A taxonomic account of non-geniculate coralline algae (Corallinophycidae, Rhodophyta) from shallow reefs of the Abrolhos Bank, Brazil

Michel B. Jesionek¹, Ricardo G. Bahia¹, Jazmín J. Hernández-Kantún², Walter H. Adey², Yocie Yoneshigue-Valentin³, Leila L. Longo⁴ and Gilberto M. Amado-Filho¹,*

¹Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Diretoria de Pesquisa Científica, Rua Pacheco Leão 915, Rio de Janeiro, RJ 22460-030, Brazil
²Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560, USA
³Departamento de Botânica, Instituto de Biologia, Universidade Federal do Rio de Janeiro (UF RJ), Av. Carlos Chagas Filho 373, Rio de Janeiro, RJ 21941-902, Brazil
⁴Departamento de Oceanografia e Ecologia, Universidade Federal do Espírito Santo, Vitória, ES 29075-910, Brazil

The Abrolhos Continental Shelf (ACS) encompasses the largest and richest coral reefs in the southern Atlantic Ocean. A taxonomic study of non-geniculate coralline algae (NGCA) from the region was undertaken using both morpho-anatomical and molecular data. Specimens of NGCA were collected in 2012 and 2014 from shallow reefs of the ACS. Phylogenetic analysis was performed using dataset of psbA DNA sequences from 16 specimens collected in the ACS and additional GenBank sequences of related NGCA species. Nine common tropical reef-building NGCA species were identified and described: *Hydrolithon boergesenii*, *Lithophyllum kaiseri*, *Lithophyllum* sp., *Lithothamnion crispatum*, *Melyvonnea erubescens*, *Pneophyllum conicum*, *Porolithon onkodes*, *Sporolithon ptychoide* and *Titanoderma prototypum*. A key for species identification is also provided in this study. Our molecular phylogenetic analyses suggest that *Lithophyllum* sp. corresponds to a new species. Our study also confirms that *Lithophyllum kaiseri* is a new record in Brazil. The psbA sequences of *Lithophyllum kaiseri* and *Melyvonnea erubescens* matched with type specimens indirectly. The taxonomic identification of the remaining species was supported by morpho-anatomical evidences as DNA sequences of their types or topotypes remain unavailable.

Key Words: Atlantic; Corallinales; Hapalidiales; psbA; Sporolithales; taxonomy

INTRODUCTION

The Abrolhos Continental Shelf (ACS), also known as the Abrolhos Bank, encompasses an area of approximately 46,000 km². It is home to the largest coralline algal formations in the southern Atlantic Ocean (Amado-Filho et al. 2012, Moura et al. 2013). The area represents the most biodiverse marine ecosystem in the South Atlantic (Dutra et al. 2005). It is a global marine conservation priority (Moura 2000). Nearly 300 species of fish and 20 species of reef-building corals are recorded in a benthic mosaic of rhodolith beds, hard calcareous reefs, soft bot-
Non-geniculate coralline red algae (NGCA) (Corallinales, Hapalidiales and Sporolithales, Rhodophyta) rank as the fifth most abundant benthic organisms in the ACS. Along with corals, NGCA are considered as the main builders of ACS reefs (Francini-Filho et al. 2013). The NGCA provide habitats for diverse flora and fauna (Nelson 2009). Some species are known to be cues for the settlement and metamorphosis of the larval stages of key invertebrate taxa such as corals and economically important mollusks (Roberts 2001, Harrington et al. 2004). NGCA have complex taxonomic history. This is mainly due to their phenotypic plasticity which can be influenced by environmental conditions and the high number of cryptic species. In addition, their identification is difficult due to their calcified thalli that require time consuming and challenging procedures to observe the essential anatomical characters (Riosmena-Rodríguez et al. 1999, Saunders 2005, Maneveldt and Keats 2008, Bittner et al. 2011, Sissini et al. 2014, Nelson et al. 2015). Identification of NGCA exclusively based on morpho-anatomy faces some limitations, including the need to examine fertile specimens of a particular life cycle stage for their identification at species level (Harvey et al. 2005).

Several recent studies have used molecular data to study coralline algae in order to reassess the classification of NGCA group at different levels, including species (Walker et al. 2009, Kato et al. 2013, Sissini et al. 2014, Bahia et al. 2015), genus (Bailey 1999, Bittner et al. 2011), subfamily (Kato et al. 2011, Rösler et al. 2016), and order levels (Le Gall et al. 2010, Nelson et al. 2015). These studies reinforce the importance of combining morpho-anatomical data with molecular data for their identification. Increasingly DNA barcoding of NGCA is used to provide more reliable taxonomic characters and the understanding of their biogeography (Kato et al. 2013, Carro et al. 2014, Hernandez-Kantun et al. 2016).

Taxonomic studies of coralline algae in the southwestern Atlantic have mostly been devoted to unattached, free-living, and rhodolith-forming species (e.g., Villas-Boas et al. 2009, Amado-Filho et al. 2010, 2012, Bahia et al. 2011, 2014, 2015, Bahia 2014). Consequently, little is known about the attached NGCA species compositions, their taxonomy, or distribution in the southwestern Atlantic (Mariath et al. 2012, Bahia et al. 2014, Crespo et al. 2014, Tâmega et al. 2014). To the best of our knowledge, only two previous studies (Figueiredo and Steneck 2000, Tâmega et al. 2014) have studied NGCA of the ACS reefs. Figueiredo and Steneck (2000) have listed 11 NGCA species. However, they did not provide any description. More importantly, their specimens could not be traced in RB (Jardim Botânico do Rio de Janeiro, Rio de Janeiro, Brazil), making positive identifications of their specimens impossible. Tâmega et al. (2014) have described four species as the main NGCA reef-forming species in the ACS, namely Lithophyllum stictaeforme (Areschoug) Hauck, Porolithon onkodes, Spongites fruticulosus Kützing, and Neogoniolithon atlanticum Tâmega, Riosmena-Rodríguez, Mariath & M. Figueiredo. However, these taxa were not analyzed molecularly.

Therefore, the aim of this study was to use both morpho-anatomical data and molecular data to improve the current knowledge of NGCA, one of the main reef-building groups of the ACS.
**MATERIALS AND METHODS**

**Sampling and study sites**

Specimens were collected from ACS by SCUBA diving in shallow reefs (2-7 m). NGCA were taken from Pedra de Leste (1), Abrolhos Archipelago (2), Parcel dos Abrolhos (3), and Recife Caliôrnia (4) (Fig. 1). Of the four sites, only Pedra de Leste is located at the inner reef arc falling outside of the AMNP marine protected area. The remaining sites are within the AMNP. They are located at the outer reef arc.

Fragments measuring around 10 cm² were collected from substrates using a hammer and chisel. Samples were brushed to remove epibionts, air dried, and preserved in silica gel, including those selected for morpho-anatomical analysis.

