion (AIC) in the software MrModeltest 2.3 (Nylander 2008), as recommended by Posada and Buckley (2004). ML analysis were conducted using RAxML (*Randomized Axelerated Maximum Likelihood*, version 7.0.4; Stamatakis 2006) with the GTR+I+G model. The best ML tree score and 500 bootstrap trees were obtained using a fast scale algorithm (Stamatakis et al. 2008).

Bayesian analysis was carried out using the software Mr. Bayes 3.1 (Ronquist & Huelsenbeck 2003) using the Marcov-Monte Carlo (MCMC) method with the selected model for the individual data sets, GTR+I+G (COI-5P, *psb*A and SSU rDNA). Five million generations in two independent races with four chains each were carried out, saving one tree every 100 generations. The *burn-in* was identified graphically to determine the period in which the likelihood values had reached a plateau. The trees sampled after stabilization were used to infer the posterior Bayesian probability.

## RESULTS

### *Molecular Analysis*

We obtained 24 partial sequences (nine *psb*A, seven SSU rDNA and eight COI-5P) that were combined with 81 sequences downloaded from GenBank (35 *psb*A, 23 SSU rDNA and 23 COI-5P). Our *psb*A final alignment comprised 44 sequences of 760 base pairs (bp), with 12 (1.6%) variable characters, and 262 (34%) parsimoniously informative

characters. Thirty SSU rDNA sequences were aligned using 1,503 bp, with 133 (9%) variable characters, and 190 (13%) parsimoniously informative characters. Thirty-one COI-5P sequences were aligned using 564 bp, with 16 (3%) variable characters, and 230 (41%) parsimoniously informative characters. Tables 2 to 4 show levels of intra and interspecific genetic variation among samples of *Neogoniolithon* species used in this study for *psb*A, SSU rDNA and COI-5P.

For the *psb*A marker, intraspecific genetic divergence varied from 0 to 10.17% (0-76bp) and the interspecific genetic divergence varied from 0.4 to 18.6% (3-139bp) (Table 2). For the SSU rDNA marker, intraspecific genetic divergence varied from 0 to 0.28% (0-4bp) and interspecific genetic divergence varied from 0.28 to 3.78% (4-54bp) (Table 3). For the COI-5P marker, intraspecific genetic divergence varied from 0 to 11% (0-58bp) and interspecific genetic divergence varied from 7.6 to 21.4% (40-113bp) (Table 4). In all phylogenetic analyses based on *psb*A and SSU data, *Neogoniolithon* was monophyletic with high support in relation to the outgroup (Fig. 1 and 2). All markers analyzed revealed three distinct and well-supported clades among members of *Neogoniolithon,* which correspond to our species circumscriptions: *Neogoniolithon* cf. *brassica-florida*, *N. rhizophorae*, and *N. crypticum* sp. nov. (Figs. 4–6, respectively).

In the *psb*A phylogeny, *Neogoniolithon* cf. *brassica-florida* specimens from Brazil grouped with *N. brassica-florida* species from New Zealand and Spain. This grouping showed strong support (100% for ML, 98% for MP and 1.0 for PP) and a genetic divergence of 0.4-1.07% (3-8 bp) (Table 2). In the COI-5P trees, *N.* cf. *brassica-florida* clade was sister with *N. brassica-florida* from France, with genetic divergence of 7.56% (40 bp) (Table 3). In the SSU rDNA tree, *N.* cf. *brassica-florida* did not form a resolved clade (Fig. 3).

Sequences of *Neogoniolithon rhizophorae* specimens collected from Bahia and Pernambuco states (northeast Brazil) revealed a minor difference of only 01 bp in *psb*A and COI-5P data; and had a difference of 04 bp (0.28%) in SSU. The Brazilian *N. rhizophorae* clade was resolved with strong support for all analyses with *psb*A and COI-5P markers (100% for ML and MP; and 1.0 for PP) and moderate support for SSU rDNA (74% for ML, 82% for MP and 1.0 for PP). These sequences clustered with *N. rhizophorae* sequences from Mexico for *psb*A data (0-1 bp, 0-0.13%), which was supported by high bootstrap values in all analyses (100% for ML and MP; and 1.0 for PP – Fig. 1).

*Neogoniolithon crypticum* specimens were previously identified as *N. accretum* due to similar morphological characters. However, it presented a large interspecific genetic

divergence varying from 15 to 18% (113-134bp) for *psb*A (Table 2); from 2.4 to 4% (35-54bp) for SSU rDNA (Table 3); and from 14.2 to 19% (75-100bp) for COI-5P (Table 4). *N. crypticum* sp. nov. presented strong support for *psb*A and SSU rDNA (100% for ML and MP; and 1.0 for PP – Fig. 1 and 2), and weak to moderate support on COI-5P analyses (55% for MP and 75% for ML – Fig. 3).

*Morphological and Anatomical Analysis*

CORALLINALES Silva and Johansen (1986: 250)

Corallinaceae Lamouroux (1812: 185)

Neogoniolithoideae A. Kato & M. Baba emend. A.Rösler, Perfectti, V.Peña & J.C.Braga (2016)

*Neogoniolithon* cf. *brassica-florida* (Harvey) Setchell & L.R. Mason

BASIONYM: *Melobesia brassica-florida* Harvey

TYPE LOCALITY: Algoa Bay, South Africa

DISTRIBUTION: Western Australia (Barry & Woelkerling 1995), Queesland (Ringeltaube & Harvey 2000), South-eastern Australia (Harvey et al. 2006), southward and eastward across southern Australia, Indian Ocean, Red Sea and Pacific Ocean (Penrose 1996).

Figures: 4A-F.

DESCRIPTION: Thallus encrusting to warty to fruticose (Fig. 4A), with branch apices often terminate in conical to dome-shaped conceptacles (Fig. 4B). The crustose portion is monomerous (Fig. 4C). Epithallial cells occurring in one layer, rounded to flattened, 4.2–11.3 µm long and 7.5–16.5 µm in diameter. Subepithallial initials (16–26 µm long and 11–18.4 µm in diameter) as long or longer than the cells immediately subtending them (Fig. 4D). Cells of the perithallial filaments, 5.2–22 µm long and 7–20 µm in diameter. Cells of the hypothallial filaments, 25–48 µm long and 9–15 µm in diameter. Trichocytes rectangular to bottle-shaped to elongate, single and in vertical rows at the surface or buried in the thallus, and measure 27–55 µm long and 19–26.1 µm in diameter (Fig. 4D). Cells of the adjacent filaments joined by cell fusions (Fig. 4D). Secondary pit-connections not observed. Gametangial thalli dioecious. Carposporangial conceptacles, apiculate (Fig. 4E), measure 140–632 µm long and 390– 708 µm in diameter. Carposporangia, measure 26–64.1 µm long and 34–75 µm in diameter, supported for short gonimoblast filaments that

arise across the dorsal surface of the discontinuous fusion cell (Fig. 4F). Conceptacle roofs 6-13 cells thick (including ephithallial cell) (Fig. 4F). The pore canal measures 40-70 µm in diameter, were lined with cells oriented more or less parallel to the roof surface and project into the canal (Fig. 4F). Tetrasporangial and spermatangial conceptacles not found.
MATERIAL EXAMINED: BRAZIL. ESPÍRITO SANTO: Guarapari, Castanheiras, 20°40'16.64"S, 40°29'46.01"W, coll. I.O. Costa et al. s.n., 20 Aug 2013, (ALCB 125631 and 125632♀); Anchieta, Castelhanos, 20°50'05.08"S, 40°37'24.84"W, coll. I.O. Costa et al. s.n., 21 Aug 2013, (ALCB 125633♀).

*Neogoniolithon rhizophorae* (Foslie & M. Howe) Setchell & L.R. Mason
BASIONYM: *Goniolithon rhizophorae* Foslie & M. Howe
TYPE LOCALITY: Bahamas, Stoking Island, Great Exuma
DISTRIBUTION: Colombia (Diaz-Pulido & Diaz-Ruiz 2003) and Mexico (Mateo-Cid et al. 2014).
Figures: 5A-G.

DESCRIPTION: Thallus encrusting sometimes with protuberances terete to 10 mm, once or twice branched (Fig. 5A and B). The crustose portion is monomerous. Epithallial cells occurring in one or two layers, rounded to flattened, 5–11 µm long and 8–19 µm in diameter. Subepithallial initials (14–20 µm long and 8–17 µm in diameter) as long or longer than the cells immediately subtending them (Fig. 5D). Cells of the perithallial filaments, 8–27 µm long and 6–17.7 µm in diameter. Cells of the hypothallial filaments, 20–27.5 µm long and 15–18 µm in diameter. Trichocytes rectangular to bottle-shaped to elongate, single and in vertical rows at the surface or buried in the thallus, and measure 23–58.1 µm long and 11.5–33 µm in diameter (Fig. 5E). Cells of the adjacent filaments joined by cell fusions (Fig. 5D). Secondary pit-connections not observed. Gametangial thalli dioecious. Carposporangial conceptacles, measure 313–537.1 µm long and 448–527.3 µm in diameter (Fig. 5F and G). Carposporangia, measure 62–152 µm long and 17.6–49 µm in diameter, supported for gonimoblast filaments that arise across the dorsal surface of the discontinuous fusion cell (Fig. 5G). Conceptacle roofs 12-20 cells thick (including ephithallial cell) (Fig. 5G). The pore canal measures 50-81 µm in diameter, were lined with cells oriented more or less parallel to the roof surface and project into the canal (Fig. 5G). Tetrasporangial and spermatangial conceptacles not found.

MATERIAL EXAMINED: BRAZIL. PERNAMBUCO: Cabo de Santo Agostinho, Gaibú, 08°18'49.5"S, 34°56'48.4"W, coll. I.O. Costa et al. s.n., 27 April 2014, (ALCB 125638 and 125639♀); BAHIA: Cairú, Morro de São Paulo, 13°23'16.8"S, 38°54'18.6"W, coll. I.O. Costa et al. s.n., 06 Oct 2013, (ALCB 125635, 125636 and 125637♀); Garapuá, 13°29'13.14"S, 38°54'25.5"W, coll. I.O. Costa et al. s.n., 03 July 2015, (ALCB 125640♀); Maraú, Ponta do Mutá, 13°52'45.61"S, 38°56'50.79"W, coll. I.O. Costa et al. s.n., 04 Oct 2013, (ALCB 125634♀).

*Neogoniolithon crypticum* I.O. Costa, P.A. Horta & J.M.C. Nunes sp. nov.

HOLOTYPE: Brazil, Rio de Janeiro State, Cabo Frio, Praia das Conchas, 22°52'16.8"S, 41°53'51.2"W; coll. I.O. Costa et al. s.n., 26 May 2013; ALCB 125630; GenBank accessions: *psb*A – XXXXXXXX / SSU rDNA – XXXXXXXX / COI-5P – XXXXXXXX.

ETYMOLOGY: The epithet refers to the cryptic nature of the new species with *N. accretum*.

DISTRIBUTION: Brazil: State of Rio de Janeiro.

DIAGNOSIS: Thallus nongeniculate encrusting; monomerous construction; trichocytes common, single at the surface or buried in the thallus. Carposporangial conceptacles conical to dome-shape, measure 110–244 µm long and 283–380 µm in diameter; carposporangia, measure 42–59 µm long and 13–15 µm in diameter, supported for three gonimoblast filaments that arise across the dorsal surface of the continuous fusion cell; conceptacle roof 7-12 cells thick.

Figures: 6A-F.

DESCRIPTION: Thallus encrusting (Fig. 6A and 6B), with monomerous construction (Fig. 6C). Epithallial cells occurring in one layer, rounded to flattened, 5.5–7.6 µm long and 8.2–13.9 µm in diameter. Subepithallial initials (9.5–24.5 µm long and 6.4–12.6 µm in diameter) as long or longer than the cells immediately subtending them (Fig. 6D). Cells of the perithallial filaments, 12–16.4 µm long and 8–16 µm in diameter. Cells of the hypothallial filaments, 18–32.4 µm long and 8.5–14.8 µm in diameter. Trichocytes rectangular to bottle-shaped to elongate, single at the surface or buried in the thallus, and measure 14.6–47 µm long and 14.2–25.6 µm in diameter (Fig. 6E). Cells of the adjacent filaments joined by cell fusions (Fig. 6E). Secondary pit-connections not observed. Gametangial thalli dioecious. Carposporangial conceptacles, conical to dome-shape (Fig.

6B), measure 110–244 µm long and 283–380 µm in diameter. Carposporangia, measure 42–59 µm long and 13–15 µm in diameter, supported for three gonimoblast filaments that arise across the dorsal surface of the continuous fusion cell (Fig. 6F). Conceptacle roofs 7-12 cells thick (including ephithallial cell) (Fig. 6F). The pore canal measures 40-50 µm in diameter, were lined with cells oriented more or less parallel to the roof surface and project into the canal. Tetrasporangial and spermatangial conceptacles not found.

MATERIAL EXAMINED: BRAZIL. RIO DE JANEIRO: Cabo Frio, Praia das Conchas, 22°52'16.8"S, 41°53'51.2"W; coll. I.O. Costa et al. s.n., 26 May 2013, (ALCB 125629 and 125630♀).

## DISCUSSION

Our molecular results based on *psb*A, SSU rDNA and COI-5P (Fig. 1 to 3) were combined with morpho-anatomical analyses (Fig. 4 to 6), which pointed out the occurrence of three distinct species of *Neogoniolithon* in the South Atlantic: *N.* cf. *brassica-florida*, *N. rhizophorae* and *N. crypticum*.

Harvey (1849) described specimens collected on Algoa Bay (South Africa) as *Neogoniolithon brassica-florida* (originally *Melobesia brassica-florida*) based on the following characters: thallus with growth-form warty; numerous trichocytes singly and in vertical rows; spermatangial conceptacles chambers with 355-519 µm in diameter, with spermatangia borne on both floor and walls of male conceptacles. Several authors considered this species as presenting various gross morphologies, many synonyms, and with cosmopolitan distribution (Woelkerling et al. 1993, Verheij 1994, Penrose 1996, Ringeltaube & Harvey 2000, Harvey et al. 2006, Kato et al. 2011, Amado-Filho et al. 2012; Villas-Boas et al. 2015). Kato et al. (2013), based in SSU and COI-5P sequences, showed that *N. brassica-florida* is polyphyletic. These authors showed that *N. fosliei* and *N. frutescens*, both synonyms of *N. brassica-florida*, were not resolved as a single clade. Hence, they concluded that crustose species with protuberances or branches belong to *N. brassica-florida* or *N. frutescens*, while species lacking any protuberances belong to *N. fosliei* . In addition, these authors have shown that under the epithet of *N. fosliei* there are three genetically different clades (which were named as clades A, B and C).

Some Brazilian specimens presented morpho-anatomical characters compatible with descriptions of *N. brassica-florida* (Table 5). The *psb*A sequences generated from our study formed clades with sequences from New Zealand and Spain (0.4-1.07%), and formed

a sister clade with sequences from France based on the COI-5P data (Fig. 1 and 2, respectively). In our study, we identified *N. brassica-florida* as *Neogoniolithon* cf. *brassica-florida* because it is considered polyphyletic (Kato et al. 2013) and because of the absence of sequences of type material to corroborate the molecular identification. Sequencing the type or topotype material of *N. brassica-florida* and of its probable synonyms is necessary for correctly applying the epithet.

*Neogoniolithon rhizophorae* was originally described based on specimens from the Bahamas (Foslie & Howe 1906, as *Goniolithon rhizophorae*), with records only for Colombia (Diaz-Pulido & Diaz-Ruiz 2003), Gulf of Mexico and the Caribbean Sea (Mateo-Cid et al. 2014). Unfortunately, sequences of the type material of this species are not available for comparisons. Confirmation of the identification on the analyzed specimens was based on congruency with the *N. rhizophorae* clade from Mexico (Mateo-Cid et al. 2014) and shared morphological diagnostic characters with the former species (Foslie & Howe 1906). Such confirmation allowed us to recognize the taxa from Brazil as belonging to *N. rhizophorae*. Morpho-anatomical features that allowed the identification of the South Atlantic specimens were: Thallus adherent, with terete protuberances to 10 mm; trichocytes present, occurring in vertical rows; carposporangial conceptacle with carposporangia supported by gonimoblast filaments that arise across the dorsal surface of the discontinuous fusion cell; and carposporangial conceptacle roof with a thickness of 7-12 cells (Table 5).

Foslie & Howe (1906) described *Neogoniolithon rhizophorae* (as *Goniolithon rhizophorae*) using only vegetative anatomical characters (measurements of the branches, perithallus and hypothallus) and superficial views of the conceptacles (without distinguishing it as asexual or sexual). Mateo-Cid et al. (2014) characterized *N. rhizophorae* using vegetative characters and measurement of the tetrasporangial conceptacle (Table 5). Regarding the SSU marker, *N. rhizophorae* was sister of *N. fosliei* from French Polynesia, *N. frutescens* from Japan, and *N. brassica-florida* from Hawaii; the sequences, however, diverged from 0.28 to 0.56% (4-8bp) – see Table 4. The present study extends the description of *N. rhizophorae* by presenting characters of the carposporangial conceptacles, which will allow a better characterization of this species. In addition, we presented the first record of *N. rhizophorae* for the South Atlantic.

Our phylogenetic analyses and both intra and interspecific divergence clearly show that *Neogoniolithon crypticum* constitutes a new taxonomic entity. *N. crypticum psb*A sequences diverged in 17.14% (128bp) from *N. accretum* from Mexico, despite their

morphological similarities. *N. crypticum* is morphologically similar to *N. accretum* Foslie & Howe (1906 - as *Goniolithon accretum* from Florida, USA), and to *N. accretum* from Mexico (Mateo-Cid et al. 2014) (Table 5). This divergence is considered high enough for segregating species in this group (Kato et al. 2011, Sissini et al. 2014, Adey et al. 2015). Recent molecular studies have shown that some groups of algae can be divided into many species, often with severely reduced geographic ranges. Even though these groups include species described as unique and with pantropical or even worldwide distributions. These algae have been determined as genetically distinct by one or more genes, even though they had no obvious morphological or anatomical differences; and have been therefore labeled "cryptic species" (Sissini et al. 2014, Araújo de Azevedo et al. 2016, Hind et al. 2016, Schneider et al. 2017).

COI-5P and *psb*A have been largely used to infer relationships on generic and specific levels in Corallinales. Both regions are also useful for identifying recently diverged taxa that could not be identified with more conserved markers (Broom et al. 2008, Bittner et al. 2011, Peña et al. 2011, Hind and Saunders 2013, Carro et al. 2014, Richards et al. 2014, Torrano-Silva et al. 2014, Vieira-Pinto et al. 2014, Hernández-Kantún et al. 2015, 2016). *Neogoniolithon* was highly supported as monophyletic only on BI analysis based on COI-5P (Fig. 3). This result is in accordance with the evidence that COI-5P does not generate well-resolved phylogenies by itself, and that it could increase resolution and support of phylogenies if analyzed simultaneously with others markers (Jesus et al. (2016). SSU rDNA has been widely used in phylogenetic analyses and, as it is more conserved, presented better resolution at orders, families and subfamilies levels (Bailey & Chapman 1996, Bailey 1999, Harvey et al. 2003, Broom et al. 2008, Vidal et al. 2008, LeGall et al. 2010, Nelson et al. 2015). In this study, we obtained high support for the deeper branches based on the SSU marker, hence we would like to recommend the use of this marker in more robust phylogenies.

Among our samples we only observed *Neogoniolithon* carposporophytic specimens, which is considered rare for some coralline algae groups (Bahia et al. 2015). This genus has been characterized as having the following reproductive features: uniporate tetrasporangial conceptacles formed by filaments to the fertile area; spermatangia borne on the floor and walls in male conceptacles; and gonimoblast filaments that arise from the dorsal surfaces of the fusion cell (Penrose 1992). The present study expanded the characterization of *Neogoniolithon* species by describing the presence of carposporophytic specimens for *N.* cf. *brassica-florida*, *N. crypticum* and *N. rhizophorae.*

Morpho-anatomical studies carried out whit *Neogoniolithon* reported carposporangial conceptacles only for *N. accretum* (Mateo-Cid et al. 2014), *N. atlanticum* (Tâmega et al. 2014), *N. brassica-florida* (Penrose 1996), *N. fosliei* (Penrose 1992), and *N. trichotomum* (Heydrich) Setchell & L.R. Mason (Kato et al. 2013). The Brazilian *N. atlanticum* was, until this study, the only representative of the genus in the South Atlantic with the description of carposporangial conceptacles. This species was recently described based only on morpho-anatomical characters and presented great similarity with *N. brassica-florida* in the shape and size of tetrasporangial conceptacles (Tâmega et al. 2014). Since *N. brassica* is known to be polyphyletic (Kato et al. 2013), sequencing of the type material of both species is needed to determine if *N. atlanticum* is a distinct and valid species.

We are in agreement with the recommendation of Sissini et al. (2014) about the use of morpho-anatomical analysis linked to the molecular evaluation of the type material, as well as the broader biogeographic taxonomic characterization of the representatives, to strengthen the knowledge about the phylogeny of this group.

## CONCLUSION

Our study indicated the occurrence of three distinct species of *Neogoniolithon* on the Brazilian coast by combining molecular and morpho-anatomical data. We described a new species: *Neogoniolithon crypticum*; the first record of carposporangial conceptacle of *N. rhizophorae*, its first occurrence in the South Atlantic. We identified the following characters as useful for segregating *Neogoniolithon* species: branched versus unbranched thalli, disposition of trichocytes, interior dimensions of carposporangial conceptacles, and number of cells forming the conceptacle roof. The current study constitutes an important advance in the knowledge of the specific diversity of this genus in a tropical and still underestimated region, as the South Atlantic. Our results emphasize the need of investments in sequencing type or topotype materials in Corallinales in order to confirm identifications and guarantee the correct use of species names. We recommend an integrated approach of molecular and morpho-anatomical analysis for the correct identification of already existing taxa and for those yet to be discovered.

## ACKNOWLEDGEMENTS

This research has been funded by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, BrBOL 564945/2010-2 and UNIVERSAL 477614/2013-2) and the Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB, PRONEM T.O.

PNE. 0020/2011 and REDES T.O. RED006/2012). We thank the Central Laboratory of Electronic Microscopy (LCME) of the Federal University of Santa Catarina (UFSC) for the availability of SEM equipment. IOC acknowledge scholarships from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). JMCN acknowledge CNPq (307368/2015-7). PAH acknowledge CNPq (PROTAX 440575/2015-0), Fundação de Apoio à Pesquisa Científica e Tecnológica do Estado de Santa Catarina (FAPESC, 2015TR1702), CAPES, Rede Clima, FAPESP, Fundação BOTICÁRIO.

REFERENCES

Amado-Filho, G.M., Moura, R.L., Bastos, A.C., Salgado, L.T., Sumida, P.Y., et al. 2012a. Rhodolith Beds Are Major CaCO3 Bio-Factories in the Tropical South West Atlantic. *PLoS ONE* 7:e35171 [online].

Bahia, R.G, Maneveldt, G.W., Amado-Filho, G.M. & Yoneshigue-Valentin, Y. 2015. New diagnostic characters for the order Sporolithales (Corallinophycidae, Rhodophyta). . *J. Phycol*. 51(6): 1137-1146.

Bailey, J.C. 1999. Phylogenetic positions of *Lithophyllum incrustans* and *Titanoderma pustulatum* (Corallinaceae, Rhodophyta) based on 18S rRNA gene sequence analyses, with a revised classification of the Lithophylloideae. *Phycologia* 38(3): 208–216.

Bailey, J.C. & Chapman, R.L. 1998. A phylogenetic study of the Corallinales (Rhodophyta) based on nuclear small-subunit rRNA gene sequences. *J. Phycol*. 34: 692–705.

Bailey, J.C. & Chapman, R.L. 1996. Evolutionary relationships among coralline red algae (Corallinaceae, Rhodophyta) determined by 18S rRNA gene sequence analysis. In: Chaudhary, B.R. & Agrawal, S.B. (Eds.) Cytology, Genetics and Molecular Biology of Algae. SPB Academic Publishing, Amsterdam. 363–376.

Bailey, J.C., Gabel, J.E. & Freshwater, D.W. 2004. Nuclear 18S rRNA gene sequence analyses indicate that the Mastophoroideae (Corallinaceae, Rhodophyta) is a polyphyletic taxon. *Phycologia* 43: 3–12.

Barry G.C. & Woelkerling W.J. 1995. Non-geniculate species of Corallinaceae (Corallinales, Rhodophyta) in Shark Bay, Western Australia: Biodiversity, salinity tolerances and biogeographic affinities. *Botanica Marina* 38: 135–149.

Bittner, L., Payri, C.E., Maneveldt, G.W., Couloux, A., Cruaud, C., de Reviers, B. and Le Gall, L. 2011. Evolutionary history of the Corallinales (Corallinophycidae, Rhodophyta) inferred from nuclear, plastidial and mitochondrial genomes. *Mol. Phylogenet. Evol.* 61: 697–713.

Broom, J. E. S., Hart, D. R., Farr, T. J., Nelson, W. A., Neil, K. F., Harvey, A. S. & Woelkerling, W. J. 2008. Utility of psbA and SSU rDNA for phylogenetic reconstruction in the Corallinales based on New Zealand taxa. *Mol. Phylogenet. Evol.* 46: 958- 973.

Chamberlain, Y. M. 1993. Observations on the crustose coralline red alga *Spongites yendoi* (Foslie) comb. nov. in South Africa and its relationship to *S. decipiens* (Foslie) comb. nov. and *Lithophyllum natalense* Foslie. *Phycologia* 32: 100–15.

Carro, B., Lopez, L., Peña, V., Bárbara, I., & Barreiro, R. 2014. DNA barcoding allows the accurate assessment of European maerl diversity: a Proof-of-Concept study. *Phytotaxa* 190(1): 176-189.

Díaz-Pulido, G. & Díaz-Ruíz, M. 2003. Diversity of benthic marine algae of the Colombian Atlantic. *Biota Colombiana* 4: 203–246.

Doyle, J. J. & Doyle, J. L. 1987. A rapid DNA isolation method for small quantities of fresh tissues. *Phytochem. Bull.* 19: 11–15.

Figueiredo, M.A.O.; Steneck, R.S. 2002. Floristic and ecological studies of crustose coralline algae on Brazil`s Abrolhos reefs. *9th International Coral Reef Symposium, Bali* 1:493-497.

Foslie, M. & Howe, M.A. 1906. New American coralline algae. *Bulletin of the New York Botanical Garden* 4: 128–136, Plates 80–93.

Foslie, M. 1909. *Algologiske notiser VI. Kongelige Norske Videnskabers Selskabs Skrifter* 2: 1–63.

Guiry, M. D. & Guiry, G. M. 2017. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Available at: http://www.algaebase.org (last accessed 30 April 2017).

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser*. 41: 95–98.

Harvey, A. S., Broadwater, S. T., Woelkerling, W. J. & Mitrovski, P. J. 2003. *Choreonema* (Corallinales, Rhodophyta): 18S rDNA phylogeny and resurrection of the Hapalidiaceae for the subfamilies Choreonematoideae, Australithoideae, and Melobesioideae. *J. Phycol.* 39: 988–98.

Harvey, A. S., Phillips, L. E., Woelkerling, W. J. & Millar, A. J. K. 2006. The Corallinaceae, subfamily Mastophoroideae (Corallinales, Rhodophyta) in south-eastern Australia. *Aust. Syst. Bot.* 19: 387–429.

Hernandez-Kantun, J. J., Rindi, F., Adey, W. H., Heesch, S., Peña, V., Le Gall, L., And Gabrielson, P. W. 2015. Sequencing type material resolves the identity and distribution of the generitype *Lithophyllum incrustans*, and related European species *L. hibernicum* and *L. bathyporum* (Corallinales, Rhodophyta). *J. Phycol*. 51(4), 791-807.

Hernández-Kantún, J.J., Gabrielson, P., Hughey, J.R., Pezzolesi, L., Rindi, F., Robinson, N.M., Peña, V., Riosmena-Rodriguez, R., Le Gall, L., and Adey, W. 2016. Reassessment of branched *Lithophyllum* spp. (Corallinales, Rhodophyta) in the Caribbean Sea with global implications. *Phycologia* 55 (6): 619-639.

