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The genus *Melobesia* (Corallinales, Rhodophyta) from the subtropical South Atlantic, with the addition of *M. rosanoffii* (Foslie) Lemoine

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Abstract

The calcareous crusted epiphytic algae are a group of extremely delicate, fragile and poorly studied algae. The subfamily Melobesioideae (Corallinophycidae, Rhodophyta) includes the genus *Melobesia*, which has no record of molecular analysis. However, thanks to measurement data, it was possible to find enough similarity for taxonomic identification of two species: *Melobesia rosanoffii* (Foslie) Lemoine, described for the first time in South Atlantic Ocean, and *Melobesia membranacea* (Esper) Lamouroux, first described in southern Brazil. The group has undergone several changes of classification from animals to plants. Today crusty coralline algae have great importance due to the possibility of easy spore dispersal between oceans.

Key words: Coralline red algae, Epiphyte crustose coralline, Hapalidiaceae, Brazil

Introduction

The subfamily Melobesioideae (Corallinophycidae, Rhodophyta) involve crustose to fruticouse plants, with cells of continuous vegetative filaments linked by cell fusions; genicula absent; multiporate plate composed of cells at maturity (Taylor 1960; Harvey 2003). The algae also present tetrasporangial and bisporangial conceptacles. This subfamily includes nine genera, and among these are the delicate epiphytes as representatives of the genus *Melobesia* J.V. Lamouroux 1812:186 (Woelkerling 1988).

Lamouroux (1812) described the genus *Melobesia* for the species *M. membranaceae*, and defined as calcified plants, largely or entirely pseudoparenchymatous, growing completely attached to the surface of various substrates (most commonly other algae), thallus construction dimerous throughout, upright filaments each normally terminating at the thallus surface in an epithelial cell, outermost epithelial cell walls rounded or flattened but not flared at the corners.

Gametangia and carposporangia develop in uniporate conceptacles. Spermatangia (male gametangia) and carpogonia (female gametangia) are produced in different conceptacles; male and female conceptacles are occasionally formed on the same plant but much more commonly formed on different plants. Spermatangia are formed on unbranched filaments arising from the conceptacle chamber floor and roof. Carpogonial branches 1–2 are celled with basal auxiliary cells, arising from the conceptacle chamber floor. Even after fertilization senescent carpogonial branches and the respectives trycogines persist in the center of the conceptacle floor. Carposporophytes develop in these female conceptacles, after fertilization.

Tetrasporangia/bisporangia formed in conceptacles on separate plants from gametangia and carposporangia. Roofs of tetrasporangial/bisporangial conceptacles multiporate. Each pore is blocked by apical plug that prevents sporangia release, which should just occur after the complete maturation of these structures. Bisporangia each contain two spores but otherwise are similar to tetrasporangia (Woelkerling 1988; Wilks & Woelkerling 1991; Woelkerling *et al.* 1993).

Despite being understudied, epiphyte coralline diversity can present key information to the understanding of biogeographical and ecological aspects of the marine biodiversity. On the one hand, the epiphytic group distribution can help in the description of the main routes of marine diversity dispersion (van den Hoek 1987), improving hypothesis about environments connectivity. On the other hand, they play important role in reducing herbivory of flesh seaweeds

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and seagrasses, a possible understudied coevolutionary process (Martin et al. 2008), that is threatened by the observed and predicted ocean acidification.

Biogeographically, *Melobesia* appears to be widespread (Pacific Islands; North, Central and South America, South Africa, Africa, Ireland, Europe, Atlantic Islands, Caribbean Islands, Western Atlantic, Indian Ocean, Islands South-west Asia, Asia, South-east Asia, Australia and New Zealand, even Antarctic and the sub Antarctic islands), but many records require confirmation, due the high complexity of performing a taxonomic study of this genus, several cases of **synonyms** and lack of molecular analysis.

