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First report of *Sporolithon ptychoides* (Sporolithales, Corallinophycidae, Rhodophyta) for the Atlantic Ocean.

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SUMMARY

Samples corresponding to *Sporolithon ptychoides* Heydrich were collected in the mesophotic zone (50 m depth) south of Espírito Santo State, Brazil. The collected material presented features characteristic of the species namely: tetrasporangia of 75-105 x 40-55 µm grouped into sori that are raised above the surrounding vegetative thallus surface; presence of a basal layer of elongate cells in areas where the tetrasporangia develop; presence of buried tetrasporangial compartments deep in the thallus; and 3-5 cells in the tetrasporangial paraphyses. These same features said to collectively characterise *S. ptychoides*, were all observed in a representative specimen and the type specimen of *Sporolithon dimotum* (Foslie & Howe) Yamaguchi-Tomita ex M.J Wynne.

This latter species is thus conspecific with *S. ptychoides* and is therefore considered a heterotypic synonym thereof as *S. ptychoides* has nomenclatural priority. This study expands the known geographical distribution of the species and may give insight into the origin of the species into other geographical regions.

Key words: Coralline algae, rhodolith, *Sporolithon dimotum*, taxonomy

Running title: *Sporolithon ptychoides* from the Atlantic.

Introduction

Sporolithon ptychoides Heydrich, the type species of the genus *Sporolithon*, is one of five extant species of *Sporolithon* for which detailed taxonomic information exists (Verheij 1993, Townsend et al. 1995, Braga and Bassi 2007). The species has been confirmed for the Mediterranean Sea (Alongi et al. 1996), Red Sea (Verheij 1993), Indian Ocean (Keats and Chamberlain 1993, Verheij 1993) and Pacific Ocean (Verheij 1993, Keats and Chamberlain 1993).

Here we report, for the first time, the presence of *S. ptychoides* in the Atlantic Ocean and provide a comparative taxonomic analysis of plants from the Atlantic against those from other known populations around the world. Also we compare the present record against some of the closely related *Sporolithon* species for which detailed modern information exists and in relation to the

records of *Sporolithon dimotum* (Foslie & M.A. Howe) Yamaguishi-Tomita ex M.J. Wynne that has been recorded for the same area (Tomita 1976).

Materials and Methods

Two techniques for coralline red algae identification were applied to check both reproductive and vegetative characters, one using scanning electron microscopy (SEM), following the procedure of Keats and Chamberlain (1993), and the other using light microscopic examination through embedding and sectioning (see Amado Filho et al. 2007 for further details).

Material for examination was collected during June of 2006 at 50 m depth in the southern region of Espírito Santo State, Brazil (21°02'972"S; 40°17'824"W, *GM Amado-Filho*, 17.vi.2006, RB 476940). Additional material from the same region (20°10' S; 40°02' W, 60 m depth), described as *Sporolithon dimotum* (Foslie & Howe) Yamaguishi-Tomita ex M.J. Wynne by Tomita (1976) (SP 113211), as well as the holotype specimen of *S. dimotum* from Puerto Rico (NY 2667) (Foslie and Howe 1906) were analyzed due to their taxonomic similarity with *S. ptychoides*.

Results

Based on our observations we have found that our record of *S. ptychoides* (Figures 1-8) is the first record for Atlantic Ocean and we also are proposing the synonymy of *S. dimotum* as a heterotypic synonym of *S. ptychoides* (Figures 9-13).

Species account

Sporolithon ptychoides Heydrich, 1897: 67-69, figs 2, 3, pl. III: figs 20-23

Figures 1-13, table 1.

LECTOTYPE: TRH (not seen, considering that Verheij 1993 has presented descriptions), El Tor, Red Sea, no number (as *Sporolithon ptychoides* f. *dura*) designated by Woelkerling and Townsend (in Woelkerling 1988, p.204, Figure 239). SYNONYMS: *Sporolithon mediterraneum* Heydrich, 1899 (based on Alongi et al. 1996) *Archaeolithothamnion mediterraneum* (Heydrich) Foslie, 1900

Archaeolithothamnion dimotum Foslie & M.A. Howe, 1906 *Sporolithon dimotum* (Foslie & Howe) Yamaguishi-Tomita ex M.J. Wynne, 1986 MATERIALS EXAMINED: Espírito Santo State, Brazil (21°02'972"S; 40°17'824"W, 50 m, *GM Amado-Filho*, 17.vi.2006, RB 476940); Espírito Santo State, Brazil (20°10'S; 40°02'W, 60 m, *NY Tomita*, unknown date, SP 113211, as *Sporolithon dimotum*); Lemon Bay near Guánica, Puerto Rico (*MA Howe*, 27.vi.1903, NY 2667, as *Archeolithothamnion dimotum*).

