



Melosira (Diatomeae) taxa from the Iguaçú River in southern Brazil

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Abstract: The study of the genus *Melosira* in plankton samples from the lower Iguaçú River revealed the presence of four taxa: *Melosira undulata* (Ehrenberg) Kützing var. *undulata*, *M. undulata* var. *normanni* Arnott, *M. varians* C. Agardh and *M. muscigena* Iwahashi. We present illustrations of the frustules using light microscopy (LM), descriptions, and comments about the morphology of the four taxa. The analysis of *Melosira muscigena* under scanning electron microscopy revealed unprecedented details of the ultrastructure, such as the shape and distribution of the rimoportulae at the valve mantle. This is the first record of *Melosira undulata* var. *undulata* and the second of *M. muscigena* in Brazil.

Keywords: diatoms, southern Brazil, taxonomy, ultrastructure.

Táxons de *Melosira* (Diatomeae) para o rio Iguaçú, sul do Brasil

Resumo: O estudo do gênero *Melosira* em amostras de plâncton do baixo rio Iguaçú revelou a presença de quatro táxons: *Melosira undulata* (Ehrenberg) Kützing var. *undulata*, *M. undulata* var. *normanni* Arnott, *M. varians* C. Agardh e *M. muscigena* Iwahashi. Apresentam-se ilustrações das frústulas usando microscopia óptica (MO), descrições e comentários sobre a morfologia dos quatro táxons. A análise de *Melosira muscigena* sob microscopia eletrônica de varredura revelou detalhes sem precedentes da ultraestrutura, como a forma e distribuição das rimopórtulas no manto da valva. Este é o primeiro registro de *Melosira undulata* var. *undulata* e o segundo de *M. muscigena* para o Brasil.

Palavras-chave: diatomáceas, sul do Brasil, taxonomia, ultraestrutura.

Introduction

Diatoms from the genus *Melosira* C. Agardh are characterized by cylindrical to subspherical frustule, usually united at the valve faces by mucilage pads and small irregular spines (Crawford 1975) in pairs or chains of three cells (Van Heurck 1896). The valve face can be flat or convex and may be bordered by a corona, with granules more or less developed, and a carina (collar-like structure) (Crawford 1975). The areolae are loculated with loculi open to the outside through small pores. The cingulum presents ligulate bands perforated by longitudinal rows of small pores (Round et al. 1990). Rimoportulae usually occur near the edge, scattered or grouped at the valve mantle (Round et al. 1990).

Previously, the genus *Melosira* included most of the centric diatoms that grew in chains with cells strongly connected by the valve face (Stoermer & Julius 2003), accommodating a wide range of taxa gradually transferred to different genera, such as: *Aulacoseira* Thwaites, *Paralia* Heiberg, *Orthoseira* Thwaites, and *Ellerbeckia* Crawford (Round et al. 1990, Houk & Klee 2007).

Melosira taxa occur in marine, brackish and freshwater benthic ecosystems (Round et al. 1990). Currently, there are at least five morphologically and ecologically heterogeneous groups within the genus: (1) **marine and brackish taxa**, this group includes the generic type, e.g., *M. nummuloides* C. Agardh and *M. arctica* Dickie, species

with a very convex valve face with prominent carina and bands with fine poroids; (2) **brackish**, e.g. *M. moniliformis* (O.F. Müller) C. Agardh, with convex valve face and bands distinctively perforated by fine poroids, in partly radiating, mostly irregular short lines; (3) **freshwater to slightly brackish**, e.g., *M. varians* C. Agardh and *M. lineata* (Dillwyn) C. Agardh, with dome-shaped valve face has smaller and much more delicate valves with inconspicuous ornamentation, difficult to see in LM.; (4) **recent fossil and freshwater**, e.g. *M. undulata* (Ehrenberg) Kützing, *M. muscigena* Iwahashi (= *M. ruttneri* Hustedt), *M. anastomosans* Grunow, with distinct areolate valve face; (5) **freshwater, epilithic, and epiphytic**, e.g., *M. dickiei* (Thwaites) Kützing, with a cingulum composed of many narrow bands, forming internal valves (Crawford 1988, Houk & Klee 2007).

