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Morphology and Taxonomy of *Melosira undulata* (Ehrenb.) Kütz. var. *normanii* Arn. (Bacillariophyta) from Rio Grande do Sul, Brazil

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Abstract

This is the first study of the freshwater diatom *Melosira undulata* (Ehrenb.) Kütz. var. *normanii* Arn. from Brazilian waters. The fine structure was studied based on sand samples from Guaíba River and plankton from Lagoa Emboaba (Rio Grande do Sul State, Brazil). The diatom attaches itself to sand grains by secreting mucilage stalks from its sessile rimoportulae, which are located in the middle of the mantle. The loculate wall structure is described in detail and the fine structure is compared to species of *Melosira* C.Agardh, *Ellerbeckia* R.M.Crawford, *Orthoseira* Thwaites and genera of Hyalodiscaceae. The features observed are in agreement with *Melosira*.

Key index words : epipsammic diatom, freshwater, Melosiraceae, morphology, phytoplankton, *Melosira undulata* var. *normanii*

Introduction

Melosira undulata (Ehrenb.) Kütz. and its varieties have been reported from fossil and recent sediments, as well from plankton. *Melosira undulata* var. *undulata* and var. *normanii* Arn. are frequently found as a fossil in European Tertiary deposits (Krammer & Lange-Bertalot 1991) and also reported in recent materials from France (Germain 1981), California-Nevada (Mahood *et al.* 1984), and tropical areas (Krammer & Lange-Bertalot 1991). In addition, Manguin (1949) summarized the geographic distribution of *M. undulata* to tropical America, Asia, Australia, and Oceania. Also, the author recorded *M. undulata* var. *undulata* from L'Ankaratra (Madagascar) with four taxa : *M. undulata* var. *fungica* Manguin, *M. undulata* var. *samoensis* Grunow, *M. undulata* var. *producta* f. *hungarica* A.W.F.Schmidt and, *M. undulata* var. *producta* f. *californica* A.W.F.Schmidt ; the last two taxa are fossils. Takano (1967) found *M. undulata* var. *undulata* in brackish water in Abashiri, Japan. According to Hustedt (1930) and Krammer & Lange-Bertalot (1991), *M. undulata* var. *normanii* was found in Denmark, Finnland and Sweden.

Several authors have found and illustrated *Melosira undulata* based on light microscopy (LM ; Hustedt 1930, Takano 1967, Germain 1981, Krammer & Lange-Bertalot 1991). Huang (1982) and Tanaka *et al.* (1984) studying fossil material from China and Japan, respectively, provided initial observations of *M. undulata* var. *undulata* and *M. undulata* var. *normanii* under scanning electron microscopy (SEM), presenting several external valve views.

In Brazil, Rosa *et al.* (1994) reported *Melosira undulata* var. *normanii* in plankton samples of Lagoa de Tramandaí (29° 56′ 46″-30° 00′ 18″S and 50° 07′ 30″-50° 11′ 00″W), a shallow brackish coastal lagoon of Rio Grande do Sul. The sample studied by Rosa *et al.* (1994) were collected in November 1977 when the salinity was 1.82 and the temperature 22℃ and an unique specimen was found.

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This is the first study of *Melosria undulata* var. *normanii* based on SEM observations from Brazilian material. The paper reports the fine structure of the specimens attached to sand grains in subtropical freshwater habitats of Brazil and discusses the taxonomic relationship among it and some genera of *Orthoseira* Thwaites, *Melosira* C.Agardh, *Paralia* Heib., *Ellerbeckia* R. M.Crawford, *Podosira* Ehrenb., and *Hyalodiscus* Ehrenb.