Adapting methodologies of coralline red algae anatomy conventionally utilized for taxonomical studies in this group, the present study report new distribution records and evaluate the significance of characters that have been used to help delimit the specie based on morphological and reproductive features

Material and Methods

Data were collected on the coast of Santa Catarina state, part of the warm temperate province on the Brazilian Coast (Horta et al. 2001), in five cities: Governador Celso Ramos—Praia Grande (27°21'26.26"S, 48°32'10.75"W) on May, 2012; Desert Island (27º16'13"S 48º19'56"W), August, 2012; Farol of Santa Marta (28°36'21.69"S 48°48'57.25"W) on January, 2013; city of Florianópolis – Sambaqui (27°29'24.61"S, 48°32'14.78"W) on February, 2013; Moleques Island (27°50'44.80"S, 48°25'46.77"W) on April 2013. All on the rocky shore, in the intertidal at low tide (<0.3 m). The studied specimens were deposited at the Phycological Herbarium of the Federal University of Santa Catarina (FLOR).

Light microscopy (LM)

The collections were placed in plastic bags, transported to the laboratory with seawater, and latter preserved in 4% formalin-seawater, and transferred to 70% alcohol-water solution with 10% added glycerin after 24 hours. For light microscopy, decalcification was made with 10% hydrochloric acid for 20–40 minutes, dehydrated in increasing series of aqueous ethanol solutions and infiltrated with Historesin (Leica Historesin, Heidelberg, Germany), according to the instructions supplied by the manufacturer. Sectioning (6–10 µm thick) was made with a microtome, stained with acidified aqueous Toluidine blue (TB-O) (Moura *et al.* 1997, Horta 2002).

Scanning electron microscope (SEM)

For the analysis in a scanning electron microscope, stubs were prepared following Horta (2002) with some modifications due the fragility of the algae were made. Conceptacle measurements were obtained from transversal fractures, perpendiculars to the thallus surface, regarding conceptacles presence, utilizing a razor blade to promote the perpendicular fracture. Conceptacle height is the distance from the conceptacle floor to the top of the chamber, and the diameter is the distance between the inner walls as described in Chamberlain (1983).

Results

During taxonomic analysis of coralline epiphytes observed in seaweeds from Brazilian coast two species belonging to the family Hapalidiaceae and subfamily Melobesioideae. Due the presence of dimerous construction, cells of adjacent filaments joined by fusion cells and multiporated tetrasporagial conceptacles these taxa was identified as being representatives of the genus *Melobesia*. Among the evaluated specimens two dietetics morpho/anatomical aspects was observed, indicating the presence of two species: one known as *M. membranaceae*, and a second as *M. rosanoffii,* a new record to South Atlantic.

Melobesia membranacea (Esper) Lamouroux 1812

Basionym: Corallina membranacea Esper

Homotypic Synonyms: Corallina membranacea Esper 1796; Epilithon membranaceum (Esper) Heydrich 1897; Lithothamnion membranaceum (Esper) Foslie 1898

Type locality: West coast of France (Dawson 1960: 8)

Distribution in the World: distributed worldwide.

References to Brazil: as *Melobesia membranacea* (Esper) J.V.Lamouroux (Taylor 1960, Oliveira Filho 1977), and as *Epilithon membranaceum* (Esper) Heydrich (Taylor, 1930).

Specimens studied Brazil, Santa Catarina, Desert Island; Farol of Santa Marta Sambaqui; Moleques Island.

Description

Vegetative structure

Calcareous plants reddish, purple or white (Figure 1A); Thallus encrusting, epigenous and completely affixes by cell adhesion. Structure pseudomarenchymatous with dorsiventral organization; Construction dimerous (Figure 1C), filaments composed of palisade or non-palisade cells 3–5 µm long and 3–6 µm high; distal wall of epithallial cells of adjacent filaments joined by cell-fusion (Figure 1D); secondary pit-connections and tricocytes not observed.

Reproductive features

Gametangia and carposporangia produced in uniporate conceptacles (Figure 1B). Tetrasporangia produced in multiporate conceptacles.

Gametangial thalli monoecious or dioecious; carpogonia and spermatangia produced in separate conceptacles. Carpogonia terminating 2–3 celled filaments arising from the female conceptacle chamber floor. Mature female carposporangial conceptacle roofs protruding above or flush with surrounding thallus surface, 18–25 µm thick, composed of 5–8 layers of short, squat cells, conceptacle chambers 90–135 µm in diameter and 55–97 µm high. Carposporophytes with 12–20 µm in diameter and 13–38 µm high; when mature apparently consisting fusion cells bearing several gonimoblast filaments.