Fifty-eight *Melosira* species are known (Novelo 2012), but the number of freshwater species are limited (Stoermer & Julius 2003). In Brazil, 10 taxa have been recorded: marine taxa - *M. borrieri* Greville, *M. nummuloides*, *M. moniliformis*, *M. octogona* Schmidt, *M. setosa* Greville and freshwater taxa- *M. dickiei*, *M. lineata*, *M. muscigena* Iwahashi (= *M. ruttneri* Hustedt), *M. undulata* var. *normanni* Arnott and *M. varians* (Rosa et al. 1994, Garcia 2009, Tremarin et al. 2009, Nardelli et al. 2014). Of these, six occurred in Paraná state: *M. dickiei*, *M. lineata*, *M. nummuloides*, *M. varians*, *M. undulata* var. *normanni*, and *M. muscigena* (Tremarin et al. 2009, Nardelli et al. 2014).

Nardelli et al. (2014) have already recorded four species of *Melosira* in Iguaçú River—*M. varians*, *M. muscigena*, *M. undulata* var. *normanni* and *Melosira* sp.

Here, we presented a taxonomic survey of *Melosira* taxa for the Iguaçú River, showing unprecedented ultrastructural details of *Melosira muscigena* and the first citation of *M. undulata* var. *undulata* to Brazil.

Material and Methods

The Iguaçú River runs westward 1275 km from its source in the Serra do Mar to its confluence with the Paraná River (Paraná 2010). Considered a large river, part of the river is located in Iguaçú National Park, a protected area important for its great biodiversity (Paraná 2010).

Station 1 (S1) is located upstream of the falls (25°39'12,8" S and 54°25'34,0" W), where the river is wider, with variable depth from 1.4 m to 3.0 m. The station was classified as oligo-mesotrophic for the sampling period, with maximum and minimum values according the Table 1 (Nardelli et al. 2016).

Station 2 (S2) is downstream from the falls (25°38'55,4" S and 54°27'31,0" W). In this region, the river is narrower, deeper (11.7 to 27.4 m), and flows faster than at Station 1. Station 2 was classified as ultra-oligotrophic to oligotrophic for the sampling period, with maximum and minimum chemical and physical values according the Table 1 (Nardelli et al. 2016).

Table 1. Maximum and minimum chemical and physical values in Station 1 and Station 2. Water temperature (T °C); pH; Dissolved oxygen (DO mg.L⁻¹); Biochemical Oxygen Demand (BOD mg.L⁻¹); Chemical Oxygen Demand (COD mg.L⁻¹); Electrical Conductivity (Cond µS.cm⁻¹); Secchi Transparency (SD m); Turbidity (NTU); Ammonium (NH₄ µg.L⁻¹); Total Nitrogen (TN µg.L⁻¹); Nitrate (NO₃ µg.L⁻¹); Total phosphorus (TP µg.L⁻¹) (Nardelli et al. 2016).

Stations Parameters	Station 1		Station 2	
	Min	Max	Min	Max
Water T °C	15.5	28.6	15.9	28.8
pH	6.1	8.7	5.9	8.3
DO mg.L ⁻¹	6.4	12.2	6.6	13.1
BOD mg.L ⁻¹	0.9	11.4	1.8	13.9
COD mg.L ⁻¹	6.8	21.6	4.7	25.9
Cond µ S.cm ⁻¹	32.8	105.0	34.0	87.0
SD m	0.9	3.6	0.7	3.6
NTU	2.0	26.1	2.8	23.2
NH ₄ ⁺ µg.L ⁻¹	0.8	123.2	0.4	70.4
TN µg.L ⁻¹	100.0	500.0	110.0	500.0
NO ₃ ⁻ µg.L ⁻¹	100.0	2140.0	100.0	1730.0
TP µg.L ⁻¹	20.0	450.0	50.0	390.0

Table 2. Herbarium data from plankton sampled in Station 1 (S1) and Station 2 (S2).