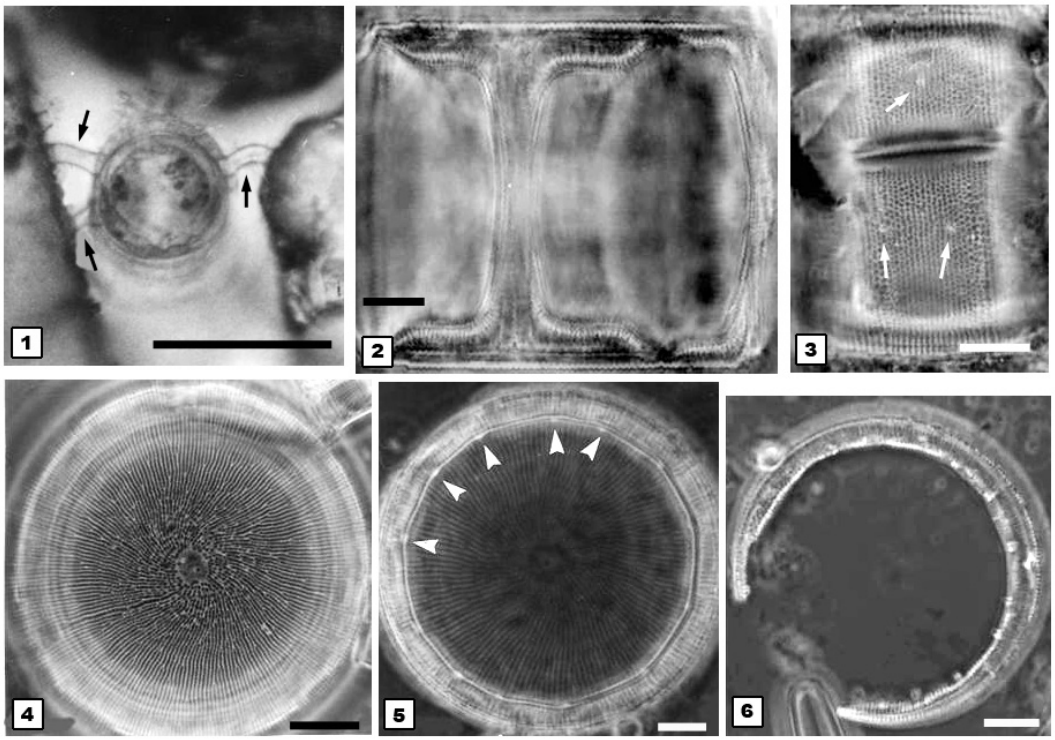
Material and Methods

Based on the herbarium collection *Melosira undulata* var. *normanii* occurs in two freshwater habitats in Rio Grande do Sul State :

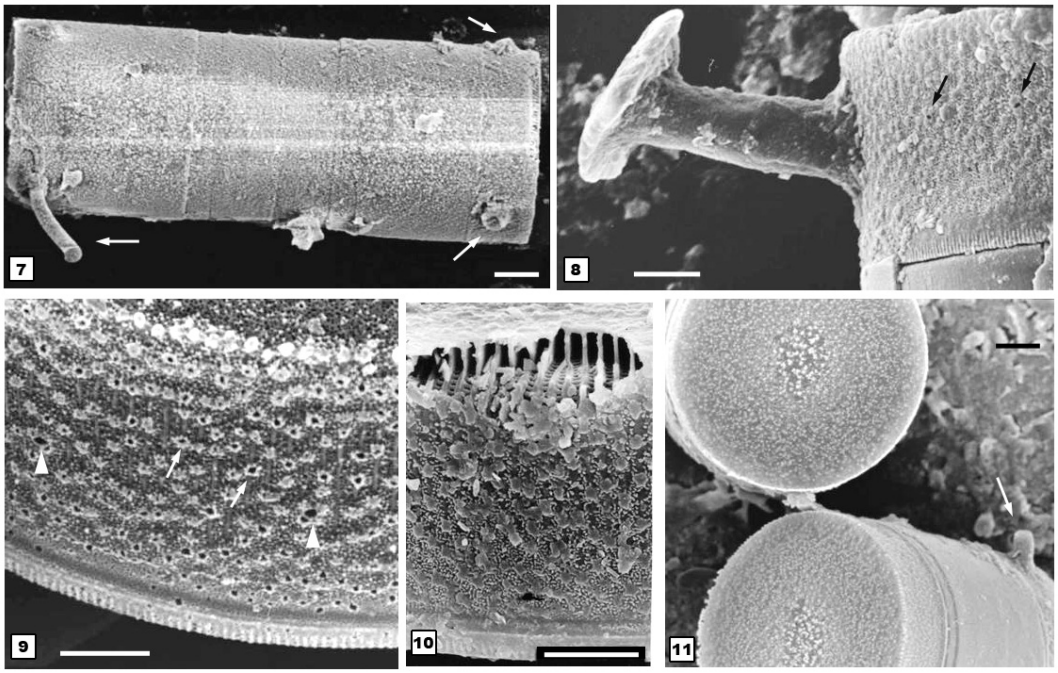
1) In Lagoa Emboaba (29° 57′ 39″-29° 58′ 32″S and 50° 13′ 37″-50° 12′ 29″W), an isolated lagoon, located 8 km from the coast, in plankton samples collected from its central part between August 1989 and August 1990. The pH and the conductivity ranged from 6.6 to 7.6 and from 57 to 91.6 μS cm^{-1}, respectively. The samples are deposited in ICN (Universidade Federal do Rio Grande do Sul Herbarium) under ICN 88018 and ICN 88019.

