

THE ESTUARINE BENTHIC DIATOM *Margaritum terebro* (BACILLARIOPHYTA, HYALODISCEAE): MORPHOLOGY AND TAXONOMY

Roseli M. de Souza-Mosimann¹; Luciano F. Fernandes² & Thelma V. Ludwig²

¹Universidade Federal de Santa Catarina
Horto Botânico - Campus Universitário, Trindade
(Caixa Postal 476, 88040-900 Florianópolis, SC, Brasil)

²Universidade Federal do Paraná, Setor de Ciências Biológicas
Departamento de Botânica, Centro Politécnico
(Caixa Postal 19031, 81531-970 Jardim das Américas, Curitiba, PR, Brasil)

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- **Abstract:** *Margaritum terebro* is an estuarine centric diatom recorded along the southeast coast of Brazil, the Atlantic coast of Africa, and in regions close to the Galapagos Islands. Previous works only give a very limited information on the morphology and taxonomy of the genus based on light microscope. In this work, the species is described with scanning electron microscope. Results show a valvar surface with specific structures such as external projections of the rimoportulae bearing a central pore and convex sides. The internal openings are sessile. The term rimoportulae "papilliformis" is proposed for this new structure. The margin of the valve is undulated and presents a ring of sessile rimoportulae and spines irregularly arranged. Comparisons between the monospecific genus *Margaritum* and other related genera (*Podosira* and *Hyalodiscus*), and its systematic positioning are made.
 - **Resumo:** *Margaritum terebro* é uma diatomácea marinha estuarina registrada apenas para a costa sul-brasileira, costa atlântica da África e adjacências das Ilhas Galápagos. Os trabalhos publicados até o momento referem-se apenas à morfologia da valva sob microscopia ótica, pouco esclarecendo sobre a posição taxonômica do gênero. No presente trabalho, a espécie é descrita sob microscopia eletrônica de varredura a partir de amostras de plâncton e perifiton de águas neríticas do sul do Brasil. Os resultados revelam uma superfície valvar provida de rimopórtulas características com projeções externas robustas, poro central e lados convexos; na superfície interna sua abertura é sésil. O termo rimopórtula "papilliformis" é proposto para este tipo inédito de rimopórtula. As estrias são constituídas por estruturas semelhantes a espinhos. A margem valvar é ondulada e apresenta um anel de rimopórtulas sésseis e espinhos irregularmente arranjados. Comparações entre o gênero monoespecífico *Margaritum* e gêneros próximos (*Podosira* e *Hyalodiscus*) permitiram posicioná-lo convenientemente na Família Hyalodiscaceae Crawford.
 - **Descriptors:** *Margaritum*, Diatom, Hyalodiscaceae, Taxonomy, Estuarine, Periphyton, Southern Brazil.
 - **Descritores:** *Margaritum*, Diatomácea, Hyalodiscaceae, Taxonomia, Estuarina, Perifiton, Sul do Brasil.
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Introduction

The centric marine diatom *Margaritum terebro* (Leuduger-Fortmorel) H. Moreira originally described as *Podosira terebro* by Leuduger-Fortmorel (1898), was transferred to the new genus *Margaritum* by Moreira-Filho (1968) based on the presence of "pearl shaped" structures or "hemispheres" on the external surface of the valve; they do not occur in other genera, including those closely related, such as *Hyalodiscus*, *Melosira* and *Podosira*. In 1969, Dr. G. Dallas Hanna from the California Academy of Sciences and Dr. N.I. Hendey sent letters to Dr. Moreira-Filho to express the acceptance of Dr. Moreira-Filho's proposal of the new genus. As pointed out by Hendey (1971), the specific epithet was misspelled by Moreira-Filho as "*tenebro*".

Margaritum terebro is an estuarine species that has been recorded along the southeast coast of Brazil (Moreira-Filho, 1968; Souza-Mosimann, 1988; Fernandes *et al.*, 1990), the Atlantic coast of Africa (Leuduger-Fortmorel, 1898; Hendey, 1958) and in the proximity of the Galapagos Islands (Hendey, 1971). These findings suggest that the species is restricted to tropical regions.

Some research on this species has been done through light microscopy, but failed to detail the morphology and taxonomy of the genus. Until now, its position in the classification of Round *et al.* (1990) was uncertain, because the internal valvar surface and the cingulum structure were not described.