Collection date	S1	S2	Collector(s)
	UNOP-Algae	UNOP-Algae	
September 2010	3161	3156	M.S. Nardelli n.11, 12, N.C. Bueno s.n.
October 2010	3225	3213	M.S. Nardelli n.14, 18, N.C. Bueno s.n.
December 2010	3342	3338	M.S. Nardelli n. 21, 25, N.C. Bueno s.n.
January 2011	3412	3408	M.S. Nardelli n. 31, 35, N.C. Bueno s.n.
February 2011	3471	3467	M.S. Nardelli n. 38, 42, N.C. Bueno s.n.
March 2011	3536	3530	M.S. Nardelli n. 45, 51, N.C. Bueno s.n.
April 2011	3606	3601	M.S. Nardelli n. 55, 60, N.C. Bueno s.n.
May 2011	3680	3670	M.S. Nardelli n. 68, 74, N.C. Bueno s.n.
June 2011	3714	3709	M.S. Nardelli n. 80, 85, N.C. Bueno s.n.
July 2011	3758	3753	M.S. Nardelli n. 92, 97, N.C. Bueno s.n.
August 2011	3775	3770	M.S. Nardelli n.101, 106, N.C. Bueno s.n.
September 2011	3784	3779	M.S. Nardelli n.110, 115, N.C. Bueno s.n.

Monthly collections were performed between September 2010 and September 2011, from the subsurface water of the river with a phytoplankton net. Samples were cleaned according to the Simonsen (1974) method modified by Moreira-Filho & Valente-Moreira (1981). Permanent slides were mounted with Naphrax® (RI = 1.73) and analyzed with an Olympus® BX60 microscope. Images were obtained with an Olympus DP71 digital camera. The slides are deposited in the herbarium of the *Universidade Estadual do Oeste do Paraná* (UNOP-Algae, Western Paraná State University), Cascavel campus (Table 2).

Part of the oxidized material was placed on aluminum stubs and sputter-coated with gold in a Balzers SCD 030 at 1 kV for 5 min. Scanning electron microscopy (SEM) analysis was performed in a JEOL JSM 6360LV microscope operating at 15 kV and 8 mm working distance. The terminology used in the species descriptions follows Round et al. (1990) and Houk & Klee (2007).