2) In Guaíba River (Barra do Ribeiro - 30° 17′ 00″S and 51° 81′ 00″W), where sand from the swash zone has been collected in March 1988. Water samples collected in February of 1995 had a pH ranging from 7.06 to 7.08 and the conductivity ranging from 42.9 to 45.5 μS cm^{-1}. Samples are deposited in PEL (Universidade Federal de Pelotas Herbarium) under PEL 22592 to 22594. In both sampling sites, Lagoa Emboaba and Guaíba River, the commonest angiosperm was *Scirpus californicus* (C.A.Mey.) Steud.

Subsamples from each site were cleaned following the technique described by Simonsen (1974). Part of the material was mounted in Naphrax and observed with a Zeiss Axioplan microscope with phase contrast optics and the specimens were photographed using Ilford F50



Figs 1-6. *Melosira undulata* var. *normanii*, LM. **Fig. 1.** A short chain attached by three mucilage stalks (arrows). **Fig. 2.** Frustules in girdle view showing undulate inner mantle surface. **Fig. 3.** Frustule in girdle view showing the rimoportulae (arrows) and parallel stria (loculi) arrangement. **Fig. 4.** Valve face showing spiral and dichotomous branched striae (loculi). **Fig. 5.** Valve face showing the position of rimoportulae (arrowheads). **Fig. 6.** A broken valve without valve face. Note the internal projections of the rimoportulae. Scale bars = 10 μm.



Figs 7-11. *Melosira undulata* var. *normanii*, SEM. Figs 7, 8, 11. Non-cleaned material, external views. Figs 9, 10. Cleaned material, external views. **Fig. 7.** Two frustules in girdle view showing three broken mucilage stalks (arrows) located at epivalves. **Fig. 8.** Girdle view showing a mucilage stalk and two external apertures of rimoportulae (arrows). **Fig. 9.** Note the external rimoportula aperture (arrowheads) and distribution of flat circular granules eroded (arrows). **Fig. 10.** Broken edge showing the vertical walls of the longer loculi. **Fig. 11.** Two frustules showing the right angle between valve and mantle face and a small broken stalk (arrow). Scale bars = 10 μm (Figs 7, 11), 5 μm (Figs 8, 9, 10).

film. For scanning electron microscopy (SEM), cleaned specimens were dried onto a stub, coated with gold at 1 kV for 4 min., and observed using a JEOL JSM-5200 microscope at an accelerating voltage of 15 kV. The working distance was of 10 mm.

In order to observe and study the relationship between rimoportulae and mucilage-stalk production, some specimens were mounted on stubs without oxidation procedures.