Recently, periphyton samples collected in Paranaguá Bay (Paraná State, Brazil) contained a reasonable amount of *Margaritum terebro* cells and permitted a more detailed study of its morphology based on scanning electron microscopy observations, elucidating the taxonomic affinities between *Margaritum* and other genera, besides placing it conveniently in higher taxonomic categories. The relationships between *Margaritum* and related genera such as *Podosira* and *Hyalodiscus* are also presented and a new type of rimoportula is described.

Material and methods

Samples containing the species *Margaritum terebro* were obtained from periphyton on glass slides and from plankton samples in Paranaguá Bay, Paraná state, Southern Brazil (25°27'S, 48°20'W). Permanent slides and the original material examined were deposited in the FLOR Herbarium at Federal

University of Santa Catarina (UFSC), number 12.759.

Samples were preserved with buffered formaldehyde (2%) and prepared for light and electron microscopy according to the method described by Hasle & Fryxell (1970). Permanent slides were prepared using Permunt as the mounting media. Light microscopy (LM) was performed with an Olympus BX40 microscope using 100x oil immersion objective. For scanning electron microscopy (SEM) samples were air-dried onto coverslips and mounted on aluminium stubs with conductive paint, coated with gold 16-20 nm thick and examined at 15-30 Kv accelerating voltage in a Phillips model XL30 scanning electron microscope.

The descriptive terminology followed that of Ross *et al.* (1979) and Round *et al.* (1990).

Results

Division Bacillariophyta

Class Coscinodiscophyceae Round & Crawford

Subclass Coscinodiscophycidae Round & Crawford

Order Melosirales Crawford

Family Hyalodiscaceae Crawford

Genus *Margaritum* H. Moreira

Species *M. terebro* (Leuduger-Fortmorel)

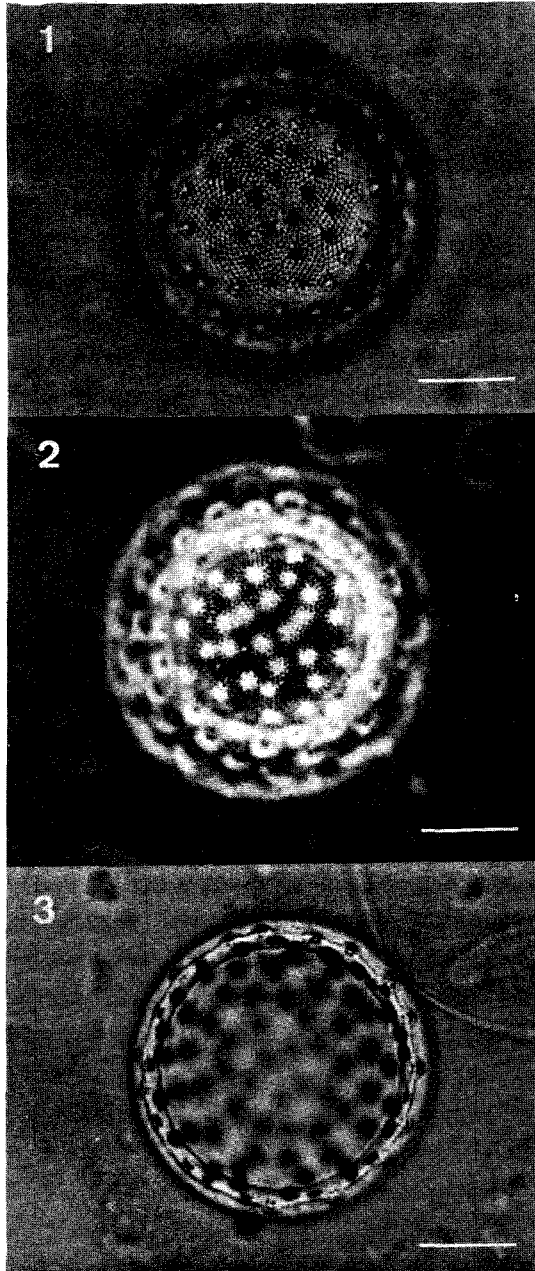
H. Moreira

Description LM: Cells are spherical; plastids are disk-like and circular. Valves circular, 30µm to 50 µm in diameter (120 valves measured); valvar surface deeply convex, with hemispheric projections arranged in diagonal rows (Fig. 1). Spines are scattered on the surface, visible as refringent small spots forming concentric striae (Fig. 1). Radial rows of areolae are visible under Phase-contrast illumination (Fig. 2). Valvar margin thick, forming an undulation separated by a concavity (Fig. 3).