**Morpho-anatomical analysis**

Samples used for light microscopy examination were prepared using histological method described by Maneveldt and van der Merwe (2012). No specific method was used for re-hydration prior to decalcification. Satisfactory results were obtained by putting dry samples directly in 10% nitric acid for decalcification following the steps for embedding and sectioning described by Maneveldt and van der Merwe (2012). Using this alternative method, exposure to formaldehyde solution (normally used for algae fixation) was avoided. For species description, growth-form terminology followed Woelkerling et al. (1993) and thallus anatomical terminology followed Adey and Adey (1973). The distance between primary pit connections was determined for cell length measurements. The maximum cell lumen at right angle to the length was measured for cell diameter. Conceptacle measurements followed Adey and Adey (1973).

**Molecular analysis**

Genomic DNA extraction and polymerase chain reaction (PCR) amplification were carried out at the Molecular Biology Laboratory of the Smithsonian National Museum of Natural History, Washington DC, USA. DNA extraction was performed using fragments of thalli (~15 mg) that were visually clean and free of epibionts. For DNA extraction, Qiagen DNeasy Blood and Tissue Kit (Qiagen, Crawley, UK) was used following the protocol of Broom et al. (2008) with slight modification. The *psbA* gene was amplified using primers *psbA*-F1 and *psbA*-R2 using published protocol of Yoon et al. (2002).

The PCR mix contained 2.5 μL of 10× buffer, 2 μL dNTPs (10 mM), 1.5 μL MgCl₂ (25 mM), 0.5 μL bovine serum albumin, 1 μL of each primer (forward and reverse) (10 μM), 0.2 μL Invitrogen Taq polymerase (5 μg μL⁻¹), 15.8 μL HyPure TM Cell Culture Grade Water (Thermo Scientific, Milwaukee, WI, USA), and 1 μL DNA template. The PCR cycle consisted of the following: an initial denaturation step at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 45 min, and elongation at 72°C for 1 min; one final cycle at 94°C for 30 s and a final extension at 72°C for 7 min. PCR products were visualized and quantified on agarose gels and sequenced commercially at Macrogen Inc. (Seoul, Korea).

**Phylogenetic construction**

The dataset comprised of 16 *psbA* sequences generated in the present study and 46 sequences selected from GenBank (Supplementary Table S1) using the Basic Local Alignment Search Tool (BLAST). Two GenBank sequences from specimens in the order of Rhodogorgonales (Corallinophycidae, Rhodophyta) were used as outgroup (Supplementary Table S1).

Phylogenetic relationships were analyzed using maximum likelihood (ML) and neighbor joining (NJ) analysis with bootstrap (BS) of 1,000 replicates for each data set (Felsenstein 1985). It was performed with Geneious R7 and MEGA6 (Tamura et al. 2013). The *jModelTest* 2.1.4 (Darriba et al. 2012) was used to estimate model parameters.

**RESULTS AND DISCUSSION**

Nine non-geniculate coralline red algal species were identified from shallow reefs of the ACS based on morpho-anatomical and DNA data. They comprised of representatives of the Corallinales (*Hydro lithon boergesenii* (Foslie) Foslie, *Lithphyllum kaiser* (Heydrich) Heydrich), *Lithophyllum* sp., *Pneophyllum conicum* (E. Y. Dawson) Keats, Y. M. Chamberlain & M. Baba, *Porolithon onkodes* (Heydrich) Foslie, and *Titanoderma prototypum* (Foslie) Woelkerling, Y. M. Chamberlain & P. C. Silva), Hapalidiales (*Melyvonnea erubescens* (Foslie) Athanasiadis & D. L. Ballantine and *Lithothamnion crispatum* Hauck), and Sporolithales (*Sporolithon ptychoides* Heydrich). The distributions of these species are shown in Table 1. Abrolhos Archipelago was the richest site. All identified NGCA species were found at this site.
A key to species, detailed species descriptions, and comparisons with related species, including molecular data, are shown below:

**Key to common non-geniculate coralline red algae in shallow reefs of the ACS**

1. Tetra/bisporangia cruciately divided, borne in calcified compartments grouped into sori ----------------- Sporolithon ptychoides
   Tetra/bisporangia zonately divided, borne in roofed conceptacles ------------------------ 2
2. Tetra/bisporangia producing apical plugs and borne in multiporate conceptacles ----------------- 3
   Tetra/bisporangia not producing apical plugs and borne in uniporate conceptacles ----------------- 4
3. Epithallial cells with distal walls flattened and flared. Roofs of mature conceptacles pitted with depressions resulting from disintegration of rosette cells surrounding the pores ----------------- Lithothamnion crispatum
   Epithallial cells with distal walls rounded or flattened but not flared. Roofs of mature conceptacles not pitted with depressions ----------------- Melyvonnea erubescens
4. Adjacent filaments joined primarily by secondary pit connections -------------------------- 5
   Adjacent filaments joined primarily by cell fusions -- 6
5. Thallus fruticose, not applanate -------------------------- Lithophyllum kaiseri or Lithophyllum sp.
   Thallus layered and applanate, giving the thallus a terraced appearance in surface view -------------------------- Titanoderma prototypum
6. Thallus dimerous, trichocytes arranged singly or in pairs -------------------------- Hydrolithon boergesenii
   Thallus monomerous, trichocytes arranged in large, tightly packed horizontal fields -------------------- 7
7. Tetrasporangial pore canals lined by a ring of conspicuous and enlarged cells that are orientated more-or-less perpendicularly (vertically orientated) to the roof surface -------------------- Porolithon onkodes
   Tetrasporangial pore canals lined by cells oriented more-or-less parallel or at a sharp angle to the conceptacle roof surface -------------------- Pneophyllum conicum

*No remarkable morpho-anatomical differentiation was observed for Lithophyllum kaiseri and Lithophyllum sp.*

**Species descriptions**

**Corallinaceae P. C. Silva & H. W. Johansen**
**Corallinaceae J. V. Lamouroux**
**Hydroolithoidea A. Kato & M. Baba in A. Kato et al.**

Hydrolithon (Foslie) Foslie
**Hydrolithon boergesenii** (Foslie) Foslie
**Hydrolithon** (Foslie) Foslie

Habit and ecological observations. Thalli attached, encrusting to lumpy (Fig. 2A). Thallus surface generally smooth. Color of living thalli whitish or pink to purple. Species limited to shallow areas (2-5 m depth) of the reef with direct exposure to sunlight. External appearance and ecological niche similar to that of Porolithon onkodes, making field identification difficult.

