Lithothamnion steneckii sp. nov. and Pneophyllum conicum: new coralline red algae (Corallinales, Rhodophyta) for coral reefs of Brazil

Rodrigo Mariath¹, Rafael Riosmena-Rodriguez²,* and Marcia Figueiredo¹

¹Programa de Pós-Graduação em Botânica, Escola Nacional de Botânica Tropical, Instituto de Pesquisas Jardim Botânico de Rio de Janeiro, Rua Pacheco Leão 2040, 22460-030, Rio de Janeiro, RJ, Brazil
²Programa de Investigación en Botánica Marina, Departamento de Biología Marina, UABCS Km 5.5 carretera al sur, La Paz, BCS 23080, México
³Instituto de Pesquisas del Jardín Botánico de Rio de Janeiro, Rua Pacheco Leão 915, 22460-030, Rio de Janeiro, RJ, Brazil

Nongeniculate coralline red algae are a common element of the Brazilian coastal zone, especially associated to coral reefs. During the course of ecological studies at Parque Municipal Marinho do Recife de Fora, two species of non-geniculate Corallinales were the major organisms covering the reef. Analyses of the vegetative and reproductive features of the species were analyzed; indicating that one new species of the genus Lithothamnion is proposed here based on the combination of several features associated with anatomy of the tetrasporangial conceptacles in relation to other species of the genus for which modern accounts are available. This new proposal along with other new species, new combinations and range extension of some species of the genus based in similar features clearly suggest that stability in species delimitation is possible. The second species found Pneophyllum conicum represents a range extension of more than 6,000 km from the Pacific in to the Atlantic Ocean suggesting that some nongeniculate species are widely distributed. The occurrence and abundance of these species supports and emphasizes the need for an extensive taxonomic reassessment of coralline red algae in the context of Brazilian coral reef biodiversity.

Key Words: biodiversity; Brazil; coralline algae; coral reefs; Lithothamnion sp. nov.; Pneophyllum conicum

INTRODUCTION

The Orders Corallinales and Sporolithales represent an evolutionary clade of red algae (Rhodophyta) that are characterized by the deposition of calcite in cell walls (e.g., Johansen 1981). Due to the natural calcification of the members of this clade, they are common in the fossil record (e.g., Aguirre et al. 2010). Species of coralline red algae are widely distributed around the world from the poles to the tropics and from intertidal areas to deep in the oceans (e.g., Steneck 1986). Because of their wide range in ecological and geographical distribution, this clade has several growth forms (e.g., Woelkerling et al. 1993), from endoparasites to free living forms (e.g., Johansen 1981), making the taxonomic delimitation in this clade complex.

Twelve genera and 42 species of coralline red algae have been identified in coastal and offshore areas of the continental shelf of Brazil (reviewed by Creed et al. 2010). From this list of taxa 10 genera and 14 species are cited for the Brazilian reefs. However, many of these species are poorly known and recent taxonomic analyses have
shown that Brazilian coralline flora is more diverse than was previously considered (e.g., Villas-Boas et al. 2009, Da Nóbrega Farias et al. 2010, Bahia et al. 2011, Horta et al. 2011, Henriques et al. 2012). Furthermore, most ecological studies were not carried out using rigorous taxonomic evaluation. During the course of ecological studies at Parque Municipal Marinho do Recife de Fora (e.g., Mariath et al. in press) two species of nongeniculate coralline red algae were found as the major species cover on the reef. When vegetative and reproductive anatomies of the species were analyzed we found a new species of the genus *Lithothamnion* and a major range extension for *Pneophyllum conicum* in the area. Here we present a morphological and taxonomic evaluation of these coralline algae.

**MATERIALS AND METHODS**

The work was carried out within the limits of the Municipal Marine Park of Recife de Fora in Porto Seguro, southeastern Brazil, which is the first Conservation Unit in this area, established in 1997. The protected area is of approximately 17.5 km² (Fig. 1). The depth surrounding this reef varies from 6 to 8 m near shore, and may reach up to 15 m in the northeast offshore area (e.g., Costa et al. 2002). The Ponto Oeste site (16°24′36″ S, 038°59′08″ W) was visited more than 6 times between Feb 27, 2007 and Jan 28, 2008.