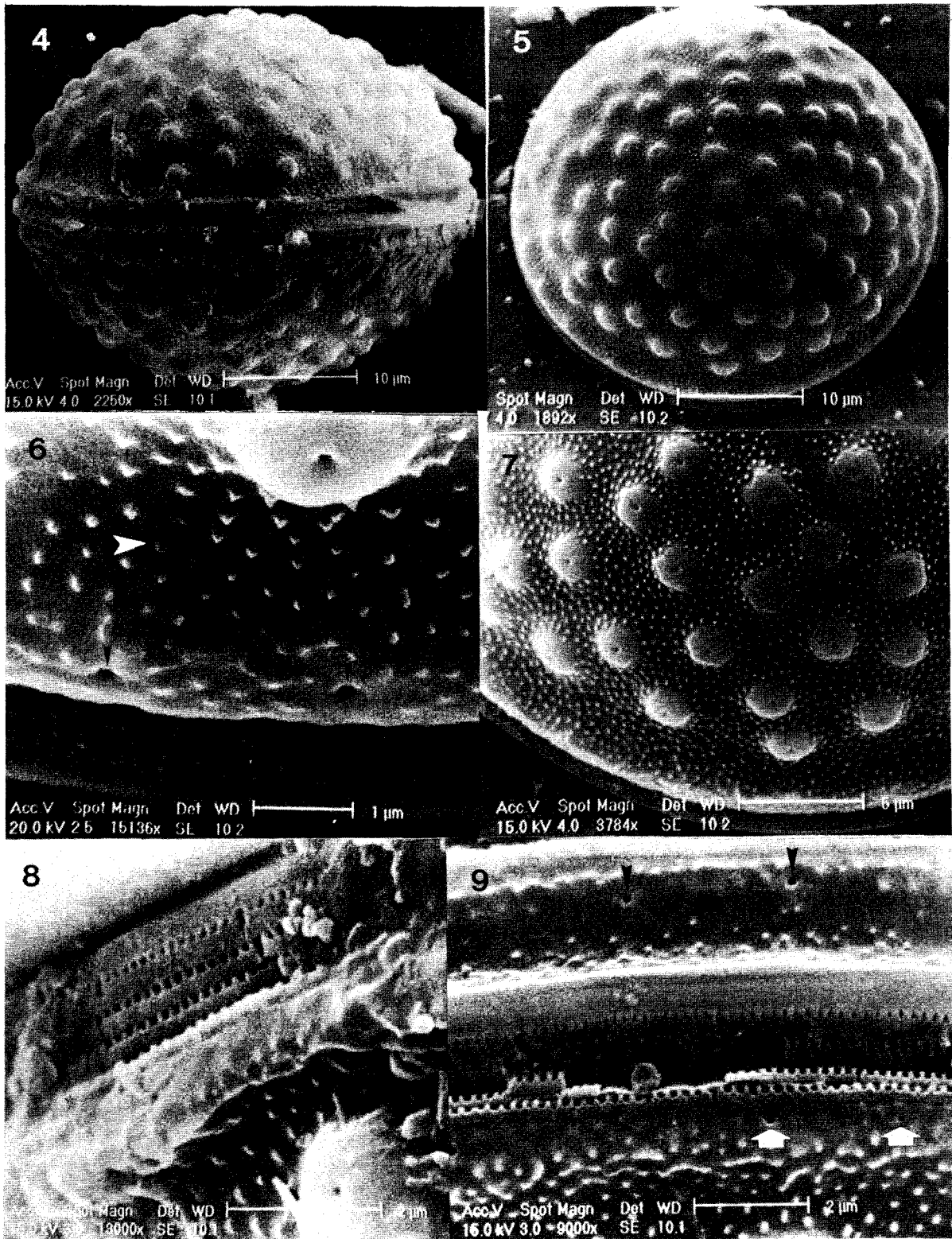
Description SEM: Frustules are robust, almost spherical; valvar diameter slightly larger than the perivalvar axis (Fig. 4). Valve is convex; valvar surface provided of small hollow spines (Figs 6-7). External projections of rimoportulae are arranged in concentric diagonal rows (Figs 5-6). Each rimoportula is formed by a robust external projection of approximately irregular morphology (almost polygonal and rounded) (Fig. 7) and a central pore that opens to the inside of the valve through a small slit surrounded by a circular thickened rim (Fig. 12). For this type of rimoportula we propose the term