Plants encrusting to warty, forming rhodoliths up to 15 cm in diameter (Fig. 1). Thallus pseudoparenchymatous; internal organization monomerous in crustose portions (Fig. 2) and radial in warty protuberances. Epithallus comprising a single layer of flattened cells with flared corners measuring 1-4 µm long and 3-8 µm in diameter (Fig. 3). Subepithallial initials, 4-12 µm long and 5-10 µm in diameter, are squarish and are as short or shorter than their inward derivatives (Fig. 3). Perithallus mainly composed of elongated cells measuring 8-18 µm long and 3-10 µm diameter (Fig. 2). Hypothallus formed by 3-4 layers of cylindrical cells measuring 15-20 µm long and 7-9 µm in diameter (Fig. 2). Cells of adjacent perithallial and hypothallial filaments

joined by both secondary pit connections and cell fusions (Fig. 4); secondary pit connections, however, predominate with a ratio of secondary pit connections to cell fusions being 2:1.

Tetrasporangia measuring 75-105 μm long and 40-55 μm in diameter, are grouped into so that are raised (Figs. 5 and 6) 2-4 cells above the surrounding vegetative thallus surface. Tetrasporangial compartments are separated from each other by 1-3 calcified filaments (paraphyses) bearing 3-5 cells each and a basal layer of elongated cells. Tetrasporangia show cruciately arranged tetraspores measuring 55-65 μm long and 35-45 μm in diameter, and are supported by a single large stalk cell measuring 8-10 μm long and 20-23 μm in diameter (Fig. 7). Tetrasporangial compartments bear apical plugs (Fig. 7) with a pore 10-14 μm in diameter and surrounded by 10-11 rosette cells (Fig. 8). Old and empty tetrasporangial compartments become buried in the thallus. Gametangial plants were not seen.

The features observed for our material correspond to *Sporolithon ptychoides* described by Verheij (1993), Keats and Chamberlain (1993), and Alongi et al. (1996) (Table 1). *Sporolithon ptychoides* identified in this study differs from other species of *Sporolithon* by the following combination of characters: 1) the size of the tetrasporangia (75-105 x 40-55 μm); 2) the presence of buried tetrasporangial compartments deep in the thallus; 3) the presence of 3-5 cells in the tetrasporangial paraphyses; 4) the ratio of secondary pit connections to cell fusions (2:1, respectively); 5) the position of tetrasporangial sori relative to the surrounding vegetative thallus surface; and 6) the presence of a basal layer of elongate cells in areas where tetrasporangia develop (Table 1). The features considered diagnostic of *S. ptychoides* were also observed in specimens designated as *S. dimotum* by Tomita (1976) as well as in the holotype of *S. dimotum* (Figs. 9-13) (Table 1). Consequently, we consider *S. dimotum* to be conspecific with *S. ptychoides* and thus a heterotypic synonym thereof, the latter taxon having nomenclatural priority.

In Brazilian waters, only one species of *Sporolithon*, namely *Sporolithon episporum* (M.A.Howe) E.Y.Dawson, has been consistently identified and described (Nunes et al. 2008), although the doctoral dissertation of Tomita (1976) reports eight species from this genus: *S. africanum* (Foslie) J.Afonso-Carillo, *S. australasicum* (Foslie) N.Yamaguishi-Tomita ex M.J.Wynne, *S. dimotum*, *S. episporum*, *S. erythraeum* (Rothpletz) Kylin, *S. howei* (Lemoine) N.Yamaguishi-Tomita ex M.J.Wynne, *S. mediterraneum* Heydrich and *S. pacificum* E.Y.Dawson. However, the identifications and descriptions of the Tomita (1976) specimens were mainly based on the size of the tetrasporangial chambers, the tetrasporangial pore densities, the number of hypothallial cells layers and the thallus texture. According to modern concepts of coralline red algal taxonomy, these characters are no longer sufficient for precise identification; some are also too variable for use in species delimitation (Verheij 1993). Although *S. mediterraneum* is regarded as a heterotypic synonym of *S. ptychoides* (Alongi et al. 1996), the specimens identified by Tomita (1976) as *S. mediterraneum* (SP 138576, SP 114638, SP 114641, and SP 114642) do not agree with the description of *S. ptychoides*. The Tomita (1976) specimens have larger tetrasporangia (150-175 X 95-120 μm) and a greater number of cells in the tetrasporangial paraphyses (8-9) (pers. obs.). Furthermore, much of the herbarium specimen collection from Tomita (1976) are not fully available and also lack representative specimens from some species. A new review with extensive

inventory of the genus *Sporolithon* in Brazil is needed to assess the real number of species in order to solve these nomenclatural issues.