Hind, K. R., & Saunders, G. W. 2013. A Molecular Phylogenetic Study of the Tribe Corallineae (Corallinales, Rhodophyta) with an Assessment of Genus-Level Taxonomic Features and Descriptions of Novel Genera. *J. Phycol*. 49(1): 103-114.

Iryu, Y. & Matsuda, S. 1994. Taxonomic studies of the *Neogoniolithon fosliei* complex (Corallinaceae, Rhodophyta) in the Ryukyu Islands. *Trans. Proc. Palaeont. Soc. Jpn. N. S.* 174: 426–48.

Kato, A., Baba, M. & Suda, S. 2011. Revision of the Mastophoroideae (Corallinales, Rhodophyta) and polyphyly in nongeniculate species widely distributed on Pacific coral reefs. *J. Phycol*. 3: 662-672.

Kato, A., Baba, M. & Suda, S. 2013. Taxonomic circumscription of heterogeneous species *Neogoniolithon brassica-florida* (Corallinales, Rhodophyta) in Japan. *Phycol. Res.*, 61: 15–26.

Le Gall, L., Payri, C. E., Bittner, L. & Saunders, G. W. 2010. Multigene phylogenetic analyses support recognition of the Sporolithales ord. nov. *Mol Phylogenet Evol*. 54(1):302-5.

Mateo-Cid, L.E., Mendoza-González, A.C. & Gabrielson, P.W. 2014. *Neogoniolithon* (Corallinales, Rhodophyta) on the Atlantic coast of México, including *N. siankanensis* Phytotaxa 190(1): 64-93.

Milstein, D. & Oliveira, M.C. 2005. Molecular phylogeny of Bangiales (Rhodophyta) based on small subunit rDNA sequencing: emphasis on Brazilian *Porphyra* species. *Phycologia* 44:212-221.

Moura, C.W.N., Kraus, J.E & Cordeiro-Marino, M. 1997. Metodologia para obtenção de cortes histológicos com historresina e coloração com azul de toluidina O para algas coralináceas (Rhodophyta, Corallinales). *Hoehnea* 24 (2): 17-27.

Nelson, W. A., Sutherland, J. E., Farr, T. J., Hart, D. R., Neill, K. F., Kim, H. J., & Yoon, H. S. 2015. Multi-gene phylogenetic analyses of New Zealand coralline algae: *Corallinapetra novaezelandiae* gen. et and recognition of the Hapalidiales ord. nov. *J. Phycol.* 51: 454–468.

Nylander, J. A. A., 2008. *MrModeltest 2.3*. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Penrose, D. 1991. *Spongites fruticulosus* (Corallinaceae, Rhodophyta), the type species of *Spongites*, in southern Australia. *Phycologia* 30:438–88.

Penrose, D. 1992. *Neogoniolithon fosliei* (Corallinaceae, Rhodophyta), the type species of Neogoniolithon, in southern Australia. *Phycologia* 31:338–50.

Penrose, D. 1996. Subfamily Mastophoroideae. In: Womersley HBSW (ed) The marine benthic flora of Southern Australia. Part IIIB, Gracilariales, Rhodymeniales, Corallinales and Bonnemaisoniales. Australian Biological Resources Study, Canberra. 237–283.

Posada, D. & Buckley, T. R. 2004. Model selection and model averaging in phylogenetics: analysis of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Syst. Biol.* 53: 793–808.

Ringeltaube, P. & Harvey, A. 2000. Non-geniculate coralline algae (Corallinales Rhodophyta) on Heron Reef Great Barrier Reef (Australia). *Botanica Marina* 43: 431–454.

Riosmena-Rodriguez, R. 1993. Una propuesta de tecnica histological para el studio de algas coralinas (Corallinales: Rhodophyta). *Rev. Inv. Cient.* 4: 65-73.

Ronquist, F. & Huelsenbeck, J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 572–1574.

Rösler, A., Perfectti, F., Peña, V. & Braga, J. C. 2016. Phylogenetic relationships of corallinaceae (Corallinales, Rhodophyta): taxonomic implications for reef-building corallines. *J. Phycol*. 52: 412–31.

Saunders, G. W. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Phil. Trans. R. Soc. B* 360: 1879–1888.

Setchell, W. A. & Mason, L. R. 1943. *Goniolithon* and *Neogoniolithon*: two genera of crustaceous coralline algae. *Proc. Natl. Acad. Sci. U.S.A.* 29: 87–92.

Silva, P.C. & Johansen, H.W. 1986. A reappraisal of the order Corallinales (Rhodophyceae). *European J. Phycol.* 21(3): 245–254.

Sissini, M. N., Oliveira, M. C., Gabrielson, P. W., Robinson, N. M., Okolodkov, Y. B., Riosmena-Rodríguez, R., & Horta, P. A. 2014. *Mesophyllum erubescens* (Corallinales, Rhodophyta)—so many species in one epithet. *Phytotaxa*, 190(1), 299-319.

Staden, R., Judge, D. P. & Bonfield, J. K. 2003. Analysing sequences using the Staden Package and EMBOSS. *In* Krawetz, S. A. & Womble, D. D. [Eds.] *Introduction to Bioinformatics. A Theoretical and Practical Approach.* Human Press Inc, pp. 393-410.

Stamatakis, A., Hoover, P. & Rougemont, J. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Syst. Biol.* 57: 758–771.

Swofford, D. L. 2002. *PAUP*: Phylogenetic Analysis Using Parsimony (*and Many Other Methods). Version 4.0b10.* Sinauer Associates, Sunderland, Massachusetts.

Tâmega, F.T.S.; Riosmena-Rodrigues, R.; Mariath, R.; Figueiredo, M.A.O. 2014. Nongeniculate coralline red algae (Rhodophyta: Corallinales) in coral reefs from Northeastern Brazil and a description of *Neogoniolithon atlanticum Phytotaxa,* 190(1): 277-298.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. 1994. Clustal, W: Improving the sensitivity of progressive weighting position-specific gap penalties and weight matrix choice. *Nucl. Acids Res.* 22: 4673–4680.

Torrano-Silva, B. N., Riosmena-Rodríguez, R., & De Oliveira, M. C. 2014. Systematic position of Paulsilvella in the Lithophylloideae (Corallinaceae, Rhodophyta) confirmed by molecular data. *Phytotaxa* 190(1): 94-111.

Verheij, E. 1994. Nongeniculate Corallinaceae (Corallinales, Rhodophyta) from the Spermonde Archipelago, SW Sulawesi, Indonesia. *Blumea* 39: 95–137.

Vidal, R., Meneses, I. & Smith, M. 2008. Phylogeography of the genus *Spongites* (Corallinales, Rhodophyta) from Chile. *J. Phycol*. 44:173–82.

Vieira-Pinto, T., Oliveira, M. C., Bouzon, J., Sissini, M., Richards, J. L., Riosmena-Rodríguez, R., & Horta, P. A. 2014. *Lithophyllum* species from Brazilian coast: range extension of *Lithophyllum margaritae* and description of *Lithophyllum atlanticum* (Corallinales, Corallinophycidae, Rhodophyta). *Phytotaxa* 190(1): 355-369.

Villas-Bôas, A.B., Riosmena-Rodriguez, R., Tâmega, F.T.S., Amado-Filho, G.M., Maneveldt, G.W. & Figueredo, M.A.O. 2015. Rhodolith-forming species of the subfamilies Neogoniolithoideae and Hydrolithoideae (Rhodophyta, Corallinales) from Espírito Santo State, Brazil. *Phytotaxa* 222(3): 169-184.

Woelkerling, W. J. 1980. Studies on Metamastophora (Corallinaceae, Rhodophyta). I. M. flabellata (Sonder) Setchell: morphology and anatomy. *Br. Phycol. J.* 15:201–25.

Woelkerling, W. J. 1998. Type collections of non-geniculate corallines housed at the Laboratoire de Cryptogamie (PC). In: Woelkerling, W.J. Eds. *Non-geniculate coralline red algae and the Paris Muséum: systematics and scientific history*. Paris: Muséum National d'Histoire naturelle / ADAC, p. 279-404.

Woelkerling, W. J., Penrose, D. & Chamberlain, Y. M. 1993. A reassessment of type collections of non-geniculate Corallinaceae (Corallinales, Rhodophyta) described by C. Montagne and L. Dufour, and of *Melobesia brassica-florida* Harvey. *Phycologia* 32: 323–31.

Zhang, J., Kapli, P., Pavlidis, P. & Stamatakis, A. 2013. A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* 29: 2869–2876.

Yoon, H.S., Hackett, J.D. & Bhattacharya, D. 2002. A single origin of the peridinin and fucoxanthin containing plastids in dinoflagellates through tertiary endosymbiosis. *Proc. Natl. Acad. Sci.*99:11724–29.

Table 1. Sequences generated in this study, with voucher information, herbarium accession numbers and GenBank accession numbers for each DNA marker. (–) no molecular data was produced, (+) generated sequences.

| Species | Voucher | Markers | | | Locality | Coordinate | Collection date |
|---|---|---|---|---|---|---|---|
| | | COI | *psb*A | SSU rDNA | | | |
| *Neogoniolithon* cf. *brassica-florida* | ALCB 125631 | + | + | - | Castanheiras, Guarapari, Espírito Santo, Brazil | 20°40'16.64"S x 40°29'46.01"W | 20/08/2013 |
| *Neogoniolithon* cf. *brassica-florida* | ALCB 125632 | + | + | + | Castanheiras, Guarapari, Espírito Santo, Brazil | 20°40'16.64"S x 40°29'46.01"W | 20/08/2013 |
| *Neogoniolithon* cf. *brassica-florida* | ALCB 125633 | - | - | - | Castelhanos, Anchieta, Espírito Santo, Brazil | 20°50'05.08"S x 40°37'24.84"W | 21/08/2013 |
| *Neogoniolithon crypticum* | ALCB 125629 | - | + | + | Praia das Conchas, Cabo Frio, Rio de Janeiro, Brazil | 22°52'16.8"S x 41°53'51.2"W | 26/05/2013 |
| *Neogoniolithon crypticum* | ALCB 125630 | + | + | - | Praia das Conchas, Cabo Frio, Rio de Janeiro, Brazil | 22°52'16.8"S x 41°53'51.2"W | 26/05/2013 |
| *Neogoniolithon rhizophorae* | ALCB 125638 | + | + | + | Gaibú, Cabo de St° Agostinho, Pernambuco, Brazil | 08°18'49.5"S x 34°56'48.4"W | 27/04/2014 |
| *Neogoniolithon rhizophorae* | ALCB 125639 | + | + | + | Gaibú, Cabo de St° Agostinho, Pernambuco, Brazil | 08°18'49.5"S x 34°56'48.4"W | 27/04/2014 |
| *Neogoniolithon rhizophorae* | ALCB 125635 | - | - | - | Morro de São Paulo, Cairú, Bahia, Brazil | 13°23'16.8"S x 38°54'18.6"W | 06/10/2013 |

| Species | Voucher | Markers | | | Locality | Coordinate | Collection date |
|---|---|---|---|---|---|---|---|
| | | COI | *psb*A | SSU rDNA | | | |
| *Neogoniolithon rhizophorae* | ALCB 125636 | + | + | + | Morro de São Paulo, Cairú, Bahia, Brazil | 13°23'16.8"S x 38°54'18.6"W | 06/10/2013 |
| *Neogoniolithon rhizophorae* | ALCB 125637 | + | + | + | Morro de São Paulo, Cairú, Bahia, Brazil | 13°23'16.8"S x 38°54'18.6"W | 06/10/2013 |
| *Neogoniolithon rhizophorae* | ALCB 125640 | + | + | - | Garapuá, Cairú, Bahia, Brazil | 13°29'13.14"S x 38°54'25.5"W | 03/07/2015 |
| *Neogoniolithon rhizophorae* | ALCB 125634 | + | - | + | Ponta do Mutá, Maraú, Bahia, Brazil | 13°52'45.61"S x 38°56'50.79"W | 03/07/2015 |

Table 2. Levels of intraspecific genetic variation (diagonal in gray), interspecific variation (in percentage, under the diagonal) and pairwise distance (over the diagonal) for *psb*A sequences, based on uncorrected p-distances between studied taxa. Intraspecific variation values are presented in bold. ( ), number of sequences/species analyzed; -, absent values.

| | *"N. brassica-florida"* | *N.* cf. *brassica-florida* | *"N. fosliei"* | *"N. fosliei"* A | *"N. fosliei"* C | *N. rhizophorae* | *N. crypticum* |
|---|---|---|---|---|---|---|---|
| *"N. brassica-florida"* (03) | **0.65% (0-5bp)** | 3-8bp | 115-132bp | 120-121bp | 128-129bp | 104-109bp | 120-122bp |
| *N.* cf. *brassica-florida* (02) | 0.40-1.07% | **0.0%** | 119-131bp | 124bp | 128bp | 104-105bp | 124bp |
| *"N. fosliei"* (02) | 15.39-17.67% | 15.93-17.54% | **10.17% (76bp)** | 126-129bp | 127-130bp | 122-139bp | 132-134bp |
| *"N. fosliei"* A (02) | 16.06-16.2% | 16.6% | 16.87-17.27% | **0.4%(3bp)** | 50-53bp | 127-129bp | 113-116bp |
| *"N. fosliei"* C (01) | 17.14-17.27% | 17.14% | 17-17.4% | 6.7-7.1% | - | 129-130bp | 117bp |
| *N. rhizophorae* (07) | 14-14.6% | 13.93-14.06% | 16.3-18.6% | 17-17.27% | 17.27-17.4% | **0.0-0.13% (0-1bp)** | 119-120bp |
| *N. crypticum* (02) | 16-16.3% | 16.6% | 15.1-17.9% | 15.13-15.53% | 15.66% | 15.93-16.07% | **0.0%** |

Table 3. Levels of intraspecific genetic variation (diagonal in gray), interspecific variation (in percentage, under the diagonal) and pairwise distance (over the diagonal) for SSU rDNA sequences, based on uncorrected p-distances between studied taxa. Intraspecific variation values are presented in bold. ( ), number of sequences/species analyzed; -, absent values.

| | *"N. brassica-florida"* | *N.* cf. *brassica-florida* | *"N. fosliei"* | *"N. fosliei"* A | *"N. fosliei"* B | *"N. fosliei"* C | *N. rhizophorae* | *N. crypticum* |
|---|---|---|---|---|---|---|---|---|
| *"N. brassica-florida"* (02) | **0.07% (1bp)** | 14-15bp | 3-37bp | 33-34bp | 35-37bp | 36-37bp | 6-8bp | 42-43bp |
| *N.* cf. *brassica-florida* (01) | 0.98-1.05% | - | 15-45bp | 36bp | 44-45bp | 43bp | 13-17bp | 41bp |
| *"N. fosliei"* (01) | 0.21-0.28% | 1.05% | - | 35bp | 35-36bp | 36bp | 4bp | 54bp |
| *"N. fosliei"* A (02) | 2.31-2.38% | 2.52% | 2.45% | **0.14% (2bp)** | 42-43bp | 39bp | 37-38bp | 35-37bp |
| *"N. fosliei"* B (02) | 2.45-2.6% | 3.08-3.15% | 2.45-2.52% | 3.01% | **0.07% (1bp)** | 7-8bp | 39-40bp | 57-58bp |
| *"N. fosliei"* C (02) | 2.52-2.6% | 3.01% | 2.52% | 2.73% | 0.49-0.56% | **0.0%** | 39-40bp | 54bp |
| *N. rhizophorae* (05) | 0.42-0.56% | 0.9-1.2% | 0.28% | 2.6-2.67% | 2.66-2.8% | 2.73-2.8% | **0.0-0.28% (0-4bp)** | 42-46bp |
| *N. crypticum* (01) | 2.9-3% | 2.89% | 3.08% | 2.45-2.6% | 4-4.06% | 3.78% | 2.9-3.2% | - |

Table 4. Levels of intraspecific genetic variation (diagonal in gray), interspecific variation (in percentage, under the diagonal) and pairwise distance (over the diagonal) for COI-5P sequences, based on uncorrected p-distances between studied taxa. Intraspecific variation values are presented in bold. ( ), number of sequences/species analyzed; -, absent values.

| | *"N. brassica-florida"* | *N.* cf. *brassica-florida* | *"N. fosliei"* | *"N. fosliei"* A | *"N. fosliei"* B | *"N. fosliei"* C | *N. rhizophorae* | *N. crypticum* |
|---|---|---|---|---|---|---|---|---|
| *"N. brassica-florida"* (01) | - | 40bp | 99bp | 79bp | 91-106bp | 96-107bp | 85-86bp | 81bp |
| *N.* cf. *brassica-florida* (02) | 7.56% | **0.0%** | 101bp | 81bp | 102-104bp | 95-113bp | 86-87bp | 90bp |
| *"N. fosliei"* (01) | 18.7% | 19.1% | - | 100bp | 77-88bp | 82-87bp | 94bp | 99bp |
| *"N. fosliei"* A (01) | 14.9% | 15.3% | 18.9% | - | 84-92bp | 96-103bp | 89-90bp | 75bp |
| *"N. fosliei"* B (02) | 17.2-18.1% | 19.1-19.7% | 14.6-16.6% | 15.9-17.4% | **11% (58bp)** | 82-88bp | 93-101bp | 94-95bp |
| *"N. fosliei"* C (02) | 18.1-20.2% | 18-21.4% | 15.5-16.4% | 18.1-19.5% | 15.5-16.6% | **8.3% (44bp)** | 96-109bp | 99-100bp |
| *N. rhizophorae* (05) | 16.07-16.26% | 16.3-16.5% | 17.8% | 16.8-17% | 17.6-19.1% | 18.1-20.6% | **0.0-0.2% (0-1bp)** | 92bp |
| *N. crypticum* (01) | 15.3% | 17% | 18.7% | 14.2% | 17.8-18% | 18.7-18.9% | 17.4% | - |

Table 5: Morphological comparison between *Neogoniolithon* taxa (- = absent; ND = not disclosed; B = branched; E = Encrusting; S = single; V = in vertical rows; H = in horizontal rows).

| Characters (measured in µm) | *N. brassica-florida* Woelkerling et al. (1993) | *N. brassica-florida* Penrose (1996) | *N. fosliei* Iryu & Matsuda (1994) | *N. rhizophorae* Foslie & Howe (1906) | *N. rhizophorae* Mateo-Cid et al. (2014) | *N. tenuicrustaceum* Iryu & Matsuda (1994) | *N. accretum* Foslie & Howe (1906) | *N. accretum* Mateo-Cid et al. (2014) | *N. atlanticum* Tâmega et al. (2014) | *N.* cf. *brassica-florida* This study | *N. rhizophorae* This study | *N. crypticum* This study |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Growth form | B | B | E | B | B | E | E | E | E | B | B | E |
| Trichocytes | V | V/H | V | ND | V | V | ND | S | - | V | V | S |
| Long of trichocytes | ND | ND | 6-27 | ND | 40-45 | 7-29 | 20-25 | 35-50 | - | 27-55 | 23-58.1 | 14.6-47 |
| Diameter of trichocytes | ND | ND | 14-22 | ND | 25-30 | 12-29 | ND | 25-35 | - | 19-26.1 | 11.5-33 | 14.2-25.6 |
| Long of epithallial cells | ND | ND | 6-10 | ND | 5-7 | 3-8 | ND | 12.5-15 | 4 | 4.2-11.3 | 5-11 | 5.5-7.6 |
| Diameter of epithallial cells | ND | ND | 7-15 | ND | 10-13 | 7-14 | ND | 6-9 | 6-7 | 7.5-16.5 | 8-19 | 8.2-13.9 |
| Long of perithallial cells | 10-40 | 10-40 | 6-16 | 24 | 18-21 | 8-15 | 10 | 18-24 | 6 | 5.2-22 | 8-27 | 12-16.4 |
| Diameter of perithallial cells | 8-19 | 10-20 | 5-13 | 8-15 | 11-13 | 5-13 | 4-9 | 6-20 | 5 | 7-20 | 6-17.7 | 8-16 |
| Long of hypothallial cells | ND | ND | 17-49 | 12-30 | 20-25 | 9-40 | 14-27 | 15-30 | 7-12 | 25-48 | 20-27.5 | 18-32.4 |
| Diameter of hypothallial cells | ND | ND | 11-20 | 8-15 | 15-17 | 5-16 | 8-14 | 9-13 | 15-29 | 9-15 | 15-18 | 8.5-14.8 |
| Long of tetrasporangial conceptacles | - | - | 140-380 | ND | 250-320 | 110-150 | ND | - | - | - | - | - |
| Diameter of tetrasporangial conceptacles | - | - | 760-1040 | 800-1000 | 600-700 | 600-760 | 300-400 | - | - | - | - | - |

| Characters (measured in µm) | *N. brassica-florida* Woelkerling et al. (1993) | *N. brassica-florida* Penrose (1996) | *N. fosliei* Iryu & Matsuda (1994) | *N. rhizophorae* Foslie & Howe (1906) | *N. rhizophorae* Mateo-Cid et al. (2014) | *N. tenuicrustaceum* Iryu & Matsuda (1994) | *N. accretum* Foslie & Howe (1906) | *N. accretum* Mateo-Cid et al. (2014) | *N. atlanticum* Tâmega et al. (2014) | *N.* cf. *brassica-florida* This study | *N. rhizophorae* This study | *N. crypticum* This study |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Long of carposporangial conceptacles | - | 500-710 | - | - | - | - | - | 140-200 | 276-515 | 140-632 | 313-537.1 | 110-244 |
| Diameter of carposporangial conceptacles | - | 460-630 | - | - | - | - | - | 200-380 | 570-890 | 390-708 | 448-527.3 | 283-380 |
| Long of carposporangia | - | 56-90 | - | ND | | - | ND | 90-100 | 47-113 | 26-64.1 | 62-152 | 42-59 |
| Diameter of carposporangia | - | 25-85 | - | ND | | - | ND | 40-60 | 20-55 | 34-75 | 17.6-49 | 13-15 |
| Long of spermatangial conceptacles | ND | 350-380 | - | ND | 150-250 | - | ND | 80-110 | - | - | - | - |
| Diameter of spermatangial conceptacles | 355-519 | 295-335 | - | ND | 350-450 | - | ND | 210-310 | - | - | - | - |
| Cells number in the roof of conceptacle | 8-15 | 10-25 | - | ND | 15-18 | 15-29 | ND | 6-7 | 11-18 | 6-13 | 12-20 | 7-12 |

Supplementary table S1. List of taxa available in GenBank and included in our analyses.

| Species | Locality | GB access nº 18S | GB access nº psbA | GB access nº COI |
|---|---|---|---|---|
| *Harveylithon rupestre* (as *Hydrolithon rupestre*) | Australia | KM073303 | KM407535 | - |
| *Hydrolithon boergesenii* | Brazil | - | KY485316 | - |
| *Hydrolithon boergesenii* (as *Hydrolithon reinboldii*) | Hawaii | - | - | HQ422709 |
| *Hydrolithon boergesenii* (as *Hydrolithon reinboldii*) | Japan | AB576017 | - | - |
| *Mastophora pacifica* | New Zealand | - | FJ361461 | - |
| *Mastophora rosea* | Japan | - | AB576041 | - |
| *Mastophora rosea* | USA | - | - | KP224298 |
| *Mesophyllum erubescens* | Hawaii | DQ629012 | - | HQ422718 |
| *Mesophyllum erubescens* | Brazil | - | KY485315 | - |
| *Metagoniolithon radiatum* | Australia | U61250 | - | - |
| *Neogoniolithon accretum* | Mexico | - | KJ637660 | - |
| *Neogoniolithon acropetum* | Mexico | - | KJ637663 | - |
| *Neogoniolithon brassica-florida* | Hawaii | DQ629008 | - | - |
| *Neogoniolithon brassica-florida* | Hawaii | DQ629007 | - | - |
| *Neogoniolithon brassica-florida* | France | - | - | KM392368 |
| *Neogoniolithon fosliei* | French Polynesia | KM073297 | - | - |
| *Neogoniolithon fosliei* | Japan | AB576020 | - | AB713922 |
| *Neogoniolithon fosliei* | Japan | AB713916 | - | AB713924 |
| *Neogoniolithon fosliei* | Japan | AB576019 | - | AB713919 |
| *Neogoniolithon fosliei* | Japan | AB713914 | - | AB713921 |
| *Neogoniolithon fosliei* | Japan | AB576018 | - | AB713920 |
| *Neogoniolithon frutescens* | Japan | AB713917 | - | AB713926 |
| *Neogoniolithon frutescens* | Japan | - | - | AB713925 |
| *Neogoniolithon megalocystum* | Japan | AB576021 | - | AB713927 |
| *Neogoniolithon mamillare* | Mexico | - | KJ637664 | - |

| | | | | |
|---|---|---|---|---|
| *Neogoniolithon propinquum* | Mexico | - | KJ637665 | - |
| *Neogoniolithon rhizophorae* | Mexico | - | KJ637670 | - |
| *Neogoniolithon rhizophorae* | Mexico | - | KJ637672 | - |
| *Neogoniolithon siankanensis* | Mexico | - | KJ637675 | - |
| *Neogoniolithon solubile* | Mexico | - | KJ637679 | - |
| *Neogoniolithon* sp. | Spain | KM073298 | - | - |
| *Neogoniolithon* sp. | Spain | KM073300 | - | - |
| *Neogoniolithon* sp | Guadeloupe | - | - | KM392369 |
| *Neogoniolithon* sp | Panama | - | - | KM392370 |
| *Neogoniolithon* sp | Guadeloupe | - | - | KM392372 |
| *Neogoniolithon* sp | Guadeloupe | - | - | KP682495 |
| *Neogoniolithon spectabile* | Mexico | - | KJ637682 | - |
| *Neogoniolithon strictum* | Mexico | - | KJ637684 | - |
| *Neogoniolithon spectabile* | Bahamas | AY234238 | - | - |
| *Neogoniolithon trichotomum* | Japan | - | - | AB713928 |
| *Pneophyllum conicum* | Hawaii | DQ628983 | - | HQ422716 |
| *Porolithon gardineri* (as *Hydrolithon gardineri*) | Hawaii | - | - | HQ423069 |
| *Porolithon onkodes* (as *Hydrolithon onkodes*) | Indonesia | KM073275 | - | - |
| *Porolithon onkodes* (as *Hydrolithon onkodes*) | Brazil | - | KY485318 | - |
| *Porolithon onkodes* (as *Hydrolithon onkodes*) | Australia | - | - | KM392359 |
| *Spongites fruticulosa* | France | - | - | KM392374 |
| *Spongites yendoi* | South Africa | U60948 | - | - |
| *Sporolithon ptychoides* | Brazil | - | KC870927 | - |
| *Sporolithon ptychoides* | Hawaii | DQ629014 | - | HQ422711 |

FIGURES

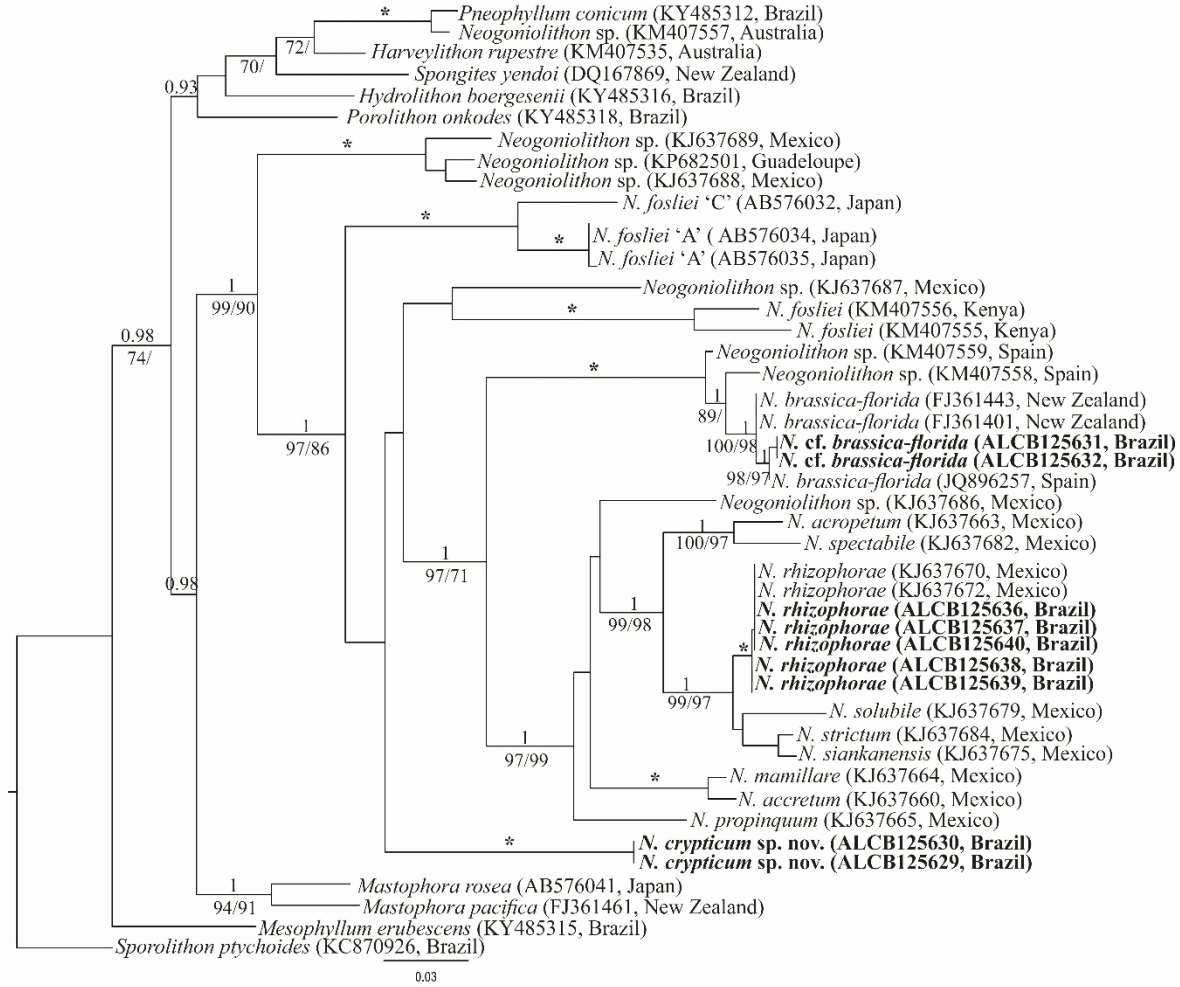


FIGURE 1: The optimal maximum likelihood (ML) topology based on the *psb*A data set. Values above each clade refer to Bayesian posterior probabilities (PP) and the ones below each clade refer to ML and MP (maximum parsimony) bootstrap values, respectively. Only bootstrap values for ML and MP ≥ 70% and PP ≥ 0.9 were plotted. New sequences produced in this study are in bold. Asterisks mark clades that are supported at 100% in all three analyses.