This site located in a portion of the reef flat ranging from 0.5 and 3 m depth and separated from most reefs flat by crevices, reaching 6 m at the reef base. This area constantly submerged and distant from reef edge therefore more protected compared to reef slope which is directly exposed to waves. Here the coral colonies are larger than on the reef flat near the reef edge, with coralline algae growing under the sediment, and among and on top of some of the coral colonies.

All material was collected by SCUBA or snorkel diving and maintained in tanks with sea water in a flow through system. Fragments with reproductive structures were fixed in 4% formalin in seawater. For light microscopy, formalin preserved specimens were first decalcified in 10% nitric acid, dehydrated in 30, 60, and 96% alcohol. Three to ten µm sections were cut with a microtome Shandon Hypercut. Individual sections were removed and stained with 1% toluidine blue as described by (e.g., Moura et al. 1997 modified where the alcoholic series was reduced to concentrations of 30, 60, and 95%).

For scanning electron microscopy we follow the methods proposed by Garbary (1978) and Garbary and Johannsen (1982) which consist in the use of air-dried material was fractured using finger nails, forceps, diagonal cutters, or a small hammer and cold chisel. The fractured pieces were mounted on stubs, using adhesive tabs double sided carbon tape and colloidal silver liquid, stored in a desiccators for at least 24 h prior to examination, coated with gold for 4-6 min in an Emitech K550X (Quorum Technologies Ltd., Kent, UK), and examined with a Zeiss EVO 40 scanning electron microscope (Carl Zeiss, Oberkochen, Germany) with an accelerating voltage of 15.34 kV. Description of the new species is following the new botanical code of nomenclature (McNeil et al. 2006) in where latin descriptions are no longer require for new species.

![Fig. 1. Location of the protected area of Parque Marinho do Recife de Fora, Bahia, Brazil, the rectangle delimits the park’s area.](http://dx.doi.org/10.4490/algae.2012.27.4.249)
Mariath et al. New Coralline Red Algae for Brazil

Isotype specimens. Recife de Fora, Bahia, Feb 27, 2007 (RB 475147, RB 475148, RB 517938, RB 517939), Rodrigo Mariath and Marcia Figueiredo collectors.

Type locality. The Municipal Marine Park of the Recife de Fora, situated in the municipal district of Porto Seguro, Bahia.

Etymology. The name of this species is to honor Dr. Robert Steneck’s work on coralline red algae and his particular interest in Brazilian coral reefs.

Description. Plants non-geniculate with an encrusting to fruticose growth form (Fig. 2A); thallus thickness 200-315 µm. Epithelial flared cells 4-5 µm long and 6-8 µm in diameter (Fig. 2B & C) and monomerous construction in a system of a single group of filaments with cell fusions (Fig. 2D) that are parallel to the substrate and giving rise to groups of vertical cells (Fig. 2E). Cells in the central area

RESULTS

Family Hapalidiaceae
   Subfamily Melobesioideae

Lithothamnion steneckii Mariath and Figueiredo (Figs 2 & 3)

Holotype specimen. Recife de Fora, Bahia, Feb 27, 2007 (RB 475146, Feb 27, 2007), Rodrigo Mariath and Marcia Figueiredo collectors.

Isotype specimens. Recife de Fora, Bahia, Feb 27, 2007 (RB 475147, RB 475148, RB 517938, RB 517939), Rodrigo Mariath and Marcia Figueiredo collectors.

Type locality. The Municipal Marine Park of the Recife de Fora, situated in the municipal district of Porto Seguro, Bahia.

Etymology. The name of this species is to honor Dr. Robert Steneck’s work on coralline red algae and his particular interest in Brazilian coral reefs.

Description. Plants non-geniculate with an encrusting to fruticose growth form (Fig. 2A); thallus thickness 200-315 µm. Epithelial flared cells 4-5 µm long and 6-8 µm in diameter (Fig. 2B & C) and monomerous construction in a system of a single group of filaments with cell fusions (Fig. 2D) that are parallel to the substrate and giving rise to groups of vertical cells (Fig. 2E). Cells in the central area

Fig. 2. Lithothamnion steneckii. (A) Holotype material of Lithothamnion steneckii sp. nov. (arrow) found on the skeleton of the coral Mussismilia hartii. (B) Transversal section showing flared epithelial cells (arrow) and cell fusions. (C) Close up of the flared epithelial cells (arrow) and cell fusions. (D) Longitudinal section showing the edge or the plant and monomerous arrangement (arrow). (E) Longitudinal section showing the middle area of the plant and monomerous arrangement. Scale bars represent: A, 1 cm; B, D & E, 100 µm; C, 16 µm.
Pneophyllum conicum (Dawson) Keats, Chamberlain and Baba (Figs 4 & 5)

Basionym. *Hydrolithon conicum* E. Y. Dawson 1960, p. 27.