Results

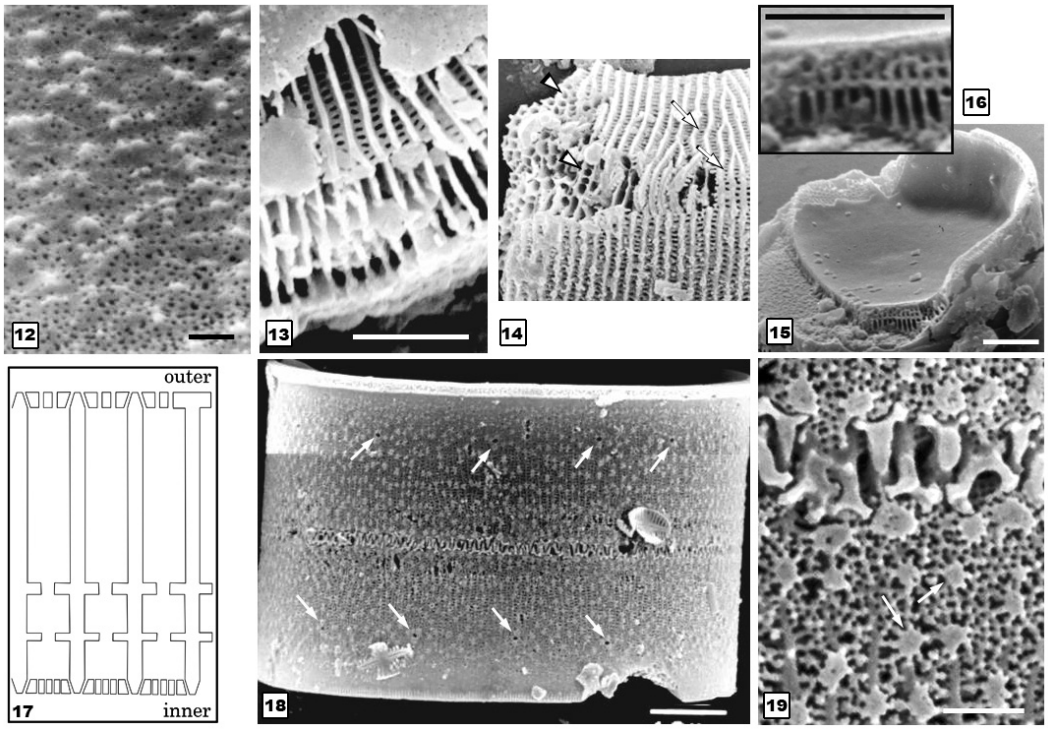
***Melosira undulata* (Ehrenb.) Kütz. var. *normanii* Arn.**, in Van Heurck, Synopsis des Diatomées de Belgique, *pl. 90. f. 7.* 1882.

LM observations

Melosira undulata var. *normanii* forms short chains of up to 4 cells fixed strongly to sand grains by mucilage stalks (Fig. 1). The rows of pores have a dichotomous and spiral arrangement on the valve face (Fig. 4), while in the mantle, the rows of pores are straight and parallel (Fig. 3). Internally, the mantle wall is undulated (Fig. 2). The diameters of the valves range from 39 to 70 μm, and the mantle height from 23 to 39 μm.

SEM observations

Terminal valves of the chains produce mucilage stalks fixing the chains to substrata (Figs 7, 8, 11). The end of the stalks is expanded (Fig. 8). The valve face is plain and is clearly distinguished from the mantle by an edge in right angle (Fig. 11). The wall is thick and loculate (cf. LM Fig. 2; Fig. 10). The rows of pores in dichotomous and spiral arrangement on the valve face (Fig. 4) observable in LM correspond to loculi (chambers) organized in dichotomous and spiral lines. The valve face and mantle wall have about the same structure (Fig. 14) but the valve face wall is thinner than the mantle wall (cf. LM



Figs 12-19. *Melosira undulata* var. *normanii*, SEM. External views and wall structure. **Fig. 12.** Note the pores randomly distributed on the valve face. **Fig. 13.** Loculate structure of the wall on the valve face. Note the elliptical loculi's foremen. **Fig. 14.** Part of an eroded valve showing the polygonal small loculi. **Fig. 15.** Fractured valve showing loculate structure of the wall. **Fig. 16.** Enlarged the wall of valve face near the face/mantle junction (in cross section ; a part of Fig. 15), showing rectangular and quadrangular/polygonal loculi. **Fig. 17.** Schematic drawing of the wall structure. **Fig. 18.** Two mantles and a ring of rimoportulae apertures (arrows). **Fig. 19.** A detailed view of interlocking spines and irregular granules on the external loculi wall (arrows). Scale bars = 10 μm (Figs 15, 18), 5 μm (Figs 13, 14), 1 μm (Figs 12, 19), 0.5 μm (Fig. 16).