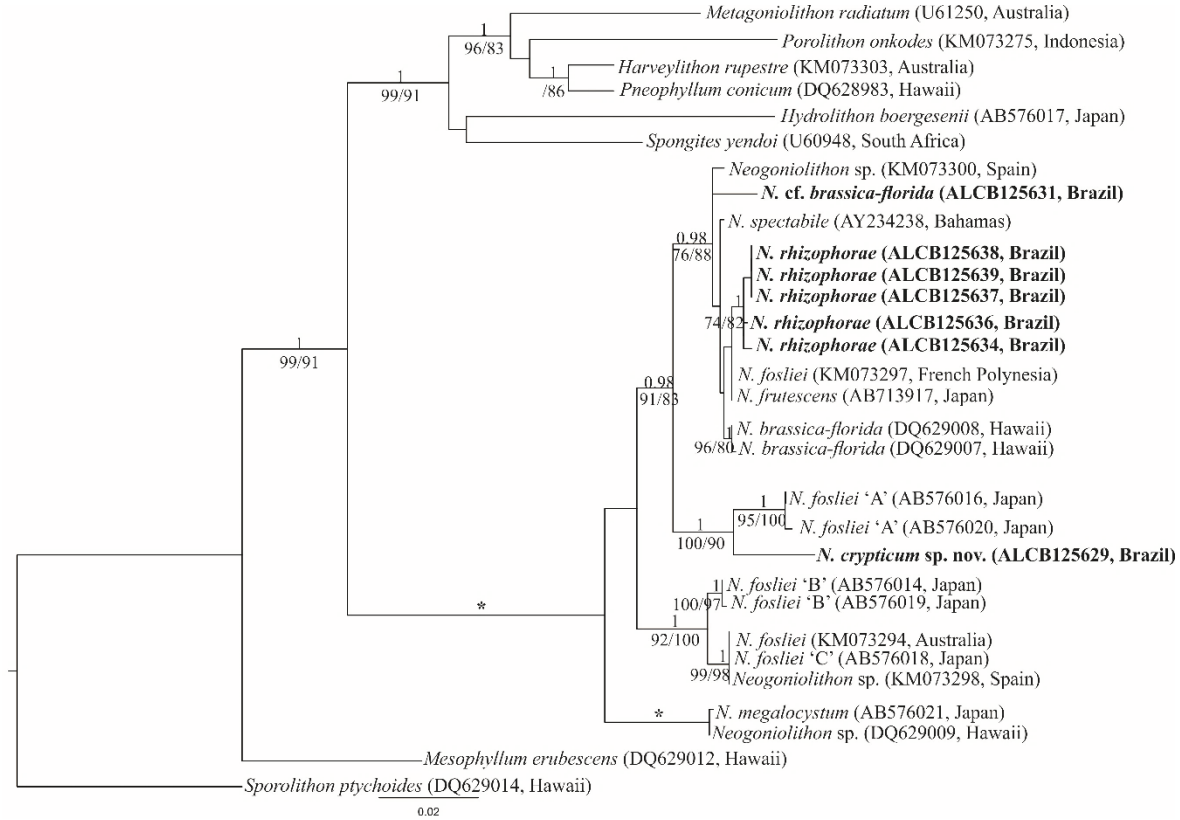


FIGURE 2: The optimal maximum likelihood (ML) topology based on the SSU rDNA data set. Values above each clade refer to Bayesian posterior probabilities (PP) and the ones below each clade refer to ML and MP (maximum parsimony) bootstrap values, respectively. Only bootstrap values for ML and MP ≥ 70% and PP ≥ 0.9 were plotted. New sequences produced in this study are in bold. Asterisks mark clades that are supported at 100% in all three analyses.

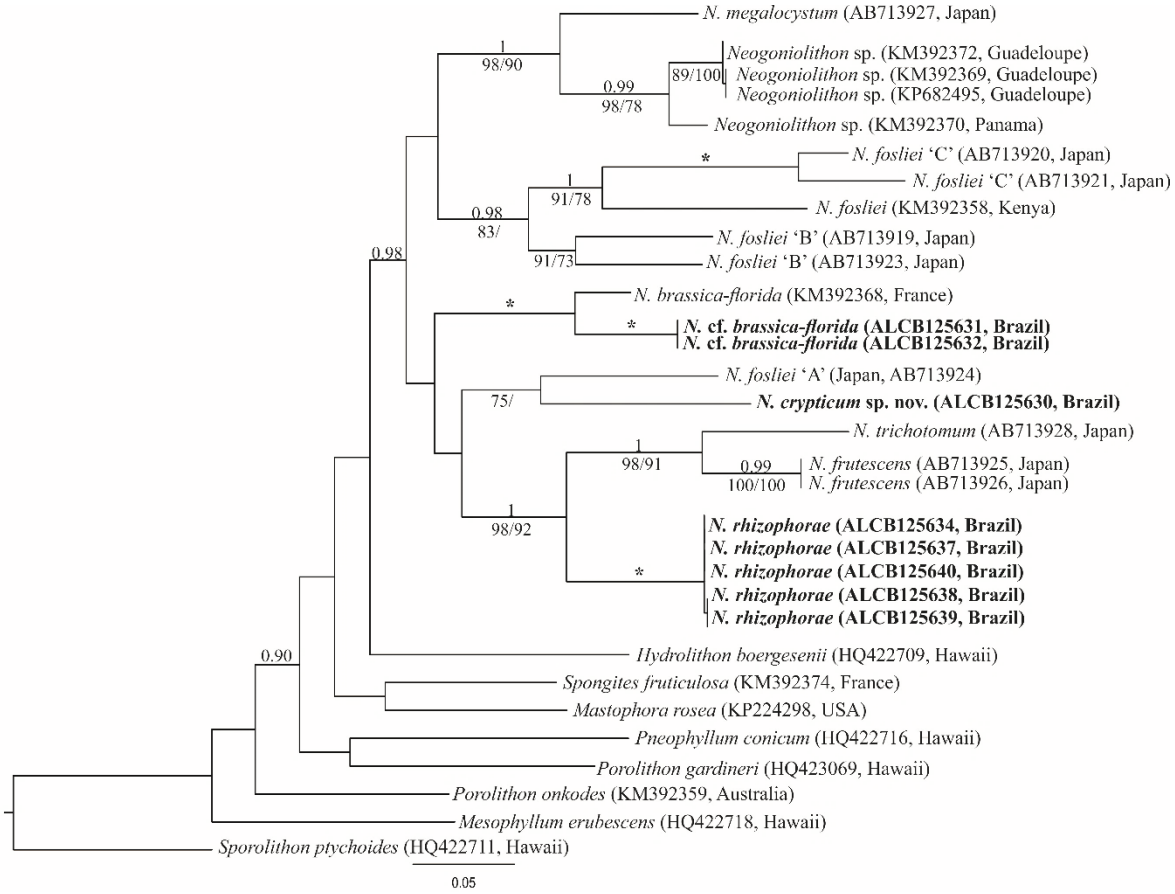


FIGURE 3: The optimal maximum likelihood (ML) topology based on the COI-5P data set. Values above each clade refer to Bayesian posterior probabilities (PP) and the ones below each clade refer to ML and MP (maximum parsimony) bootstrap values, respectively. Only bootstrap values in ML and MP ≥ 70% and PP ≥ 0.9 were plotted. New sequences produced in this study are in bold. Asterisks mark clades that are supported at 100% in all three analyses.

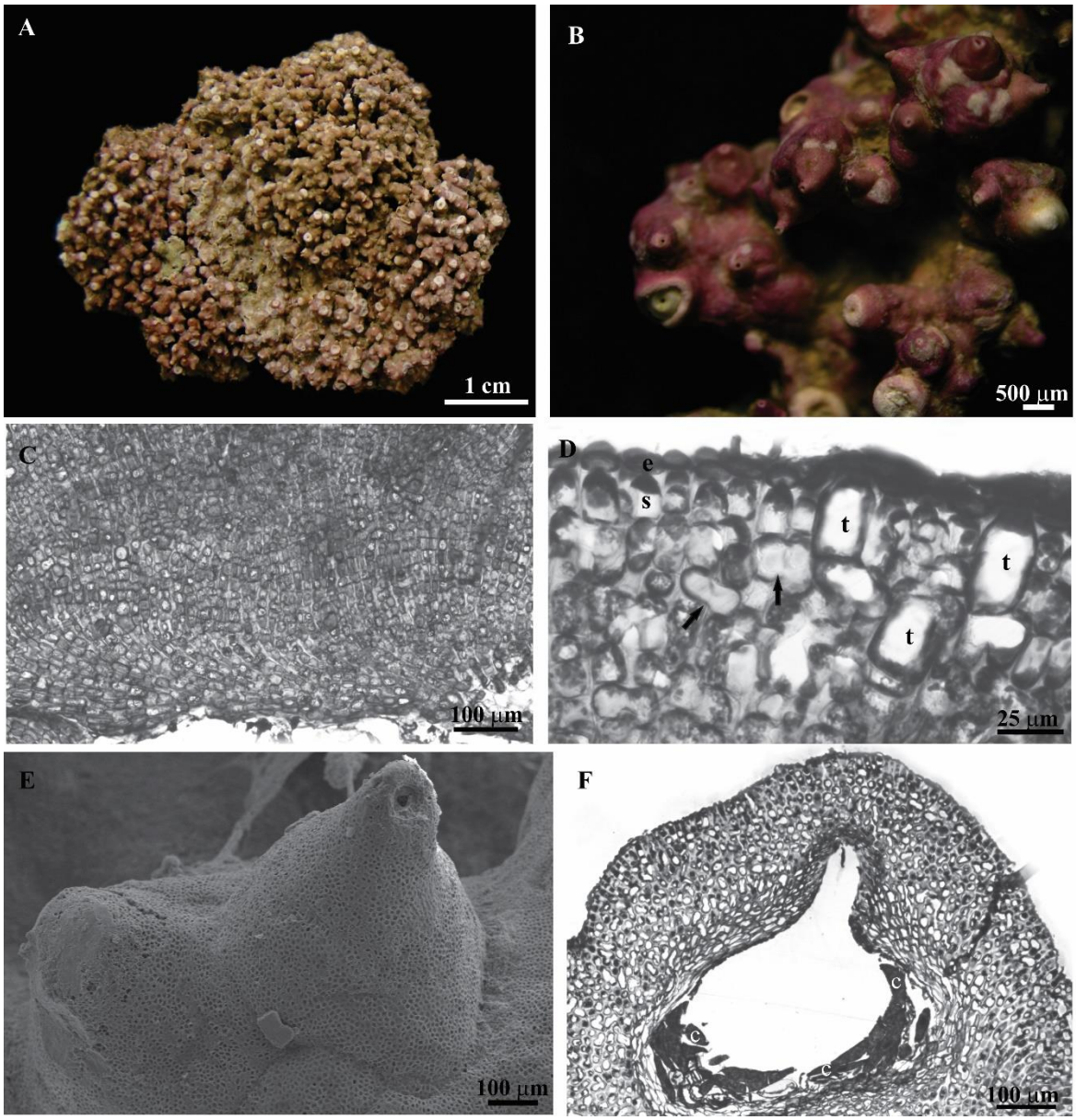


FIGURE 4 A-F: *Neogoniolithon* cf. *brassica-florida*. A. Encrusting growth-form with protuberances. B. Detail of the branch with conical uniporate conceptacles on the apice. C. Longitudinal section through the thallus showing monomerous construction. D. Longitudinal section through the thallus showing epithallial cells (e), rounded to flattened; subepithallial initial cells (s) longer than subtending ones; cells of adjacent filaments linked by lateral cell fusions (arrows); and trichocytes (t). E. Carposporangial conceptacle uniporate in surface view, SEM. F. Longitudinal section through carposporangial conceptacle showing carposporangia (c) scattered across conceptacle floor.

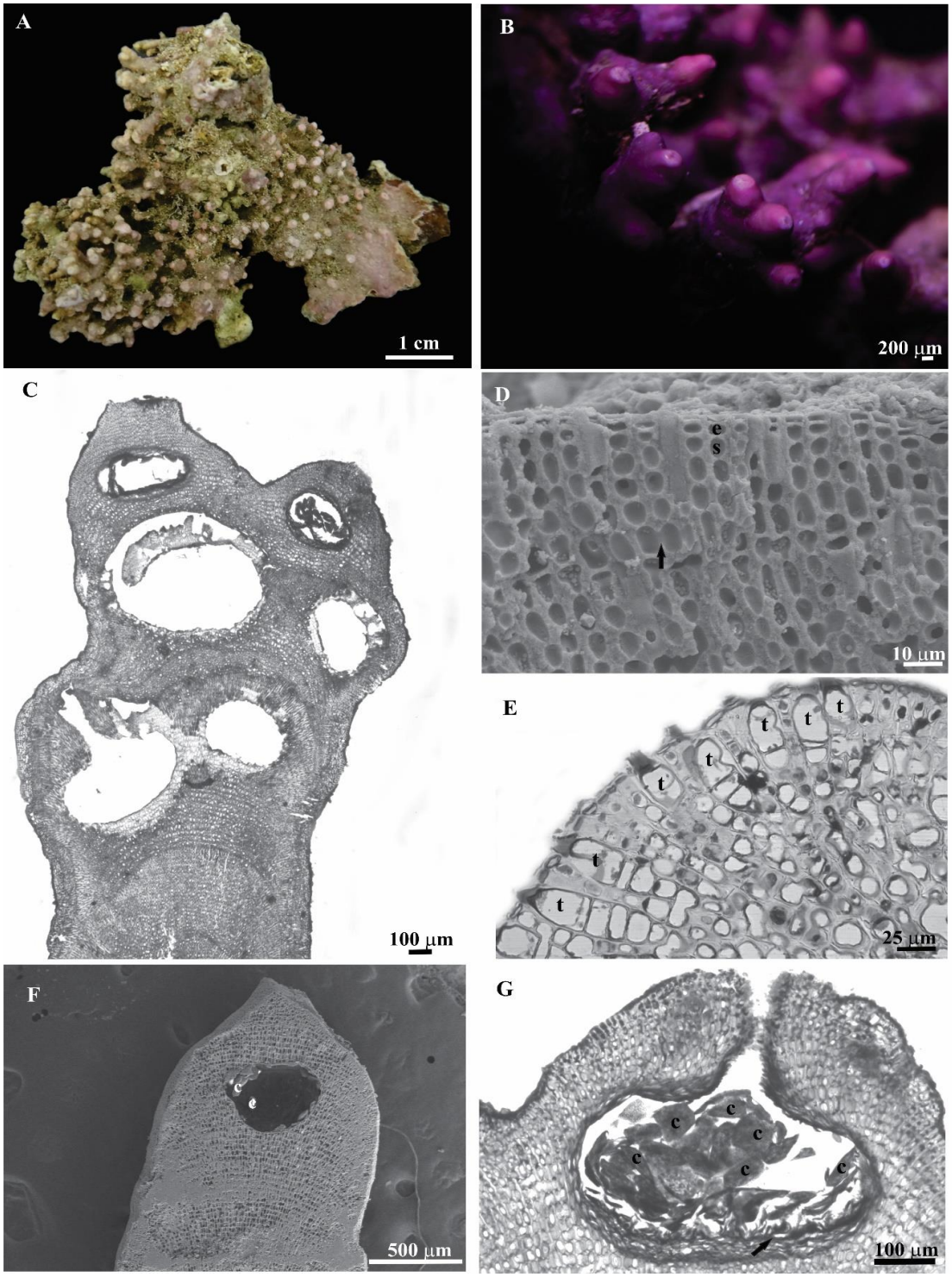


FIGURE 5 A-G: *Neogoniolithon rhizophorae*. A. Encrusting growth-form with protuberances. B. Detail of the branch with conical uniporate conceptacles on the apice. C. Longitudinal section through branch with carposporangial conceptacles. D. Longitudinal fracture, SEM, showing epithallial cells (e) occurring in one or two layers, rounded to flattened; subepithallial initial cells (s) longer than subtending ones, and cells of adjacent filaments linked by lateral cell fusions (arrow). E. Longitudinal section through the thallus showing trichocytes (t) in vertical rows. F. Longitudinal fracture, SEM, showing carposporangial conceptacles with carposporangium (c). G. Longitudinal section through the carposporangial conceptacle showing carposporangia (c) supported for gonimoblast filaments that arise across the dorsal surface of the discontinuous fusion cell (arrow).

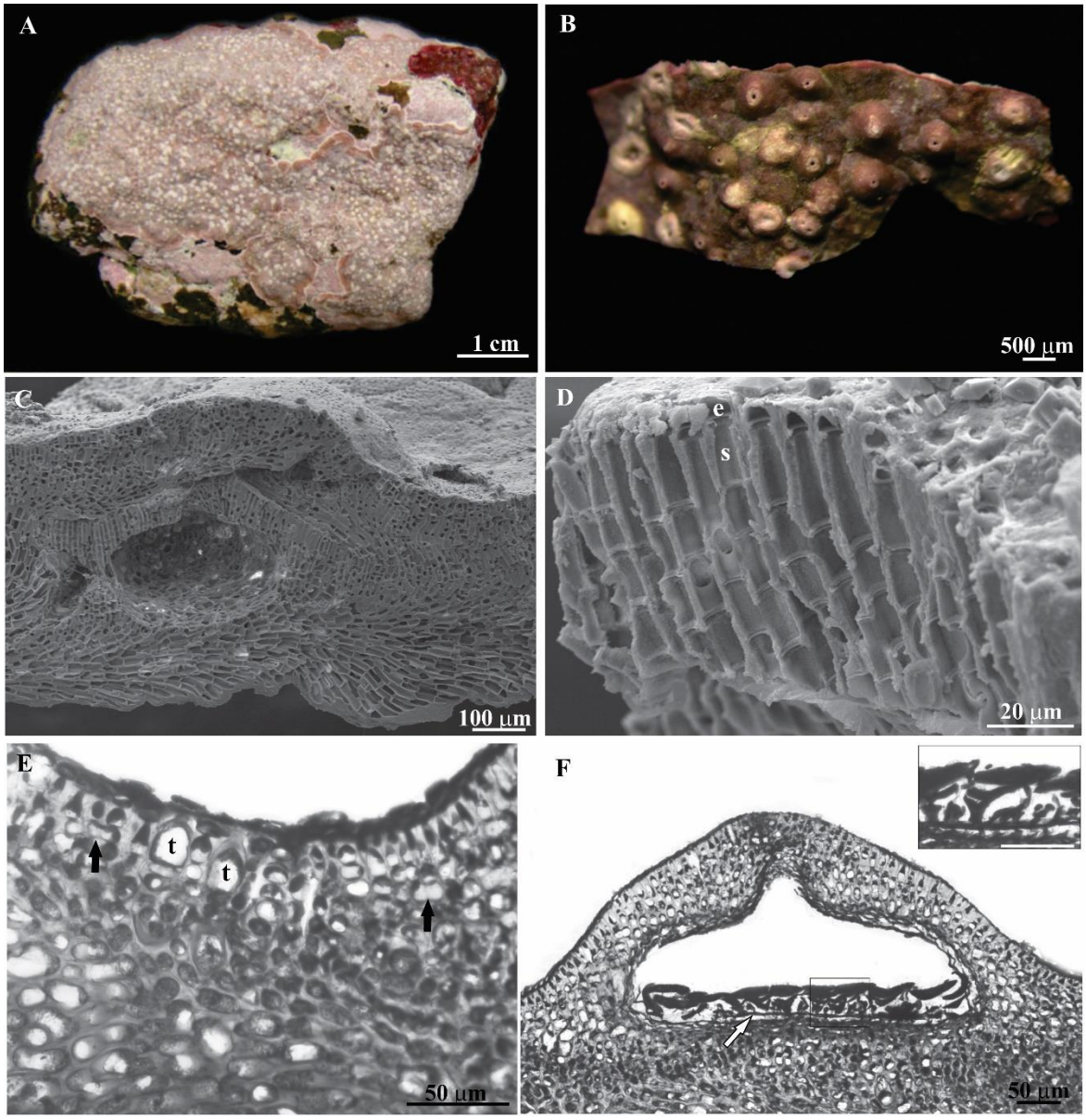


FIGURE 6 A-F: *Neogoniolithon crypticum* sp.nov. A. Encrusting growth-form. B. Detail of the conical uniporate conceptacles. C. Longitudinal fracture, SEM, showing monomerous construction. D. Longitudinal fracture, SEM, showing epithallial cells (e), rounded to flattened; subepithallial initial cells (s) longer than subtending ones. E. Longitudinal section through the thallus showing trichocytes (t) single at the surface of the thallus, and cells of adjacent filaments linked by lateral cell fusions (arrows). F. Longitudinal section through the carposporangial conceptacle showing carposporangia (c) supported for gonimoblast filaments that arise across the dorsal surface of the continuous fusion cell (arrow), and detail of the carposporangium supported by three-celled filaments of gonimoblast.

# CAPÍTULO 3

## PHYLOGENETIC RELATIONSHIPS WITHIN CORALLINACEAE (CORALLINALES – RHODOPHYTA)*

IARA OLIVEIRA COSTA, PRISCILA BARRETO DE JESUS, PAULO ANTUNES HORTA, JOSÉ MARCOS DE CASTRO NUNES

***Manuscrito a ser submetido ao periódico Molecular Phylogenetic Evolution** (normas disponíveis em: https://www.elsevier.com/journals/molecular-phylogenetics-and-evolution/1055-7903/guide-for-authors)

# PHYLOGENETIC RELATIONSHIPS WITHIN CORALLINACEAE (CORALLINALES – RHODOPHYTA)

Iara Oliveira Costa [a,b*], Priscila Barreto de Jesus [c], Paulo Antunes Horta [d] and José Marcos de Castro Nunes [b]

[a] *Programa de Pós-Graduação em Botânica, Departamento de Ciências Biológicas, Universidade Estadual de Feira de Santana, Av. Universitária, CEP 44031-460, Feira de Santana, Bahia, Brazil*

[b] *Laboratório de Algas Marinhas (LAMAR), Departamento de Botânica, Instituto de Biologia, Campus de Ondina, Universidade Federal da Bahia, CEP 40210-730, Salvador, Bahia, Brazil*

[c] *Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, Rua do Matão 277, CEP 05508-090, São Paulo, Brazil*

[d] *Departamento de Botânica, Universidade Federal de Santa Catarina, Caixa Postal 476, 88010-970 Florianópolis, Santa Catarina, Brazil*

* Corresponding author.

*Email address: iaraoc@hotmail.com* (Iara Oliveira Costa)

**Abstract**

The taxonomy of the Corallinales has been under constant revision, due to the variability of the morphological characters frequently used to distinguish among genera and species. The incorporation of new approaches, such as molecular analysis, has provided an advance in knowledge of the diversity of coralline algae in recent decades. In this study, we analyzed two distinct markers (*psb*A and SSU rDNA) and morpho-anatomical analysis to establish the phylogenetic relationships between Brazilian species of non-geniculate Corallinaceae. We identified thirteen taxa and proposed the amendment of Metagoniolithoideae that now contains *Tomitolithon* gen. nov., with *T. deniquerum* sp. nov. and *T. polistratum* sp. nov.

**Keywords:** Brazil, *Tomitolithon* gen. nov., Non-geniculate coralline algae, *psb*A, SSU rDNA.

## 1. Introdution

More than 100 years ago, Foslie and Heydrich proposed around 700 coralline red algae species (Corallinophycidae, Rhodophyta), all circumscribed based on morphological features (Woelkerling, 1988; Irvine and Chamberlain, 1994; Horta 2002). However, the first molecular phylogeny of Corallinales was proposed by Bailey and Chapman only in 1996 (updated in 1998). Since then, the diversity of these cosmopolitan, economic and ecologically important group has received new incites due the utilization of molecular approaches. New species (Vieira-Pinto et al., 2014; Bahia et al., 2014, 2015; Hernandez-Kantun et al., 2016), genera (Hind et al., 2016; Rosler et al., 2016), subfamilies (Kato et al., 2011), orders (Le Gall et al., 2010; Nelson et al., 2015) or even a sub class (Le Gall and Saunders, 2007) were described based on molecular data accomplished of morphoanatomical characterization.

The high phenotypic plasticity and the overlapping of many taxonomic characters among species and genera made difficult delimit taxa in Corallinophycidae (Maneveldt and Keats, 2008; Vidal et al., 2003). Despite the high morphological variation, this type of data keep being a precious tool to systematic of coralline algae. Johansen (1969) recognized three subfamilies of geniculate corallines (Amphiroideae, Corallinoideae and Metagoniolithoideae), arguing the fundamental importance of the presence of genicula to classify corallines algae. Cabioch (1971) emphasized the importance of cellular connections (secondary pit-connections vs. cell fusions) to group geniculate and non-geniculate taxa in the same tribes and subfamilies. Recent molecular studies shown, in agreement with Cabioch (1971), that genicula evolved independently in the three subfamilies recognized by Johansen and that each geniculate subfamily likely arose from different non-geniculate ancestors (Bailey and Chapman, 1998; Hind et al., 2013; Rösler et al., 2016).

Two decades ago, the order Corallinales was composed of two families: Corallinaceae, with eight subfamilies, and Sporolithaceae (Woelkerling, 1996). The relationships within Corallinales has undergone significant changes. Bailey (1999), using nuclear SSU rDNA, merged the subfamily Amphiroideae (geniculate) into the Lithophylloideae (non-geniculate). Harvey et al. (2003) resurrected Hapalidiaceae, and transferred to it three subfamilies previously classified under of Corallinaceae (Austrolithoideae, Choreonematoideae, Melobesoideae). The family Corallinaceae had, then, four subfamilies: Corallinoideae, Lithophylloideae, Masthophoroideae and

Metagoniolithoideae (Harvey et al. 2003). Bailey et al. (2004) and Broom et al. (2008), based on SSU rDNA and *psb*A, showed that, while three of these subfamilies were monophyletic, Mastophoroideae was polyphyletic.