**Table 1.** Non-geniculate coralline algae species distribution along the shallow reefs of the Abrolhos Continental Shelf

<table>
<thead>
<tr>
<th>Species</th>
<th>Inner reef arc</th>
<th>Outer reef arc</th>
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<tr>
<td></td>
<td>Pedra de Leste</td>
<td>Abrolhos Archipelago</td>
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<td><strong>Corallinaceae</strong></td>
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<td>Hydrolithon boergesenii (Foslie) Foslie</td>
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<td>Lithophyllum kaiseri (Heydrich) Heydrich</td>
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<td>Lithophyllum sp.</td>
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<td>Pneophyllum conicum (E. Y. Dawson) Keats, Y. M. Chamberlain &amp; M. Baba</td>
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<td>Porolithon onkodes (Heydrich) Foslie</td>
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<td>Titanoderma prototypum (Foslie) Woelkerling, Y. M. Chamberlain &amp; P. C. Silva</td>
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<td><strong>Hapalidiaceae</strong></td>
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<td>Lithothamnion crispatum Hauck</td>
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<tr>
<td>Melyvonnea erubescens (Foslie) Athanasiadis &amp; D. L. Ballantine</td>
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<td>x</td>
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<tr>
<td><strong>Sporolithaceae</strong></td>
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<td>Sporolithon ptychoides Heydrich</td>
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https://doi.org/10.4490/algae.2016.31.11.16
Fig. 2. (A-F) Vegetative and reproductive features of *Hydro lithon boergesenii* (specimen RB 621493). (A) General view of the specimen with encrusting to lumpy growth form. (B) Section through the vegetative thallus showing the dimerous thallus construction and numerous trichocytes, singly or in pairs, both at the thallus surface (arrow) and buried (arrowhead). (C) Basal portion of the thallus showing its dimerous construction and cell fusions connecting adjacent filaments (arrows). (D) Details of the thallus surface showing rounded or flattened epithallial cells (e), subepithallial initials (i) as long as or longer than their immediate inward derivatives and trichocytes arranged singly (arrow) or in pair (arrowhead). (E) Section through a protruding mature uniporate tetrasporangial conceptacle showing the sunken pore opening (d) and enlarged cells (arrows) lining the base of the pore canal. (F) Section through a young uniporate tetrasporangial conceptacle showing the remains of filaments (arrows) interspersed among the tetrasporangial initials that formed the conceptacle roof. Scale bars represent: A, 2 cm; B, 170 μm; C, 60 μm; D, 50 μm; E, 125 μm; F, 100 μm.
Vegetative and reproductive anatomy. Thallus dimerous (Fig. 2B), basal cells square to elongate (Fig. 2C), erect filaments composed of cells at 7-15 μm in diameter and 5-25 μm in length. Subepithallial initials usually as long as or longer than immediate inward derivatives (Fig. 2D). Epithallial cells occur in a single layer with outer walls rounded or flattened, but not flared (Fig 2D). Cells of adjacent filaments connected by cell fusions; secondary pit connections not observed (Fig. 2C). In large portions of thallus, outline of erect filaments entirely lost due to widespread and extensive cell fusions, giving thallus a distinct appearance in cross-section (Fig. 2B & C). Trichoocytes common, occur singly or in pairs at both thallus surface and buried in thallus (Fig. 2B & D). Only tetrasporangial thalli observed. Tetrasporangial conceptacles uniporate, raising above surrounding thallus surface (Fig. 2E). Conceptacle chambers are spherical to elliptical, 215-255 μm in diameter and 125-145 μm in height. Floors are located 10-15 cells below surrounding thallus surface. Conceptacle roofs are 5-6 cells thick (including epithallial cells) (Fig. 2E & F). Conceptacle roofs formed from filaments peripheral to and interspersed among tetrasporangial initials (Fig. 2F). Pores sunken, pore canal lined by a ring of conspicuous, enlarged cells without protruding into pore canal, orientated more-or-less perpendicularly (vertically orientated) to roof surface (Fig. 2E & F).


Comments. H. boergesenii has been previously recorded from Abrolhos reefs in the checklist provided by Figueiredo and Steneck (2000). However, this species is described for the first time in details for the region. The examined specimen of H. boergesenii is morphologically (externally) similar to P. onkodes. Anatomically, H. boergesenii can be differentiated from P. onkodes mainly by four anatomical features. H. boergesenii has a dimerous thallus construction and a single layer of epithallial cells. It bears trichoocytes arranged singly or in pairs. It has uniporate tetrasporangial conceptacles with a sunken pore opening in relation to the surrounding conceptacle roof surface (Maneveldt 2005). On the other hand, P. onkodes possesses a monomorous thallus construction. It has more than one layer of epithallial cells. It bears trichoocytes arranged in large, tightly packed horizontal fields. It has uniporate tetrasporangial conceptacles with pore opening at the same level as the surrounding conceptacle roof surface (Maneveldt and Keats 2014). H. boergesenii might have been overlooked in previous studies in Abrolhos due to its superficial resemblance to P. onkodes.

Lithophylloideae Setchell
Lithophyllum Philippi
Lithophyllum kaiseri (Heydrich) Heydrich

Habit and ecological observations. Thalli fruticose, attached to corals, NGCA or other biogenic reef structure. Color of living thalli pink to dark red (Fig. 3A). Protruberances with smooth surface, usually compressed, some fused at distal ends (Fig. 3A). This species occurs like small shrubs spread along shallow areas (2-5 m depth) of ACS reefs. External appearance and ecological niche similar to those of Lithophyllum sp., making field identification difficult.

Vegetative and reproductive anatomy. Only protuberant branches were sectioned, exhibiting radial internal organization. Since no crustose portion was analyzed, information on thallus construction could not be provided. Vegetative filaments composed of cells at 5-12 μm in diameter and 5-20 μm in length. Subepithallial initials usually as long or longer than immediate inward derivatives (Fig. 3B). Epithallial cells elliptical to rounded, forming a unistratose layer (Fig. 3B). Cells of adjacent filaments joined by secondary pit connections (Fig. 3C). Trichoocytes single or in pair, abundantly present in either thallus surface or buried (Fig. 3B). Only tetrasporangial thalli observed. Tetrasporangial conceptacles uniporate, flush or slightly raised above the surrounding thallus surface (Fig. 3D-F). Conceptacle chambers at 200-350 μm in diameter and 115-150 μm in height. Conceptacle roofs formed by 4-6 cells layers (including epithallials) (Fig. 3E & F). Conceptacle floor located 12-16 cells below surrounding thallus surface. Conceptacle pore canals lined by cells that may protrude laterally into pore canal, not completely occluding canal (canal may be blocked by mucilaginous material) (Fig. 3E & F). Tetrasporangia formed peripheral to a central columella (Fig. 3E).