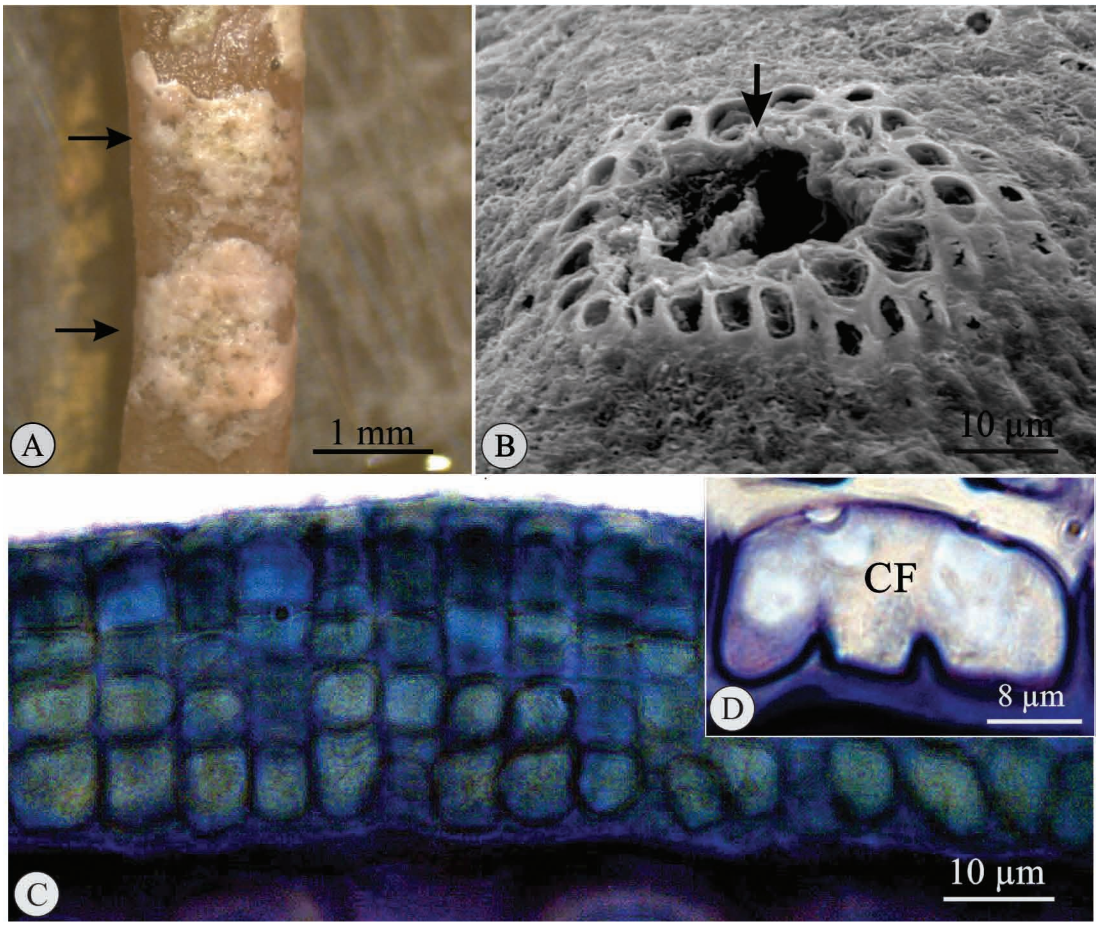

FIGURE 1 Epiphytic coralline algae *Melobesia membranacea*. Image 1B. From scanning electron microscope; Images 1C and 1D from light microscopy. Fig. 1A. Plants growing on *Pterocladiella capillacea* (S.G.Gmelin) Santelices & Hommersand. Fig. 1B. Uniporate conceptacle (arrow), note the detail of epithallial concavities raised above the thallus. Fig. 1C. Section through older portion of thallus showing dimerous organization. Fig. 1D. Detail of a hipothallus cell, CF indicates cell fusion.