Molecular studies have confirmed ancient circumscriptions or sometimes, resigned new taxa to marine and freshwater environments (Bailey and Chapman, 1996; Bailey. 1999; Kato et al., 2011, 2013; Hind and Saunders, 2013; Zuljevic et al., 2016). Kato et al. (2011) reviewed Mastophoroideae proposing the amendment of this subfamily and the establishment of three new subfamilies: Hydrolithoideae, Neogoniolithoideae and Porolithoideae. More recently, Rösler et al. (2016) proposed the amendment of Metagoniolithoideae and Neogoniolithoideae, and the rejection of Porolithoideae as an independent subfamily. Thus, Metagoniolithoideae now includes geniculate and non-geniculate taxa, inclusive with representatives of the former Porolithoideae.

Currently, Corallinales includes one family – Corallinaceae – and six subfamilies: Corallinoideae, Hydrolithoideae, Lithophylloideae, Mastophoroideae, Metagoniolithoideae, and Neogoniolithoideae (Rosler et al., 2016). However, the taxonomic status of some coralline taxa is still polemic (Bailey and Champan, 1998; Kato et al. 2013, Nelson et al. 2015).

To date, 30 valid species of non-geniculate Corallinaceae have been recorded to the Brazilian coast (Horta, 2000; Figueiredo and Steneck, 2000; Villas-Boas et al., 2005; Tâmega and Figueiredo, 2005; Nunes et al., 2008; Villas-Boas et al., 2009; Amado-Filho et al., 2012a,b; Henriques et al., 2012, 2014; Mariath et al., 2012; Pereira Filho et al., 2012; Bahia et al., 2014; Costa et al., 2014a,b; Crespo et al., 2014; Tâmega et al., 2014; Torrano-Silva et al., 2014; Vieira-Pinto et al., 2014; Villas-Boas et al., 2015; Jesionek et al., 2016; Moura et al., 2016), including the description of new species (Villas-Boas et al., 2009; Henriques et al., 2014; Tâmega et al., 2014; Vieira-Pinto et al., 2014; Costa et al., 2017 a, b – submitted). Despite the great attention that the Brazilian coast has received in recent years, there are still few studies combining morpho-anatomical and molecular data to assess the biodiversity of non-geniculate Corallinaceae (Torrano-Silva et al., 2014; Vieira-Pinto et al., 2014; Jesionek et al., 2016; Costa et al., 2017 a, b - submitted), which probably remains underestimated (Rösler et al., 2016).

The present study contributes to the knowledge of the taxonomy and systematic of non-geniculate Corallinaceae from the Brazilian coast. For this, we evaluated two molecular markers (SSU rDNA and *psb*A) and performed a morpho-anatomical characterization. The inclusion of the Brazilian sequences improves the understanding of

the relationship between species, within the distinct subfamilies of Corallinaceae. Herein, we propose the amendment the Metagoniolithoideae, with the description of *Tomitolithon* gen. nov. to accommodate two news species: *T. deniquerum* sp. nov. and *T. polistratum* sp. nov.

## 2. Materials and Methods

### *2.1. Sampling*

We collected 52 samples at 19 localities along the Brazilian coast (Table 1), on intertidal regions during syzygy low tides, and from the subtidal zone (3–11m depth) by SCUBA diving and Petersen dredge. Cleaned samples were air dried for at least 24 hours and then stored on silica gel. Samples were examined using a stereomicroscope (Leica® Zoom 2000, Wetzlar, Germany) for the separation of the fragments for molecular and morpho-anatomical analysis. Materials for morpho-anatomical observations were subsequently preserved in formaldehyde 4%.

### 2.2. *Morphological and anatomical observations*

Fragments were decalcified with 0.6M HNO3 for 24 hours, followed by dehydration in an ethanol series (30, 50, 70, 90 and 100%) and by infiltration and inclusion in "Historesin embedding Kit" (Leica, Heidelberg, Germany), following the instructions provided by the manufacturer. Sectioning (4-10 μm thickness) was performed with a Thermo Scientific microtome (model HM 325, Walldorf, Germany), stained with toluidine blue acidified with borax (Riosmena-Rodriguez, 1993; Moura et al., 1997). Images were obtained using a photomicroscope (Olympus trinocular CX31RTS5®, Tokyo, Japan) coupled with a digital camera (QImaging GO-3). For scanning electron microscopy (SEM), dry specimens were fractured for the observation of diagnostic features and placed in stubs using carbon tapes, following the procedures described by Chamberlain (1993). Specimens were deposited at the Herbarium Alexandre Leal Costa (ALCB) of the Universidade Federal da Bahia (UFBA/Brazil).

### *2.3. DNA extraction, PCR and sequencing*

Total DNA was extracted from fragments of dry samples using the Chelex resin extraction protocol (Goff and Moon, 1993) or the CTAB method (Cetyl Trimethyl Ammonium Bromide), modified from Doyle and Doyle (1987). 52 samples were sequenced for *psb*A and SSU rDNA (Table 1).

Molecular markers were PCR-amplified in a final volume of 25 μL: 1x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.2 μM primer, 5 ng DNA, and 1.25 U Taq DNA polymerase (Invitrogen, São Paulo, SP, Brazil). For the amplification and sequencing reactions of each marker, specific primer pairs were used. For plastidial *psb*A, PCR was conducted following Sissini et al. (2014). For nuclear SSU rDNA,we used the primers 18S5' and 1055R, and 1055F and 18S3' (Milstein and Oliveira, 2005). PCR was performed with an initial denaturation at 94°C for 4 min, 35 cycles at 94°C for 30 sec. (denaturation), 60°C for 1 min (primer annealing), and 72° C (extension) for 2 min; and a final extension at 72° C for 7 min. Reactions were performed on the Vertic® 96-Well Thermal Cycler (Applied Biosystems, Foster City, California, USA). The company GeneWiz (Cambridge, Massachusetts, USA, http://www.genewiz.com/) carried out the purifications and sequencing reactions of the PCR products.

*2.4. Molecular analyses*

Electropherograms were manually assembled using the Staden package (Staden et al., 2003) and edited in BioEdit 5.0.6 (Hall, 1999). For each marker, a multiple alignment was generated with ClustalW tool (Thompson et al., 1994), available in BioEdit 5.0.6 (sequences of the PCR primers were excluded from alignment). In addition to the sequences generated in this study, sequences of other species of Corallinales available from GenBank were used and included in the analyses (www.ncbi.nlm.nih.gov/genbank/, searched in April 2017 - Supplementary Table S1).

Analyses of Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI) were performed to infer phylogenetic relationships. Analyses were based on the data set of each marker (*psb*A and SSU rDNA), and on the dataset of the two markers (concatenated). Outgroup taxa for the *psb*A and SSU rDNA analyses were chosen from Sporolithales and Hapalidiales (Table S1). For the concatenated analyses, the outgroup chosen was *Sporolithon ptychoides* (Sporolithales - Table S1).

MP trees were constructed on PAUP* 4.0b.10 (Swofford, 2002), using a heuristic search algorithm with the following definitions: initial tree(s) obtained by stepwise addition; tree-bisection-reconnection (TBR) as *branch-swapping* algorithm and 10 trees retained by replication. All characters were given equal weights, their states were considered unordered, and the character-optimization criterion was ACCTRAN (*Accelerated Transformation*). Branches with a maximum length of zero collapsed to produce polytomies. Strict consensus analysis was calculated and the bootstrap values were

accessed using 1,000 replicates with random addition of terminals. The consistency index (CI), retention index (RI), and rescaling consistency index (CR) resulting from MP analysis were calculated (Supplementary Table S2).

The most suitable evolutionary model was selected using the software MrModeltest 2.3 (Nylander, 2008), using the Akaike Information Criterion (AIC), as recommended by Posada and Buckley (2004). ML analysis was conducted using RAxML (*Randomized Axelerated Maximum Likelihood*, version 7.0.4; Stamatakis, 2006) with the GTR+I+G model. The best ML tree score and 500 bootstrap trees were obtained using a fast scale algorithm (Stamatakis et al., 2008).

Bayesian analysis was carried out using the software Mr. Bayes 3.1 (Ronquist and Huelsenbeck, 2003) using the Marcov - Monte Carlo (MCMC) method with the selected model GTR+I+G. Five million generations in two independent races with four chains each were carried out, saving one tree every 100 generations. The *burn-in* was identified graphically to determine the period in which the likelihood values had reached a plateau. Trees sampled after stabilization were used to infer the posterior Bayesian probability.

## 3. Results

Based on an extensive sampling along the Brazilian coast and a combination of morpho-anatomical (Figs. 1, 2 and S1; Table 2) and molecular (Figs. 3, S2 and S3) data, we identified 13 Corallinaceae species. These species were distributed in three subfamilies: Lithophylloideae – *Lithophyllum atlanticum*, *L. margaritae* and *Lithophyllum* sp.; Metagoniolithoideae – *Harveylithon catarinense*, *H. maris-bahiensis*, *H. riosmenum*, *Pneophyllum conicum*, *Porolithon onkodes*, *Tomitolithon deniquerum* sp. nov. and *T. polistratum* sp. nov.; Neogoniolithoideae – *Neogoniolithon* cf. *brassica-florida*, *N. crypticum* and *N. rhizophorae*.

### *3.1. Taxonomic and morphological results*

CORALLINALES Silva and Johansen (1986: 250)

Corallinaceae Lamouroux (1812: 185)

**Metagoniolithoideae** (H.W. Johansen) emend. I.O. Costa, P.A. Horta et J.M.C. Nunes

DESCRIPTION: Thallus either geniculate with conspicuous mucilaginous caps, genicula of many-celled untiered filaments, or non-geniculate with monomerous or dimerous construction, lateral cell fusions present, secondary pit-connections restricted to basal filaments in dimerous thallus, trichocytes present or absent. Tetrasporangial

uniporate conceptacle roofs are formed by filaments peripheral to the fertile area and interspersed among tetrasporangial initials. Carpogonial filaments 2-celled, carposporangia terminating short gonimoblast filaments arising from the margins of a thin basal fusion cell. Spermatangia develop on the floor of male conceptacle.

Type genus: *Metagoniolithon* Weber-van Bosse 1904: 86, 101.

*Tomitolithon* I.O. Costa, P.A. Horta et J.M.C. Nunes **gen. nov.**

ETYMOLOGY: The name is in honor of Noemi Tomita, a pioneer in studies of crustose coralline algae in Brazil.

DIAGNOSIS: Thallus encrusting and smooth; dimerous construction; consisting of a single ventral layer of branched filaments of non-palisade cells and multicellular simple or branched filaments arising perpendicularly from the ventral layer. Cells of the basal filament with quadratic to rectangular shape. Cells of the adjacent filaments joined by cell fusions. Secondary pit connections only in basal filaments. Trichocytes presented or absent. Spermatangial conceptacles small, with spermatangia restricted to the conceptacle chamber floor. Carposporangial conceptacles with carposporangia supported for gonimoblast filaments that arise peripherally to surface of the continuous fusion cell. Tetrasporangial conceptacle roof 2–4 cells thick, with a single collunar meristematic cell.

TYPE SPECIES: *Tomitolithon deniquerum* I.O. Costa, P.A. Horta et J.M.C. Nunes sp. nov. We chose *T. deniquerum* as the generitype of *Tomitolithon* based on the availability of numerous specimens for the analyses.

COMMENTS: Specimens of this genus have been sampled in the Northeastern region of the Brazilian coast. Thin sections are required to observe the suite of characters that define the genus, but these characters are rarely sufficient to differentiate species.

*Tomitolithon deniquerum* I.O. Costa, P.A. Horta et J.M.C. Nunes **sp. nov.**

HOLOTYPE: Brazil, Pernambuco State, Goiana, Ponta das Pedras, 07°37'47.9"S, 34°48'31.2"W, coll. I.O. Costa et al. s.n., 30 April 2014; ALCB 125644; GenBank accessions: psbA – XXXXXXXX / SSU rDNA – XXXXXXXX.

ISOTYPE: Brazil, Pernambuco State, Goiana, Ponta das Pedras, 07°37'47.9"S, 34°48'31.2"W, coll. I.O. Costa et al. s.n., 30 April 2014; ALCB 125647; GenBank accessions: psbA – XXXXXXXX / SSU rDNA – XXXXXXXX.

ETYMOLOGY: The epithet refers to the characteristic thin nature of the vegetative thallus.

DIAGNOSIS: Thallus encrusting and smooth; dimerous construction; consisting of a single ventral layer of branched filaments of non-palisade cells and multicellular simple or branched filaments arising perpendicularly from the ventral layer, up to 20 cell layers thick. Secondary pit connections only in basal filaments. Trichocytes single at the surface of the thallus. *psb*A and SSU rDNA sequences unique.
Figure: 1A-F.

DESCRIPTION: Thallus encrusting and smooth; pink color (Fig. 1A). Structure pseudoparenchymatous; construction dimerous (up to 20 cell layers), consisting of a single ventral layer of branched filaments of non-palisade cells and multicellular simple or branched filaments arising perpendicularly from the ventral layer (Fig. 1B and 1C). Epithallial cells in one or two layers, squat to elliptical to spherical, 3.2–9 µm long and 3.6–7.9 µm in diameter (Fig. 1B). Subepithallial initials (3.8–11.8 µm long and 4–8 µm in diameter) as long or longer than the cells immediately subtending them (Fig. 1B). Cells of the perithallial filaments, 3.6–7.9 µm long and 3.6–12.5 µm in diameter. Cells of the hypothallial filaments, 3.2–9.2 µm long and 3.9–11.2 µm in diameter. Trichocytes rectangular to bottle-shaped to elongate, single at the surface of the thallus, and measure 10.9–23.5 µm long and 6.6–10 µm in diameter (Fig. 1B). Cells of the adjacent filaments joined by cell fusions (Fig. 1B). Secondary pit connections were observed only in basal filaments (Fig. 1C). Spermatangial conceptacles small and inconspicuous, chambers measure 39–47 µm long and 53–55 µm in diameter. Simple spermatangial systems restricted to the conceptacle chamber floor (Fig. 1D). Female conceptacles, with 41.5–87.9 µm long and 74.2–111.9 µm in diameter, containing a fusion cell across the chamber floor with gonimoblast filaments occurring at the margins and bearing terminal carposporangia (Fig. 1E). Uniporate tetrasporangial conceptacles flush in the thallus surface, chambers elliptical to spherical, measure 41.4–100.2 µm long and 60.8–120.1 µm in diameter (Fig. 1F). Central columella absent. Conceptacle roofs 2-4 cells thick, comprising a single squat to elliptical to spherical epithallial cell, a single collunar meristematic cell that is 2–4 times the length of the epithallial cell, and a small inner cell (Fig. 1F). Zonately divided tetrasporangia, 20.7–45.7 µm long and 5.7–15.3 µm in diameter (Fig. 1F).

MATERIAL EXAMINED: BRAZIL. PERNAMBUCO: Goiana, Ponta das Pedras, 07°37'47.9"S, 34°48'31.2"W, coll. I.O. Costa et al. s.n., 30 April 2014, (ALCB 125643, ALCB 125644, ALCB 125645, ALCB 125646 and ALCB 125647).

*Tomitolithon polistratum* I.O. Costa, P.A. Horta et J.M.C. Nunes **sp. nov.**

HOLOTYPE: Brazil, Ceará State, Caucaia, Praia do Pacheco, 03°41'09.18"S, 38°37'57.19"W, coll. I.O. Costa et al. s.n., 26 March 2013; ALCB 125641; GenBank accessions: psbA – XXXXXXXX / SSU rDNA – XXXXXXXX.

ETYMOLOGY: The epithet refers to the multiple layers of cells that compose the vegetative thallus.

DIAGNOSIS: Thallus encrusting and smooth; dimerous construction; consisting of a single ventral layer of branched filaments of non-palisade cells and multicellular simple or branched filaments arising perpendicularly from the ventral layer, more than 20 cell layers thick. Secondary pit connections only in basal filaments. Trichocytes absent. *psb*A and SSU rDNA sequences unique.

Figure: 2A-H.

DESCRIPTION: Thallus encrusting and smooth; pink color (Fig. 2A and 2B). Struture pseudoparenchymatous; construction dimerous (Fig. 2C), consisting of a single ventral layer of branched filaments of non-palisade cells and multicellular simple or branched filaments arising perpendicularly from the ventral layer (Fig. 2D). Epithallial cells single, squat to elliptical to spherical, 3.9–6.5 µm long and 3.6–6 µm in diameter. Subepithallial initials (3.2–5.3 µm long and 3.9–5.7 µm in diameter) as long or longer than the cells immediately subtending them (Fig. 2E). Cells of the perithallial filaments, 3.2–5.3 µm long and 3.9–5.7 µm in diameter. Cells of the hypothallial filaments, 6.4–7.8 µm long and 4.1–6.5 µm in diameter. Trichocytes not observed. Cells of the adjacent filaments joined by cell fusions (Fig. 2D). Secondary pit connections were observed only in basal filaments (Fig. 2D). Gametangial thalli monoecious (Fig. 2C). Spermatangial conceptacles small and inconspicuous, chambers measure 25.5–57.6 µm long and 35.9–56.1 µm in diameter. Simple spermatangial systems restricted to the conceptacle chamber floor (Fig. 2F). Carposporangial conceptacles with 41.5–87.9 µm long and 74.2–111.9 µm in diameter, and carposporangia (19.4–36.1 µm long and 6.6–18 µm in diameter) supported for gonimoblast filaments that arise peripherally to surface of the continuous fusion cell (Fig. 2G). Uniporate tetrasporangial conceptacles flush in the thallus surface, chambers elliptical to spherical, measure 42.8–86.3 µm long and 66.3–118.3 µm in diameter (Fig. 2H). Central columella absent. Conceptacle roofs 2-4 cells thick, comprising a single squat to elliptical to spherical epithallial cell, a single collunar meristematic cell that is 2–4 times

the length of the epithallial cell, and a small inner cell (Fig. 2H). Zonately divided tetrasporangia, 30.6–67.2 µm long and 7.9–30.7 µm in diameter.

MATERIAL EXAMINED: BRAZIL. CEARÁ: Caucaia, Praia do Pacheco, 03°41'09.18"S, 38°37'57.19"W, coll. I.O. Costa et al. s.n., 26 March 2013, (ALCB 125641 and ALCB 125642).

### *3.2. Phylogenetic datasets*

We obtained 66 partial sequences (37 *psb*A and 29 SSU rDNA) that were combined with 201 sequences downloaded from GenBank (122 *psb*A and 79 SSU rDNA). A multiple alignment was generated to each individual dataset: *psb*A included 159 sequences of 760 bp, with 12 (1.6%) variable, and 276 (36%) parsimoniously informative characters; SSU rDNA included 108 of 1,496 bp, with 95 (6%) variable, and 367 (25%) parsimoniously informative characters. Single datasets did not showed enough resolution or strong support (Figs. S2 and S3, respectively) to clarify phylogenetic relationships among the sampled groups. A concatenated dataset of the two gene regions resulted in better resolution and robust support, which we used to discuss the relationships among the group (Fig. 3). Inconsistencies found in analyzes of isolated data are also discussed below.

#### *3.2.1. Analyses of combined plastidial and nuclear data.*

We presented the topology of the most likelihood tree (Fig. 3), with ML and MP bootstrap and PP values plotted above each clade. The final alignment of the concatenated data set comprised 64 sequences for the two gene regions (*psb*A and SSU rDNA) of 2,269 base pairs (bp), with 148 (6.5%) variable, and 546 (24%) parsimoniously informative characters.

The six subfamilies within Corallinaceae also were resolved as monophyletic (Fig. 3). Corallinoideae, Lithophylloideae, Mastophoroideae were strongly supported (ML and MP=100%, PP=1.0); Neogoniolithoideae had moderate to strong support (ML=74%, MP=90% and PP=0.99); Metagoniolithoideae had strong support only in ML (94%) and BI (PP=1.0); while Hydrolithoideae had weak support in all analyses (ML=43%, MP=56% and PP=0.56).

Despite Lithophylloideae being high resolved, its internal relationships remain unclear. The members assigned to the genus *Lithophyllum* were polyphyletic with well-supported sub-clades. The Brazilian *Lithophyllum* species (*L. atlanticum*, *L. margaritae*,

and *Lithophyllum* sp.) were monophyletic with strong support (ML and MP=100%, PP=1.0).

In Neogoniolithoideae, the Brazilian *Neogoniolithon* (*N.* cf. *brassica-florida*, *N. crypticum* and *N. rhizophorae*) were monophyletic with moderate to strong support (ML and MP=100%, PP=1.0). Genbank sequences identified as *Pneophyllum confevicola* and *Spongites fruticulosus* grouped with *Neogoniolithon*, but the relationship of *Spongites fruticulosus* was poorly supported in all analyses.

Metagoniolithoideae was composed of five monophyletic groups: *Metagoniolithon*, "*Pneophyllum conicum*", and *Porolithon onkodes*, both with strong support (ML and MP=100%, PP=1.0); *Harveylithon* with weak support, although formed by several well-supported species clusters; and *Tomitolithon* gen. nov. with strong support (ML and MP=99%, PP=1.0), and formed by two well-resolved species.

3.2.2. *Analyses of individual psbA and SSU rDNA datasets.*

Both *psb*A and SSU rDNA phylogeny, recovered Corallinaceae as monophyletic with moderate to strong support (*psb*A – ML=74% and PP=0.97; SSU – PP=0.91). All the six subfamilies of Corallinaceae were resolved as monophyletic lineages, with moderate to strong support in all analyses (Figs. S2 and S3). Some species attributed to *Spongites* and *Pneophyllum* formed a clade with uncertain positioning (Figs. S2 and S3). Here we discussed the inconsistencies between the two markers.

Lithophylloideae: For *psb*A, *L. atlanticum* was sister of *Lithophyllum* sp. from Panama, with divergence of 3.88 to 4.34% (25-28bp). The Brazilian *L. margaritae* was grouped with *L. margaritae* from Mexico, diverging in 2.17% (14bp). Our *Lithophyllum* sp. specimens form a well-supported clade with *Lithophyllum* sp. from Abrolhos (Brazil), diverging in only 0.16% (1bp), and were sister of *L. neocongestum* from Panamá (ML=86%). For SSU, *L. atlanticum* was sister of *L. kotschyanum* from Australia and Japan, with divergence of 2.75% (35bp). Our *Lithophyllum* sp. formed a cluster with *L. kotschyanum* from Fiji, Hawaii and Japan, diverging in 1.25-1.33% (16-17bp). We did not obtain SSU rDNA sequences for the species *L. margaritae*.

Neogoniolithoideae: For *psb*A, the Brazilian *Neogonioltihon* cf. *brassica-florida* formed a cluster with *N. brassica-florida* from Spain and New Zealand (ML=97%, MP=99%, PP=0.98). *N. rhizophorae* was strongly supported (ML and MP=100%, PP=1.0), and was a sister of *Neogoniolithon* species from Mexico. For SSU rDNA, the Brazilian *Neogonioltihon* cf. *brassica-florida* was grouped with *N. brassica-florida*, *N. foslie*, *N.*

*spectabile*, *N. rhizophorae* and *Neogoniolithon* sp. in the same clade with moderate to strong support (ML=80%, MP=85% and PP=1.0).

Metagoniolithoideae: For *psb*A, *Harveylithon* formed two distinct clades with moderate to strong support. The Brazilian *Harveylithon* species (*H. catarinense*, *H. maris-bahiensis*, and *H. riosmenum*) were monophyletic and had strong support. For SSU rDNA, *Harveylithon* was monophyletic with strong support for BI (PP=0.98). *Tomitolithon* had strong support for the two individual markers. Sequences of *T. deniquerum* and *T. polistratum* showed interspecific divergence of 2.79% (18bp) for *psb*A marker, and of only 0.31% (4bp) for SSU rDNA.

## 4. Discussion

Investigating the diversity of non-geniculate Corallinales, we found taxa with similarities in reproductive characters to species of the genus *Harveylithon*, but with distinct vegetative characters. *Tomitolithon* is distinct from all other taxa in the subfamily Metagoniolithoideae, by presenting thallus with dimerous construction, and the presence of secondary pit connections restricted to the basal filaments. Intergeneric sequence divergence values indicate *Tomitolithon* as distinct genus. In all analyses and for all markers *Tomitolithon* was well-supported as monophyletic. This new genus was closely related to the genus *Harveylithon*, forming a sister clade. Based on these data, we amended here the concept and circumscription of Metagoniolithoideae.

Only vegetative characters, such as presence or absence of trichocytes, and thallus thickness distinguish *Tomitolithon deniquerum* and *T. polistratum*. Comparing the two markers used in our analyses, SSU rDNA presented low genetic divergence between *T. deniquerum* and *T. polistratum* (0.31%). Despite this, these species were clearly distinguished on *psb*A analyses (2.79%). Some studies have shown that SSU has not been efficient to segregate species and its use is more efficient for the delimitation of orders, families and subfamilies (Hind and Saunders, 2013; Adey et al., 2015; Hind et al., 2016). The *psb*A has been largely used to infer relationships on generic and specific levels in Corallinales. This region is also useful for identifying recently diverged taxa that could not be identified with more conserved markers (Broom et al., 2008; Bittner et al., 2011; Hind and Saunders, 2013; Torrano-Silva et al., 2014; Hernández-Kantún et al., 2015, 2016; Hind et al., 2016).

Our phylogenetic analyses based on independent and combined *psb*A and SSU rDNA datasets confirmed the monophyletism of Corallinaceae, corroborating previous

findings (Broom et al., 2008; Bittner et al., 2011; Nelson et al., 2015; Rösler et al., 2016). Our data recovered the six current subfamilies of Corallinaceae as monophyletic lineages. This result is consistent with the phylogenies inferred by Rösler et al. (2016).

Our phylogeny based on the combined dataset, recovered Corallinoideae as sister of Neogoniolithoideae, but with weak to moderate support depending on the method used to infer the trees. The relationship between these two subfamilies has already been pointed out in previous studies (Bailey et al., 2004; Kato et al., 2011; Hernandez-Kantun et al., 2015; Hind et al., 2016). Members of these two subfamilies share some reproductive characters: spermatangia born on the floor and walls of male conceptacles; gonimoblast filaments arising from dorsal and/or marginal surfaces of the fusion cell; and tetrasporangial conceptacles formed by filaments peripherical to the fertile area (Bailey et al., 2004; Bittner et al., 2011; Hind and Saunders, 2013; Hind et al., 2016).

Neogoniolithoideae was proposed by Kato et al. (2011) to include only the genus *Neogoniolithon*. Rosler et al. (2016) emended this subfamily and proposed the inclusion of *Spongites fruticulosus* (generitype of *Spongites*) and *Pneophyllum confervicola* in Neogoniolithoideae. In our analyses *Pneophyllum confervicola* cluster with *Neogoniolithon*, with moderate to strong support. *Spongites fruticulosus* cluster with *Neogoniolithon* and *Pneophyllum confevicola*, but with weak support. In the single analyses of the SSU rDNA marker, the position of *Spongites fruticulosus* was uncertain. Thus, we recommend the maintenance of only *Neogoniolithon* in the subfamily Neogoniolithoideae until additional molecular data related to the species *Pneophyllum confevicola* and *Spongites fruticulosus* are accessed. Costa et al. (2017b, submitted) discussed the relationships between the Brazilian *Neogoniolithon* species, and presented detailed description with illustrations.

Phylogenetic trees based on single markers and on the combined dataset recovered Lithophylloideae as sister of Metagoniolithoideae, with support values depending on the method used to infer the trees. The relationships between the representatives of these two subfamilies was evidenced in previous studies (Harvey et al., 2003; Bailey et al., 2004; Broom et al., 2008; Hernandez-Kantun et al., 2015; Hind et al., 2016). However, the analyses performed by Rosler et al. (2016) recovered Lithophylloideae as sister of a clade formed by species of *Spongites* and *Pneophyllum*, but with weak support.