rimoportula "papilliformis". The valvar margin presents a thickened undulation projected outward and includes a ring of rimoportulae lacking external projections (Figs 4, 6, 9). The internal valvar surface possesses openings of rimoportulae and foramina (Figs 11-13). The foramina are circular with slightly thickened edges, and arranged in radial rows (Figs 10-13). As the basal siliceous layer is thickened, the foramina clearly form elongated channels through it

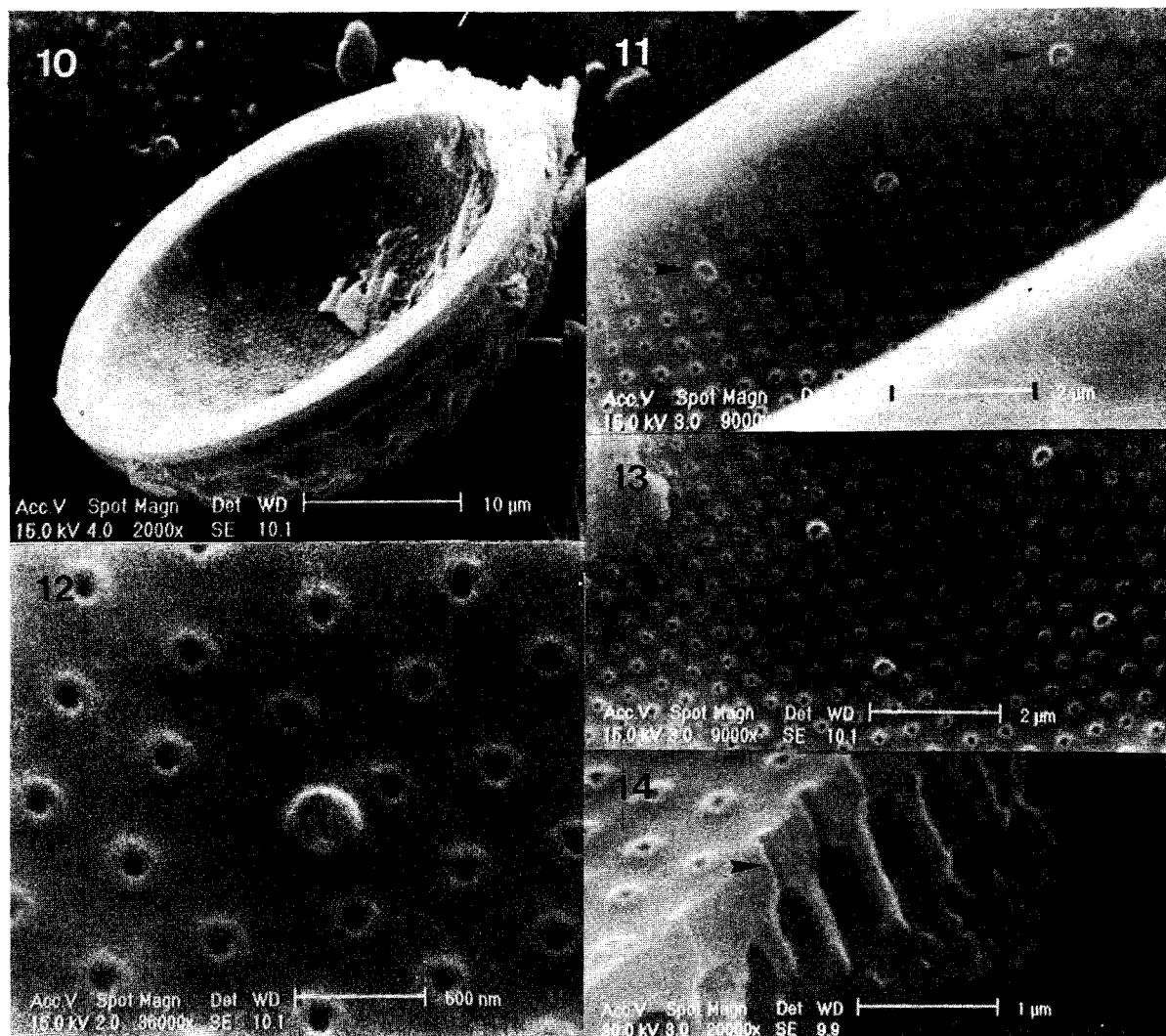
(Fig. 14). Areolae loculate lacking rotae, non-bululate (Fig. 14). The valvar edge is thickened, slightly concave, and its borders show a small groove. Cingulum is composed of 4-5 narrow bands. Each copula is formed by a longitudinal smooth ring and numerous longitudinal pores (Figs 8-9); ligulae were not observed.