Comments. Hernandez-Kantun et al. (2016) have reassessed the branched Lithophyllum spp. in the Caribbean Sea by combining DNA sequencing of type and field-collected specimens with morpho-anatomical data and recognized four Lithophyllum species for specimens previously assigned to Lithophyllum congestum (Foslie) (Foslie). Surprisingly, Lithophyllum kaiseri (= Lithophyllum congestum) was confirmed to be widespread through the tropical Indo-West Pacific Oceans, Red Sea, and Ca-

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Fig. 3. (A–F) Vegetative and reproductive features of *Lithophyllum kaiseri*. (A) General view of a fruticose specimen (RB 623160). (B) Transversal section showing trichocytes arranged singly or in pair at the thallus surface (arrow) or buried (arrowheads). (C) Transversal section through the vegetative thallus showing secondary pit connections (arrows). (D) Surface view of flush to slightly raised uniporate tetrasporangial conceptacles. (E) Section through tetrasporangial conceptacles showing central collumela (c) in the center of chamber floor. (F) Longitudinal section showing trasporangial conceptacle roof composed of 4-6 layers of cells and pore canal lined by cells that do not occlude the canal. Scale bars represent: A, 2 cm; B, 60 μm; C, 15 μm; D, 1 mm; E, 150 μm; F, 65 μm.
ribbean Sea. It exhibits identical or nearly identical sequences of both rbcl and psbA markers over these regions. *L. kaiser i* is now being confirmed to be in the South Atlantic by this study (see molecular results), expanding its known geographical distribution. The single specimen (RB 623160) analyzed in the present study with compressed protuberances is morphologically similar to the syntype specimen of *Lithophyllum congestum* (TRH A23-1381, S13) illustrated by Hernandez-Kantun et al. (2016, Fig. 5). All other measured morpho-anatomical features of *L. kaiseri* from the Abrolhos overlapped with those of species lectotype from the Red Sea (Basso et al. 2015) and *L. kaiseri* from the Caribbean (Hernandez-Kantun et al. 2016). No central colulma was observed in tetrasporangial conceptacles of the lectotype (Basso et al. 2015). However, this structure was preset in either the Brazilian (the present study) or the Caribbean (Hernandez-Kantun et al. 2016) specimens.

**Lithophyllum sp.**

**Habit and ecological observations.** Thalli fruticose, attached to corals of NGCA or other biogenic reef structure. Color of living thalli pink to dark red (Fig. 4A & B). Protuberances with smooth surface, generally cylindrical, terminating with plump apex, some fused at distal ends (Fig. 4A & B). This species occurs like small shrubs spreading along shallow areas (2-5 m depth) of ACS reefs.

**Vegetative and reproductive anatomy.** Thallus dimerous, consisting of a single ventral layer of more or less quadratic cells (non-palisade), erect filaments arising perpendicularly to ventral layer (Fig. 4C). Erect filaments composed of cells at 5-11 μm in diameter and 4-20 μm in length. Subepithallial initials usually as long or longer than immediate inward derivatives (Fig. 4D & E). Epithallial cells elliptical to rounded, forming a unistratose layer (Fig. 4E). Cells of adjacent filaments joined by secondary pit connections (Fig. 4E). Trichocytes single, abundantly present in thallus surface or buried (Fig. 4D). Only tetrasporangial thalli observed. Tetrasporangial conceptacles uniporate, flush or slightly raised above surrounding thallus surface (Fig. 4F & G). Conceptacle chambers with 330-345 μm in diameter and 115-130 μm height. Conceptacle roofs formed by 4-6 layers cells (including epithallials) (Fig. 4G). Conceptacle floor located 9-12 cells below surrounding thallus surface. Conceptacle pore canals lined by cells that may protrude laterally into pore canal, not completely occluding canal (Fig. 4G). Tetrasperangia formed peripheral to a central columella (Fig. 4G).


**Comments.** Lithophyllum sp. is morpho-anatomically similar to *L. kaiseri*. All traditional features used to distinguish *Lithophyllum* species are overlapping. It is premature to speculate which characters or combinations of characters may be used to differentiate the two species considering the limited number of specimens analyzed. Further studies using more samples and DNA sequencing could be used to identify diagnostic morpho-anatomical features. It is worth noting that we have observed the presence of trichocytes in both *L. kaiseri* and *Lithophyllum* sp. Prior to Basso et al. (2014), trichocytes are generally considered as rare or absent in Lithophyllum (Harvey et al. 2009). Hernandez-Kantun et al. (2016) have noted that trichocytes are present in all tropical Red Sea and Caribbean *Lithophyllum* species, but not in temperate European species. Morphologically speaking, the fruticose growth-form of the branched *Lithophyllum* sp. and *L. kaiseri* found in the present study with their cylindrical or compressed protuberances notably resemble that of other *Lithophyllum* species, including *L. affine* (Foslie) Foslie, *L. kotschyanum*, *L. neocongestum* J. J. Hernandez-Kantun, W. H. Adey & P. W. Gabrielson, *L. platyphyllum* (Foslie) Foslie, *L. pseudoplatyphyllum* J. J. Hernandez-Kantun, W. H. Adey & P. W. Gabrielson *L. subplicatum* (Foslie) Basso, Caragnano, Le Gall & Rodondi, *L. subreduncum* Foslie, and *L. yemenense* Basso, Caragnano, Le Gall & Rodondi (Basso et al. 2015, Hernandez-Kantun et al. 2016). Anatomically, these species can be distinguished by minor differences in tetrasporangial conceptacle dimensions, number of cells in the conceptacle roof, and depth of the conceptacle chamber floor relative to the thallus surface (Basso et al. 2015, Hernandez-Kantun et al. 2016). Previous studies about ACS reefs have considered that the typical fruticose morphotype of *Lithophyllum* with cylindrical or compressed protuberances (here reported for *Lithophyllum* sp. and *L. kaiseri*) belongs to a single species, i.e., *L. congestum* (currently confirmed to be an heterotypic synonym of *L. kaiseri* by Hernandez-Kantun et al. 2016) (Figueiredo and Steneck 2000).

**Titanoderma Nägeli**

**Titanoderma prototypum** (Foslie) Woelkerling, Y. M. Chamberlain & P. C. Silva

**Habit and ecological observations.** Thalli attached, pink to purple with layered growth-form, 2-7 m in depth. Thallus surface marked by successive layers of planate
Fig. 4. (A-G) Vegetative and reproductive features of Lithophyllum sp. (A) General view of a fruticose specimen (RB 623158) in the field. (B) Fragments of another fruticose specimen (RB 623159). (C) Basal portion of the thallus showing the dimerous thallus construction. (D) Vertical section showing trichocytes arranged singly at the thallus surface or buried (arrows). (E) Section through the thallus surface showing elliptical to rounded epithallial cells (e) and secondary pit connections (arrows). (F) Surface view of flush to slightly raised uniporate tetrasporangial conceptacles. (G) Section through a tetrasporangial conceptacle showing vestiges of a central collumela (c) and a zonately divided tetrasporangium (arrow) peripherally arranged. Note that the conceptacle pore canal is lined by cells that do not occlude the canal. Scale bars represent: A, 3 cm; B, 2 cm; C & G, 50 μm; D, 70 μm; E, 30 μm; F, 500 μm.
branches with swirled margins, giving thallus a terraced appearance in surface view (Fig. 5A).