In our analyses, Lithophylloideae was represented by four genera (*Amphiroa*, *Lithophyllum*, *Paulsilvella* and *Titanoderma*). In all analyses, *Lithophyllum* was not resolved as monophyletic. This genus formed at least three distinct lineages, and one of

them was strictly close to the genera *Amphiroa*, *Paulsilvella* and *Titanoderma*. This result was in agreement with previous phylogenetic analyses (Bittner et al., 2011; Hernadez-Kantun et al., 2015; Nelson et al., 2015). Brazilian *Lithophyllum* species were monophyletic with strong support. *L. atlanticum* is morpho-anatomically similar to *L. margaritae*, differing only in the number of cells of the tetrasporangial conceptacle roof. In addition, these two species showed high interspecific genetic divergence. *Lithophyllum* sp. formed a clade well-supported in all analyses performed here. For *psb*A, *Lithophyllum* sp. was placed as sister of *L. neocongestum* from Panama. In SSU rDNA, *Lithophyllum* sp. was sister of *L. kotschyanum* from Hawaii and Japan, with high interspecific genetic divergence. This suggests that *Lithophyllum* sp. corresponds to a new species. However, no fertile specimens were found in our research. Future investigations, including analyses of more specimens, are required before proposing *Lithophyllum* sp. as a new species. Sequences attributed to *L. kotschyanum* occurred in different clades, indicating that they are not the same species.

Metagoniolithoideae was represented by five different lineages: *Metagoniolithon*, *Porolithon*, "*Pneophyllum conicum*", *Harveylithon*, and the new genus *Tomitolithon*. *Metagoniolithon* was sister of *Porolithon*; and "*Pneophyllum conicum*"Was sister of *Harveylithon* and *Tomitolithon*. In Rösler et al. (2016), "*Pneophyllum conicum*"Was sister of *Harveylithon. Tomitolithon* formed a clade monophyletic well-supported, and was sister of *Harveylithon* in concatenated and single SSU rDNA phylogenies. Costa et al. (2017a, submitted) discussed the relationship between the Brazilian *Harveylithon* species, and presented detailed description with illustrations. The inclusion of the Brazilian sequences improved the understanding of the relationship between species of Metagoniolithoideae.

## 5. Conclusion

This is the first analysis of the phylogenetic relationships within Corallinales that included non-geniculate representatives of the Southwestern Atlantic. The inclusion of the Brazilian sequences improves the understanding of the relationship between species, within the distinct subfamilies of Corallinaceae. Our analyses confirmed the monophyletism of Corallinaceae, and their respective current subfamilies. The subfamily Metagoniolithoideae was amended, with the inclusion of the new genus *Tomitolithon* with two news species. We emphasize the need for revision of specimens and type materials of species from the genera *Spongites* and *Pneophyllum* for the formal definition of this clade.

**Acknowledgements**

This research has been funded by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, BrBOL 564945/2010-2 and UNIVERSAL 477614/2013-2) and the Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB, PRONEM T.O. PNE. 0020/2011 and REDES T.O. RED006/2012). We thank the Central Laboratory of Electronic Microscopy (LCME) of the Federal University of Santa Catarina (UFSC) for the availability of SEM equipment. IOC and PBJ acknowledge scholarships from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and CNPq, respectively. JMCN acknowledge CNPq (307368/2015-7). PAH acknowledge CNPq (PROTAX 440575/2015-0), Fundação de Apoio à Pesquisa Científica e Tecnológica do Estado de Santa Catarina (FAPESC, 2015TR1702), CAPES, Rede Clima, FAPESP, Fundação BOTICÁRIO.

**References**

Amado-Filho, G.M., Moura, R.L., Bastos, A.C., Salgado, L.T., Sumida, P.Y., et al. 2012a. Rhodolith Beds Are Major CaCO3 Bio-Factories in the Tropical South West Atlantic. *PLoS ONE* 7:e35171 [online].

Amado-Filho, G.M., Moura, R.L., Bastos, A.C., Salgado, L.T., Sumida, P.Y.; Guth, A.Z., Francini-Filho, R.B., Pereira-Filho, G.H.; Abrantes, D.P., Brasileiro, P.S., Bahia, R.G., Leal, R.N., Kaufman, L., Kleypas, J.A.; Farina, M., Thompson, F.L. 2012b. Rhodolith Beds Are Major CaCO3 Bio-Factories in the Tropical South West Atlantic. PLoS ONE, 7: e35171 [online].

Bahia, R.G., Amado-Filho, G.M.; Azevedo, J., Maneveldt, G.W. 2014. *Porolithon improcerum* (Porolithoideae, Corallinaceae) and *Mesophyllum macroblastum* (Melobesioideae, Hapalidiaceae): new records of crustose coralline red algae for the Southwest Atlantic Ocean. *Phytotaxa*, 190: 38-44.

Bailey, J.C. 1999. Phylogenetic positions of *Lithophyllum incrustans* and *Titanoderma pustulatum* (Corallinaceae, Rhodophyta) based on 18S rRNA gene sequence analyses, with a revised classification of the Lithophylloideae. *Phycologia* 38(3): 208–216.

Bailey, J.C. and Chapman, R.L. 1998. A phylogenetic study of the Corallinales (Rhodophyta) based on nuclear small-subunit rRNA gene sequences. *J. Phycol*. 34: 692–705.

Bailey, J.C. and Chapman, R.L. 1996. Evolutionary relationships among coralline red algae (Corallinaceae, Rhodophyta) determined by 18S rRNA gene sequence analysis. In: Chaudhary, B.R. and Agrawal, S.B. (Eds.) *Cytology, Genetics and Molecular Biology of Algae.* SPB Academic Publishing, Amsterdam. 363–376.

Bailey, J.C., Gabel, J.E. and Freshwater, D.W. 2004. Nuclear 18S rRNA gene sequence analyses indicate that the Mastophoroideae (Corallinaceae, Rhodophyta) is a polyphyletic taxon. *Phycologia* 43: 3–12.

Barry G.C. and Woelkerling W.J. 1995. Non-geniculate species of Corallinaceae (Corallinales, Rhodophyta) in Shark Bay, Western Australia: Biodiversity, salinity tolerances and biogeographic affinities. *Botanica Marina* 38: 135–149.

Bittner, L., Payri, C.E., Maneveldt, G.W., Couloux, A., Cruaud, C., de Reviers, B. and Le Gall, L. 2011. Evolutionary history of the Corallinales (Corallinophycidae, Rhodophyta) inferred from nuclear, plastidial and mitochondrial genomes. *Mol. Phylogenet. Evol.* 61: 697–713.

Broom, J. E. S., Hart, D. R., Farr, T. J., Nelson, W. A., Neil, K. F., Harvey, A. S. and Woelkerling, W. J. 2008. Utility of psbA and SSU rDNA for phylogenetic reconstruction in the Corallinales based on New Zealand taxa. *Mol. Phylogenet. Evol.* 46: 958- 973.

Cabioch, J. 1971. Essai d'une nouvelle classification des Corallinacees actuelles. *Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences, Paris* 272D: 1616-1619.

Cabioch, J. 1988. Morphogenesis and generic concepts in coralline algae - a reappraisal. *Helgoltinder Meeresuntersuchungen* 42: 493- 509.

Chamberlain, Y. M. 1983. Studies in the Corallinaceae with special reference to *Fosliella* and *Pneophyllum* in the British Isles. *Bulletin of the British Museum (Natural History) Botany*, 11: 291-463.

Chamberlain, Y. M. 1993. Observations on the crustose coralline red alga *Spongites yendoi* (Foslie) comb. nov. in South Africa and its relationship to *S. decipiens* (Foslie) comb. nov. and *Lithophyllum natalense* Foslie. *Phycologia* 32: 100–15.

Costa, I.O., Horta P.A., Nunes, J.M.C. 2014a. *Spongites yendoi* (Foslie) Chamberlain (Corallinales, Rhodophyta) on the coast of Bahia, Brazil. *Braz. J. Bot.* 37(4): 637-641.

Costa, I. O., Horta, P. A., Bergstrom, E. R., Nunes, J. M. C. 2014b. Taxonomic study of crustose coralline algae off the northeastern Brazilian coast. *Phytotaxa*, 190(1): 130-161.

Costa, I.O., Jesus, P.B., Jesus, T.S., Souza, P.S., Horta P.A., Nunes, J.M.C. 2017a - submitted. Reef-building coralline algae from the South Atlantic: filling gaps with the recognition of *Harveylithon* (Corallinaceae, Rhodophyta) on the Brazilian coast. *J. Phycol*.

Costa, I.O., Jesus, Araújo, V.L., Horta P.A., Nunes, J.M.C. 2017b - submitted. *Neogoniolithon* from the Southwestern Atlantic: molecular and morpho-anatomical analyses reveal taxonomic novelty. *J. Phycol*.

Crespo, T. M., Bahia, R. G., Maneveldt, G. W., Amado Filho, G. M. 2014. Floristic composition of crustose coralline algae from the St. Peter and St. Paul Archipelago, a summit of the Mid-Atlantic Ridge. *Phytotaxa*, 190(1): 17-37.

Doyle, J. J. and Doyle, J. L. 1987. A rapid DNA isolation method for small quantities of fresh tissues. *Phytochem. Bull.* 19: 11–15.

Farr, T., Broom, J., Hart, D., Neill, K., Nelson, W. 2009. Common coralline algae of northern New Zealand: an identification guide. *NIWA Information Series*, 70: 125p.

Figueiredo, M.A.O.; Steneck, R.S. 2002. Floristic and ecological studies of crustose coralline algae on Brazil`s Abrolhos reefs. *9th International Coral Reef Symposium, Bali* 1:493-497.

Foslie, M. and Howe, M.A. 1906. New American coralline algae. *Bulletin of the New York Botanical Garden* 4: 128–136, Plates 80–93.

Foslie, M. 1909. *Algologiske notiser VI. Kongelige Norske Videnskabers Selskabs Skrifter* 2: 1–63.

Foster, M.S. 2001. Rhodoliths: between rocks and soft places - Minireview. *J. Phycol*., 37: 659-667.

Guiry, M. D. and Guiry, G. M. 2017. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Available at: http://www.algaebase.org (last accessed 30 April 2017).

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser*. 41: 95–98.

Harvey, A. and Woelkerling, W. 2007. A guide to non geniculate coralline red algal (Corallinales, Rhodophyta) rhodolith identification. *Cienc. Mar.*, 33: 411-426.

Harvey, A. S., Broadwater, S. T., Woelkerling, W. J. and Mitrovski, P. J. 2003. *Choreonema* (Corallinales, Rhodophyta): 18S rDNA phylogeny and resurrection of the Hapalidiaceae for the subfamilies Choreonematoideae, Australithoideae, and Melobesioideae. *J. Phycol.* 39: 988–98.

Harvey, A. S., Phillips, L. E., Woelkerling, W. J. and Millar, A. J. K. 2006. The Corallinaceae, subfamily Mastophoroideae (Corallinales, Rhodophyta) in south-eastern Australia. *Aust. Syst. Bot.* 19: 387–429.

Hernandez-Kantun, J. J., Rindi, F., Adey, W. H., Heesch, S., Peña, V., Le Gall, L., And Gabrielson, P. W. 2015. Sequencing type material resolves the identity and distribution of the generitype *Lithophyllum incrustans*, and related European species *L. hibernicum* and *L. bathyporum* (Corallinales, Rhodophyta). *J. Phycol*. 51: 791-807.

Hernández-Kantún, J.J., Gabrielson, P., Hughey, J.R., Pezzolesi, L., Rindi, F., Robinson, N.M., Peña, V., Riosmena-Rodriguez, R., Le Gall, L., and Adey, W. 2016. Reassessment of branched *Lithophyllum* spp. (Corallinales, Rhodophyta) in the Caribbean Sea with global implications. *Phycologia* 55: 619-639.

Hind, K. R., and Saunders, G. W. 2013. A Molecular Phylogenetic Study of the Tribe Corallineae (Corallinales, Rhodophyta) with an Assessment of Genus-Level Taxonomic Features and Descriptions of Novel Genera. *J. Phycol*. 49(1): 103-114.

Hind, K. R., Gabrielson, P. W., P Jensen, C., and Martone, P. T. 2016. *Crusticorallina* gen. nov., a non-geniculate genus in the subfamily Corallinoideae (Corallinales, Rhodophyta). *J. Phycol.* 52:929-41.

Horta, P.A. 2000. *Macroalgas do infralitoral do sul e sudeste do Brasil: Taxonomia e Biogeografia.* Tese de Doutorado. Universidade de São Paulo, São Paulo. 301p.

Horta, P.A. 2002. Bases para a identificação das coralináceas não articuladas do litoral brasileiro – uma síntese do conhecimento. *Biotemas*. 15: 7-44.

Irvine, L. M. and Chamberlain, Y. M. 1994. *Seaweeds of the British Isles. vol I Rhodophyta Part 2B Corallinales, Hildenbrandiales.* HMSO, London, 276pp.

Iryu, Y. and Matsuda, S. 1994. Taxonomic studies of the *Neogoniolithon fosliei* complex (Corallinaceae, Rhodophyta) in the Ryukyu Islands. *Trans. Proc. Palaeont. Soc. Jpn. N. S.* 174: 426–48.

Johansen, H .W. 1969. Morphology and systematics of coralline algae with special reference to *Calliarthron. University of California Publications in Botany* 49: 1-98.

Kato, A., Baba, M. and Suda, S. 2011. Revision of the Mastophoroideae (Corallinales, Rhodophyta) and polyphyly in non-geniculate species widely distributed on Pacific coral reefs. *J. Phycol*. 3: 662-672.

Kato, A., Baba, M. and Suda, S. 2013. Taxonomic circumscription of heterogeneous species *Neogoniolithon brassica-florida* (Corallinales, Rhodophyta) in Japan. *Phycol. Res.*, 61: 15–26.

Le Gall, L. and Saunders, G. W. 2007. A nuclear phylogeny of the Florideophyceae (Rhodophyta) inferred from combined EF2, small subunit and large subunit ribosomal DNA: establishing the new red algal subclass Corallinophycidae. *Mol. Phylogenet. Evol.* 43:1118–30.

Le Gall, L., Payri, C. E., Bittner, L. and Saunders, G. W. 2010. Multigene phylogenetic analyses support recognition of the Sporolithales ord. nov. *Mol Phylogenet Evol*. 54(1):302-5.

Mariath, R., Riosmena-Rodriguez R., Figueiredo, M. 2012. *Lithothamnion steneckii* sp. nov. and *Pneophyllum conicum*: new coralline red algae (Corallinales, Rhodophyta) for coral reefs of Brazil. *Algae,* 27: 249-258.

Mateo-Cid, L.E., Mendoza-González, A.C. and Gabrielson, P.W. 2014. *Neogoniolithon* (Corallinales, Rhodophyta) on the Atlantic coast of México, including *N. siankanensis* sp. nov. *Phytotaxa* 190(1): 64-93.

Milstein, D. and Oliveira, M.C. 2005. Molecular phylogeny of Bangiales (Rhodophyta) based on small subunit rDNA sequencing: emphasis on Brazilian *Porphyra* species. *Phycologia* 44:212-221.

Moura, C.W.N., Kraus, J.E and Cordeiro-Marino, M. 1997. Metodologia para obtenção de cortes histológicos com historresina e coloração com azul de toluidina O para algas coralináceas (Rhodophyta, Corallinales). *Hoehnea* 24 (2): 17-27.

Moura, R.L., Amado-Filho, G.M., Moraes, F.C., Brasileiro, P.S., Salomon, P.S., Mahiques, M.M., Bastos, A.C., Almeida, M.G., Silva, J.M., Araujo, B.F., Brito, F.P., Rangel, T.P., Oliveira, B.C.V., Bahia, R.G., Paranhos, R.P., Dias, R.J.S., Siegle, E., Figueiredo, A.G., Pereira, R.C., Leal, C.V., Hajdu, E., Asp, N.E., Gregoracci, G.B., Neumann-Leitao, S., Yager, P.L., Francini-Filho, R.B., Froes, A., Campeão, M., Silva, B.S., Moreira, A.P.B., Oliveira, L., Soares, A.C., Araujo, L., Oliveira, N.L., Teixeira, J.B., Valle, R.A.B., Rezende, C.E., Thompson, C.C. 2016. An extensive reef system at the Amazon River mouth. *Science Advances,* 2: e1501252-e1501252.

Nelson, W. A., Sutherland, J. E., Farr, T. J., Hart, D. R., Neill, K. F., Kim, H. J., and Yoon, H. S. 2015. Multi-gene phylogenetic analyses of New Zealand coralline algae: *Corallinapetra novaezelandiae* gen. et sp. nov. and recognition of the Hapalidiales ord. nov. *J. Phycol.* 51: 454–468.

Nunes, J.M.C., Guimarães, S.M.P.B., Donnangelo, A., Farias, J., Horta, P.A. 2008. Aspectos taxonômicos de três espécies de coralináceas não geniculadas do litoral do Estado da Bahia, Brasil. *Rodriguesia* 59(1): 75-86.

Nylander, J. A. A., 2008. *MrModeltest 2.3*. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Posada, D. and Buckley, T. R. 2004. Model selection and model averaging in phylogenetics: analysis of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Syst. Biol.* 53: 793–808.

Riosmena-Rodriguez, R. 1993. Una propuesta de tecnica histological para el studio de algas coralinas (Corallinales: Rhodophyta). *Rev. Inv. Cient.* 4: 65-73.

Ronquist, F. and Huelsenbeck, J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 572–1574.

Rösler, A., Perfectti, F., Peña, V. and Braga, J. C. 2016. Phylogenetic relationships of corallinaceae (Corallinales, Rhodophyta): taxonomic implications for reef-building corallines. *J. Phycol*. 52: 412–31.

Saunders, G. W. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Phil. Trans. R. Soc. B* 360: 1879–1888.

Setchell, W. A. and Mason, L. R. 1943. *Goniolithon* and *Neogoniolithon*: two genera of crustaceous coralline algae. *Proc. Natl. Acad. Sci. U.S.A.* 29: 87–92.

Silva, P.C. and Johansen, H.W. 1986. A reappraisal of the order Corallinales (Rhodophyceae). *European J. Phycol.* 21(3): 245–254.

Sissini, M. N., Oliveira, M. C., Gabrielson, P. W., Robinson, N. M., Okolodkov, Y. B., Riosmena-Rodríguez, R., and Horta, P. A. 2014. *Mesophyllum erubescens* (Corallinales, Rhodophyta)—so many species in one epithet. *Phytotaxa*, 190(1): 299-319.

Staden, R., Judge, D. P. and Bonfield, J. K. 2003. Analysing sequences using the Staden Package and EMBOSS. *In* Krawetz, S. A. and Womble, D. D. [Eds.] *Introduction to Bioinformatics. A Theoretical and Practical Approach.* Human Press Inc, pp. 393-410.

Stamatakis, A., Hoover, P. and Rougemont, J. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Syst. Biol.* 57: 758–771.

Swofford, D. L. 2002. *PAUP*: Phylogenetic Analysis Using Parsimony (*and Many Other Methods). Version 4.0b10.* Sinauer Associates, Sunderland, Massachusetts.

Tâmega, F.T.S.; Riosmena-Rodrigues, R.; Mariath, R.; Figueiredo, M.A.O. 2014. Non-geniculate coralline red algae (Rhodophyta: Corallinales) in coral reefs from Northeastern Brazil and a description of *Neogoniolithon atlanticum* sp. nov. *Phytotaxa*, 190(1): 277-298.

Thompson, J. D., Higgins, D. G. and Gibson, T. J. 1994. Clustal, W: Improving the sensitivity of progressive weighting position-specific gap penalties and weight matrix choice. *Nucl. Acids Res.* 22: 4673–4680.

Torrano-Silva, B. N., Riosmena-Rodríguez, R., and De Oliveira, M. C. 2014. Systematic position of Paulsilvella in the Lithophylloideae (Corallinaceae, Rhodophyta) confirmed by molecular data. *Phytotaxa* 190(1): 94-111.

Verheij, E. 1994. Non-geniculate Corallinaceae (Corallinales, Rhodophyta) from the Spermonde Archipelago, SW Sulawesi, Indonesia. *Blumea* 39: 95–137.

Vidal, R., Meneses, I. and Smith, M. 2008. Phylogeography of the genus *Spongites* (Corallinales, Rhodophyta) from Chile. *J. Phycol*. 44:173–82.

Vieira-Pinto, T., Oliveira, M. C., Bouzon, J., Sissini, M., Richards, J. L., Riosmena-Rodríguez, R., and Horta, P. A. 2014. *Lithophyllum* species from Brazilian coast: range extension of *Lithophyllum margaritae* and description of *Lithophyllum atlanticum* sp. nov. (Corallinales, Corallinophycidae, Rhodophyta). *Phytotaxa* 190(1): 355-369.

Villas-Bôas, A.B., Riosmena-Rodriguez, R., Tâmega, F.T.S., Amado-Filho, G.M., Maneveldt, G.W. and Figueredo, M.A.O. 2015. Rhodolith-forming species of the subfamilies Neogoniolithoideae and Hydrolithoideae (Rhodophyta, Corallinales) from Espírito Santo State, Brazil. *Phytotaxa* 222(3): 169-184.

Villas-Boas, A.B.; Riosmena-Rodriguez, R.; Amado-Filho, G.M.; Maneveldt, G.W.; Figueiredo, M.A.O. 2009. Rhodolith-forming species of *Lithophyllum* (Corallinales; Rhodophyta) from Espírito Santo, Brazil, including the description of *L. depressum* sp. nov. *Phycologia*, 48: 237-248.

Woelkerling, W. J. 1980. Studies on *Metamastophora* (Corallinaceae, Rhodophyta). I. *M. flabellata* (Sonder) Setchell: morphology and anatomy. *Br. Phycol. J.* 15:201–25.

Woelkerling, W. J. 1985. A taxonomic reassessment of *Spongites* (Corallinaceae, Rhodophyta) based on studies of Kützing's original collections. Br. Phycol., 20: 123-153.

Woelkerling, W. J. 1988. The coralline red algae: an analysis of the genera and sub-families of nongeniculate Corallinaceae. British Museum Natural History and Oxford University Press, London.

Woelkerling, W. J. 1996. The order Corallinales. In: Womersley HBSW (ed) The marine benthic flora of Southern Australia. Part IIIB, Gracilariales, Rhodymeniales, Corallinales and Bonnemaisoniales. Australian Biological Resources Study, Canberra. 237–283.

Woelkerling, W. J. 1998. Type collections of non-geniculate corallines housed at the Laboratoire de Cryptogamie (PC). In: Woelkerling, W.J. Eds. Non-geniculate coralline red algae and the Paris Muséum: systematics and scientific history. Paris: Muséum National d'Histoire naturelle / ADAC, p. 279-404.

Woelkerling, W. J., Penrose, D. and Chamberlain, Y. M. 1993. A reassessment of type collections of non-geniculate Corallinaceae (Corallinales, Rhodophyta) described by C. Montagne and L. Dufour, and of *Melobesia brassica-florida* Harvey. *Phycologia* 32: 323–31.

Zhang, J., Kapli, P., Pavlidis, P. and Stamatakis, A. 2013. A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* 29: 2869–2876.

Zuljevic, A.; Kaleb, S.; Peña, V.; Despalatovic, I.; Cvitkovic, I.; De Clerck, O.; Le Gall, L.; Falace, A.; Vita, F.; Braga, J.C.; and Antolic, B. 2016. First freshwater coralline alga and the role of local features in a major biome transition. *Scientific Reports* 6(19642): 1-12.

Yoon, H.S., Hackett, J.D. and Bhattacharya, D. 2002. A single origin of the peridinin and fucoxanthin containing plastids in dinoflagellates through tertiary endosymbiosis. *Proc. Natl. Acad. Sci.*99:11724–29.

Table 1. Sequences generated in this study, with voucher information, herbarium accession numbers and GenBank accession numbers for each DNA marker. (–) no molecular data was produced, (+) generated sequences.

| Species | Voucher | Markers | | Locality | Coordinate | Collection date |
|---|---|---|---|---|---|---|
| | | *psb*A | 18S | | | |
| *Harveylithon catarinense* | ALCB 125627 | + | + | Ilha Deserta, Bombinhas, Santa Catarina | 27°27'54.22"S x 48°35'91.44"W | 02/03/2016 |
| *Harveylithon catarinense* | ALCB 125628 | + | + | Ilha Deserta, Bombinhas, Santa Catarina | 27°27'54.22"S x 48°35'91.44"W | 02/03/2016 |
| *Harveylithon maris-bahiensis* | ALCB 125620 | + | + | Morro de São Paulo, Cairú, Bahia | 13°22' 44"S x 38°54' 46"W | 06/10/2013 |
| *Harveylithon maris-bahiensis* | ALCB 125621 | - | + | Morro de São Paulo, Cairú, Bahia | 13°22' 44"S x 38°54' 46"W | 06/10/2013 |
| *Harveylithon maris-bahiensis* | ALCB 125622 | + | + | Morro de São Paulo, Cairú, Bahia | 13°22' 44"S x 38°54' 46"W | 06/10/2013 |
| *Harveylithon riosmenum* | ALCB 125625 | + | - | Praia de Pina, Boa Viagem, Pernambuco | 8°05'25.52"S x 34°52'46.59"W | 29/04/2014 |
| *Harveylithon riosmenum* | ALCB 125626 | - | - | Praia de Pina, Boa Viagem, Pernambuco | 8°05'25.52"S x 34°52'46.59"W | 29/04/2014 |
| *Harveylithon riosmenum* | ALCB 125624 | + | + | Ens. dos Corais, C. St° Agostinho, Pernambuco | 8°18'50.3"S x 34°56'47.8"W | 26/04/2014 |
| *Harveylithon riosmenum* | ALCB 125612 | + | + | Pontal de Guaibura, Guarapari, Espírito Santo | 20°43'40.86"S x 40°31'19.32"W | 20/08/2013 |
| *Harveylithon riosmenum* | ALCB 125613 | - | - | Pontal de Guaibura, Guarapari, Espírito Santo | 20°43'40.86"S x 40°31'19.32"W | 20/08/2013 |
| *Harveylithon riosmenum* | ALCB 125614 | + | + | Pontal de Guaibura, Guarapari, Espírito Santo | 20°43'40.86"S x 40°31'19.32"W | 20/08/2013 |
| *Harveylithon riosmenum* | ALCB 125615 | + | + | Pontal de Guaibura, Guarapari, Espírito Santo | 20°43'40.86"S x 40°31'19.32"W | 20/08/2013 |
| *Harveylithon riosmenum* | ALCB 125616 | - | - | Praia de Ubú, Anchieta, Espírito Santo | 20°48'49.36"S x 40°36'42.45"W | 21/08/2013 |

| Species | Voucher | Markers | | Locality | Coordinate | Collection date |
|---|---|---|---|---|---|---|
| | | *psb*A | 18S | | | |
| *Harveylithon riosmenum* | ALCB 125617 | + | + | Praia de Ubú, Anchieta, Espírito Santo | 20°48'49.36"S x 40°36'42.45"W | 21/08/2013 |
| *Harveylithon riosmenum* | ALCB 123210 | + | + | Praia da Conchas, Cabo Frio, Rio de Janeiro | 22°52'16.8"S x 41°53'51.2"W | 26/05/2013 |
| *Harveylithon riosmenum* | ALCB 125611 | - | - | Praia da Conchas, Cabo Frio, Rio de Janeiro | 22°52'16.8"S x 41°53'51.2"W | 26/05/2013 |
| *Harveylithon riosmenum* | ALCB 125618 | + | + | Praia Rasa, Búzios, Rio de Janeiro | 23°02'34.4"S x 43°30'29.1"W | 25/05/2013 |
| *Harveylithon riosmenum* | ALCB 125619 | - | + | Praia Rasa, Búzios, Rio de Janeiro | 23°02'34.4"S x 43°30'29.1"W | 25/05/2013 |
| *Harveylithon riosmenum* | ALCB 123166 | + | + | Ilha Deserta, Bombinhas, Santa Catarina | 27°27'54.22"S x 48°35'91.44"W | 02/03/2016 |
| *Lithophyllum atlanticum* | IBC1527 | + | - | Ilha da Rapada, Ubatuba, São Paulo | 23°51'76.79"S x 45°04'10.72"W | 12/01/2012 |
| *Lithophyllum atlanticum* | ALCB 125751 | + | + | Praia do Miguel, Ilha do Mel, Paraná | 25°33'43.8"S x 48°18'05.4"W | 15/05/2014 |
| *Lithophyllum atlanticum* | ALCB 125752 | + | + | Praia do Miguel, Ilha do Mel, Paraná | 25°33'43.8"S x 48°18'05.4"W | 15/05/2014 |
| *Lithophyllum atlanticum* | ALCB 125756 | + | + | Praia do Miguel, Ilha do Mel, Paraná | 25°33'43.8"S x 48°18'05.4"W | 15/05/2014 |
| *Lithophyllum atlanticum* | ALCB 125753 | - | - | Praia do Miguel, Ilha do Mel, Paraná | 25°33'43.8"S x 48°18'05.4"W | 15/05/2014 |
| *Lithophyllum atlanticum* | ALCB 125754 | + | + | Praia do Miguel, Ilha do Mel, Paraná | 25°33'43.8"S x 48°18'05.4"W | 15/05/2014 |
| *Lithophyllum atlanticum* | ALCB 125755 | + | + | Praia do Miguel, Ilha do Mel, Paraná | 25°33'43.8"S x 48°18'05.4"W | 15/05/2014 |
| *Lithophyllum atlanticum* | ALCB 125757 | + | + | Praia do Miguel, Ilha do Mel, Paraná | 25°33'43.8"S x 48°18'05.4"W | 15/05/2014 |