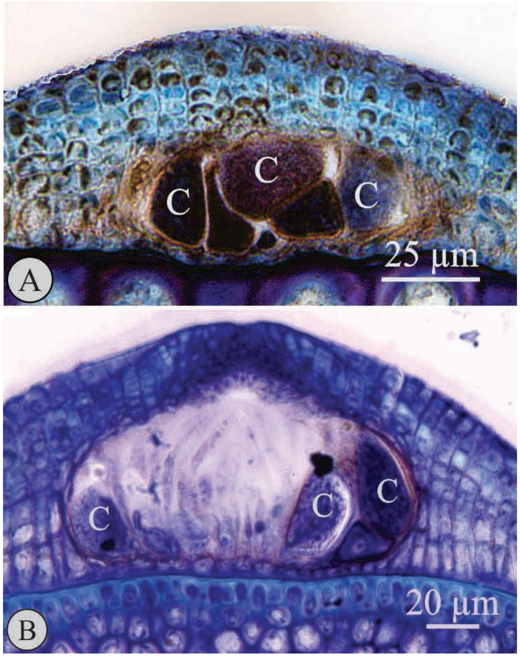

FIGURE 2 Reproductive features of *Melobesia membranacea*. Images from light microscopy. Fig. 2A. Carposporophytic conceptacle with mature carpospores (C). Fig. 2B. Image of a carposporophytic conceptacle with senescent carpogonial branches in the central region and carpospores (C) in the periphery.

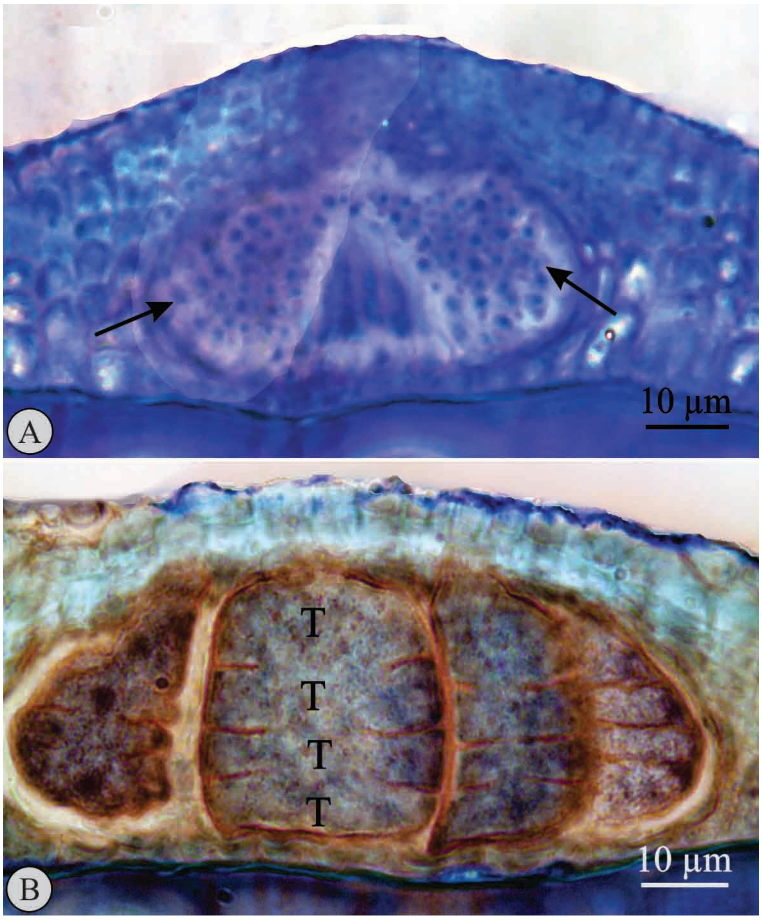

FIGURE 3 Reproductive features of *Melobesia membranacea*. Images from light microscopy. Fig. 3A. Detail of a male conceptacle with mature, unbranched spermatangial filaments arising from the chamber floor and roof (arrows) showing central filaments that resemble mature carpogonial branches. Fig. 3B. Section of a mature tetrasporangial conceptacle with mature tetrasporagium sporangium (T).

Spermatangial filaments arising from the floor and roof (Figure 3A), mature male conceptacles protruding above or flush with surrounding surface, 15–21 µm thick, composed of 6–12 layers of more or less squat cells, conceptacle chambers 50–75 µm in diameter and 47–55 µm high.

Tetrasporangial conceptacle roofs protruding above or flush with surrounding surface, commonly but not always differentiated into a central sunken pore-plate; pore-plate composed of cells that are similar in size and shape to other roof cells, roof 5–6 cells thick above the chamber, with 10–14 µm thick, conceptacle chambers 78–102 µm in diameter and 60–102 µm high; tetrasporangial initial situated one cell layer below the thallus surface, each mature sporangium with 14–21 µm in diameter and 44–51 µm long, containing zonately arranged tetraspores and possessing an apical plug that blocks a roof pores. Bisporangia with 35–38 µm in diameter and 24–39 µm long (Figure 3B).