Results and Discussion

Coscinodiscophyceae Round & Crawford in Round et al. 1990

Melosirales Crawford 1990 in Round et al. 1990

Melosiraceae Kützing 1844

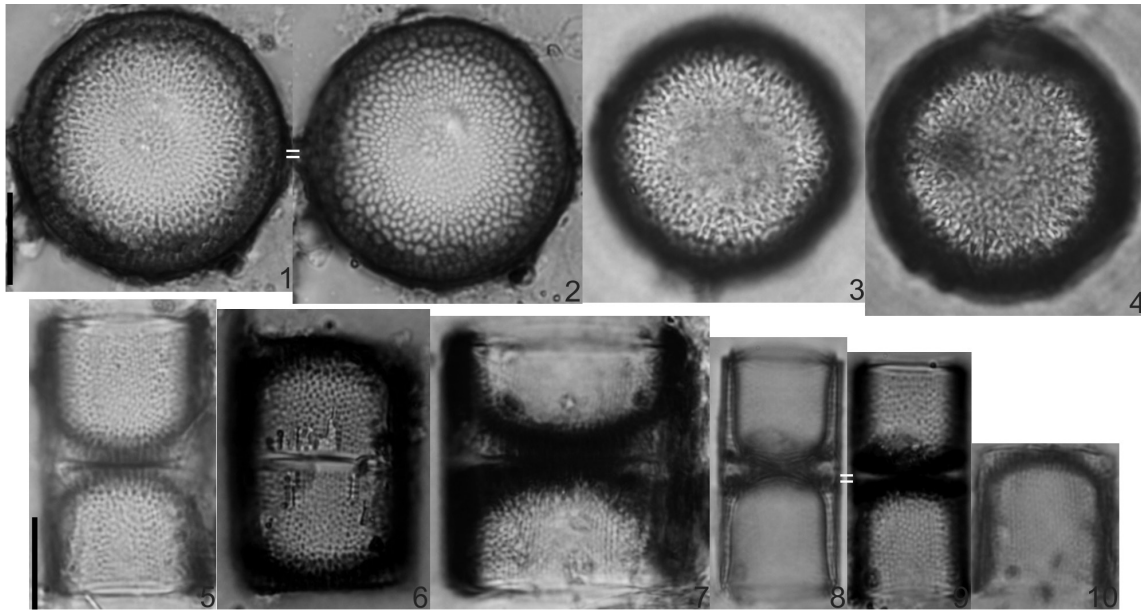
Melosira C. Agardh 1824

Melosira muscigena Iwahashi. Journal of Japanese Botany, 12(2): 121-127, p.121, fig.17. 1936.

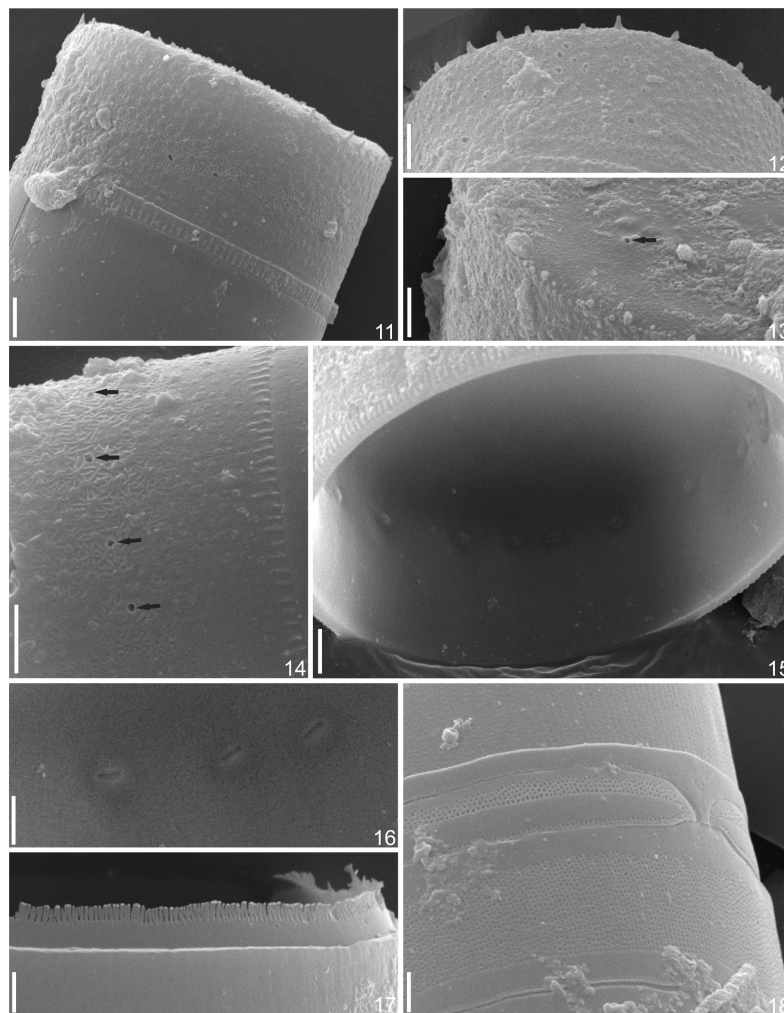
Synonym: *Melosira rutneri* Hustedt. Archiv für Hydrobiologie 15: 140-141, pl. 9, fig. 11-16. 1937.