| Species | Voucher | Markers | | Locality | Coordinate | Collection date |
|---|---|---|---|---|---|---|
| | | *psb*A | 18S | | | |
| *Lithophyllum margaritae* | ALCB 125648 | + | - | Praia do Forte, Mata de São João, Bahia | 12°34'41"S x 38°00'06"W | 25/07/2013 |
| *Lithophyllum margaritae* | IBC1726 | + | - | Coroa, Ilha de Itaparica, Bahia | 13°00' 35.30"S x 38°38' 27.4"W | 24/05/2013 |
| *Lithophyllum* sp. | ALCB 125756 | + | + | Morro de São Paulo, Cairú, Bahia | 13º23'04.4"S x 38º54'00.1"W | 23/03/2014 |
| *Lithophyllum* sp. | IBC1872 | + | - | Ilhote de Ubú, Anchieta, Espírito Santo | 20°80'83.66"S x 40°59'88.26"W | 08/09/2014 |
| *Lithophyllum* sp. | IBC1873 | + | - | Ilhote de Ubú, Anchieta, Espírito Santo | 20°80'83.66"S x 40°59'88.26"W | 08/09/2014 |
| *Neogoniolithon* cf. *brassica-florida* | ALCB 125632 | + | + | Praia das Castanheiras, Guarapari, Espírito Santo | 20°40'19.64"S x 40°29'46.01"W | 20/08/2013 |
| *Neogoniolithon* cf. *brassica-florida* | ALCB 125631 | + | - | Praia das Castanheiras, Guarapari, Espírito Santo | 20°40'19.64"S x 40°29'46.01"W | 20/08/2013 |
| *Neogoniolithon crypticum* | ALCB 125629 | + | + | Praia da Conchas, Cabo Frio, Rio de Janeiro | 22°52'16.8"S x 41°53'51.2"W | 26/05/2013 |
| *Neogoniolithon crypticum* | ALCB 125630 | + | - | Praia da Conchas, Cabo Frio, Rio de Janeiro | 22°52'16.8"S x 41°53'51.2"W | 26/05/2013 |
| *Neogoniolithon rhizophorae* | ALCB 125638 | + | + | Praia do Cabo, C. St° Agostinho, Pernambuco | 8°18'49.5"S x 34°56'48.4"W | 27/04/2014 |
| *Neogoniolithon rhizophorae* | ALCB 125639 | + | + | Praia do Cabo, C. St° Agostinho, Pernambuco | 8°18'49.5"S x 34°56'48.4"W | 27/04/2014 |
| *Neogoniolithon rhizophorae* | ALCB 125635 | - | + | Morro de São Paulo, Cairú, Bahia | 13°22' 44"S x 38°54' 46"W | 06/10/2013 |
| *Neogoniolithon rhizophorae* | ALCB 125636 | + | | Morro de São Paulo, Cairú, Bahia | 13°22' 44"S x 38°54' 46"W | 06/10/2013 |
| *Neogoniolithon rhizophorae* | ALCB 125637 | + | + | Morro de São Paulo, Cairú, Bahia | 13°22' 44"S x 38°54' 46"W | 06/10/2013 |
| *Neogoniolithon rhizophorae* | ALCB 125640 | + | + | Garapuá, Cairú, Bahia | 13°29'13.14"S x 38°54'25.5"W | 03/07/2015 |

| Species | Voucher | Markers | | Locality | Coordinate | Collection date |
|---|---|---|---|---|---|---|
| | | *psb*A | 18S | | | |
| *Neogoniolithon rhizophorae* | ALCB 125634 | - | + | Ponta do Mutá, Maraú, Bahia | 13°52'45.61"S x 38°56'50.79"W | 04/10/2013 |
| *Pneophyllum conicum* | ALCB 125658 | + | - | Praia de Itapuã, Salvador, Bahia | 12°57' 22"S x 38°21' 31"W | 29/09/2011 |
| *Porolithon onkodes* | ALCB 125642 | + | + | Praia de Parati, Anchieta, Espírito Santo | 20°48'19.28"S x 40°36'07.45"W | 21/08/2013 |
| *Tomitolithon deniquerum* | ALCB 125643 | + | - | Ponta das Pedras, Goiana, Pernambuco | 7°37'47.9"S x 34°48'31.2"W | 30/04/2014 |
| *Tomitolithon deniquerum* | ALCB 125644 | + | + | Ponta das Pedras, Goiana, Pernambuco | 7°37'47.9"S x 34°48'31.2"W | 30/04/2014 |
| *Tomitolithon deniquerum* | ALCB 125645 | - | - | Ponta das Pedras, Goiana, Pernambuco | 7°37'47.9"S x 34°48'31.2"W | 30/04/2014 |
| *Tomitolithon deniquerum* | ALCB 125646 | + | + | Ponta das Pedras, Goiana, Pernambuco | 7°37'47.9"S x 34°48'31.2"W | 30/04/2014 |
| *Tomitolithon deniquerum* | ALCB 125647 | + | + | Ponta das Pedras, Goiana, Pernambuco | 7°37'47.9"S x 34°48'31.2"W | 30/04/2014 |
| *Tomitolithon polistratum* | ALCB 125641 | + | + | Praia do Pacheco, Caucaia, Ceará | 3°41'09.18"S x 38°37'57.19"W | 26/03/2013 |
| *Tomitolithon polistratum* | ALCB 125642 | - | - | Praia do Pacheco, Caucaia, Ceará | 3°41'09.18"S x 38°37'57.19"W | 26/03/2013 |

Table 2: Morphological comparison between *Tomitolithon* and *Harveylithon* taxa (- = absent; += present; D=Dimerous; M=Monomerous). [1]Maneveldt et al. (2015), [2]Costa et al. (2017a – submitted), [3]this study.

| Characters (measured in µm) | Lectotype *H. samöense*[1] | *H. samöense*[1] | *H. riosmenum*[2] | *H. maris-bahiensis*[2] | *H. catarinense*[2] | *T. polistratum*[3] | *T. deniquerum*[3] |
|---|---|---|---|---|---|---|---|
| Organization | M | M | M | M | M | D | D |
| Height of epithallial cells | 4 - 6 | 2-7 | 2.6-6.5 | 4.3-7.5 | 2.7-4.7 | 3.9–6.5 | 3.2-9 |
| Diameter of epithallial cells | 5 - 7 | 3-10 | 7.5-9 | 4.8-8.5 | 4.3-7.7 | 3.6–6 | 3.6-7.9 |
| Height of perithallial cells | 3 - 15 | 3-15 | 2.4-14.5 | 4.6-10 | 5.9-18.6 | 3.2–5.3 | 3.6-7.9 |
| Diameter of perithallial cells | 3 - 9 | 3-13 | 3.5-8.5 | 4.4-8.6 | 4.5-7.1 | 3.9–5.7 | 3.6-12.5 |
| Height of hypothallial cells | 5 - 19 | 4-31 | 4.1-12.8 | 4-12 | 4.9-20 | 6.4–7.8 | 3.2-9.2 |
| Diameter of hypothallial cells | 3 - 17 | 3-17 | 8-11 | 8-10 | 5-10.8 | 4.1–6.5 | 3.9-11.2 |
| Presence of trichocytes | + | + | + | + | + | - | + |
| Height of tetrasporangial conceptacles | 31 - 60 | 30-80 | 43-126 | 50-71.6 | 43-174 | 42.8–86.3 | 41.4-100.2 |
| Diameter of tetrasporangial conceptacles | 56 - 130 | 55-185 | 72-164.8 | 70-111 | 127-250 | 66.3–118.3 | 60.8-120.1 |
| Height of tetrasporangia | | 18-60 | 11.5-89 | 30-69.3 | 25.5-71.7 | 30.6–67.2 | 20.7-45.7 |
| Diameter of tetrasporangia | | 6-35 | 8-34 | 12-27.6 | 18-26 | 7.9–30.7 | 5.7-15.3 |
| Cells number in the roof of tetrasporangial conceptacle | 2 - 4 (3) | 2 - 4 (3) | 2 - 4 (3) | 2-3 | 4-8 | 2 - 4 (3) | 2 - 4 (3) |
| Gametangial thalli | Dioecious | Dioecious | Monoecious | - | Dioecious | Monoecious | Monoecious |
| Height of carposporangial conceptacles | 25-30 | 16-30 | 48-66.1 | - | 94.8 | 41.5–87.9 | 41.5-87.9 |
| Diam. of carposporangial conceptacles | 56-80 | 35-84 | 90-125 | - | 237.5 | 74.2–111.9 | 74.2-111.9 |
| Height of spermatangial conceptacles | 19-29 | 15-30 | 24.4-26 | - | 21.4-25.6 | 25.5–57.6 | 39-47 |
| Diameter of spermatangial conceptacles | 37-62 | 32-71 | 46-54 | - | 30-31.2 | 35.9–56.1 | 53-55 |

Supplementary table S1. List of taxa available in GenBank and included in our analyses.

| Species | Locality | GB access nº 18S | GB access nº psbA |
|---|---|---|---|
| *Amphiroa fragilissima* | Brazil | KM044027 | KM044017 |
| *Amphiroa* sp. | Brazil | KM044030 | KM044020 |
| *Amphiroa* sp. | Fiji | GQ917416 | - |
| *Amphiroa* sp. | Guadeloupe | GQ917380 | - |
| *Arthrocardia corymbosa* | Canada | - | JQ917408 |
| *Bossiella plumosa* | Canada | - | KT782167 |
| *Corallina officinalis* | Scotland | FM180104 | - |
| *Crusticorallina adhaerens* | Canada | KU983205 | - |
| *Crusticorallina muricata* | Canada | KU983206 | - |
| *Crusticorallina painei* | Canada | KX036713 | JQ422241 |
| *Ellisolandia elongata* | South Africa | U60946 | JQ422231 |
| *Haliptilon roseum* | New Zealand | EF628229 | - |
| *Harveylithon canariense* (as *Neogoniolithon accretum*) | Canary Island | - | KM407560 |
| *Harveylithon munitum* (as *Hydrolithon munitum*) | Australia | - | KM407531 |
| *Harveylithon rupestre* (as *Hydrolithon rupestre*) | Australia | KM073303 | KM407535 |
| *Harveylithon samoënse* (as *Hydrolithon samoënse*) | South Australia | AY234236 | - |
| *Hydrolithon boergesenii* | Guadeloupe | KM073301 | KM407536 |
| *Hydrolithon boergesenii* | Guadeloupe | KP030741 | KM407537 |
| *Hydrolithon boergesenii* | Brazil | - | KY485316 |
| *Hydrolithon boergesenii*(as *Hydrolithon reinboldii*) | Hawaii | DQ628999 | - |
| *Hydrolithon boergesenii* (as *Hydrolithon reinboldii*) | Japan | AB576017 | - |
| *Hydrolithon boergesenii* (as *Hydrolithon reinboldii*) | Hawaii | DQ629003 | - |
| *Hydrolithon boergesenii* (as *Hydrolithon* cf. *reinboldii*) | Hawaii | DQ629001 | - |
| *Hydrolithon boergesenii* (as *Hydrolithon* cf. *reinboldii*) | Hawaii | DQ629000 | - |
| *Hydrolithon pachydermum* | Caribe | AY234235 | - |
| *Jania crassa* | South Africa | U62113 | - |

| | | | |
|---|---|---|---|
| *Jania rubens* | Brazil | KM044029 | - |
| *Lithophyllum acrocamptum* | Australia | KM073277 | KM407538 |
| *Lithophyllum atlanticum* | Brazil | KP192386 | - |
| *Lithophyllum atlanticum* | Brazil | KP192390 | - |
| *Lithophyllum* cf. b*amleri* | Fiji | GQ917406 | - |
| *Lithophyllum* cf. *bamleri* | Fiji | GQ917417 | - |
| *Lithophyllum byssoides* | Spain | - | JQ896252 |
| *Lithophyllum byssoides* | Croatia | - | KX130631 |
| *Lithophyllum carpophylli* | New Zealand | - | DQ167984 |
| *Lithophyllum carpophylli* | New Zealand | - | FJ361555 |
| *Lithophyllum corallinae* | New Zealand | - | FJ361544 |
| *Lithophyllum dentatum* | Spain | - | JQ896255 |
| *Lithophyllum dentatum* | Spain | KM073281 | KP030737 |
| *Lithophyllum dentatum* | Spain | - | KP708613 |
| *Lithophyllum hibernicum* | Ireland | - | KR708594 |
| *Lithophyllum hibernicum* | Ireland | - | KR708598 |
| *Lithophyllum hibernicum* | Ireland | - | KR708600 |
| *Lithophyllum hibernicum* | France | - | KR708612 |
| *Lithophyllum hibernicum* | United Kingdom | - | KR708615 |
| *Lithophyllum hibernicum* | Spain | - | KR736256 |
| *Lithophyllum incrustans* | Spain | KM073283 | KM407543 |
| *Lithophyllum incrustans* | Spain | KM073286 | KM407546 |
| *Lithophyllum incrustans* | Spain | KM073284 | JQ896236 |
| *Lithophyllum incrustans* | United Kingdom | AF093410 | JQ896256 |
| *Lithophyllum incrustans* | France | GQ917385 | KR708601 |
| *Lithophyllum insipidum* | Japan | AB576007 | - |
| *Lithophyllum johansenii* | New Zealand | - | DQ167963 |
| *Lithophyllum johansenii* | New Zealand | - | FJ361549 |

| | | | |
|---|---|---|---|
| *Lithophyllum kaiseri* | Madagascar | - | KX020445 |
| *Lithophyllum kaiseri* | Brazil | - | KY485306 |
| *Lithophyllum kotschyanum* | Hawaii | KM073296 | KM407548 |
| *Lithophyllum kotschyanum* | Australia | KM073287 | KM407547 |
| *Lithophyllum kotschyanum* | Japan | AB576009 | AB576030 |
| *Lithophyllum kotschyanum* | Hawaii | DQ628974 | - |
| *Lithophyllum kotschyanum* | Japan | AB576008 | AB576029 |
| *Lithophyllum kotschyanum* | Japan | AB576010 | AB576031 |
| *Lithophyllum kotschyanum* | Fiji | U62117 | - |
| *Lithophyllum kotschyanum* | Japan | AB576011 | - |
| *Lithophyllum margaritae* | Brazil | KP192392 | - |
| *Lithophyllum margaritae* | Mexico | JQ896280 | JQ896253 |
| *Lithophyllum neocongestum* | Panama | - | KX020466 |
| *Lithophyllum orbiculatum* | France | - | KR708609 |
| *Lithophyllum pustulatum* | New Zealand | - | DQ167872 |
| *Lithophyllum pustulatum* | New Zealand | - | FJ361746 |
| *Lithophyllum pustulatum* | Spain | KM073290 | KM407551 |
| *Lithophyllum pygmaeum* | New Caledonia | GQ917403 | - |
| *Lithophyllum racemus* | Spain | KM073292 | KM407552 |
| *Lithophyllum riosmenae* | New Zealand | - | FJ361615 |
| *Lithophyllum* sp. | Fiji | GQ917413 | - |
| *Lithophyllum* sp. | Vanuatu | GQ917397 | - |
| *Lithophyllum* sp. | Ireland | - | KC819247 |
| *Lithophyllum* sp. | Panama | - | KJ418411 |
| *Lithophyllum* sp. | USA | - | KJ418415 |
| *Lithophyllum* sp. | New Zealand | - | DQ167958 |
| *Lithophyllum* sp. | Ireland | - | JQ896239 |
| *Lithophyllum* sp. | Spain | - | KR736254 |

| | | | |
|---|---|---|---|
| *Lithophyllum* sp. | France | - | KR736255 |
| *Lithophyllum* sp. | Brazil | - | KY485308 |
| *Lithophyllum stictaeforme* | Spain | KM073293 | KM407553 |
| *Lithophyllum stictaeforme* | New Zealand | - | DQ167970 |
| *Lithophyllum stictaeforme* | New Zealand | - | FJ361442 |
| *Lithophyllum stictaeforme* | New Zealand | - | FJ361446 |
| *Lithophyllum stictaeforme* | Greece | - | KX020442 |
| *Lithophyllum stictaeforme* | Italy | - | KX020443 |
| *Lithophyllum stictaeforme* | Italy | - | KX020444 |
| *Lithophyllum stictaeforme* | Italy | - | KX020464 |
| *Lithophyllum stictaeforme* | Italy | - | KX020465 |
| *Lithophyllum yemenense* | Yemen | - | KP976408 |
| *Lithothamnion corallioides* | France | JQ896261 | - |
| *Lithothamnion crispatum* | New Zealand | - | KC963420 |
| *Mastophora pacifica* | New Zealand | FJ361365 | FJ361461 |
| *Mastophora pacifica* | Japan | AB576026 | AB576041 |
| *Mastophora pacifica* | FrenchPolynesi | GQ917430 | - |
| *Mastophora rosea* | Japan | AB576024 | AB576041 |
| *Mastophora rosea* | Japan | - | AB576042 |
| *Mesophyllum engelhartii* | South Africa | U61256 | - |
| *Mesophyllum erubescens* | Spain | JQ896273 | - |
| *Mesophyllum erubescens* | USA | DQ629012 | - |
| *Mesophyllum erubescens* | Brazil | - | KM983046 |
| *Mesophyllum erubescens* | Brazil | - | KY485315 |
| *Mesophyllum lichenoides* | Spain | KP142819 | KP142728 |
| *Mesophyllum lichenoides* | France | GQ917384 | - |
| *Metagoniolithon chara* | Australia | U60743 | - |
| *Metagoniolithon radiatum* | Australia | U61250 | GQ917496 |

| | | | |
|---|---|---|---|
| *Metagoniolithon stelliferum* | Australia | U61251 | - |
| *Metamastophora flabellata* | SouthAustralia | AY234239 | - |
| *Metamastophora flabellata* | SouthAustralia | AY234240 | - |
| *Neogoniolithon accretum* | Mexico | - | KJ637660 |
| *Neogoniolithon accretum* | Mexico | - | KM407560 |
| *Neogoniolithon acropetum* | Mexico | - | KJ637663 |
| *Neogoniolithon brassica-florida* | Hawaii | DQ629008 | - |
| *Neogoniolithon brassica-florida* | Hawaii | DQ629007 | - |
| *Neogoniolithon brassica-florida* | Hawaii | DQ629009 | - |
| *Neogoniolithon brassica-florida* | New Zealand | - | FJ361401 |
| *Neogoniolithon brassica-florida* | Spain | - | JQ896257 |
| *Neogoniolithon fosliei* | Australia | - | KM407555 |
| *Neogoniolithon fosliei* | Australia | KM073294 | KM407556 |
| *Neogoniolithon fosliei* | Moorea | KM073297 | - |
| *Neogoniolithon fosliei* | Japan | AB576020 | AB576034 |
| *Neogoniolithon fosliei* | Japan | AB713916 | - |
| *Neogoniolithon fosliei* | Japan | AB576019 | AB576033 |
| *Neogoniolithon fosliei* | Japan | AB713914 | - |
| *Neogoniolithon fosliei* | Japan | AB576018 | AB576032 |
| *Neogoniolithon frutescens* | Japan | AB713917 | AB576038 |
| *Neogoniolithon megalocystum* | Japan | AB576021 | - |
| *Neogoniolithon mamillare* | Mexico | - | KJ637664 |
| *Neogoniolithon propinquum* | Mexico | - | KJ637665 |
| *Neogoniolithon propinquum* | Mexico | - | KJ637667 |
| *Neogoniolithon rhizophorae* | Mexico | - | KJ637670 |
| *Neogoniolithon rhizophorae* | Mexico | - | KJ637672 |
| *Neogoniolithon siankanensis* | Mexico | - | KJ637675 |
| *Neogoniolithon solubile* | Mexico | - | KJ637679 |

| | | | |
|---|---|---|---|
| *Neogoniolithon spectabile* | Mexico | JQ896267 | - |
| *Neogoniolithon* sp. | Mexico | - | KJ637686 |
| *Neogoniolithon* sp. | Mexico | - | KJ637687 |
| *Neogoniolithon* sp. | Mexico | - | KJ637688 |
| *Neogoniolithon* sp. | Mexico | - | KJ637689 |
| *Neogoniolithon* sp. | Spain | KM073298 | KM407558 |
| *Neogoniolithon* sp. | Spain | KM073300 | KM407559 |
| *Neogoniolithon* sp. | Guadeloupe | - | KP682501 |
| *Neogoniolithon* sp. | Hawaii | DQ629009 | - |
| *Neogoniolithon* sp.(as Uncultured Corallinales) | Vanuatu | GQ917387 | - |
| *Neogoniolithon* sp. (as Uncultured Corallinales) | New Caledonia | GQ917424 | - |
| *Neogoniolithon* sp. (as Uncultured Corallinales) | Vanuatu | GQ917396 | - |
| *Neogoniolithon* sp. (as Uncultured Corallinales) | New Caledonia | GQ917425 | - |
| *Neogoniolithon* sp. (as Uncultured Corallinales) | New Caledonia | GQ917426 | - |
| *Neogoniolithon* sp. (as Uncultured Corallinales) | New Caledonia | GQ917434 | - |
| *Neogoniolithon* sp.(as Uncultured Corallinales) | Fiji | GQ917410 | - |
| *Neogoniolithon* sp.(as Uncultured Corallinales) | New Caledonia | GQ917403 | - |
| *Neogoniolithon spectabile* | Mexico | - | JQ896240 |
| *Neogoniolithon spectabile* | Mexico | - | KJ637682 |
| *Neogoniolithon strictum* | Mexico | - | JQ896254 |
| *Neogoniolithon strictum* | Mexico | - | KJ637684 |
| *Neogoniolithon spectabile* | Bahamas | AY234238 | - |
| *Paulsilvella huveorum* | Brazil | KM044031 | KM044018 |
| *Paulsilvella huveorum* | Brazil | - | KM044021 |
| *Phymatolithon calcareum* | United Kingdom | JQ896258 | - |
| *Phymatolithon lenormandii* | Ireland | - | JQ896249 |
| *Pneophyllum confervicola* | Spain | KM073305 | KP030738 |
| *Pneophyllum* cf. *conicum* | Hawaii | DQ628989 | - |

| | | | |
|---|---|---|---|
| *Pneophyllum* cf. *conicum* | Hawaii | DQ628994 | - |
| *Pneophyllum conicum* | Hawaii | DQ628985 | - |
| *Pneophyllum conicum* | Hawaii | DQ628983 | - |
| *Pneophyllum conicum* | Australia | - | KM407561 |
| *Pneophyllum conicum* | Brazil | - | KY485312 |
| *Pneophyllum conicum* | Japan | AB576022 | AB576040 |
| *Pneophyllum conicum*(as Uncultured Corallinales) | Fiji | GQ917414 | - |
| *Pneophyllum conicum*(as Uncultured Corallinales) | Fiji | GQ917408 | GQ917464 |
| *Pneophyllum conicum*(as Uncultured Corallinales) | Vanuatu | GQ917389 | - |
| *Pneophyllum conicum* | Hawaii | DQ628983 | - |
| *Pneophyllum conicum* | Hawaii | DQ628985 | - |
| *Pneophyllum coronatum* | New Zealand | - | DQ168007 |
| *Pneophyllum coronatum* | New Zealand | - | DQ168008 |
| *Pneophyllum fragile* | New Zealand | - | DQ167967 |
| *Pneophyllum fragile* | New Zealand | - | DQ167969 |
| *Pneophyllum* sp. | New Zealand | - | FJ361545 |
| *Porolithon gardineri* (as *Hydrolithon gardineri*) | Hawaii | - | - |
| *Porolithon onkodes* (as *Hydrolithon* cf. *onkodes*) | Hawaii | DQ628996 | - |
| *Porolithon onkodes* (as *Hydrolithon onkodes*) | Indonesia | KM073275 | KM407532 |
| *Porolithon onkodes* (as *Hydrolithon onkodes*) | Indonesia | - | KM407534 |
| *Porolithon onkodes* (as *Hydrolithon onkodes*) | Japan | AB576013 | AB576037 |
| *Porolithon onkodes* (as *Hydrolithon onkodes*) | Japan | - | AB576037 |
| *Porolithon onkodes* (as *Hydrolithon onkodes*) | Australia | AY234237 | KM407532 |
| *Porolithononkodes* (as *Hydrolithon onkodes*) | Brazil | - | KY485318 |
| *Porolithon onkodes* (as *Hydrolithon onkodes*) | New Zealand | AY234237 | - |
| *Porolithon onkodes* (as Uncultured Corallinales) | New Caledonia | GQ917372 | - |
| *Porolithon onkodes* (as Uncultured Corallinales) | New Caledonia | GQ917373 | - |
| *Porolithon onkodes* (as Uncultured Corallinales) | New Caledonia | GQ917371 | GQ917480 |

| | | | |
|---|---|---|---|
| *Porolithon pachydermum* (as *Hydrolithon pachydermum*) | Japan | AY234235 | - |
| *Serraticardia macmillanii* | USA | U62114 | - |
| *Spongites fruticulosa* | Spain | KM073306 | - |
| *Spongites hyperellus* (as Uncultured Corallinales) | Australia | GQ917431 | - |
| *Spongites yendoi* | South Africa | U60948 | - |
| *Spongites yendoi* | New Zealand | EF628234 | DQ167869 |
| *Spongites yendoi* | New Zealand | - | DQ167903 |
| *Spongites yendoi* | New Zealand | - | DQ167905 |
| *Spongites yendoi* | New Zealand | - | DQ167907 |
| *Spongites yendoi* | New Zealand | - | DQ167952 |
| *Spongites yendoi* | New Zealand | - | DQ167982 |
| *Spongites yendoi* | New Zealand | - | FJ361440 |
| *Spongites yendoi* | New Zealand | - | FJ361459 |
| *Sporolithon durum* | New Zealand | - | - |
| *Sporolithon* cf. *ptychoides* | Brazil | KP142840 | - |
| *Sporolithon ptychoides* | Hawaii | DQ629014 | KC870926 |
| *Sporolithon ptychoides* | Brazil | - | KY485313 |
| *Sporolithon ptychoides* | Brazil | - | KC870927 |
| *Sporolithon tenue* | Brazil | KP142838 | KP142751 |
| *Titanoderma prototypum* | Hawaii | DQ628981 | - |
| *Titanoderma pustulatum* | UK | AF093409 | - |
| *Titanoderma* sp. (as Uncultured Corallinales) | Fiji | GQ917421 | - |
| *Titanoderma* sp. | USA | - | KJ418412 |
| *Titanoderma* sp. | USA | - | KJ418413 |

Supplementary table S2. Summary of parsimony analysis for four genes and concatenated data sets.