This is the first report of *S. ptychoides* from the Atlantic Ocean. Our study has expanded the known geographical distribution of the species and may give insight into the origin of the species into other geographical regions. In a study on the Neogene history of *Sporolithon* from the Mediterranean region, Braga and Bassi (2007) assumed that representatives from the genus *Sporolithon* disappeared from the Mediterranean region during the Messinian Salinity Crisis. They went on to conclude that later on, in the Early Pliocene, a single species of *Sporolithon*, *S. ptychoides*, re-invaded the Mediterranean Basin and continues to inhabit this sea till today. Braga and Bassi (2007) suggested that the most probable source of the Mediterranean re-invasion was the Atlantic Ocean, the only open ocean to which the Mediterranean Sea has been connected since the Mid Miocene. Till now, no evidence to support this suggestion was forthcoming and so the discovery of *S. ptychoides* in the Atlantic Ocean represents an important finding, one that possibly validates the Braga and Bassi (2007) hypothesis.

Acknowledgements

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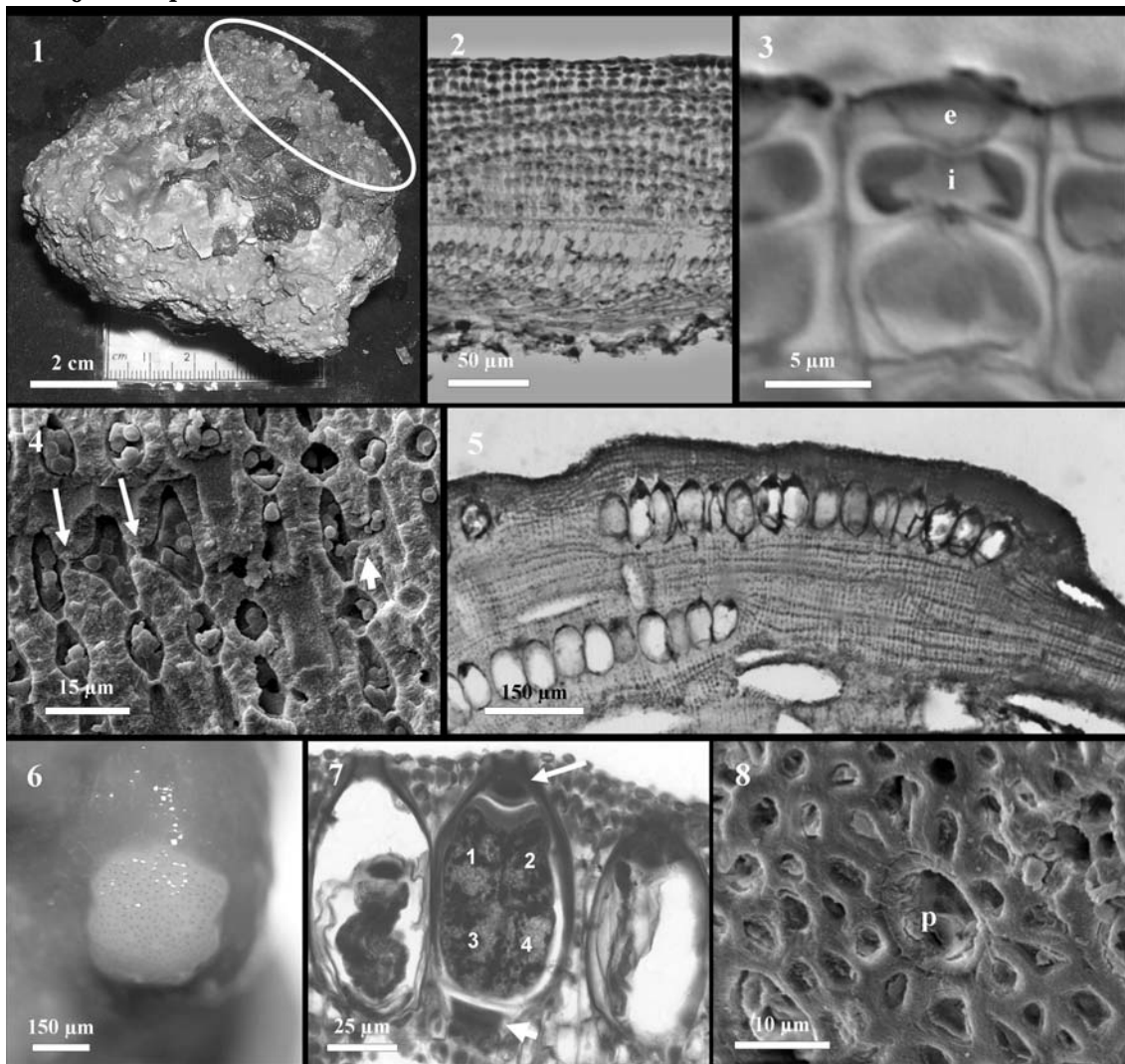
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Figure captions