LM observations. Cylindrical frustules, with thick wall of uneven structure forming two layers, the external view cylindrical and the internal view more or less elliptical. Valve mantle formed with a reticulated pattern in polygonal chambers, decreasing toward collar. Circular valve view, reticulated striation pattern, composed of polygonal areolae decreasing in diameter toward the center, changing to radial rows of small pattern, scored lengthwise, hyaline central area. Cells solitary or joined by the valve face, forming pairs or short chains of three cells. Mantle height: 14.3–15.5 µm; diameter: 15.7–27.9 µm; 19–21 striae in 10 µm (Figures 1-10).

SEM observations. Flat valve face with short marginal spines. Rimoportulae scattered on the valve face and disposed in a ring around the mantle, not equidistant from each other. Rimoportula with external openings rounded or elongated, 0.5 µm in diameter, and internally sessile. Collar with short perivalvar wrinkles at the valve margin, and often oblique. Connective bands open, ornamented by delicate striae, 9-10 per 1 µm, composed by rounded pores, 10-11 per 1 µm. Prominent ligule and rounded antiligule. Fimbriate valvocopula and pleura thinner than the other bands (Figures 11-18).

Melosira taxa from the Iguaçu River

Figures 1–10. *Melosira muscigena*, LM. Figures 1–4. Valve view. Figures 5–10. Girdle view. Scales: 10 μm .



Figures 11–18. *Melosira muscigena*, SEM. Figure 11. Girdle view. Figure 12. Marginal spines located in the valve face/mantle junction. Figure 13. External rimoportula opening in the valve face (arrow). Figure 14. External rimoportula openings in the mantle (arrows). Figure 15. Internal rimoportula openings in the mantle. Figure 16. Detail of internal rimoportula openings. Figure 17. Fimbriate valvocopula. Figure 18. Detail of ligulate pleura and cingulum bands perforated by porous. Scales: 2 μm (Figs 11–15), 1 μm (Figs 16–18).

Comments. This is the first ultrastructure documentation of a Brazilian population of *Melosira muscigena*. Analysis via SEM revealed unprecedented details of the ultrastructure, such as the shape and distribution of the rimoportulae at the valve mantle. The species was recorded by Koide (1987), as *M. ruttneri*, by Houk & Klee (2007) and Nardelli et al. (2014). Koide (1987) compared individuals identified as *M. ruttneri* from Japan (pl. 1, figures 1–7; diameter: 7–33 µm; mantle height: 5–25 µm) with the type material illustrations present by Iwahashi (1936) of *M. muscigena* (diameter: 16–33 µm; mantle height: 12–20 µm) and verified that it was the same taxon. The only difference found in the Hustedt (1937) and Iwahashi (1936) descriptions was regarding the presence or absence of thin spines on the rim of the valve face. The first describes the spines as not being well distinguished, and the latter makes no mention of them. As the *M. muscigena* name is older, it has priority over *M. ruttneri*.

We suggested the investigation of *Melosira muscigena* and *Melosira ruttneri* type materials since they were originally described based on optically limited characteristics. Also the original illustrations are uninformative. The

two taxa are ambiguous, showing many similarities in their morphology. The frustule ultrastructure would properly ascertain other morphologically diagnostic features between the species.