Vegetative and reproductive anatomy. Thallus dimerous, with overlapping layers as a result of secondary growth, resulting in appplanate branching. Vegetative thalli consist of a single basal layer of palisade cells, 10-21 μm in diameter and 20-40 μm in length, and a single layer of epithallial cells, 7-11 μm in diameter and 4-5 μm in length. Applanate branches are adjoined to each other (Fig. 5B) or more or less separated (Fig. 5C). Epithallial cells rounded or triangular (Fig. 5B & C). Basal cells of each branch connected laterally by secondary pit connections (Fig. 5B). Trichocytes not found. Only tetrasporangial plants observed. Tetrasporangial conceptacles uniporate, protruding above surrounding vegetative thallus surface (Fig. 5D). Conceptacle chambers 330-415 μm in diameter and 100-190 μm in height. Conceptacle floor located only one cell below surrounding thallus surface (Fig. 5E). Conceptacle roofs formed by 2-3 cells layers (including epithallial). Conceptacle roof formed from filaments peripheral to and interspersed among tetrasporangial initials. Pore canals lined by cells that may project slightly into canal without completely occluding entire canal. A mucilage plug may occludes pore opening (Fig. 5F).

Examined specimens. Brazil, Bahia, Caravelas, Parcel das Paredes, Pedra de Leste (17°47′00″ S, 39°03′05″ W, RG Bahia, Jun 29, 2014, RB 632601). Brazil, Bahia, ACS (16°54′36″ S, 38°40′37″ W, GM Amado-Filho, Jul 26, 2009, RB 534878, as Lithophyllum prototypum); Espírito Santo, Abrolhos Bank (19°32′23″ S, 38°46′06″ W, GM Amado-Filho, Nov 20, 2010, RB 525331, as Lithophyllum prototypum).

Comments. Titanoderma prototypum was first reported from the South Atlantic Ocean by Pereira-Filho et al. (2011). However, they did not present a description for this species. Therefore, our finding represents the first documented record of the species from the South Atlantic Ocean by Pereira-Filho, Abrolhos Bank (19°32′23″ S, 38°46′06″ W, GM Amado-Filho, Nov 20, 2010, RB 525331, as Lithophyllum prototypum). In addition, we report the thallus of this species is pink to purple in color, covering large areas of top and walls of reefs at 2-7 m in depth. Often overgrowing, consequently killing hydrocorals, stony corals, and other NGCA species.

Vegetative and reproductive anatomy. Thallus monomeric with medulla arranged in a coaxial manner (Fig. 6C), filaments (both medullary and cortical) composed of cells 3-11 μm in diameter and 5-25 μm in length. Sub-epithallial initials usually as long or longer than immediate inward derivatives (Fig. 6D). Epithallial cells occur in a single layer with outer walls rounded but not flared (Fig. 6D). Cells of adjacent filaments connected by cell fusions. Secondary pit connections not observed (Fig. 6D). Trichocytes are abundant and arranged in large and tightly packed horizontal fields (Fig. 6D). Trichocyte fields not buried. Tetrasporangial and carpogonial (female) thalli observed. Tetrasporangial conceptacles uniporate, raising above surrounding thallus surface (Fig. 6E). Chambers elliptically flattened, 260-360 μm in diameter and 90-115 μm in height. Floors located 6-12 cells below surrounding thallus surface. Roofs 5-10 cells thick (including epithallial cells) (Fig. 6E). No conceptacle primordia observed. From orientation of conceptacle roof cells, roof appearing formed from filaments peripheral to and interspersed among tetrasporangial initials. Conceptacle pore canals lined by cells oriented more-or-less parallel or at a sharp angle to roof surface (Fig. 6E). Zonately divided tetrasporangia arranged peripherally to a central columella (Fig. 6E). Buried conceptacles not found. Carpogonial (female) conceptacles uniporate, raising above surrounding thallus surface (Fig 6F). Chambers 270-300 μm in diameter and 70-115 μm in height. Floors located 0-8 cells below surrounding thallus surface. Conceptacle roof with 7-9 layers of cells (including epithallial). Two-celled carpogonial branches terminate in a carpogonium that extends into an elongate trichogyne, may project through pore canal (Fig. 6F).
Fig. 5. (A-F) Vegetative and reproductive features of *Titanoderma prototypum*. (A) Surface view of an encrusting specimen (RB 632601) showing applanate branches with swirled margins (arrow). (B) Section through the thallus showing numerous layers of vertically adjoined applanate branches (1-7). Note that each branch is composed of a basal layer of palisade cells and a layer of dark staining triangular epithallial cells. Note also secondary pit connections (arrow) between cells of adjoining filaments. (C) Vertical section of the thallus showing more-or-less separated applanate branches. (D) Longitudinal section through a uniporate tetrasporangial conceptacle. Note the remains of filaments interspersed among the tetrasporangial initials (arrow) originated from the conceptacle roof. (E) Details of the tetrasporangial conceptacle floor located only one cell below the surrounding thallus surface. (F) Details of a tetrasporangial pore canal lined by cells that project slightly into the canal without completely occluding the entire canal (arrows). A mucilage plug occludes the pore opening (arrowhead). Scale bars represent: A, 3 mm; B & E, 80 μm; C, 100 μm; D, 130 μm; F, 60 μm.
Fig. 6. (A-F) Vegetative and reproductive features of *Pneophyllum conicum*. (A) Warty specimen (RB 621932) in the field growing on the wall of a pinnacle reef of Parcel dos Abrolhos. (B) Encrusting to warty specimens (RB 621924) collected at 4 m depth in the Abrolhos Archipelago. (C) Vertical section through the thallus showing monomerous internal construction with a predominantly coaxial medulla. (D) Vertical section through the thallus surface showing rounded epithallial cells (e), cell fusions (arrow), and trichocytes arranged in a large and tightly packed horizontal field (arrowhead). (E) Longitudinal section through a uniporate tetrasporangial conceptacle showing a central columella (arrow). (F) Longitudinal section through a uniporate carpogonial conceptacle showing carpogonial branches at the center of the conceptacle floor, some extends into a trichogyne (arrow) that projects through the conceptacle pore canal. Scale bars represent: A, 5 cm; B, 2 cm; C, 140 μm; D, 70 μm; E, 120 μm; F, 75 μm.