Holotype specimen. (E. Y. Dawson, Nov 19, 1953, Dawson 12148). This material was listed by Dawson (1960) as being in the herbarium of the Baudette Foundation, with an isotype in the herbarium of the A. Hancock Foundation but all the material are now housed in the Herbaria of UC Berkeley.

Type locality. Intertidal reef at Biners Cove, Isla So-corro, Revillagigedo Archipelago, Mexico.

Specimens examined. Recife de Fora, Bahia, Feb 27, 2007 (RB 475144, RB 517933, RB 517934, RB 517935, RB 517936).

Description. Plants non-geniculate with an encrusting...

Fig. 3. *Lithothamnion steneckii*. (A) Scanning electron microscopy (SEM) showing tetrasporangial multipore conceptacle in surface view (arrow). (B) Longitudinal section of roof structure showing the pore plug delimited by two cells different from cell layers in size and shape (arrow). (C) SEM of a chamber of multiporate conceptacle (arrow). (D) SEM showing surface view of pore of a multiporate tetrasporangial conceptacle with rosettes formed (arrow). Scale bars represent: A-C, 80 μm; D, 30 μm.
The conceptacle chamber is 3-4 cells below the level of the surrounding thalli. The conceptacle roof is formed from filaments that are both interspersed among the sporangial initials and peripheral to them. The pore canal is lined by papillae cells which project slightly into the canal, and a broad central collumela is present in mature conceptacles. Tetraspores are zonately arranged, 55-60 µm long and 25-40 µm in diameter. They are restricted to the conceptacle periphery.

Female (Fig. 5B & C) and carposporangial conceptacles (Fig. 5D) are small and inconspicuous, 45-90 µm long and 60-125 µm in diameter; carposporangial conceptacle roofs are formed only from filaments located peripheral to the fertile area. The initiation of a carpogonial conceptacle begins when groups of subepithelial initials elongate, forming a small disc of cell sat the thalli surface, which begin to shed an epithelial layer (Fig. 5B). Cells

Fig. 4. Pneophyllum conicum. (A) Specimen with encrusting growth form (arrow). (B) Superficial view showing one tricocysts field (arrows). (C) Superficial view showing one germination disc (arrow). (D) Longitudinal section showing monomerous growth (arrow). Scale bars represent: A, 1.5 cm; B, 1 cm; C & D, 200 µm.
near its edge. Gonimoblast filaments are borne peripherally, and originate from the lower edge of the fusion cell. Male plants were not seen.

Ecological distribution. This is the first report of the occurrence of this species in the Atlantic Ocean. The samples were collected to a depth of 4 m. This species grows over live coral (*Mussismilia hartii* Millepora sp.) or, less commonly, on other live corals, rocks and dead coral skeletons.

**DISCUSSION**

The taxonomic history of the genus *Lithothamnion* (subfamily Melobesioideae) is full of conflicts in their delimitation from other genera in the same subfamily (Harvey et al. 2003). However, modern anatomical analyses
along with molecular data have delimited this genera and other related (Harvey et al. 2003). Anatomically, is characterized by the presence of flared cells (Fig. 2B), adjacent filament cells united by cell fusions, absence of secondary pit connections and multiporate tetra / bisporangial conceptacles, with plugs in the pores (e.g., Wilks and Woelkerling 1995, Harvey et al. 2003). Lithothamnion is distinguished from other genera of Melobesioideae by the absence of arborescent or unconsolidated growth forms, and absence of haustoria. The thallus has a monomerous construction, and flared epithelial cells. Initial vegetative cells equal in height and width or elongated in their immediate inward thallus derivates and tetra / bisporangial conceptacles develop from the initial cells (e.g., Wilks and Woelkerling 1995).

There are 429 species (and infraspecific) names in the database at present, of which 84 have been flagged as currently accepted taxonomically (Guiry and Guiry 2012). Only few new species of the genus, such as Lithothamnion carpoklonion Athanasiadis et Ballentine has been recently described (Athanasiadis and Ballantine 2011); review their taxonomic status, like in Lithothamnion crispatum Hauk (Basso et al. 2011) or represents a range extension (Henriques et al. 2012). There are a group of features reliable to delimit species within the genus.