Melobesia rosanoffii (Foslie) Lemoine 1912: LXI

Basionym: *Lithothamnion rosanoffii* Foslie 1908:5. Woelkerling 1993:191 *Epilithon rosanoffii* (Foslie) Foslie 1909:55

Type locality: Port Phillip Bay, Victoria, Australia (Womersley 1996: 173).

Holotype: TRH; includes slides 1342, 1641, and one unnumbered slide) (Womersley 1996: 173).

Distribution in the World: New Zealand (Nelson 2012), South Australia (Wilks & Woelkerling 1991, Womersley 1996), Tasmania (Wilks & Woelkerling 1991, Womersley 1996), and Victoria (Wilks & Woelkerling 1991, Womersley 1996, Morcom *et al*. 2005).

Specimens studied: Brazil, Santa Catarina, Governador Celso Ramos-Praia Grande.

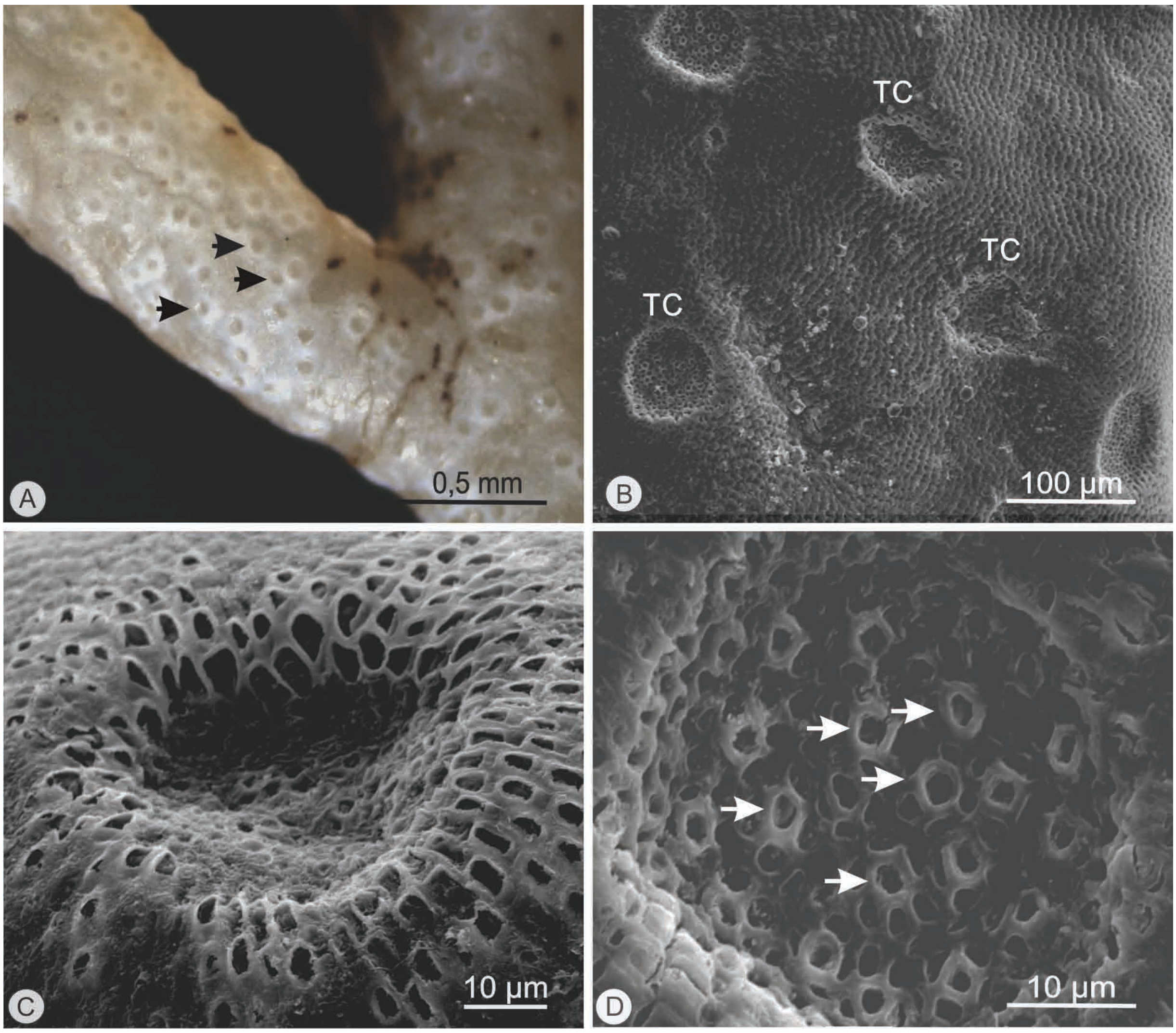

FIGURE 4. Epiphytic coralline algae. Images 4B, 4C and 4D from scanning electron microscope. Fig. 4A. Plants growing on *Laurencia* sp. Arrows denote conceptacles. Fig 4B. Note the cell wall surface view of tetrasporangial