Figs 1 - 3. *Margaritum terebro*, Light microscope, valve views. Scale bar = 10 μ m. Fig. 1. Upper focus showing rimoportulae papilliformis and disposition of spines. Fig. 2. Upper focus under phase-contrast. Fig. 3. Lower focus showing a side view of the rimoportulae near the margin.



Figs. 4 - 9. *Margaritum terebro*, SEM, external views. Fig. 4. Lateral view of the frustule. Valvar diameter is slightly larger than pervalvar axis. Arrowhead indicates the undulation of the valve. Scale bar = 10 μ m. Fig. 5. Oblique orientation of a valve illustrating the disposition of the rimoportulae. Scale bar = 10 μ m. Fig. 6. Detail of an undulation showing two sessile external apertures of rimoportulae (arrowheads). Note the spines on the surface. White arrowhead indicates a broken spine. Scale bar = 1 μ m. Fig. 7. Morphology of rimoportulae on the external side. Each projection is approximately polygonal with a central aperture. Many refringent spines are scattered over the valve. Scale bar = 5 μ m. Fig. 8. Detail of an intact cingulum showing four bands. Scale bar = 2 μ m. Fig. 9. Lateral view of a frustule to show the cingulum and undulations of the valves. Arrowheads and white arrows indicate the undulations bearing sessile apertures of rimoportulae. Scale bar = 2 μ m.



Figs. 10 - 14. *Margaritum terebro*, SEM, internal view. Fig. 10. General view of the valve. Scale bar= 10 μ m. Fig. 11. Detail of the valvar margin with three rimoportulae aligned to form a ring (arrowheads). Note areolae in radial rows. Scale bar= 2 μ m. Fig. 12. Internal structure of a rimoportula. Scale bar= 500 nm. Fig. 13. Disposition of the rimoportulae on the surface (except for the valvar margin; see Fig. 11). Scale bar = 2 μ m. Fig. 14. Broken valve showing non-bululate areolae. Arrowhead indicates an elongated foramen. Scale bar = 1 μ m.

Discussion

The main feature of *Margaritum terebro* is the unusual external aperture of each rimoportula, which is robust and polygonal. It was named "hemispherical projections" by Moreira-Filho (1968)

and "wart-like structures" by Hendey (1958, 1971). Its morphology does not fit into any of the known types of rimoportulae (Hasle, 1972; Ross *et al.*, 1979; Round *et al.*, 1990), and therefore we propose the term rimoportula "papilliformis" for this new type. In addition, the

marginal ring of rimoportulae, and the undulation of the valve make the species well distinct from others. In order to better circumscribe the genus *Margaritum* some comparisons with related genera were made. Table 1 summarizes the main features of the genus *Margaritum* compared with the genera *Hyalodiscus* and *Podosira*.

The genus *Podosira* does not pose a marginal ring of rimoportulae, as seen in the genus *Margaritum*. On the valvar surface of *Podosira* the rimoportulae occur in greater numbers and are not ordered. Moreover, their external openings are sessile, i.e., lacking projections. In *Margaritum* the rimoportulae are ordered and characteristic (papilliformis). The foramina of the areolae are arranged in radial rows and lack rotae. Observations of the areolar structure in *Podosira* by Round *et al.* (1990) showed rotae occluding foramina. Furthermore, these authors recorded no defined pattern in the areolar disposition, and closely packed areolae. The minute external pores of the areolae, as cited by Round *et al.* (*op. cit.*) for *Podosira* were not present, or were not discernible in our samples of *Margaritum* with SEM. In relation to the spines, these structures have not yet been found in *Podosira*, although they have been observed in *Margaritum*.