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Comments. *Pneophyllum conicum* has been reported to be a widespread Indo-Pacific NGCA that can overgrow and kill live coral (Keats et al. 1997, Antonius 2001). This species has been recently reported in Brazil by Mariath et al. (2012) in Porto Seguro, a region about 150 km north of the ACS. The specimens of *Pneophyllum conicum* analyzed in this study are morpho-anatomically similar to those described by Mariath et al. (2012). Because of its coral killer behavior, *P. conicum* could represent a threat to the ACS coral community. Since this species has been positively identified in the ACS reefs, further studies are needed to determine this species’ abundance, functional dynamics, and potential threat in this important Atlantic region. In a five-marker molecular phylogeny analysis for family Corallinaceae, Rösler et al. (2016) have found that specimens morpho-anatomically attributed to *Pneophyllum* (including *P. conicum*) are distributed in separate clades, concluding that this genus requires reassessment. *Pneophyllum conicum* should be considered as uncertain genus until unambiguous attribution of DNA sequences to type material or epitypes from the type locality are obtained for this species and the generitype *Pneophyllum fragile* Kützing (Rösler et al. 2016).

*Porolithon Foslie*

*Porolithon onkodes* (Heydrich) Foslie

Habit and ecological observations. Thalli attached, encrusting to lumpy (Fig. 7A) with surface generally smooth. Color of living thalli whitish or pink to purple. Crusts thick (>1 mm), generally capable of overgrowing thinner ones such as those from *T. prototypum* (Fig. 7B). Species limited to shallow areas (2-5 m depth) of reef with direct exposure to sunlight. Borer holes frequently seen at thallus surface.

Vegetative and reproductive anatomy. Thallus monomorphic and non-coaxial (Fig. 7C), filaments (medullary and cortical) composed of cells 4-13 μm in diameter and 6-26 μm in length. Subepithallial initials usually as long as or longer than immediate inward derivatives. Epithallial cells occur in 1-3 layers with outer walls elliptical to rounded, but not flared (Fig. 7D). Cells of adjacent filaments connected by cell fusions; secondary pit connections not observed. Trichocytes abundant and arranged in large and tightly packed horizontal fields at thallus surface (Fig. 7C) or buried in thallus (Fig. 7C & D). Only tetrasporangial thalli observed. Tetrasporangial conceptacles uniporate, more-or-less flush, only slightly raising above surrounding thallus surface. Conceptacle chambers elliptical, 220-230 μm in diameter and 100-120 μm in height. Floors located 10-17 cells below surrounding thallus surface. Conceptacle roofs 4-6 cells thick (including epithallial cells) (Fig. 7F). Conceptacle primordia not observed. From orientation of conceptacle roof cells, roof appearing formed from filaments peripheral to and interpolated among tetrasporangial initials. Conceptacle pore canals lined by a ring of conspicuous and enlarged cells that do not protrude into pore canal, orientated more-or-less perpendicularly (vertically orientated) to roof surface (Fig. 7F).

Examined specimen. Brazil, Bahia, Parcel das Paredes, Pedra de Leste (17°47′00″ S, 39°03′05″ W, RG Bahia, Jun 29, 2014, RB 623155).

Comments. According to Maneveldt and Keats (2014), *P. onkodes* is one of the most widespread tropical to subtropical NGCA. Although generally encrusting to smooth surfaced, it has a various morphologies largely resulting from grazing. *Porolithon onkodes* (as *P. pachydernum*) has been cited by Figuieredo and Steneck (2000) and Tâmega et al. (2014) as one of the dominant NGCA species in the shallow reefs of the ACS.

*Hapalidiales W. A. Nelson, J. E. Sutherland, T. J. Farr & H. S. Yoon in Nelson et al.*

*Hapalidaceae J. E. Gray*

*Melobesioidae Bizzozero*

*Lithothamniaceae Heydrich*

*Lithothamnion crispatum* Hauck

Habit and ecological observations. Thalli attached or free-living, lumpy to fruticose (Fig. 8A). In most cases, protuberances short and nodular (Fig. 8A). Color of living thalli pink to purple.

Vegetative and reproductive anatomy. Thallus monomorphic non-coaxial (Fig. 8B), filaments (both medullary and cortical) composed of cells at 6-15 μm in diameter and 6-35 μm in length. Subepithallial initials usually as long or longer than immediate inward derivatives (Fig. 8C). Epithallial cells with distal walls flattened and flared, disposed in a single layer (Fig. 8C). Cells of adjacent filaments connected by cell fusions (Fig. 8C). Trichocytes not found. Only tetrasporangial plants observed. Tetrasporangial conceptacles multiporate, protruding above surrounding vegetative thallus surface (Fig. 8D). Concep-
Fig. 7. (A-F) Vegetative and reproductive features of *Porolithon onkodes* (specimen RB 623155). (A) Specimen in the field (arrow) covering the hydrocoral *Millepora alcicornis* (arrowhead). (B) *Porolithon onkodes* (PO) on *Titanoderma prototypum* (TP). (C) Vertical section through the thallus showing monomeres and non-coaxial construction. Note buried trichocytes arranged in large and tightly packed horizontal fields (arrows). Note also that the thallus of *Porolithon onkodes* (PO) is on the top of a thallus of *Titanoderma prototypum* (TP). (D) Vertical section through the thallus surface showing elliptical to rounded epithallial cells occurring in multiple layers (arrow), cell fusions (arrowhead), and a tightly packed horizontal field of trichocytes (T). (E) Thallus surface showing trichocyte fields (arrows) and more-or-less flush to slightly raised uniporate tetrasporangial conceptacles (arrowheads). (F) Section through a uniporate tetrasporangial conceptacle showing enlarged cells (arrows) lining the base of the pore canal. Scale bars represent: A, 5 cm; B, 3 mm; C, 250 μm; D, 75 μm; E, 500 μm; F, 70 μm.
Fig. 8. (A–F) Vegetative and reproductive features of *Lithothamnion crispatum*. (A) General view of a lumpy specimen (RB 623157). (B) Section through the thallus showing monomerous and non-coaxial thallus construction. (C) Section through the thallus surface showing flared epithallial cells (arrows) and cell fusions (arrowhead) connecting adjacent filaments. (D) Surface view showing multiporate tetrasporangial conceptacles. (E) Section through a multiporate conceptacle showing zonately divided tetrasporangia distributed across the chamber floor. Note that conceptacle roof is pitted with depressions (arrows) resulting from disintegration of rosette cells surrounding the pores. (F) Details of depressions (arrows) surrounding pore canal (p) in conceptacle roof. Scale bars represent: A, 2 cm; B, 30 μm; C, 15 μm; D, 500 μm; E, 75 μm; F, 20 μm.
tacle chambers at 250-450 μm in diameter and 140-200 μm in height. Conceptacle floor located 8-12 cells below surrounding thallus surface. Roof filaments 3-5 cells long (including epithallial) (Fig. 8E). Roofs of mature conceptacles pitted with depressions (Fig. 8E & F) resulting from disintegration of rosette cells surrounding pores. Filaments lining pore canal composed of cells differing in size and shape from other roof cells (Fig. 8F).