| | SSU rDNA | *psb*A | Concatenated |
|---|---|---|---|
| Number of taxa | 107 | 159 | 64 |
| Number of nucleotide (bp) included in analysis | 1,496 | 760 | 2,269 |
| Number of parsimony informative characters | 365 | 276 | 546 |
| Tree length | 1,675 | 3,342 | 2,787 |
| Consistency index (CI) | 0.4263 | 0.1553 | 0.38 |
| Retention index (RI) | 0.8377 | 0.7439 | 0.7237 |
| Rescaled consistency index (RC) | 0.3571 | 0.1155 | 0.2750 |

FIGURES

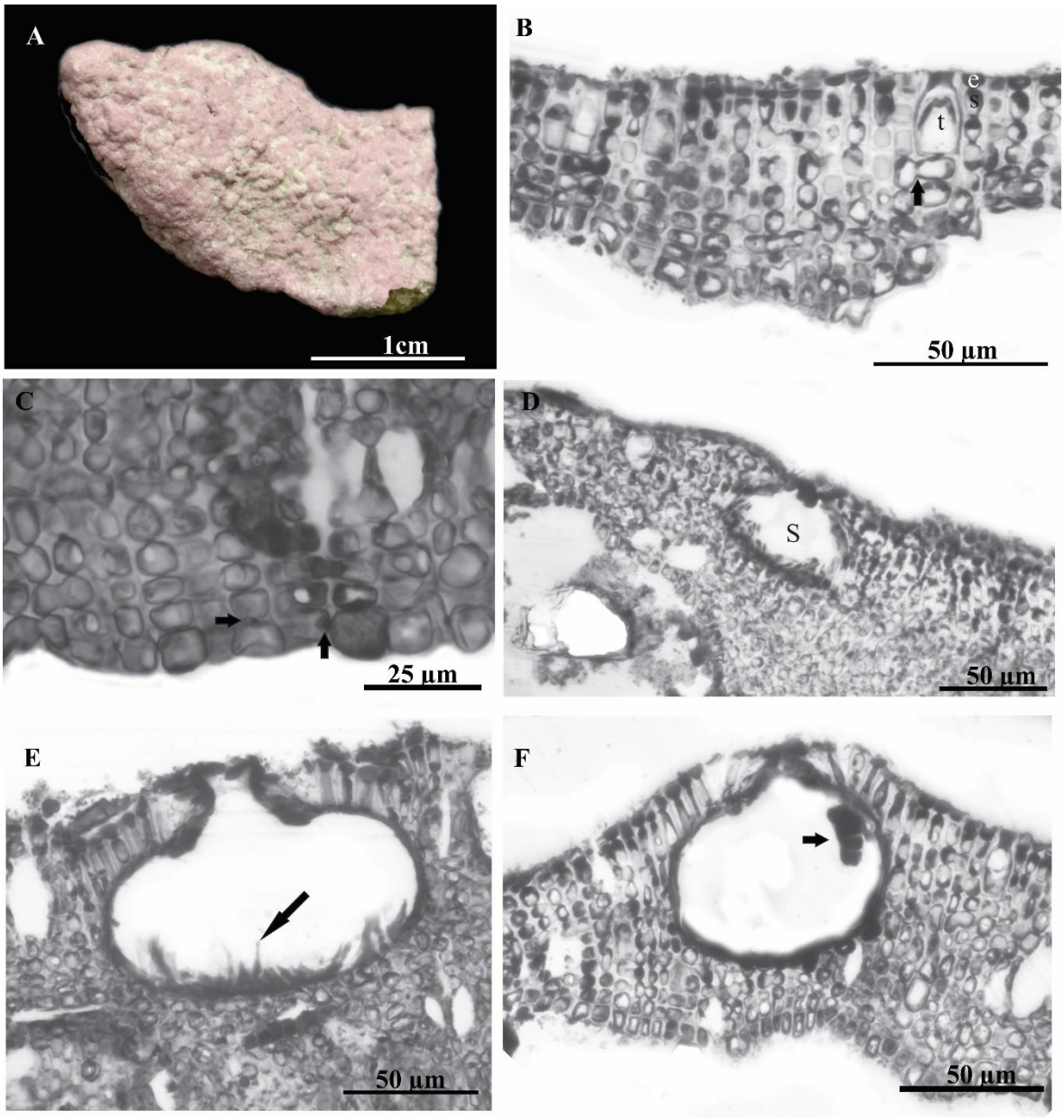


FIGURE 1 A-F: *Tomitolithon deniquerum* sp.nov. (ALCB125644). A. Encrusting growth-form. B. Longitudinal section through the thallus showing epithallial cells (e), rounded to squat; subepithallial initial cells (s) longer than subtending ones; trichocytes (t) single at the surface of the thallus; and cells of adjacent filaments linked by lateral cell fusions (arrows). C. Longitudinal section through the thallus showing dimerous construction, consisting of a single ventral layer of branched filaments of non-palisade cells and multicellular simple or branched filaments arising perpendicularly from the ventral layer, with primary and secondary pit connections in basal filaments (arrows). D. Longitudinal section through male conceptacle (S) showing unbranched spermatangial filaments across

the chamber floor only. E. Longitudinal section through female conceptacle showing central fusion cell and gonimoblast filaments (arrow). F. Longitudinal section through uniporate tetrasporangial conceptacle, showing pore canals lined by cells orientated perpendicularly to the thallus surface, and sporangium zonately divided (arrow).

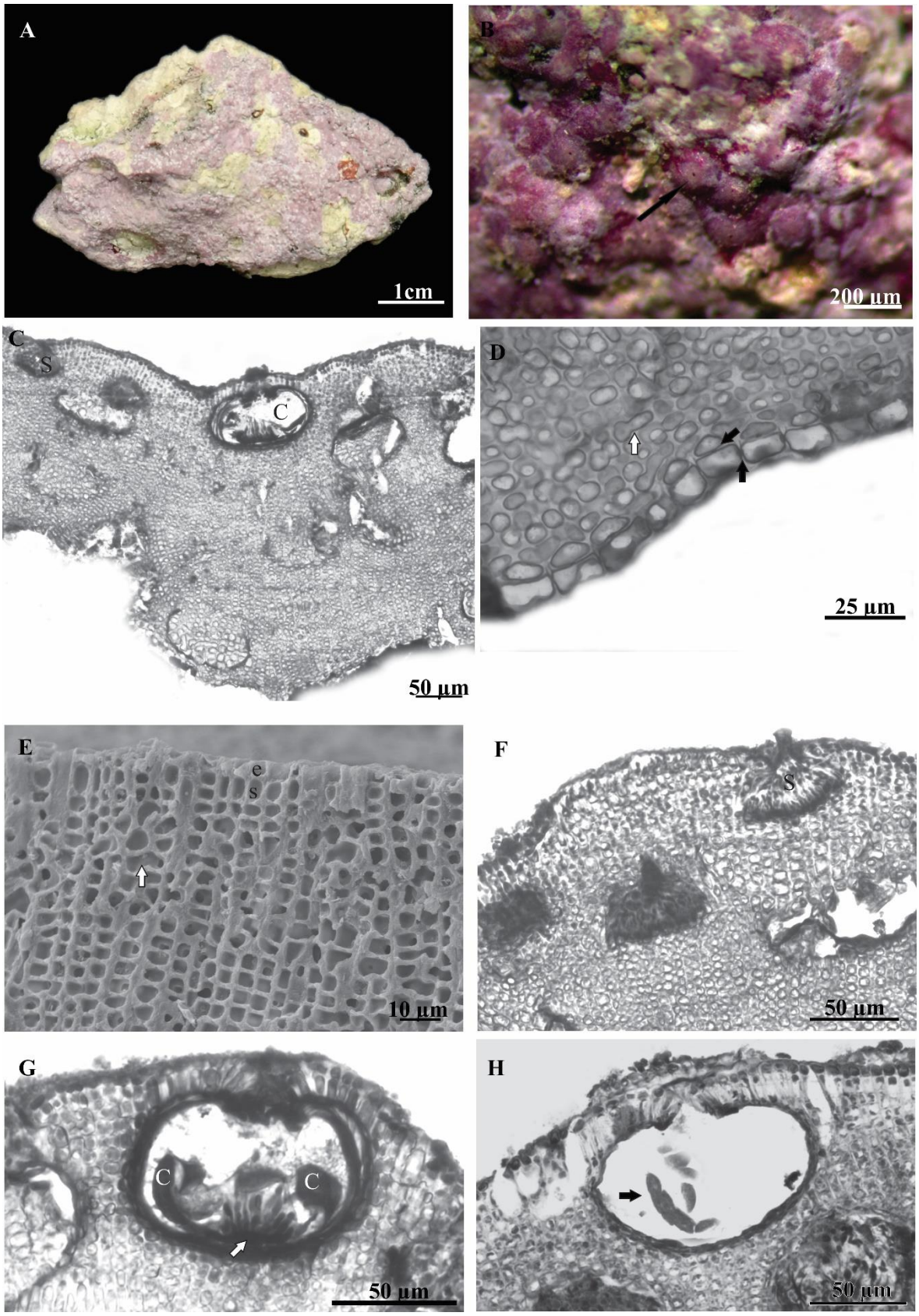


FIGURE 2 A-H: *Tomitolithon polistratum* sp.nov. (ALCB125641). A. Encrusting growth-form. B. Superficial view of the uniporate conceptacles (arrow). C. Longitudinal section through monoecius thallus. D. Longitudinal section through the thallus showing dimerous

construction, consisting of a single ventral layer of branched filaments of non-palisade cells and multicellular simple or branched filaments arising perpendicularly from the ventral layer, with primary and secondary pit connections in basal filaments (black arrows). E. Longitudinal fracture, SEM, showing epithallial cells (e), rounded to squat; subepithallial initial cells (s) longer than subtending ones; and cells of adjacent filaments linked by lateral cell fusions (arrow). F. Longitudinal section through male conceptacle (S) showing unbranched spermatangial filaments across the chamber floor only. G. Longitudinal section through the carposporangial conceptacle showing central fusion cell (arrow) and peripheral gonimoblast filaments bearing terminal carposporangia (C). H. Longitudinal section through uniporate tetrasporangial conceptacle, showing pore canals lined by cells orientated perpendicularly to the thallus surface, and sporangium zonately divided (arrow).

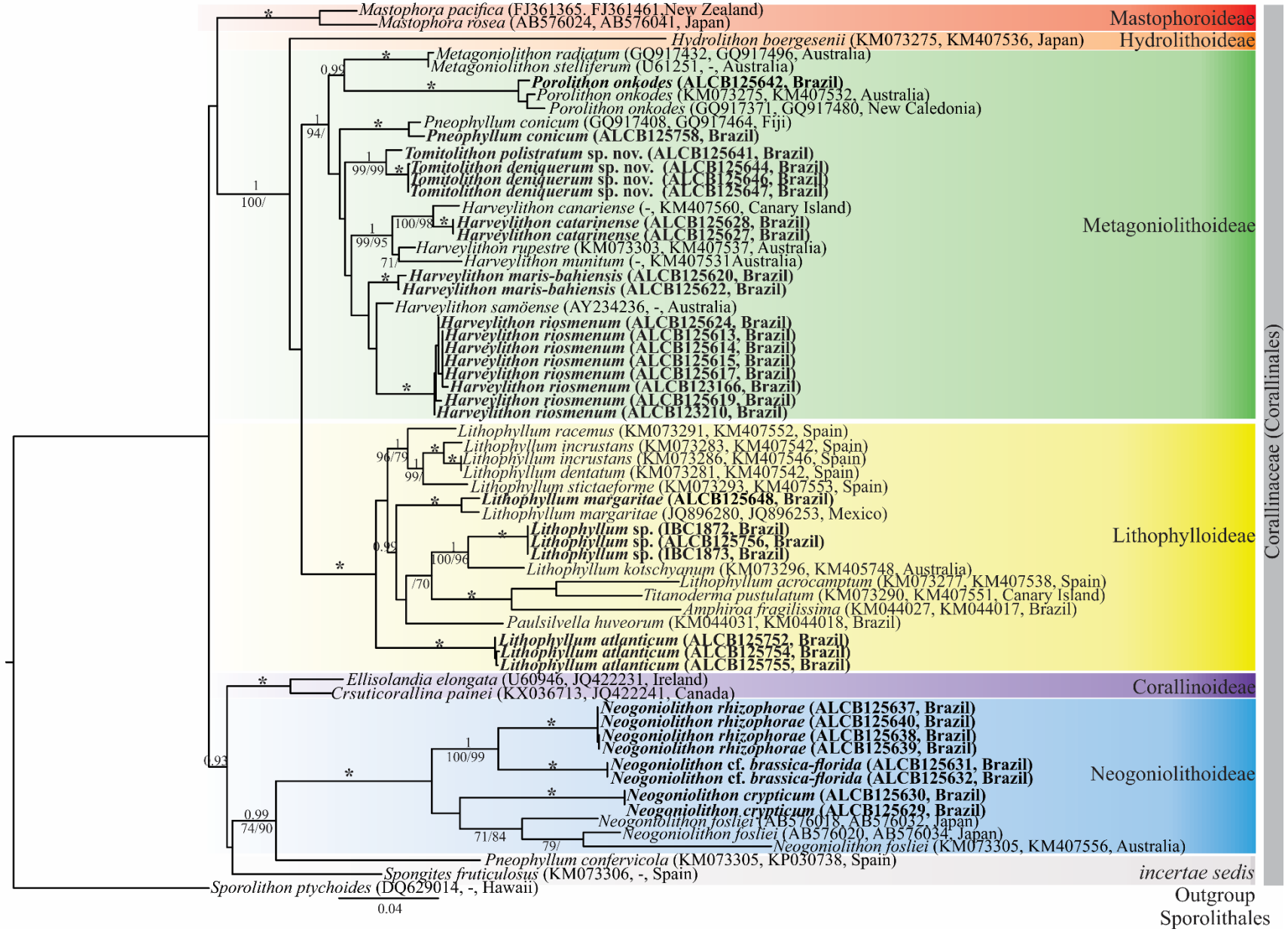
Mastophora pacifica (FJ361365, FJ361461,New Zealand)
Mastophora rosea (AB576024, AB576041, Japan)
Mastophoroideae
Hydrolithon boergesenii (KM073275, KM407536, Japan)
Hydrolithoideae
Metagoniolithon radiatum (GQ917432, GQ917496, Australia)
Metagoniolithon stelliferum (U61251, -, Australia)
Porolithon onkodes (ALCB125642, Brazil)
Porolithon onkodes (KM073275, KM407532, Australia)
Porolithon onkodes (GQ917371, GQ917480, New Caledonia)
Pneophyllum conicum (GQ917408, GQ917464, Fiji)
Pneophyllum conicum (ALCB125758, Brazil)
Tomitolithon polistratum sp. nov. (ALCB125641, Brazil)
Tomitolithon deniquerum sp. nov. (ALCB125644, Brazil)
Tomitolithon deniquerum sp. nov. (ALCB125646, Brazil)
Tomitolithon deniquerum sp. nov. (ALCB125647, Brazil)
Harveylithon canariense (-, KM407560, Canary Island)
Harveylithon catarinense (ALCB125628, Brazil)
Harveylithon catarinense (ALCB125627, Brazil)
Harveylithon rupestre (KM073303, KM407537, Australia)
Harveylithon munitum (-, KM407531Australia)
Harveylithon maris-bahiensis (ALCB125620, Brazil)
Harveylithon maris-bahiensis (ALCB125622, Brazil)
Harveylithon samöense (AY234236, -, Australia)
Harveylithon riosmenum (ALCB125624, Brazil)
Harveylithon riosmenum (ALCB125613, Brazil)
Harveylithon riosmenum (ALCB125614, Brazil)
Harveylithon riosmenum (ALCB125615, Brazil)
Harveylithon riosmenum (ALCB125617, Brazil)
Harveylithon riosmenum (ALCB123166, Brazil)
Harveylithon riosmenum (ALCB125619, Brazil)
Harveylithon riosmenum (ALCB123210, Brazil)
Metagoniolithoideae
Lithophyllum racemus (KM073291, KM407552, Spain)
Lithophyllum incrustans (KM073283, KM407542, Spain)
Lithophyllum incrustans (KM073286, KM407546, Spain)
Lithophyllum dentatum (KM073281, KM407542, Spain)
Lithophyllum stictaeforme (KM073293, KM407553, Spain)
Lithophyllum margaritae (ALCB125648, Brazil)
Lithophyllum margaritae (JQ896280, JQ896253, Mexico)
Lithophyllum sp. (IBC1872, Brazil)
Lithophyllum sp. (ALCB125756, Brazil)
Lithophyllum sp. (IBC1873, Brazil)
Lithophyllum kotschyanum (KM073296, KM405748, Australia)
Lithophyllum acrocamptum (KM073277, KM407538, Spain)
Titanoderma pustulatum (KM073290, KM407551, Canary Island)
Amphiroa fragilissima (KM044027, KM044017, Brazil)
Paulsilvella huveorum (KM044031, KM044018, Brazil)
Lithophyllum atlanticum (ALCB125752, Brazil)
Lithophyllum atlanticum (ALCB125754, Brazil)
Lithophyllum atlanticum (ALCB125755, Brazil)
Lithophylloideae
Ellisolandia elongata (U60946, JQ422231, Ireland)
Crsuticorallina painei (KX036713, JQ422241, Canada)
Corallinoideae
Neogoniolithon rhizophorae (ALCB125637, Brazil)
Neogoniolithon rhizophorae (ALCB125640, Brazil)
Neogoniolithon rhizophorae (ALCB125638, Brazil)
Neogoniolithon rhizophorae (ALCB125639, Brazil)
Neogoniolithon cf. brassica-florida (ALCB125631, Brazil)
Neogoniolithon cf. brassica-florida (ALCB125632, Brazil)
Neogoniolithon crypticum (ALCB125630, Brazil)
Neogoniolithon crypticum (ALCB125629, Brazil)
Neogoniolithon fosliei (AB576018, AB576032, Japan)
Neogoniolithon fosliei (AB576020, AB576034, Japan)
Neogoniolithon fosliei (KM073305, KM407556, Australia)
Neogoniolithoideae
Pneophyllum confervicola (KM073305, KP030738, Spain)
Spongites fruticulosus (KM073306, -, Spain)
incertae sedis
Sporolithon ptychoides (DQ629014, -, Hawaii)
0.04
Outgroup
Sporolithales
Corallinaceae (Corallinales)

FIGURE 3: The optimal maximum likelihood (ML) topology inferred from the concatenated data set of SSU rDNA and *psb*A. Values above each clade refer to Bayesian posterior probabilities (PP) and the ones below each clade refer to ML and MP (maximum parsimony) bootstrap values, respectively. Only bootstrap values in MP and ML ≥ 70% and Bayesian posterior probability (PP) ≥ 0.9 were plotted. New sequences produced in this study are in bold, others are from GenBank. Asterisks mark clades that are supported at 100% in all three analyses.

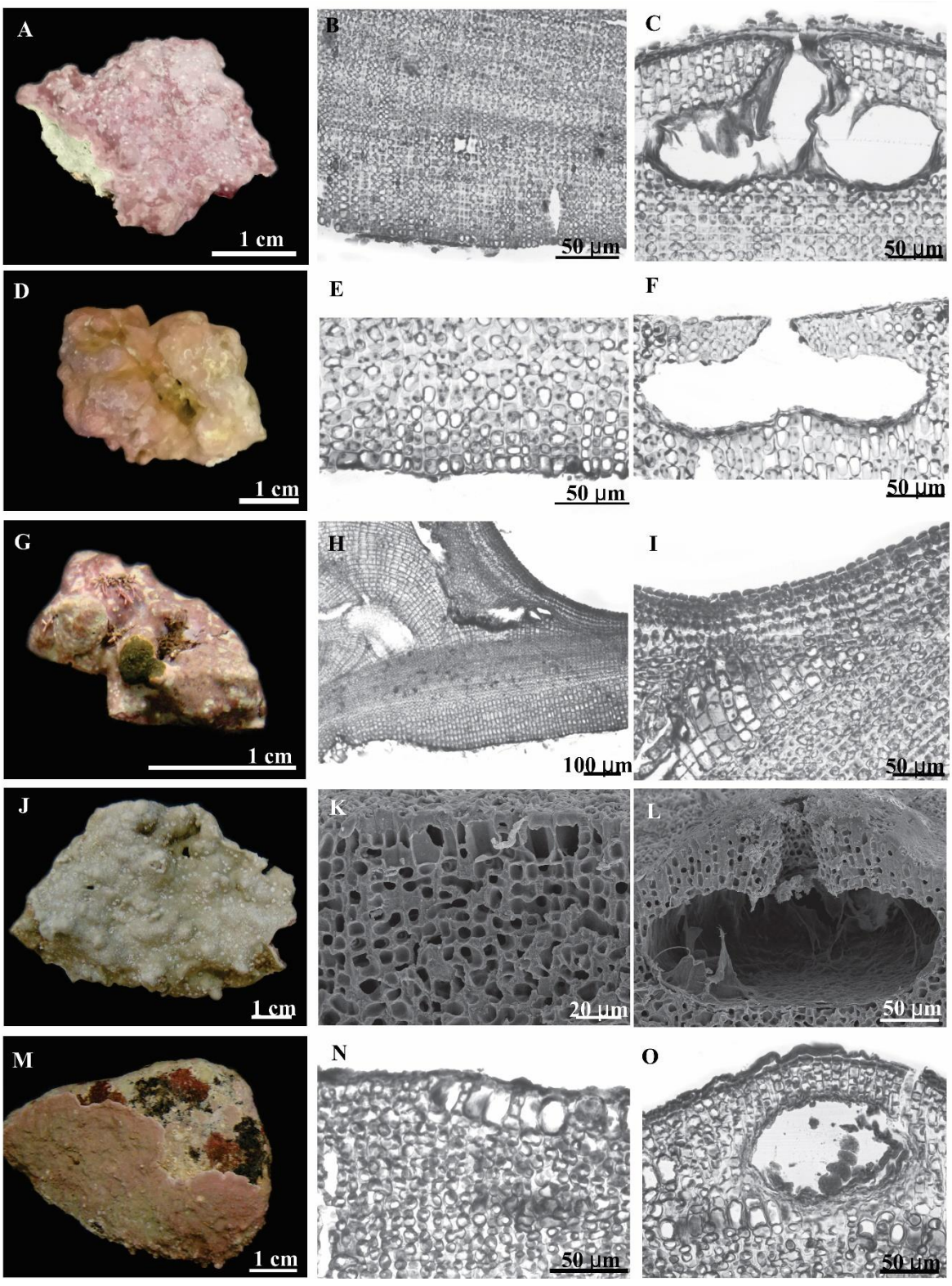


FIGURE S1: *Lithophyllum atlanticum* (ALCB125751). A. Encrusting growth-form. B. Longitudinal section through the thallus showing dimerous construction, and cells of adjacent filaments linked by secondary pit connections. C. Longitudinal section through uniporate tetrasporangial conceptacle with collumela. *Lithophyllum margaritae* (ALCB125648). D. Encrusting growth-form. E. Longitudinal section through the thallus

showing dimerous construction, and cells of adjacent filaments linked by secondary pit connections. F. Longitudinal section through uniporate tetrasporangial conceptacle. *Lithophyllum* sp. (ALCB125756). G. Encrusting growth-form. H. Longitudinal section through the thallus showing dimerous construction, and cells of adjacent filaments linked by secondary pit connections. I. Longitudinal section through epithallial cells, rounded; subepithallial initial cells longer than subtending ones. *Pneophylum conicum* (ALCB125758). J. Encrusting growth-form. K. Longitudinal fracture, SEM, showing trichocytes occurring in horizontal fields, and cells of adjacent filaments linked by cell fusions. L. Longitudinal fracture, SEM, showing uniporate tetrasporangial conceptacle. *Porolithon onkodes* (ALCB125642). M. Encrusting growth-form. N. Longitudinal section through the thallus showing trichocytes occurring in horizontal fields, and cells of adjacent filaments linked by cell fusions. O. Longitudinal section through tetrasporangial conceptacle with sporangium zonately divided.