On the other hand, both genera show non-bullulate areolae that, as suggested by Round *et al.* (*op. cit.*), could be a potential criterion of separation between *Hyalodiscus*, *Margaritum* and *Podosira*. Copulae are also similar, although less developed in *Margaritum*, with 4-5 bands. Judging by the location of the copulae and the structure of the margin edge, it is likely that the species presented 2 cells joined by the cingulum, as in the case of *Podosira*. However, we were not able to find any specimen forming cell diplets in preserved samples.

The genus *Hyalodiscus* shows characteristics that are not present in *Margaritum* as a conspicuous central area and bullulate areolae (closely packed). In addition, the rimoportulae of *Hyalodiscus* show differences in morphology regarding to *Margaritum*, although a marginal ring of rimoportulae is present in both genera. Stidolph (1993) describes the species *H. pustulatus* A. Schmidt under SEM reporting ligulate open bands, spinules, peculiar baciliform structures on the inner side of the valve, and microlabiate processes. However, the species shows dissimilarities when compared with more typical representatives like *H. subtilis* and *H. scoticus*. Indeed, it seems to bear characteristics of *Melosira* Agardh, as special loculate areolae with external ridges encircling the pores, granules, spines and bands of cingulum (see figures 12, 13 and 23 of Stidolph, 1993).

We decided to place the genus *Margaritum* within the Family Hyalodiscaceae, because its frustule morphology is more closely related to those of *Podosira* and *Hyalodiscus*. For instance, in all of them the loculate areolae and/or the disposition of the rimoportulae are similar.

As demonstrated from the SEM study in the present work, we support the maintenance of the genus *Margaritum* as first proposed by Moreira-Filho (1968), due to the presence of typical rimoportulae papilliformis, marginal ring of rimoportulae (as in *Hyalodiscus*) and the non-bullulate areolae and bands of the cingulum (as in *Podosira*).

Margaritum terebro has been found in estuarine environments from tropical regions of the World. It was collected from the stomachs of benthic invertebrates (Leuduger-Fortmorel, 1898; Valente-Moreira *et al.*, 1994), epiphytic on macroalgae (Moreira-Filho & Valente-Moreira, 1980, 1981; Valente-Moreira *et al.*, 1980; Moreira-Filho *et al.*, 1990), and from the sand gravels of intertidal zones (Hendey, 1958 and 1971). In the present study the species was fairly common on glass slides used to analyze the seasonal dynamics of the periphyton in the Paranaguá Bay (Brandini, pers. com.*). All of these findings lead us to believe that *M. terebro* grows in a benthic habitat, an environment that could be a unifying characteristic among the representatives of the Family Hyalodiscaceae. Other authors have encountered *M. terebro* in plankton samples, but always in shallow, turbulent regions of the sea (Fernandes *et al.*, 1990; Moreira-Filho *et al.*, 1975; Souza-Mosimann, 1984, 1985 and 1988; Souza-Mosimann *et al.*, 1989).

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(*)Brandini, F. P. Universidade Federal do Paraná. Centro de Estudos do Mar.

Table 1. Comparative morphology between the Genus *Margaritum* and the related Genera *Hyalodiscus* and *Podosira*. (1) from Round *et al.* (1990).

<i>Margaritum</i>	<i>Hyalodiscus</i> ¹	<i>Podosira</i> ¹
external apertures of the rimoportulae projected, characteristic (papilliformis)	external apertures of the rimoportulae inconspicuous	external apertures of the rimoportulae inconspicuous
internal apertures of the rimoportulae single and sessile with a longitudinal slit	similar	similar
presence of a marginal ring of rimoportulae	presence of a marginal ring of rimoportulae	marginal ring of rimoportulae absent
non bullulate areolae	bullulate areolae	non bullulate areolae
spines on the valvar surface	no spines	no spines
central area absent	central area large, evident	central area absent
bands of cingulum open (?)	bands of cingulum open (?)	bands of cingulum open
areolar pores not evident on outer surface (present ?)	areolar pores evident, arranged in rows	areolar pores minute, hardly visible with SEM

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