Figs 1-8. *Sporolithon ptychoides* (RB 476940): vegetative and reproductive features. 1. A rhodolith bearing many species including a somewhat warty *S. ptychoides* specimen (ellipse). 2. Vertical section of the vegetative thallus showing the monomerous internal construction. 3. Vertical section of the vegetative thallus showing a flared epithallial cell (e) and squarish subepithallial initial (i). 4. SEM image of a fracture showing secondary pit connections (arrows) and a cell fusion (arrowhead) between cells of adjacent perithallial filaments. 5. Transverse

section of the thallus showing tetrasporangial compartments both raised above the surrounding vegetative thallus surface and buried deep in the thallus. 6. Surface view of sori in stereomicroscopy. 7. Vertical section of the outer thallus showing a tetrasporangium bearing a pore plug (arrow) and cruciately arranged tetraspores (1, 2, 3 and 4), subtended by a single large stalk cell (arrowhead). 8. SEM image of the thallus surface showing a tetrasporangial compartment pore (p) surrounded by 10 rosette cells.

Figs 9-13. Holotype specimen of *Sporolithon dimotum* (NY 2667): vegetative and reproductive features. 9. General view of the holotype. 10. Vertical section of outer thallus showing a single layer of flared epithelial cells. 11. Vertical section of the outer thallus showing calcified filaments (paraphyses) (arrows) between two tetrasporangial compartments. 12. Vertical section of the thallus showing tetrasporangial compartments buried deep in the thallus. 13. SEM image of a fracture showing secondary pit connections (arrows) and a cell fusion (arrowhead) between cells of adjacent perithallial filaments.



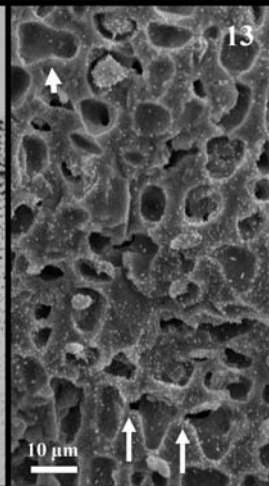
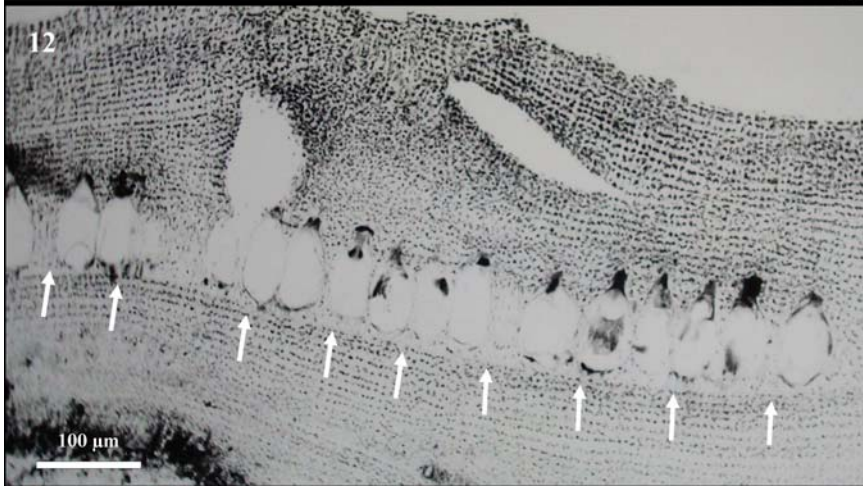
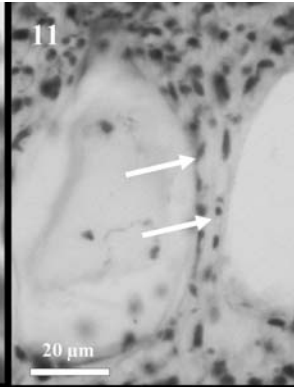
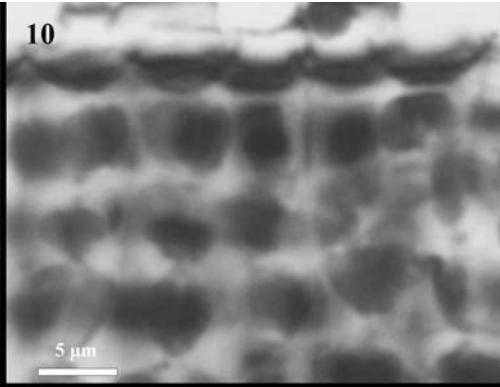
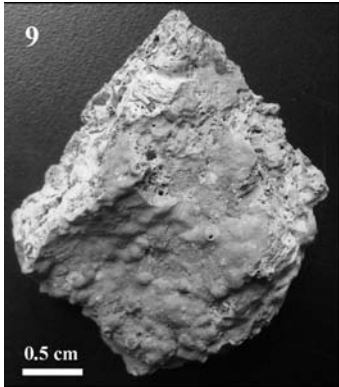