conceptacles (TC). Fig 4C. Detail of epithallial concavities of a tetrasporangial conceptacle. Fig 4D. Note the pore plate of a young tetrasporangial multiporate conceptacle. Arrows denote pores.

Description:

Vegetative structure

Plants nongeniculate, entirely attached to the host alga by cell adhesion, encrusting (Figure 4A); construction dimerous (Figure 5); vegetative thallus 25–40 µm, with epithelial cell 4–7 µm in diameter and 4–8 µm long and additional subtending cells 4–8 µm in diameter and 5–7 µm long; distal walls of the epithelial cells rounded or flattened (Figure 4, and figure 5A and 5B).

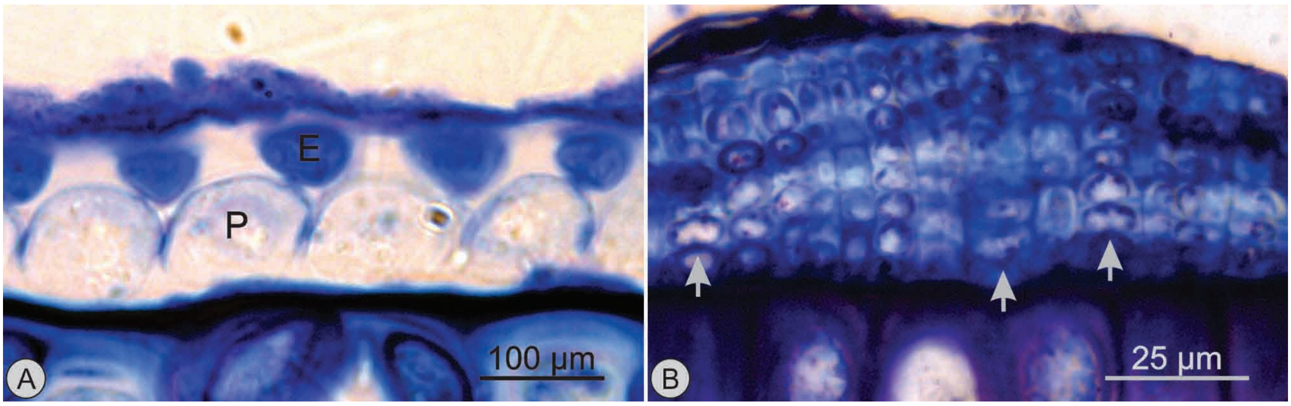

FIGURE 5. Vegetative features of *Melobesia rosanoffii* Fig 5A. Detail of margin of thallus observed by light microscopy. Primigenous cell (P) and epithallial cell (E). Fig 5B. Section through older portion of thallus, showing cell fusion (arrows). Dimerous organization.