Comments. The specimens of *L. crispatum* found in this study showed unique tetrasporangial conceptacle roof structure pitted with depressions which were considered as diagnostic features of this species (Basso et al. 2011). These features are displayed when observing multiporate conceptacles under stereoscopy microscope, usually dispensing anatomical sectioning for species identification. *L. crispatum* has been previously recorded in Brazil only as free-living rhodoliths (Bahia et al. 2010, Da Nóbrega Farias et al. 2010, Bahia 2014). This is the first time that this species is found to grow attached on a reef in Brazil.

**Melyvonnea Athanasiadis & D. L. Ballantine**

*Melyvonnea erubescens* (Foslie) Athanasiadis & D. L. Ballantine

Habit and ecological observations. Thalli fruticose, pink to red in color (Fig. 9A). Most protuberances cylindrical with rounded apex (Fig. 9A) being ramified and / or anastomosing. This species occurs like small shrubs spreading along shallow areas (2-5 m) of Abrolhos reefs.

Vegetative and reproductive anatomy. Thallus monomeros with coaxial medula (Fig. 9B), filaments (medullary and cortical) composed of cells at 4-10 μm in diameter and 7-30 μm in length. Subepithallial initials usually as long or longer than immediate inward derivatives (Fig. 9C). Epithallial cells rounded or flattened and disposed in a single layer (Fig. 9C). Cells of adjacent filaments connected by cell fusions (Fig. 9C). Trichocytes not found. Only tetrasporangial plants observed. Tetrasporangial conceptacles multiporate, flat topped, protruding above surrounding vegetative thallus surface (Fig. 9D & E). Conceptacle chambers at 315-400 μm in diameter and 150-200 μm in height, commonly filled with large sterile vegetative cells. Roof filaments 4-7 cells long (including epithallial cells), formed by filaments peripheral to and interspersed amongst tetrasporangial initials. Filaments lining pore canals composed of 3 to 5 cells. More elongate than other roof cells, especially near base of pore (Fig. 9F).

Examined specimen. Brazil, Bahia, Abrolhos Archipelago, Ilha de Santa Bárbara, Porto Norte (17°57′45″ S, 38°41′43″ W, GM Amado-Filho, Oct 23, 2012, RB 621495, as *Mesophyllum erubescens*).

Comments. *M. erubescens* is the only species of *Melyvonnea* recorded from Brazil. The ascription of our specimens into genus *Melyvonnea* is based on the following combined features: fruticose thalli with branched protuberances, predominantly coaxial, medulla and filaments lining canals of multiporate roofs composed of 3 to 5 cells with distinctively elongate basal cells (Athanasiadis and Ballantine 2014). Other features related to gametophytes and carposporophytes complement the genus diagnosis. However, these life stages were not investigated here. Beyond *M. erubescens*, genus *Melyvonnea* is represented by other three species, namely *M. aemulans* (Foslie & M. Howe) Athanasiadis & D. L. Ballantine, *M. canariensis* (Foslie) Athanasiadis & D. L. Ballantine (type species), and *M. madagascariensis* (Foslie) Athanasiadis & D. L. Ballantine. *M. erubescens* can be distinguished from these species mainly by the number of cells lining the tetrasporangial pore canal and the distribution of spermatangia on male conceptacles (floor or floor and roofs) (Athanasiadis and Ballantine 2014).

*Sporolithales Le Gall, Payri, Bittner & G. W. Saunders* Sporolithaceae E. Verheij

*Sporolithon Heydrich* Sporolithon ptychoides Heydrich

Habit and ecological observations. Thalli attached, pink to purple with encrusting to warty growth-form (Fig. 9A). Species mostly found in shaded environments protected from direct sunlight exposure.

Vegetative and reproductive anatomy. Thallus monomeros non-coaxial (Fig. 10B), filaments composed of cells at 5-11 μm in diameter and 8-23 μm in length. Subepithallial initials usually with same size as immediate inward derivatives (Fig. 10C). Epithallial cells with distal walls flared and disposed in a single layer (Fig. 10C). Cells of adjacent filaments joined by both secondary pit connections and cell fusions with a ratio of 2:1. Trichocytes not found. Only tetrasporangial plants observed. Tetrasporangia formed within calcified compartments grouped into sori (Fig. 10D-F). Sori raised 2-4 layers of cells above surrounding vegetative thallus surface (Fig. 10D). Tetrasporangial compartments elliptical to rounded at 43-50 μm in diameter and 75-100 μm in height (Fig.
Fig. 9. (A-F) Vegetative and reproductive features of *Melyvonnea erubescens*. (A) General view of a fruticose specimen (RB 621495) in the field. (B) Section through the thallus showing the coaxial and monomerous thallus construction. (C) Section through the thallus surface showing rounded epithallial cells (arrows) and subepithallial initials (i) as long as or longer than their immediate inward derivatives and cell fusions (arrowheads) connecting adjacent filaments. (D) Surface view of flat topped multiporate tetrasporangial conceptacles. (E) Section through a multiporate conceptacle showing remnants of tetrasporangia within the chamber. (F) Details of the filaments lining the tetrasporangial conceptacle pore canal. Note that they are composed of cells different from other roof cells. They are consisted of cells that are more elongate, especially near the base of the pore canal (arrows). Note also remains of a tetrasporangium (T). Scale bars represent: A, 4 cm; B & E, 150 μm; C, 15 μm; D, 1 mm; F, 40 μm.
Fig. 10. (A-F) Vegetative and reproductive features of *Sporolithon ptychoides*. (A) General view of an encrusting to warty specimen (RB 621750). (B) Section through the thallus showing monomerous and non-coaxial thallus construction. (C) Section through the thallus surface showing epithallial cells (arrows) with flared distal walls. (D) Surface view of a raised sorus. (E) Vertical section through the thallus showing old buried and empty tetrasporangial compartments. (F) Longitudinal section through two tetrasporangial compartments showing a tetrasporangium with cruciately divided tetraspores (1-4) with pore plug (arrow) and single stalk cell at the base of tetrasporangium (arrowhead). Scale bars represent: A, 1.5 cm; B, 60 μm; C, 20 μm; D, 0.5 cm; E, 200 μm; F, 30 μm.
10E & F), separated from each other by 1-3 calcified filaments (paraphyses), bearing 3-5 cells each with a basal layer of elongated cells (Fig. 10F). Tetrasporangia cruciately divided, with apical plugs, supported by a single stalk cell (Fig. 10F). Senescent tetrasporangial compartments buried in thallus (Fig. 10E).