Fig. 2) and non-loculate wall is present at the valve centre (cf. LM Fig. 4). As it was illustrated in Figures 15-17, the wall is organized in two main parts : in the first there are longer and rectangular (in cross-section) loculi and in the second part is divided in two or three smaller quadrangular/polygonal (in cross-section) loculi. The loculi connect each other by elliptical foramen (Fig. 13). Studding the wall from the outside to the inside in section it is possible to describe : 1. the outer layer of the wall with small simple pores (Fig. 12) ; 2. the vertical wall of the longer loculi (Fig. 10) ; 3. two or three smaller loculi (Figs 15, 16) ; 4. the surface of the inner wall layer (Figs 23, 25). Externally, the valve face is perforated by tiny and simple pores which are distributed among flat and irregular granules (Fig. 12). On the mantle, these granules lie above the loculi walls (Figs 18, 19) and when the granules are eroded they form holes (Fig. 9 arrows) that can be confused with the aperture of rimoportulae (Fig. 9 arrowheads). The inner wall is smooth with randomly arranged small simple pores (Fig. 25). Interlocking and dichotomous branched spines similar in shape and in size are positioned around the edge of the valve face (Figs 18, 19).

Valves from Barra do Ribeiro (Guaíba River) have 12-14 rimoportulae per valve and from Lagoa Emboaba have 14-16 situated in a ring at the middle of the mantle (Figs 18, 23). Internally, the rimoportulae are simple and sessile (Figs 23-25).

The complete epi-cingulum is composed of three bands, one valvocopula and two copulae that have straight rows of fine pores (Figs 21,

22). The valvocopula is wider than the other bands (Fig. 22) and it has a fimbriate margin (Fig. 20). On some frustules, the cingula of adjacent parent cells are observed (Fig. 20).

Occurrence in the samples studied

In the Guaíba River samples *Melosira undulata* var. *normanii* was found attached to sand grains and never observed in plankton samples. In Lagoa Emboaba, however, it was always present in plankton samples throughout the year but as dead frustules.

Discussion

Krammer & Lange-Bertalot (1991) stated that the discerning characters between *Melosira undulata* var. *undulata* and *M. undulata* var. *normanii* are the mantle inner contour and the radial spiral pattern of the striae on the valve face. In var. *undulata*, the mantle contour is circular and in var. *normanii* it is polygonal. The latter feature seems to be related to the number of rimoportulae. Manguin (1949) illustrates several varieties and forms of *M. undulata* with one or two rings of rimoportulae and distinct patterns of striation on the mantle. The var. *undulata* was illustrated by Manguin (1949, pl. 1, fig. 19) showing a cross section of the valve with no indication of rimportulae on the mantle but Schmidt (1874-1959, pl. 180, figs 1-6, 16) show a ring of rimoportulae on the mantle and circular inner contour. Huang (1982) and Tanaka *et al.* (1984) recorded *M. undulata* var. *normanii*, and Schauderna (1983) and Houk (2003) recorded *M. undulata* var. *undulata*, both varieties presented a ring of rimoportulae on the mantle. If the two varieties have a ring of rimoportulae on the mantle, they will be in agreement with the ultrastructure of several *Melosira* species such as in *M. varians* C.Agardh, *M. lineata* (Dillwyn) C. Agardh, *Melosira nummuloides* (Dillwyn) C. Agardh, and *M. moniliformis* (O.F.Müll.) C. Agardh described by Crawford (1975, 1977 and 1978). The stria arrangement is main characteris-