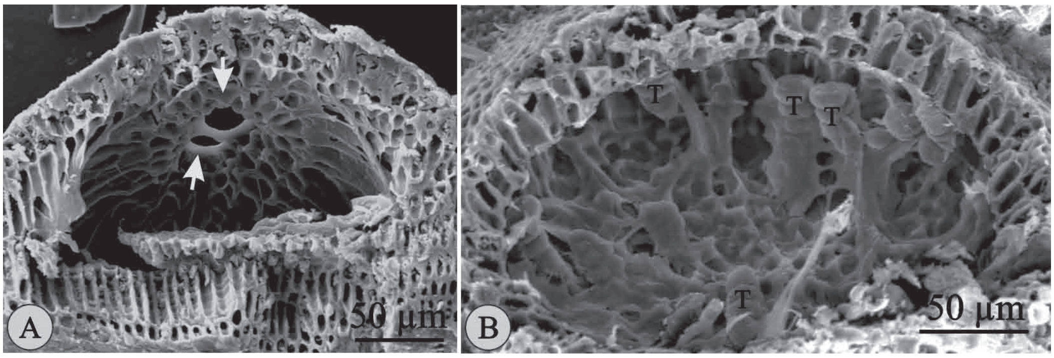

FIGURE 6. Reproductive features of *Melobesia rosanoffii*. Both images from scanning electron microscope. Fig. 6A. Multiporate conceptacle, with evident two pores (arrows). Fig. 6B. Detail of tetrasporangia produced inside de conceptacle (T).

Reproductive features

Gametangia and carposporangia produced in uniporate conceptacles (Figure 4B, 4C and 4D). Tetrasporangia produced in multiporate conceptacles (Figure 6). Carpogonia and spermatangia produced in separate conceptacles. Carpogonia terminating 2-celled filaments arising from the female conceptacle chamber floor. Mature female-carposporangial conceptacles roofs more or less flush with surrounding thallus surface, 17–21 µm thick, composed of 5 layers of densely packed cells; conceptacle chambers 90–120 µm in diameter and 60–74 µm high. Carposporophytes when mature apparently lacking an evident fusion cell and consisting of several-celled gonimoblast filaments bearing terminal carposporangia 18–28 µm in diameter and 16–32 µm high. Spermatangial filaments unbranched, arising from the floor, walls and roofs of male conceptacle chambers, mature male conceptacle roofs more or less flush with surrounding thallus surface; conceptacle chambers 50–75 µm in diameter and 34–38 µm high (figure 7)

Tetrasporangial conceptacle roofs more or less flush with or slightly sunken below surrounding surface, central sunken pore-plate but pore-plate sharply differentiated anatomically from the remainder of roof and composed of cells that differ in size and shape from other roof cells, roof 12–18 µm thick above the chamber; conceptacle chamber 51–83 µm in diameter and 80–100 µm high; tetrasporangial initials situated five or more cell layers below the thallus surface, scattered across the conceptacle chamber floor, each mature sporangium 23–35 µm in diameter and 42–58 µm long, containing zonately arranged tetraspores and possessing an apical plug that blocks a roof pore prior to spore release.

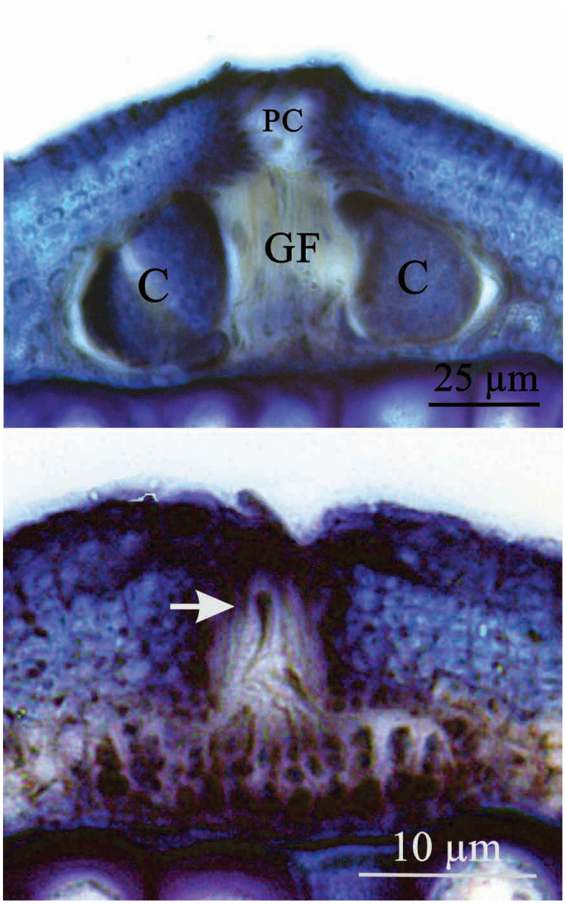

FIGURE 7. Reproductive features of *Melobesia rosanoffii* Fig 7A. Section of female-carpogonial conceptacle in vertical section with senescent carpogonial branches with persistent trychogina (GF). Fig 7B. Female conceptacle in vertical section, with carpogonial branches, arising from the floor with trychogine projected in the pore chanel (arrow). Note the roof flush.