Table 1. Comparative analysis of species of *Sporolithon* for which detailed descriptions are available. Information sources are cited at the bottom of the table.

Character	<i>S. ptychooides</i> (present study)	<i>S. ptychooides</i> ¹	<i>S. ptychooides</i> ²	<i>S. ptychooides</i> ³	<i>S. dimotum</i> ⁴	<i>S. dimotum</i> ⁵ (holotype)	<i>S. durum</i> ⁶	<i>S. episporum</i> ^{1&2}	<i>S. episoredion</i> ¹	<i>S. molle</i> ¹
Locality	Southwestern Atlantic (Brazil)	Red Sea and Indian Ocean (Hawaii)	Indian Ocean (South Africa)	Mediterranean Sea	Southwestern Atlantic (Brazil)	Puerto Rico (Caribbean)	Southern Australia	Panama (Caribbean coast) and Indian Ocean	Hawaii and Indonesia	Red Sea and Indian Ocean
Relative abundance of secondary connections of cell vs. pit fusions (proportion)	Mainly secondary pit connections (2:1)	Mainly secondary pit connections (2-3:1)	Mainly cell fusions	No data	Mainly secondary pit connections (3-1) †	Mainly secondary pit connections (2:1) †	Mainly cell fusions	Mainly secondary pit connections (4-5:1)	Mainly secondary pit connections (2-4:1)	Mainly secondary pit connections (2-3:1)
Tetrasporangia length	75 -105 µm	85-105 µm	77-108µm	85 -130 µm	88-125 µm	70-85 µm	92-105 µm	70 – 96 µm	180 - 220 µm	70 - 85µm
Tetrasporangia diameter	40-55 µm	35-45 µm	29-53 µm	40 -60 µm	35-50 µm	40-60 µm	38–54 µm	30 – 55 µm	100 - 135 µm	25 - 45µm
Tetrasporangial compartment pore diameter	10-14 µm	No data	10-14 µm	12-15 µm	No data	No data	13–21 µm	16-20 µm	No data	No data
Number of rosette cells surrounding tetrasporangial	10-11	No data	8-11	No data	No data	No data	14-15	8-12	No data	No data

compartment pore											
Layer of elongate cells at the base of tetrasporangia	Present	Present	Present	Present	Present †	Present †	Present	Present	Present	Present	Absent
Number of paraphyses between tetrasporangial compartments	1-3	1-2	1-several	1-2	1-3 †	1-3 †	No data	1-several	3-5		1
Number of cells in paraphyses between tetrasporangial compartments	3-5	3-5	7-9	4-6	3-5 †	4-6 †	6-7	3-8	6-9		3-4
Buried tetrasporangial compartments	Present	Present	Present	Present	Present †	Present †	Absent / rarely present	Absent	Present		Present
Position of tetrasporangial compartments relative to surrounding vegetative surface (number of cells)	Raised (2-4 cells)	Raised (1 - 2 cells)	Raised (2 - 8 cells)	Raised	Raised (3-5 cells) †	Raised (3-4 cells) †	Raised (2-3 cells)	Raised (1-4 cells)	Raised (3-5 cells)		Sunken

¹ Verheij (1992, 1993); ² Keats and Chamberlain (1993); ³ Alongi et al. (1996); ⁴ Tomita (1976); ⁵ Foslie & Howe (1906); ⁶ Townsend et al. (1995); † This study.