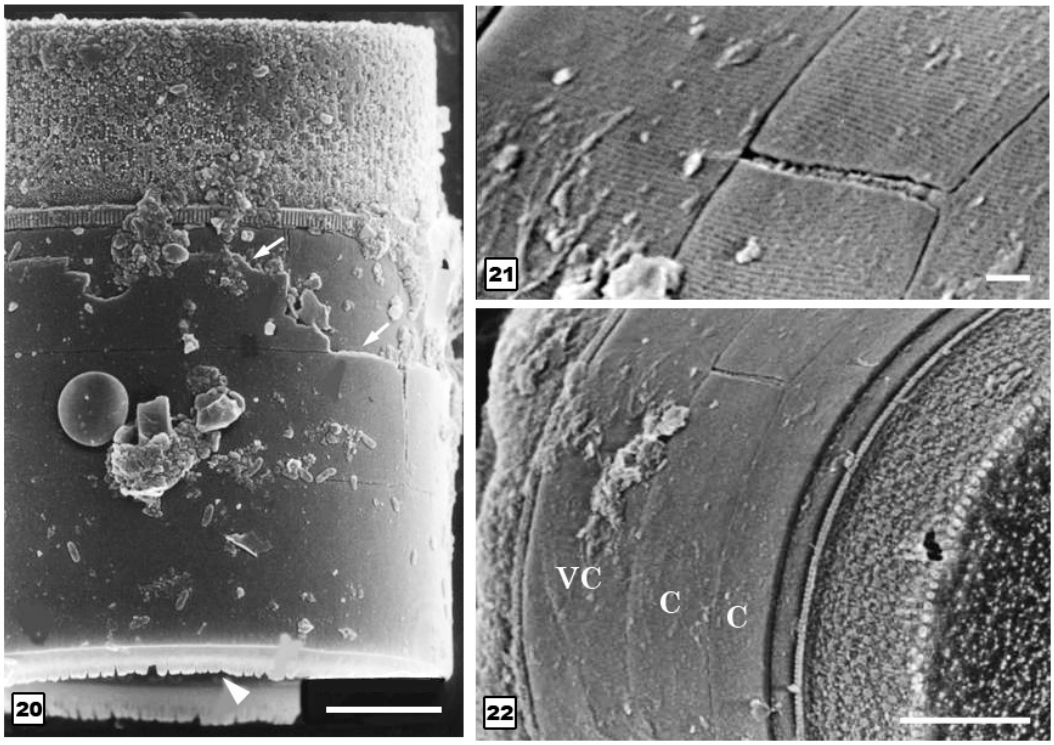
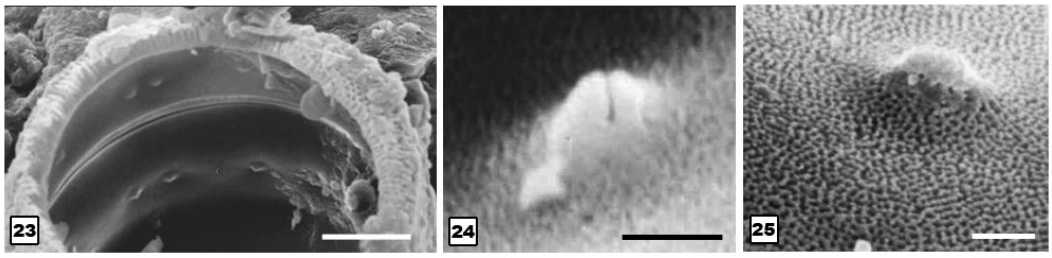


Fig. 20-22. *Melosira undulata* var. *normanii*, SEM. External views. **Fig. 20.** Parent cell girdle bands overlapping daughter cell girdle bands (arrows), note the valvocopula with fimbriae (arrowhead). **Fig. 21.** Detail of Figure 22; note rows of fine pores on girdle bands. **Fig. 22.** A whole frustule. Epicingulum is composed of a valvocopula (VC) and two copulae (C). Scale bars = 10 μm (Figs 20, 22), 1 μm (Fig. 21).



Figs 23-25. *Melosira undulata* var. *normanii*, SEM. Internal views. **Fig. 23.** Two valves without valve face showing a rimoportula arrangement. **Fig. 24.** A simple and sessile rimoportula. **Fig. 25.** Other inside view of a rimoportula and pores. Scale bars = 10 μm (Fig. 23), 1 μm (Figs 24, 25).

tic left to identify *M. undulata* and its varieties safely, the radial striae pattern, dichotomic sometimes, is found in the var. *undulata* and the spiral and dichotomous striae pattern is found in the var. *normanii*. As a circular mantle contour and radial striation pattern are not present in the specimens studied, it must be identified as *M. undulata* var. *normanii*.

Crawford (1975) has suggested that rimoportulae can be involved in secretion of mucilage and our observation on *M. undulata* var. *normanii* suggests a close relationship between rimoportulae and mucilage stalks (Figs 7, 8).

Among the species of *Melosira* C.Agardh, *Melosira undulata* var. *normanii* is similar to *M. varians*, *M. lineata*, *M. nummuloides*, and *M. moniliformis* described by Crawford (1975, 1977 and 1978). *Melosira undulata* var. *normanii* has a flat valve face covered with granules and internal mantle surface with small pores. Another feature shared with *Melosira* is the copulae ligulate with regular longitudinal rows of small pores. However, the ligula is somewhat thinner than in *M. varians*, *M. lineata* and *M. moniliformis*. The wall in *M. undulata* var. *normanii* is more complex than the wall found in *M. moniliformis* and *M. nummuloides*; the wall is composed of two types of loculi while in other *Melosira* species only one type of loculi was described. However, in *M. nummuloides* and *M. undulata* var. *normanii* have in common the centre of the valve face with non-loculate structure (see also Tanaka *et al.* 1984, pl. 16, fig. 3, where the absence of loculi at the centre of the valve face is clear).