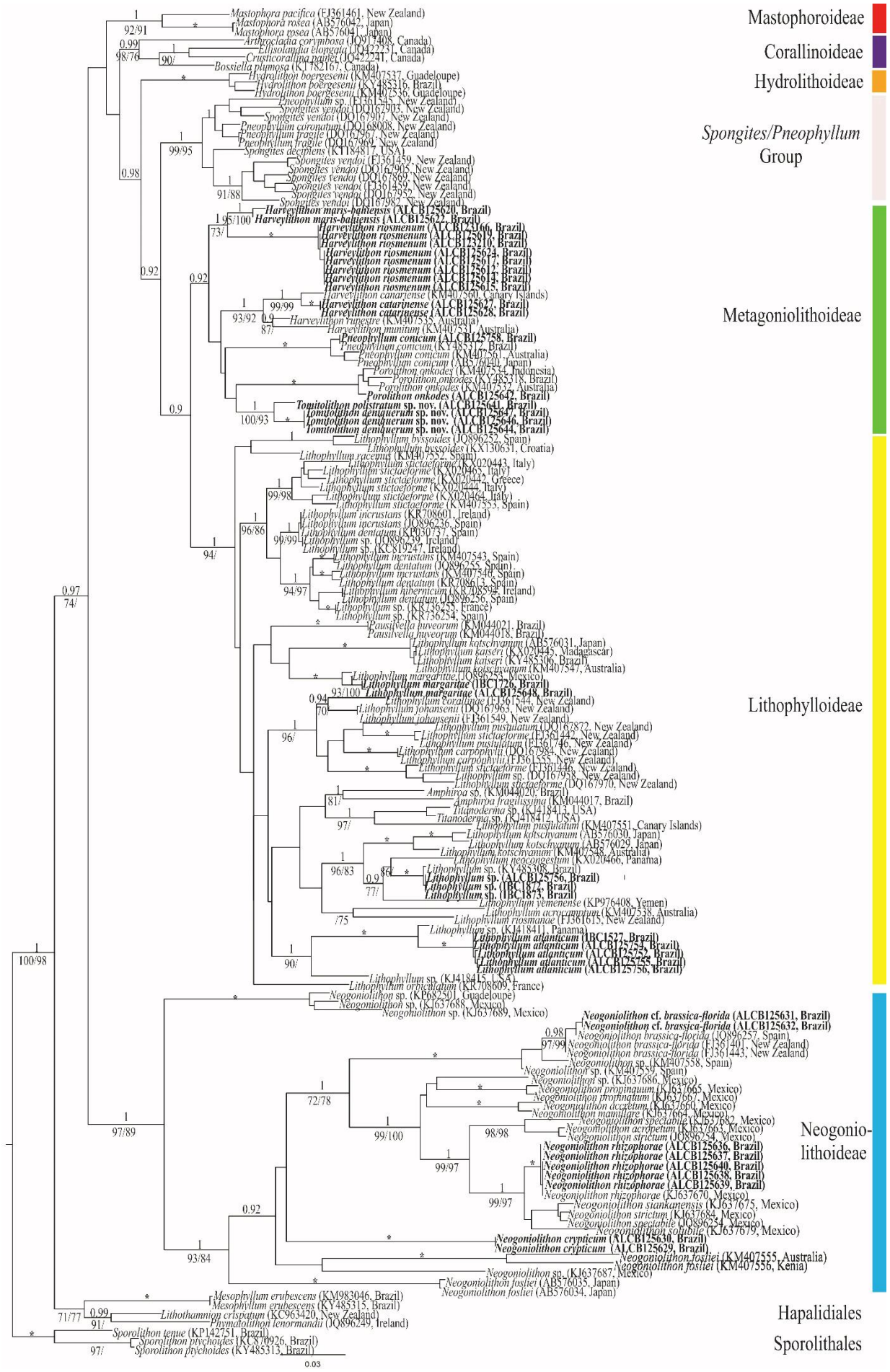
Mastophoroideae
Corallinoideae
Hydrolithoideae
Spongites/Pneophyllum Group
Metagoniolithoideae
Lithophylloideae
Neogonio-lithoideae
Hapalidiales
Sporolithales
Mastophora pacifica (FJ361461, New Zealand)
Mastophora rosea (AB576042, Japan)
Mastophora rosea (AB576041, Japan)
Arthrocladia corymbosa (JQ917408, Canada)
Lithsolandia elongata (JQ422231, Canada)
Crusticoralina gaulei (JQ422241, Canada)
Bossiella plumosa (KJ782167, Canada)
Hydrolithon boergesenii (KM407537, Guadeloupe)
Hydrolithon boergesenii (KY485316, Brazil)
Hydrolithon boergesenii (KM407536, Guadeloupe)
Pneophyllum sp. (FJ361455, New Zealand)
Spongites yendoi (DQ167905, New Zealand)
Spongites yendoi (DQ167907, New Zealand)
Pneophyllum coronatum (DQ168008, New Zealand)
Pneophyllum fragile (DQ167967, New Zealand)
Pneophyllum fragile (DQ167969, New Zealand)
Spongites decipiens (KT184817, USA)
Spongites yendoi (FJ361459, New Zealand)
Spongites yendoi (DQ167905, New Zealand)
Spongites yendoi (DQ167869, New Zealand)
Spongites yendoi (FJ361459, New Zealand)
Spongites yendoi (DQ167952, New Zealand)
Spongites yendoi (DQ167982, New Zealand)
Harveyllithon maris-bahiensis (ALCB125620, Brazil)
Harveyllithon maris-bahiensis (ALCB125622, Brazil)
Harveyllithon riosmenum (ALCB123166, Brazil)
Harveyllithon riosmenum (ALCB125619, Brazil)
Harveyllithon riosmenum (ALCB123210, Brazil)
Harveyllithon riosmenum (ALCB125624, Brazil)
Harveyllithon riosmenum (ALCB125617, Brazil)
Harveyllithon riosmenum (ALCB125612, Brazil)
Harveyllithon riosmenum (ALCB125614, Brazil)
Harveyllithon riosmenum (ALCB125615, Brazil)
Harveyllithon canariense (KM407560, Canary Islands)
Harveyllithon catarinense (ALCB125627, Brazil)
Harveyllithon catarinense (ALCB125628, Brazil)
Harveyllithon rugestre (KM407535, Australia)
Harveyllithon muricatum (KM407531, Australia)
Pneophyllum conicum (ALCB125758, Brazil)
Pneophyllum conicum (KY485317, Brazil)
Pneophyllum conicum (KM407561, Australia)
Pneophyllum conicum (AB576040, Japan)
Porolithon onkodes (KM407544, Indonesia)
Porolithon onkodes (KY485318, Brazil)
Porolithon onkodes (KM407537, Australia)
Porolithon onkodes (ALCB125642, Brazil)
Tomiolithon polistratum sp. nov. (ALCB125641, Brazil)
Tomiolithon denquerum sp. nov. (ALCB125647, Brazil)
Tomiolithon denquerum sp. nov. (ALCB125646, Brazil)
Tomiolithon demquerum sp. nov. (ALCB125644, Brazil)
Lithophyllum byssoides (JQ896252, Spain)
Lithophyllum byssoides (KX130631, Croatia)
Lithophyllum racemus (KM407552, Spain)
Lithophyllum stictaeforme (KX020443, Italy)
Lithophyllum stictaeforme (KX020465, Italy)
Lithophyllum stictaeforme (KX020442, Greece)
Lithophyllum stictaeforme (KX020444, Italy)
Lithophyllum stictaeforme (KX020464, Italy)
Lithophyllum stictaeforme (KM407553, Spain)
Lithophyllum incrustans (KR708601, Ireland)
Lithophyllum incrustans (JQ896226, Spain)
Lithophyllum dentatum (KP030737, Spain)
Lithophyllum sp. (JQ896229, Ireland)
Lithophyllum sp. (KC819247, Ireland)
Lithophyllum incrustans (KM407543, Spain)
Lithophyllum dentatum (JQ896265, Spain)
Lithophyllum incrustans (KM407548, Spain)
Lithophyllum dentatum (KR708613, Spain)
Lithophyllum hibernicum (KR708592, Ireland)
Lithophyllum dentatum (JQ896256, Spain)
Lithophyllum sp. (KR736255, France)
Lithophyllum sp. (KR736254, Spain)
Pausilvella nuveorum (KM044021, Brazil)
Pausilvella nuveorum (KM044018, Brazil)
Lithophyllum katschyanum (AB576031, Japan)
Lithophyllum kaiseri (KX020445, Madagascar)
Lithophyllum kaiseri (KY485306, Brazil)
Lithophyllum kotschyanum (KM407547, Australia)
Lithophyllum margaritae (JQ896253, Mexico)
Lithophyllum margaritae (IBC1726, Brazil)
Lithophyllum margaritae (ALCB125648, Brazil)
Lithophyllum corallinae (FJ361544, New Zealand)
Lithophyllum johansenii (DQ167963, New Zealand)
Lithophyllum johansenii (FJ361549, New Zealand)
Lithophyllum pustulatum (DQ167872, New Zealand)
Lithophyllum stictaeforme (FJ361442, New Zealand)
Lithophyllum pustulatum (FJ361746, New Zealand)
Lithophyllum carpophylli (DQ167984, New Zealand)
Lithophyllum carpophylli (FJ361555, New Zealand)
Lithophyllum stictaeforme (FJ361446, New Zealand)
Lithophyllum sp. (DQ167958, New Zealand)
Lithophyllum stictaeforme (DQ167970, New Zealand)
Amphiroa sp. (KM044020, Brazil)
Amphiroa fragilissima (KM044017, Brazil)
Titanoderma sp. (KJ418413, USA)
Titanoderma sp. (KJ418412, USA)
Lithophyllum pustulatum (KM407551, Canary Islands)
Lithophyllum kotschyanum (AB576030, Japan)
Lithophyllum kotschyanum (AB576029, Japan)
Lithophyllum kotschyanum (KM407548, Australia)
Lithophyllum neocongestum (KX020466, Panama)
Lithophyllum sp. (KY485308, Brazil)
Lithophyllum sp. (ALCB125756, Brazil)
Lithophyllum sp. (IBC1874, Brazil)
Lithophyllum sp. (IBC1873, Brazil)
Lithophyllum yemenense (KP976408, Yemen)
Lithophyllum acrocamptum (KM407538, Australia)
Lithophyllum riosmanae (FJ361615, New Zealand)
Lithophyllum sp. (KJ418411, Panama)
Lithophyllum atlanticum (IBC1527, Brazil)
Lithophyllum atlanticum (ALCB125754, Brazil)
Lithophyllum atlanticum (ALCB125752, Brazil)
Lithophyllum atlanticum (ALCB125755, Brazil)
Lithophyllum atlanticum (ALCB125756, Brazil)
Lithophyllum sp. (KJ418415, USA)
Lithophyllum orbiculatum (KC708809, France)
Neogoniolithon sp. (KP682501, Guadeloupe)
Neogoniolithon sp. (KJ637688, Mexico)
Neogoniolithon sp. (KJ637689, Mexico)
Neogoniolithon cf. brassica-florida (ALCB125631, Brazil)
Neogoniolithon cf. brassica-florida (ALCB125632, Brazil)
Neogoniolithon brassica-florida (JQ896257, Spain)
Neogoniolithon brassica-florida (FJ361401, New Zealand)
Neogoniolithon brassica-florida (FJ361443, New Zealand)
Neogoniolithon sp. (KM407558, Spain)
Neogoniolithon sp. (KM407559, Spain)
Neogoniolithon sp. (KJ637686, Mexico)
Neogoniolithon propinquum (KJ637665, Mexico)
Neogoniolithon proptinatum (KJ637661, Mexico)
Neogoniolithon accretum (KJ637660, Mexico)
Neogoniolithon mamillare (KJ637664, Mexico)
Neogoniolithon speciabile (KJ637682, Mexico)
Neogoniolithon acropetum (KJ637663, Mexico)
Neogoniolithon strictum (JQ896254, Mexico)
Neogoniolithon rhizophorae (ALCB125636, Brazil)
Neogoniolithon rhizophorae (ALCB125637, Brazil)
Neogoniolithon rhizophorae (ALCB125640, Brazil)
Neogoniolithon rhizophorae (ALCB125638, Brazil)
Neogoniolithon rhizophorae (ALCB125639, Brazil)
Neogoniolithon rhizophorae (KJ637670, Mexico)
Neogoniolithon siankanensis (KJ637675, Mexico)
Neogoniolithon strictum (KJ637684, Mexico)
Neogoniolithon speciabile (JQ896254, Mexico)
Neogoniolithon solubile (KJ637679, Mexico)
Neogoniolithon crypticum (ALCB125630, Brazil)
Neogoniolithon crypticum (ALCB125629, Brazil)
Neogoniolithon fosliei (KM407555, Australia)
Neogoniolithon fosliei (KM407556, Kenia)
Neogoniolithon sp. (KJ637687, Mexico)
Neogoniolithon fosliei (AB576035, Japan)
Neogoniolithon fosliei (AB576034, Japan)
Mesophyllum erubescens (KM983046, Brazil)
Mesophyllum erubescens (KY485315, Brazil)
Lithothamnion crispatum (KC963420, New Zealand)
Phymatolithon lenormandii (JQ896249, Ireland)
Sporolithon tenue (KP142751, Brazil)
Sporolithon ptychoides (KU870926, Brazil)
Sporolithon ptychoides (KY485313, Brazil)
0.03

FIGURE S2: The optimal maximum likelihood (ML) topology based on the *psb*A data set. Values above each clade refer to Bayesian posterior probabilities (PP) and the ones below each clade refer to ML and MP (maximum parsimony) bootstrap values, respectively. Only bootstrap values for ML and MP ≥ 70% and PP ≥ 0.9 were plotted. New sequences produced in this study are in bold. Asterisks mark clades that are supported at 100% in all three analyses.


Mastophoroideae
Corallinoideae
incertae sedis
Hydrolithoideae
incertae sedis
Metagoniolithoideae
Lithophylloideae
Neogoniolithoideae
Hapalidiales
Sporolithales
Mastaphora pacifica (AB576025, Japan)
Mastaphora pacifica (AB576026, Japan)
Mastaphora rosea (AB576024, Japan)
Metamastaphora flabellata (AY234239, Australia)
Serraticardia macmillanii (U62114, USA)
Ellisolandia elongata (U60946, South Africa)
Corallina officinalis (FM180104, Scotland)
Crusticorallina muricata (KU983206, Canada)
Crusticorallina adhaerens (KU983205, Canada)
Crusticorallina painei (KX036713, Canada)
Jania crassa (U62113, South Africa)
Jania rosea (EF628229, New Zealand)
Jania rubens (KM044029, Brazil)
Spongites fruticulosus (KM073306, Spain)
Hydrolithon boergesenii (DQ628999, Hawaii)
Hydrolithon cf. boergesenii (DQ629001, Hawaii)
Hydrolithon boergesenii (DQ629003, Hawaii)
Hydrolithon cf. boergesenii (DQ629000, Hawaii)
Hydrolithon boergesenii (AB576017, Japan)
Spongites yendoi (U60948, South Africa)
Spongites hyperellus (GQ917431, Australia)
Metagoniolithon stelliferum (U61251, Australia)
Metagoniolithon radiatum (U61250, Australia)
Metagoniolithon chara (U60743, Australia)
Porolithon onkodes (AB576013, Japan)
Porolithon onkodes (DQ628996, Hawaii)
Porolithon onkodes (KM073275, Indonesia)
Porolithon onkodes (ALCB125642, Brazil)
Porolithon onkodes (AY234235, Puerto Rico)
Porolithon onkodes (AY234237, Australia)
Harveylithon catarinense (ALCB125628, Brazil)
Harveylithon rupestre (KM073303, Australia)
Harveylithon samoense (AY234236, Australia)
Harveylithon riosmenum (ALCB125624, Brazil)
Harveylithon riosmenum (ALCB125617, Brazil)
Harveylithon riosmenum (ALCB125612, Brazil)
Harveylithon riosmenum (ALCB125614, Brazil)
Harveylithon riosmenum (ALCB125615, Brazil)
Harveylithon riosmenum (ALCB123166, Brazil)
Harveylithon riosmenum (ALCB123210, Brazil)
Harveylithon riosmenum (ALCB125619, Brazil)
Harveylithon maris-bahiensis (ALCB125621, Brazil)
Harveylithon maris-bahiensis (ALCB125620, Brazil)
Harveylithon maris-bahiensis (ALCB125622, Brazil)
Tomitolithon polistratum sp. nov. (ALCB125641, Brazil)
Tomitolithon deniquerum sp. nov. (ALCB125647, Brazil)
Tomitolithon deniquerum sp. nov. (ALCB125646, Brazil)
Tomitolithon deniquerum sp. nov. (ALCB125644, Brazil)
Pneophyllum conicum (ALCB125758, Brazil)
Pneophyllum cf. conicum (DQ628989, Hawaii)
Pneophyllum conicum (AB576022, Japan)
Pneophyllum conicum (DQ628983, Hawaii)
Pneophyllum conicum (DQ628985, Hawaii)
Pnepohyllum cf. conicum (DQ628994, Hawaii)
Paulsilvella huveorum (KM044031, Brazil)
Lithophyllum insipidum (AB576007, Japan)
Titanoderma pustulatum (AF093409, UK)
Titanoderma prototypum (DQ628981, Haw
Amphiroa sp. (KM044030, Brazil)
Amphiroa fragilissima (KM044027, Brazil)
Lithophyllum racemus (KM073292, Spain)
Lithophyllum stictaeforme (KM073293, Spain)
Lithophyllum incrustans (KM073284, Spain)
Lithophyllum incrustans (AF093410, UK)
Lithophyllum dentatum (KM073281, Spain)
Lithophyllum atlanticum (ALCB125751, Brazil)
Lithophyllum atlanticum (ALCB125754, Brazil)
Lithophyllum atlanticum (KP192386, Brazil)
Lithophyllum atlanticum (KP192390, Brazil)
Lithophyllum kotschyanum (AB576011, Japan)
Lithophyllum kotschyanum (KM073287, Australia)
Lithophyllum margaritae (KP192391, Brazil)
Lithophyllum margaritae (KP192392, Brazil)
Lithophyllum kotschyanum (U62117, Fiji)
Lithophyllum sp. (ALCB125756, Brazil)
Lithophyllum kotschyanum (DQ628974, Hawaii)
Lithophyllum kotschyanum (AB576008, Japan)
Lithophyllum kotschyanum (AB576010, Japan)
Neogoniolithon spectabile (AY234238, Bahamas)
Neogoniolithon brassica-florida (DQ629007, Hawaii)
Neogoniolithon brassica-florida (DQ629008, Hawaii)
Neogoniolithon fosliei (KM073297, French Polynesia)
Neogoniolithon rhizophorae (ALCB125636, Brazil)
Neogoniolithon rhizophorae (ALCB125634, Brazil)
Neogoniolithon rhizophorae (ALCB125637, Brazil)
Neogoniolithon rhizophorae (ALCB125638, Brazil)
Neogoniolithon rhizophorae (ALCB125639, Brazil)
Neogoniolithon cf. brassica-florida (ALCB125632, Brazil)
Neogoniolithon sp. (KM073300, Spain)
Neogoniolithon crypticum (ALCB125629, Brazil)
Neogoniolithon fosliei (AB713916, Japan)
Neogoniolithon fosliei (AB713920, Japan)
Neogoniolithon fosliei (AB713919, Japan)
Neogoniolithon fosliei (AB713914, Japan)
Neogoniolithon sp. (KM073298, Spain)
Neogoniolithon fosliei (AB713918, Japan)
Neogoniolithon fosliei (KM073294, Australia)
Neogoniolithon megalocystum (AB713921, Japan)
Neogoniolithon sp. (DQ629009, Hawaii)
Pneophyllum confervicola (KM073305, Spain)
Phymatolithon calcareum (JQ896258, United Kingdom)
Lithothamnion corallioides (JQ896261, France)
Mesophyllum lichenoides (KP142819, Spain)
Mesophyllum erubescens (JQ896273, Spain)
Sporolithon tenue (KP142838, Brazil)
Sporolithon cf. ptychoides (KP142840, Brazil)
Sporolithon ptychoides (DQ629014, Hawaii)
0.03

FIGURE S3: The optimal maximum likelihood (ML) topology based on the SSU rDNA data set. Values above each clade refer to Bayesian posterior probabilities (PP) and the ones below each clade refer to ML and MP (maximum parsimony) bootstrap values, respectively. Only bootstrap values for ML and MP ≥ 70% and PP ≥ 0.9 were plotted. New sequences produced in this study are in bold. Asterisks mark clades that are supported at 100% in all three analyses.

## CONSIDERAÇÕES FINAIS

Este estudo abordou as algas coralináceas incrustantes pertencentes à ordem Corallinales na costa brasileira, combinando dados morfoanatômicos e moleculares. A grande plasticidade fenotípica dos membros desta ordem torna a sua taxonomia complexa e a incorporação de novas abordagens, como análises moleculares, tem proporcionado um crescente avanço no conhecimento da diversidade de algas coralináceas incrustantes nas últimas décadas, nesse sentido o presente estudo traz uma considerável contribuição.

No primeiro capítulo, apresentamos a confirmação da ocorrência de espécies do gênero *Harveylithon* no Atlântico Sul. Dado que se trata de um gênero com espécies crípticas, utilizamos diversos testes para a delimitação específica (ABGD, mABGD, PTP e SPN) com base em quatro marcadores moleculares (COI-5P, *psb*A, rbcL-3P e SSU rDNA). A análise conjunta dos dados morfoanatômicos e moleculares resultou na descrição de três espécies novas para a Ciência: *H. catarinense*, *H. maris-bahiensis* e *H. riosmenum*.

No segundo capítulo, o nosso estudo se concentrou no gênero *Neogoniolithon*. As análises de dados morofoanatômicos e moleculares indicaram a ocorrência de três espécies distintas do referido gênero na costa brasileira. Identificamos pela primeira vez a ocorrência de exemplares carposporofíticos de *N. rhizophorae* e o primeiro registro deste táxon para o Atlântico Sul. Confirmamos o polifiletismo de *Neogoniolithon brassica-florida*, e a ocorrência de espécimes morfologicamente semelhantes a este táxon na costa brasileira. Descrevemos uma nova espécie – *N. crypticum* – que apresenta características morfoanatômicas similares a *N. accretum* mas com alta taxa de divergência genética.

As relações filogenéticas entre os membros de Corallinales foram analisadas no capítulo 3. A análise combinada de dois marcadores moleculares (*psb*A e SSU rDNA), juntamente com a análise morfoanatômica de amostras coletadas ao longo da costa brasileira, resultou na confirmação do monofiletismo da família Corallinaceae e de suas respectivas subfamílias (Corallinoideae, Hydrolithoideae, Lithophylloideae, Mastophoroideae, Metagoniolithoideae e Neogoniolithoideae). Foi proposta, ainda, uma nova circunscrição da subfamília Metagoniolithoideae e outras novidades taxonômicas: *Tomitolithon* foi descrito como novo gênero com ocorrência registrada para a costa nordeste do Brasil, incluindo duas novas espécies, *T. deniquerum* e *T. polistratum*. Além disso, ressaltamos a necessidade da revisão das espécies atribuídas aos gêneros

*Pneophyllum* e *Spongites* que ainda se encontram como *incertae sedis* dentro de Corallinaeceae.

Neste trabalho os quatro marcadores testados (COI-5P, *psb*A, rbcL-3P e SSU rDNA) mostraram-se eficazes para a delimitação de gêneros e espécies. Mas ressaltamos, que a região nuclear SSU rDNA deve ser utilizada com cautela, por ser mais eficiente em níveis de ordens, famílias e subfamílias. Em grupos que divergiram recentemente o SSU rDNA pode não apresentar variabilidade suficiente para objetivos de delimitação específica.

Os caracteres morofanatômicos consistentes para a identificação dos táxons estudados foram: tipo de crescimento do talo, tipos de conexões celulares entre células de filamentos adjacentes, forma e tamanho do conceptáculo tetrasporangial, forma e número de células do teto do conceptáculo tetrasporangial, localização dos espermatângios no conceptáculo masculino, e a posição dos carposporângios em relação a célula de fusão no conceptáculo carposporangial. Os citados caracteres mostraram maior eficiência para a delimitação dos táxons ao nível de subfamília e gênero. Para a separação de espécies os caracteres morfoanatômicos foram imprecisos, principalmente devido à existência de espécies crípticas no grupo estudado, necessitando do auxílio da biologia molecular para a confirmação dos táxons.

Nossos resultados demonstraram que a diversidade de Corallinales no litoral do Brasil ainda é subestimada. Estudos anteriores referiram para o Brasil 26 espécies não-geniculadas pertencentes a esta ordem, mas apenas dez foram analisadas por meio de técnicas biomoleculares. Neste estudo, foram gerados dados moleculares para 13 diferentes espécies pertencentes à Corallinales, sendo seis destas espécies novas para a Ciência, além e uma nova adição para o Atlântico Sul. *Harveylithon riosmenum* é a espécie com distribuição mais ampla, ocorrendo de Pernambuco à Santa Catarina. *H. catarinense* está restrita ao litoral de Santa Catarina, *H. maris-bahiensis* ao litoral da Bahia, *Neogoniolithon crypticum* ao litoral do Rio de Janeiro, *Tomitolithon deniquerum* ao litoral de Pernambuco e *T. polistratum* ao litoral do Ceará. Registramos pela primeira vez a ocorrência de *Neogoniolithon rhizophorae* no Atlântico Sul, ocorrendo nos estados da Bahia e Pernambuco. Confirmamos a presença de espécies morfologicamente semelhantes a *N. brassica-florida* no litoral brasileiro. Referimos pela primeira vez *Lithophyllum margaritae* para o litoral da Bahia.

Salientamos que, apesar dos recentes esforços e interesse no conhecimento desse complexo grupo de algas, ainda há grande dificuldade na concatenação dos dados gerados.

Grande parte das informações geradas sobre a diversidade e relações entre as algas coralináceas incrustantes do Brasil está na forma de dissertações ou teses e a disponibilização de sequências nos bancos de dados digitais ainda não foi realizada. Quando for possível ter acesso a estes dados, poderemos ter um melhor entendimento sobre a diversidade do grupo no Atlântico Sul.

Enfatizamos ainda a necessidade da obtenção de sequências dos tipos ou topotipos das espécies já descritas para o melhor entendimento das relações dentro da ordem. Porém, nem sempre é possível a revisitação das localidades de ocorrência das espécies e muitas vezes o estado de conservação dos tipos impossibilita a obtenção de sequências viáveis. A disponibilidade dos tipos por Herbários e trâmites burocráticos como: impedimento de entrada do material no país e extravio do material, tem dificultado que alguns tipos sejam examinados através de técnicas modernas.

Por meio de ampla amostragem da distribuição das espécies e com a combinação de dados morfoanatômicos e moleculares, contribuímos para o início do desvendamento da diversidade de espécies de Corallinales no litoral brasileiro. Os resultados obtidos enfatizam a importância da realização de pesquisas na costa brasileira no intuito de conhecer a real biodiversidade das algas coralináceas nesta área, bem como a compreensão das relações evolutivas desse complexo grupo de algas.

## RESUMO

A ordem Corallinales é, atualmente, composta por uma única família – Corallinaceae – que apresenta seis subfamílias: Corallinoideae, Hydrolithoideae, Lithophylloideae, Mastophoroideae, Metagoniolihoideae e Neogoniolithoideae. As algas coralináceas incrustantes construtoras de recifes são representadas principalmente por táxons da família Corallinaceae. Estas algas produzem importantes depósitos sedimentares, aumentando a heterogeneidade do habitat e disponibilidade de nichos, o que resulta em maior diversidade de espécies. A costa brasileira é notável por ter extensas áreas de recife. Apesar da grande importância deste grupo de algas para a resiliência da biodiversidade, muitas espécies de algas coralináceas incrustantes conhecidas para o Atlântico Sul, como os representantes de Metagoniolithoideae e Neogoniolithoideae, precisam ser amplamente revisadas, além da necessidade de revisão à luz da biologia molecular. Diante disto, o presente estudo teve por objetivo contribuir para o conhecimento da taxonomia das espécies de algas coralináceas incrustantes pertencentes à ordem Corallinales no Atlântico Sul, baseado em estudos morfoanatômicos e moleculares. Foram realizadas coletas em 75 localidades ao longo da costa brasileira. Os táxons foram descritos e identificados com base em características morfoanatômicas, por meio de observações em microscopia óptica e eletrônica de varredura, e com o uso de dados moleculares baseados em quatro marcadores (COI-5P, *psb*A, *rbc*L-3P e SSU rDNA). Foram identificados 13 táxons infragenéricos pertencentes a três subfamílias de Corallinaceae. A subfamília Lithophylloideae foi representada por três espécies (*Lithophyllum atlanticum*, *L. margaritae* e *Lithophyllum* sp.). Neogoniolithoideae foi representada também por três espécies (*Neogoniolithon* cf. *brassica-florida*, *N. rhizophorae* e *N. crypticum* sp. nov.), sendo uma nova espécie (*N. crypticum* sp. nov.) e uma nova ocorrência para o Atlântico Sul (*N. rhizophorae*). Foi proposto o novo gênero *Tomitolithon*, classificado em Metagoniolithoideae, incluindo duas novas espécies (*T. deniquerum* e *T. polistratum*); três novas espécies foram descritas e classificadas no gênero *Harveylithon* (*H. catarinense*, *H. maris-bahiensis* e *H. riosmenum*), além da identificação das espécies *Porolithon onkodes* e *Pneophyllum conicum*. Ademais, uma nova circunscrição da subfamília Metagoniolithoideae foi proposta. Os quatro marcadores testados mostraram-se eficazes para a delimitação de gêneros e espécies na ordem

estudada. No entanto, a região nuclear SSU rDNA deve ser utilizada com cautela para fins de delimitação específica, uma vez que é mais eficiente em níveis maiores, como ordens, famílias e subfamílias. Estes resultados representam uma importante contribuição para o conhecimento da diversidade desse grupo de algas coralináceas incrustantes.

**Palavras chaves:** Algas não-geniculadas, Brasil, Corallinaceae, COI-5P, *psb*A, *rcb*L, SSU rDNA, Taxonomia.

**ABSTRACT**

Corallinales currently includes a single family – Corallinaceae – which presents six subfamilies: Corallinoideae, Hydrolithoideae, Lithophylloideae, Mastophoroideae, Metagoniolihoideae and Neogoniolithoideae. The reef-building crustose coralline algae are mainly represented by taxa classified in Corallinaceae. These algae produce important sedimentary deposits, increasing the habitat heterogeneity and availability of niches, which results in a greater diversity of species. The Brazilian coast is notable for having extensive reef areas. Despite the great importance of this group of algae for the resilience of biodiversity, many species of crustose coralline algae referred for the South Atlantic, as the representatives of Metagoniolithoideae and Neogoniolithoideae, need to be extensively reviewed, besides the need for revision in the light of molecular biology. The present study aimed to contribute to the knowledge of the taxonomy of non-geniculate species of Corallinales in the South Atlantic, based on morpho-anatomical and molecular studies. Sampling was carried out in 75 localities along the Brazilian coast. Taxa were described and identified based on morpho-anatomical characteristics, using optical and scanning electron microscopy observations, and molecular data based on four markers (COI-5P, psbA, rbcL-3P and SSU rDNA). Thirteen infrageneric taxa belonging to three subfamilies of Corallinaceae were identified. The subfamily Lithophylloideae was represented by three species (*Lithophyllum atlanticum*, *L. margaritae* and *Lithophyllum* sp.). Neogoniolithoideae was also represented by three species (*Neogoniolithon* cf. *brassica-florida*, *N. rhizophorae* and *N. crypticum* sp. nov.), including one new to Science (*N. crypticum* sp. nov.), and one new occurrence for the South Atlantic (*N. rhizophorae*). We described a new genus, *Tomitolithon*, classified in Metagoniolithoideae including two new species (*T. deniquerum* and *T. polistratum*); three new species were described and classified in the genus *Harveylithon* (*H. catarinense*, *H. maris-bahiensis* and *H. riosmenum*); and we also identified the species *Porolithon onkodes* and *Pneophyllum conicum*. In addition, a new circumscription of the subfamily Metagoniolithoideae was proposed. The four markers tested were effective for the delimitation of genera and species. However, the nuclear region SSU rDNA should be used with caution for species delimitation, as it is more efficient at higher levels such as orders, families and subfamilies.

These results represent an important contribution to the knowledge of the diversity of this group of crustose coralline algae.

**Keywords:** Nongeniculate algae, Brazil, Corallinaceae, COI-5P, *psb*A, *rcb*L, SSU rDNA, Taxonomy.