The characteristics used to determine the species (e.g., Wilks and Woelkerling 1995, Harvey et al. 2003, Woelkerling et al. 2005, Wynne 2005, Da Nórega Farias et al. 2010, Athanasiadis and Ballantine 2011, Basso et al. 2011, Table 1 in Henriques et al. 2012), do not allow our material of Lithothamnion to be assigned to any known species. Nonetheless, Lithothamnion muelleri Lenormand ex Rosanoff and L. crispatum known for Brazilian shores are discussed here for comparative purposes to delimit our new species. L. steneckii is characterized by sunken pores forming a rosette composed of 6-7 cells at the surface of conceptacles. In L. crispatum the pores of the tetrasporangial conceptacles are marked by areas with depressions seen around the region of the pores. These depressions do not occur on the pores of L. muelleri which possess pores aligned with the surface of the conceptacle. Besides this, L. steneckii, has both elongated and short cells along the margins of the pore canal, as seen in L. crispatum, which

<table>
<thead>
<tr>
<th>Features</th>
<th>L. steneckii (Brazil)</th>
<th>L. muelleri (Western Australia)</th>
<th>L. crispatum (South Australia)</th>
<th>L. crispatum (Southeast Australia)</th>
<th>L. crispatum (Brazil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth form</td>
<td>Encrusting to fruticose</td>
<td>Warty</td>
<td>Flat or branched</td>
<td>Warty and branched</td>
<td>Lumpy to fruticose</td>
</tr>
<tr>
<td>Height of initial cells (µm)</td>
<td>7-14</td>
<td>7-11</td>
<td>5-22</td>
<td>7-14</td>
<td>ND</td>
</tr>
<tr>
<td>Diameter of initial cells (µm)</td>
<td>3-6</td>
<td>4-7</td>
<td>2-10</td>
<td>2-10</td>
<td>ND</td>
</tr>
<tr>
<td>Height of epithelial cell (µm)</td>
<td>4-5</td>
<td>4-6</td>
<td>1-8</td>
<td>2-5</td>
<td>3-5</td>
</tr>
<tr>
<td>Diameter of epithelial cell (µm)</td>
<td>6-8</td>
<td>5-7</td>
<td>2-8</td>
<td>2-10</td>
<td>8-11</td>
</tr>
<tr>
<td>Tetrasporangial conceptacle chamber height (µm)</td>
<td>195-330</td>
<td>200-320</td>
<td>150-200</td>
<td>150-170</td>
<td>125-170</td>
</tr>
<tr>
<td>Tetrasporangial conceptacle diameter (µm)</td>
<td>195-330</td>
<td>375-755</td>
<td>280-750</td>
<td>450-600</td>
<td>390-440</td>
</tr>
<tr>
<td>Tetrasporangial conceptacle roof thickness (µm)</td>
<td>ND</td>
<td>ND</td>
<td>30-70</td>
<td>25-60</td>
<td>ND</td>
</tr>
<tr>
<td>No. of cells in tetrasporangial roof filaments</td>
<td>4-6</td>
<td>ND</td>
<td>5-9</td>
<td>3-8</td>
<td>4-5</td>
</tr>
<tr>
<td>Pore lining the plugs in tetrasporangial conceptacle</td>
<td>Rosettes under depression by 6-7 cells</td>
<td>Aligned with the surface</td>
<td>Aligned with the surface</td>
<td>Pitted with depressions</td>
<td>Pitted with depressions</td>
</tr>
</tbody>
</table>

ND, no data.
contrasts with *L. muelleri*, where the cells along the margin of the pore canal do not differ in size and shape from the other filament cells of the roof of the conceptacle. Characteristics such as the diameter of the conceptacles reinforce the differences between these species. As such *L. steneckii* has the least variation in the size of the conceptacle than other species (Table 1).