Regarding the presence of rimoportulae and carinoportulae, Crawford (1977, 1978 and 1981) describes *Melosira* species with rimoportulae scattered or grouped on the valve face and the carinoportulae are restricted to *Orthoseira* Thwaites (Table 1). The carinoportula is circular, has coarse structures on the valve face, and its external aperture is surrounded by a raised collar. Usually, the carinoportula is bigger than the rimoportula.

Krammer & Lange-Bertalot (1991) considered the structure of *Orthoseira* similar to *Melosira undulata* var. *normanii*, but the former has carinoportulae, a unique characteristic of this genus. On the other hand, a centric marine benthic diatom *Podosira* Ehrenb. has loculate structure while *Hyalodiscus* Ehrenb. has bullulate structure (Table 1). The former genus has very small and sessile rimoportulae scattered over the whole valve, and the latter has a ring of rimportulae next to the mantle edge and other rimoportulae scattered over the valve, as well.

Melosira undulata var. *normanii* is comparable to *Ellerbeckia* R.M.Crawford (1988) only by their heavily silicified wall and plain valve face. The most important characteristics distinguishing them are the rings of unique type of tubular process and the presence of heterovalvy with sibling valves, the 'cameo' valve and 'intaglio' valve on *Ellerbeckia*, while in *M. undulata* the frustule is isovalvar with one ring of simple rimoportulae on the mantle, and there are no marks on the valve face. Tanaka *et al.* (1984) presented three SEM micrographs showing *M. undulata* var. *normanii* in valve view and no indication of heterovalvy is evidenced. In conclusion, *Melosira undulata* var. *normanii* does not belong to Paraliaceae R.M.Crawford because it does not exhibit heterovalvy as *Paralia* and *Ellerbeckia*.

Considering the number of cells in the chains, it is interesting to notice that true marine ben-

Table 1. Summarizing data relating genera mentioed in the text and their respective characteristic features.

Taxa/Features	Valves	Wall structure	Process	References
Paralia	Heterovalve	thick with chambers	rimoportula	Crawford (1979)
Ellerbeckia	Heterovalve	thick with tubes	tube process	Crawford (1988)
Podosira	Isovalve	loculate	rimoportula	Round *et al.* (1990)
Hyalodiscus	Isovalve	bullulate	rimoportula	Round *et al.* (1990)
Orthoseira	Isovalve	Simple laminate layer	carinoportula	Round *et al.* (1990)
Melosira *	Isovalve	pseudoloculate	rimoportula	Crawford (1975, 1977, 1978)
M. undulata var. *normanii*	Isovalve	loculate	rimoportula	This study

**M. lineata*, *M. varians, M. moniliformis, M. nummuloides*

thic diatoms as *Podosira*, *Hyalodiscus*, *Melosira moniliformis*, and *Melosira nummuloides* have a few cells in their chains. Takano (1967) illustrates four cells in a chain of var. *undulata* and Huang (1982) does three cells of var. *normanii*. The specimens studied here have been observed with a maximum of four cells.

This study confirms that *M. undulata* var. *normanii* should belong to the genus *Melosira*, since it lives in freshwater habitats as *M. varians*, forms short chains as observable in *M. nummuloides* and, more importantly, it has a single ring of rimoportulae on the mantle and loculate valve structure. These are characteristics that put it apart from *Ellerbeckia*, *Orthoseira*, *Podosira*, and *Hyalodiscus*.

Melosira undulata var. *normanii* has probably a wide distribution in Rio Grande do Sul since it has been found in different environments (Lagoa Emboaba and Guaíba River) but seems to be mainly related to sandy sediments. Its occurrence in the plankton of Lagoa de Tramandaí (Rosa *et al.* 1994), seems to be unlikely once it is a brackish lagoon and *M. undulata* var. *normanii* is a fresh water species.

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