**Comments.** The samples of *Sporolithon ptychoides* identified here are morpho-anatomically in accordance with the type collection of this species found in the Red Sea (Verheij 1993) as well as with previous records of this species collected from Brazil (Bahia et al. 2011, Henries et al. 2014). However, in these previous records, *S. ptychoides* was found only in free-living (rhodolith) habit. This is the first time that *S. ptychoides* is found as attached crust in Brazil.

**Molecular results**

This study generated 16 sequences from nine morpho-anatomically identified species (Supplementary Table S1). The resulting phylogenetic tree from concatenated ML and NJ analyses are shown in Fig. 11.

The *psbA* sequence from the material identified as *L. kaiseri* (RB 623160) from Abrolhos was identical to those from conspecific specimens collected from Madagascar, Martinique, and Australia, forming a fully supported clade (100% BS in both analysis, NJ and ML). As these specimens had their *rbcL* sequences matching those from type material of *L. kaiseri* (Hernández-Kantum et al. 2016), the presence of *L. kaiseri* in Abrolhos is confirmed. Two *psbA* sequences corresponding to branched species *Lithophyllum* sp. from Abrolhos formed an isolated clade (97/98% BS) within the low supported clade that grouped others branched species of *Lithophyllum* (*L. yemenense, L. neocongestum, L. platyphyllum,* and *L. pseudoplatyphyllum*). This suggests that *Lithophyllum* sp. corresponds to a new species. However, further investigations including analyses of more specimens are needed before proposing it as a new species.

*M. erubescens* was another species whose identification was confirmed based on indirect DNA sequences comparison with type material. The *psbA* sequence of *M. erubescens* from Abrolhos was identical to those of topotype material (KM 983035 and KM 983036) collected from Fernando de Noronha Island of northeastern Brazil with *rbcL* sequences matching those of species holotype used in Sissini et al. (2014).

The specimen identified as *T. prototypum* (RB 632601) from Abrolhos and *T. pustulatum* from New Zealand did not form a monophyletic clade. Instead, they grouped with other *Lithophyllum* species, suggesting that the morpho-anatomical features used to distinguish these genera (e.g., presence or absence of palisade cells in basal filaments) should be reviewed or at least these genera should be placed as synonym as previously proposed (Campbell and Woelkerling 1990). Whether *Titanoderma* and *Lithophyllum* are distinct genera remain unclear. DNA sequences of type / topotype material of theirs type species (*T. pustulatum* and *L. incrustans*) are needed for comparison.

The material identified in this study as *Porolithon onkodes* from Abrolhos formed a clade with *P. onkodes* from Indonesia with moderate to strong support (87/97% BS). *Porolithon onkodes* from Japan was isolated in another clade with full support, suggesting that the Brazilian and Indonesian specimens correspond to species different from that of Japan. *Pneophyllum conicum* from Abrolhos formed a clade with *Pneophyllum conicum* from Japan and Australia with strong support (99/99% BS). However, within this clade, four *Pneophyllum conicum* specimens from Abrolhos formed an independent clade with high support (90/93% BS). Specimen identified as *H. boergeseni* (RB 621493) grouped with *H. boergesenii* from El Salvador, Phillippines, and Guadeloupe with high support (80/95% BS). The sequence from specimen identified as *S. ptychoides* from Abrolhos (RB 621750) was identical to two others (KC 870926 and KC 870927) from Fernando de Noronha Island of northeastern Brazil.

Specimens of *L. crispatum* from ACS (RB 623156 and 623157) grouped with a specimen of *L. crispatum* from Balearic Sea (KJ 710356) (87/88% BS) which was collected relatively close (~1,000 km away) to the type locality of *L. crispatum*, Adriatic Sea. However, *L. crispatum* from New Zealand formed an isolate clade with strong support (98/100% BS), suggesting that it is a different species. Both attached (encrusting) (RB 623157) and free-living (RB 623156) *L. crispatum* specimens from Abrolhos were grouped into the same clade. Specimens of *S. ptychoides* from Abrolhos (RB 621750, attached) and Fernando de Noronha (KC 870926, KC 870927, free-living) had identical *psbA* sequences. Hernández-Kantún et al. (2015) have also observed that rhodolith-forming individuals are genetically similar to encrusting forms in the genera *Lithothamnion, Phymatolithon, Mesophyllum, Hydrolithon, Spongites,* and *Sporolithon,* concluding that rhodolith habit cannot be used to delimit species for taxonomic or identification purposes.
Fig. 11. Phylogram inferred from psbA sequences (62 specimens). Species with sequenced type material, complementary rbcL sequences matching type material in other studies (Sissini et al. 2014, Hernandez-Kantun et al. 2016), and sequences from topotypes are represented in bold. Scale represents the number of substitutions per site. Values in the branches nodes represent bootstrap (for 1,000 replicates) for maximum likelihood on the left and neighbor-joining analysis on the right (e.g., 90/98%). Bootstraps below 80 are not indicated. *Sequences generated in this study.
Gabrielson et al. (2015) have obtained partial rbcL sequences from the type material of *S. ptychoides* and observed that the type sequences are identical to topotype specimens collected from the Red Sea during their study. From these field-collected topotype specimens, they also obtained *psbA*, COI, and LSU sequences to compare with published sequences of specimens labeled as *S. ptychoides*. According to their results, a comparison of the sequence divergence values obtained in their study showed that none of the published sequences from material identified as *S. ptychoides* from Hawaii, New Caledonia, or Brazil corresponded to the topotype material of *S. ptychoides* (Gabrielson et al. 2015), indicating that they are likely to have different taxa. However, the sequences mentioned by Gabrielson et al. (2015) are unavailable at GenBank for comparison. Until those sequences can be accessed, we will continue to apply the name of *S. ptychoides* for Brazilian specimens morpho-anatomically identified as such.

CONCLUSION

In summary, this study provided detailed taxonomic analysis of the NGCA from shallow reefs of the ACS using both morpho-anatomical data and molecular data. Among the nine species identified, only *L. kaiseri* (new record from Brazil) and *M. erubescens* had *psbA* sequences that matched those of type and/or topotype specimens. The phylogenetic analyses presented herein supports the taxon *Lithophyllum* sp. as a new species to science, confirming the existence of at least two branched *Lithophyllum* species for the ACS (*L. kaiseri* and *Lithophyllum* sp.) rather than only one as previously thought (Figueiredo and Steneck 2000, Tâmega et al. 2014). However, further investigations using more specimens are recommended prior to formal new species description. The taxonomic identifications for the remaining six species were supported by morpho-anatomical evidences. However, their identifications remain doubtful because DNA sequences of their type/topotype are currently unavailable. Sequencing of type material is outside the scope of this study. This study represents the first assessment of phenotypical and genetic diversity of NGCA in the ACS reefs. This study provides useful information for future monitoring and conservation of this important reef system of the South Atlantic.

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SUPPLEMENTARY MATERIAL

Supplementary Table S1. *psbA* sequences information (www.e-algae.org).

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