Discussion

The coralline red algal genus *Melobesia* J.V.F. Lamouroux, 1812, Hapalidiaceae, Corallinales, Rhodophyta (see Harvey *et al.* 2003 for classification details), was established for three taxa: *M. membranacea*, *M. orbiculata*, and *M. verrucata*. Lamouroux considered *membranacea* to be an animal, but under the current ICBN, the name is treated as validly published as a plant in 1796 (Art. 45.4).

To the subfamily Melobesioideae consists now a total of 116 species (and infraspecific) names in the database, of which 17 are flagged as currently accepted taxonomically. At present, most species assigned to *Melobesia* are poorly or incompletely known (Guiry 2014). The record of *M. rosanoffii* is represented by a single collection from the northern tip of the North Island, growing as an epiphyte on *Lessonia* sp. and there were multi and uniporate conceptacles on adjacent plants. The identity of the specimen was confirmed by Adele Harvey based on sections. This is the first time that this specie is described from the Brazilian Coast.

Using diagnostic features employed in the taxonomy of non-geniculate coralline algae, the specimens were compared with the same taxa described from other parts of the world (Table 1), and identifications based on a combination of characteristics (Lamouroux 1812; Wilks and Woelkerling 1991; Womersley 1996).

Considering that epiphytic organisms may disperse more easily, we can start from the hypothesis that the diversity of the group would be higher than the presently described. Besides being cryptic, the diffusion of the equipment and the specific methodological approaches should improve the efforts investigating the diversity of this group. We can not forget that we are passing through a very dynamic moment where climatic changes, that increase the influence of the Indic in the South Atlantic Ocean (Beal et al. 2011), and the intensification of global marine transport (Whinam et al. 2005, Gregory 2009), can represent the necessary conditions to this group take more frequently hitchhiking from Indo/Pacific oceans to southwestern Atlantic coast. Thereby, the occurrence of M. *rosanoffii* probably results from the lack of studies about epiphytic coralline algae on the Brazilian coast. However, we cannot ignore the possibility of recent arrival, as has been observed in the invasion of other taxa (Macreadie et al. 2011). These each time more connected oceans represent important challenge to the epiphytic coralline algae current taxonomists, once traditional approaches should be limited to provide robust answers requested nowadays.

Despite the modern analyses of the material provide good morphoanatomical information, the systematics should consider the possibility of the occurrence of cryptic species, as observed in other coralline red algae taxa (Bittner et al 2011). This particularity reinforces the need of additional molecular data for a better characterization of this group.

TABLE 1. Comparative table. A.Present study (*M. rosanoffii*); B. Present study (*M. membranacea*). C and D. Data from Wilks and Woelkerling (1991), about the species concerned. ND – no data.

Character	A	B	C	D
Thallus thickness	25–40	13–60	25–113	10–75
Epitelial cell length	3–5	3–5	2–3	2–8
Epitelial cell diameter	3–5	3–6	2–3	2–10
Postigenous cell length	5–7	4–10	2–15	2–15
Postigenous cell diameter	4–8	6–21	2–15	2–15
Tetrasporangial conceptacle chamber diameter	51–83	78–102	45–118	50–252
Tetrasporangial conceptacle chambre high	50–90	60–102	40–80	15–83
Tetrasporangial conceptacle roof thickness	12–18	10–14	12–25	ND
Number of cell layers in tetrasporangial conceptacle roof	4–6	5–6	2–7	2–5
Lengh of tetrasporangia	42–58	44–51	25–63	10–53
Diameter of tetrasporangia	23–35	14–21	10–25	22–65
Male conceptacle chamber diameter	50–75	50–75	100–138	57–130
Male conceptacle chamber high	34–38	47–55	30–75	25–40
Carposporangial conceptacle chamber diameter	90–120	90–135	100–193	50–250
Carposporangial conceptacle chamber high	60–74	55–97	30–55	25–80
Carposporangial conceptacle roof thickness	17–21	18–25	35–63	5–25
Number of cell layers in carposporangial conceptacle roof	5–8	5–8	5–14	2–8
Diameter of carposporangia	18–28	12–20	12–20	18–60
Length of carposporangia	16–32	13–38	12–25	ND

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