*Pneophyllum* is segregated from *Foselila* and *Spongites* by the 8-celled sporeling and by trichocytes that are intercalary in first-order filaments. However, studies are needed to verify the discreteness of these genera, and additional data may come from studies of reproductive features. There are 26 species (and infraspecific) names in the database at present, of which 18 have been flagged as currently accepted taxonomically (Guiry and Guiry 2012). However, in many of the known species ontogenetic and molecular studies are urgently needed to evaluate the limits between species and related genera. The material collected in Brazil represents a range extension of *P. conicum* from the Eastern Pacific into the Atlantic Ocean who is more than 6,000 km in distance. This is consistent with the series of new records and species that have been recently found along the coast of Brazil (e.g., Nunes et al. 2008, Villas-Boas et al. 2009, Amado-Filho et al. 2010, Da Nóbrega Farias et al. 2010, Bahia et al. 2011, Henriques et al. 2012) when a proper and detailed analysis is made of the coraline algae flora (Creed et al. 2010).

According to Keats et al. (1997), *P. conicum* is identifiable by the following characteristics: 1) monomerous thallus; 2) trichocyte arranged in circle at surface level and horizontal fields; 3) pore canal of the tetrasporangial conceptacles aligned by cells that are orientated more-or-less parallel to the surface of the roof; 4) roof of the elevated conceptacles, slightly conical; 5) diameter of the chamber of the tetra / bisporangial conceptacles measuring 220–400 µm; 6) senescent conceptacles are not found buried in the thallus. The specimens collected in Arrecife de Fora have all the features of *P. conicum* studied and clearly show that this is a species with a very wide geographical distribution. The data of vegetative and reproductive anatomy of this alga were compared with other species of *Pneophyllum*: *P. fragile* (type species), *Spongites fruticulosus* (type species) and *Paragoniolithon conicum* (Dawson) Adey, Townsend and Boykins (Table 2) and found consistent with the original description and very different from the other species and genera. However, in the present paper we describe the vegetative and reproductive anatomy of *P. conicum* and found some inconsistencies with the generic circumscription of the species because the position of the filaments near the pore seems

### Table 2. Comparison *Pneophyllum conicum* vegetative and reproductive anatomical features from recent studies

<table>
<thead>
<tr>
<th>Features</th>
<th><em>P. conicum</em> (Brazil)</th>
<th><em>P. conicum</em> (Hawaii)</th>
<th><em>P. conicum</em> (Fiji)</th>
<th><em>P. conicum</em> isotype (México)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Form of conceptacles tetrasporangial over the surface of the talus</td>
<td>High rounded</td>
<td>Convex / tapered</td>
<td>High / slightly tapered</td>
<td>High / tapered</td>
</tr>
<tr>
<td>Diameter of the chamber of tetrasporangial conceptacles (µm)</td>
<td>308-390</td>
<td>140-360</td>
<td>220-400</td>
<td>273-310</td>
</tr>
<tr>
<td>Long of the tetrasporangial conceptacles (µm)</td>
<td>65-80</td>
<td>50-160</td>
<td>42-75</td>
<td>81-105</td>
</tr>
<tr>
<td>Thickness of the roof of tetrasporangial conceptacles (cells)</td>
<td>4-9</td>
<td>4-11</td>
<td>5-11</td>
<td>11-13</td>
</tr>
<tr>
<td>Diameter of carpogonial conceptacles (µm)</td>
<td>52-125</td>
<td>ND</td>
<td>110-125</td>
<td>ND</td>
</tr>
<tr>
<td>Long of carpogonial conceptacles (µm)</td>
<td>47-60</td>
<td>ND</td>
<td>31-90</td>
<td>ND</td>
</tr>
<tr>
<td>Diameter of carposporangial conceptacles (µm)</td>
<td>197-211</td>
<td>ND</td>
<td>190-235</td>
<td>ND</td>
</tr>
<tr>
<td>Long of carposporangial conceptacles (µm)</td>
<td>98-110</td>
<td>ND</td>
<td>91-125</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, no data.
to be more similar to what is described for *Spongites* than for *Pneophyllum*. But, positions of gonimoblast filaments are consistent with the generic circumscription. Analyses of the origin of tetrasporangial and carposporangial conceptacles are not clearly known and more research is needed in addition to records to support a transfer of this species to *Spongites*.

**ACKNOWLEDGEMENTS**

We would like to thanks the staff of the Municipal Marine Park who supported field work in special Renata Arantes and Leones Lopes and Clarice Martins Ribeiro for her help in the herbaria activities. Petrobras Ambiental and Coral Vivo Project gave the MSc scholarship to the first author. We would like to acknowledge the comments of Dr. David Garbary, Dr. Robert Steneck and anonymous reviewer that improved our manuscript.

**REFERENCES**