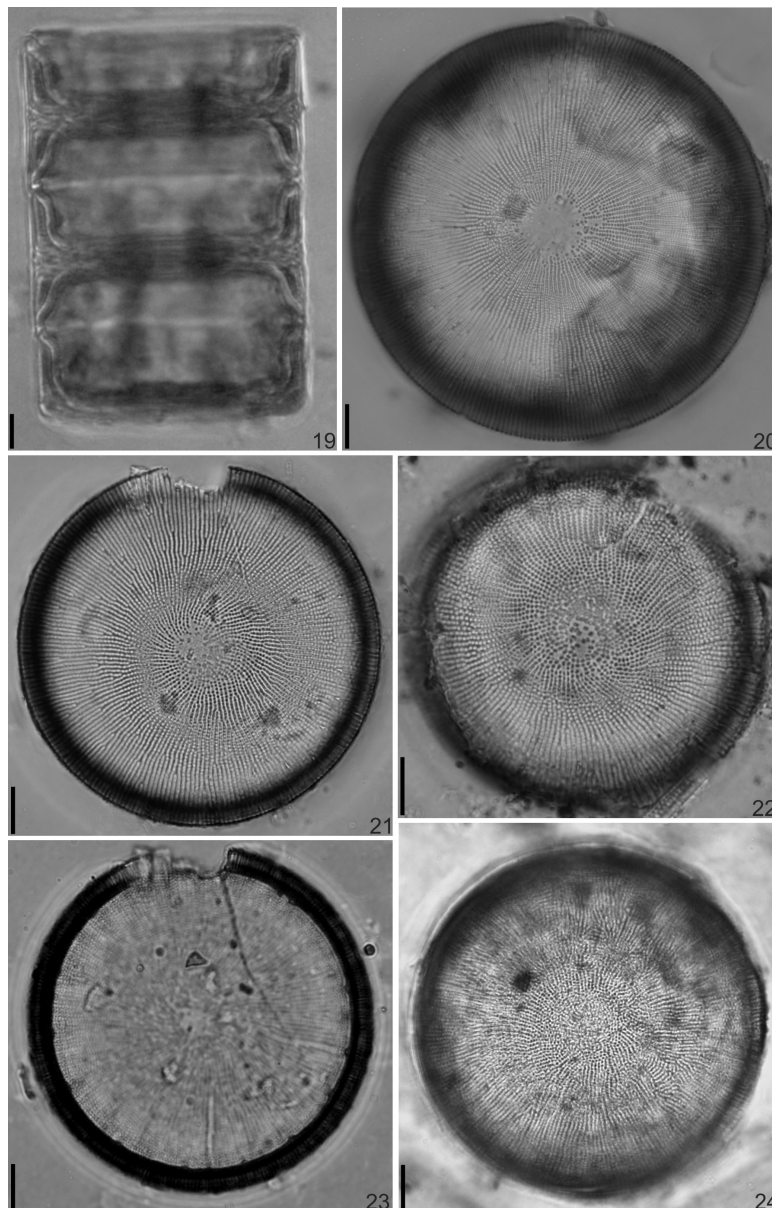
Melosira muscigena is morphologically similar to *M. anastomosans* Grunow in the reticulate pattern of the valve face but differs in the mantle outline, which is rounded and more similar to *M. undulata* (Houk & Klee 2007).

Houk & Klee (2007) describe *M. ruttneri* as rare in waterfalls, springs, and streams. It is frequently found in moss, and also a good indicator of alkaline waters (Koide 1987). However, in the present study, the species was found in the Iguaçú River, a place of flow and large water falls.

Occurrence in samples: (UNOP-Algae, Herbarium accession numbers) 3213, 3606, 3709, 3753, 3758, 3770, 3784.

Melosira undulata (Ehrenberg) Kützing var. *undulata*. Bacillarien, p.54, 1844.

LM observations. Cylindrical frustules (Figure 19), usually solitary or united in pairs or short chains, valves wider than higher, internally



Figures 19–20. *Melosira undulata* var. *undulata*, LM. Figure 19. Frustules in girdle view showing undulate inner mantle surface. Figure 20. Valve view. **Figures 21–24.** Valve view of *Melosira undulata* var. *normanii*, LM. Figure 23. Note the position of rimoportulae in the internal valve margin. Scales: 10 µm.

constricted at the mantle midregion. Internal margin the of valve face with polygonal circumference and areolae disposed in radial rows which do not reach the valve center, forming a small hyaline central area (Figure 20). Mantle height: 21.5–32.0 μm ; diameter: 93.5–94.0 μm ; 10–11 striae in 10 μm on the valve face; 17–18 areolae in 10 μm on the valve face; 10 internal projections on the wall.

Comments. Krammer & Lange-Bertalot (1991) describe *M. undulata* var. *undulata* as presenting a rounded valve, with internal margin the of valve face with polygonal circumference different from the internal rounded shape of *M. undulata* var. *normanii* Arnott. Ferguson Wood et al. (1959) described individuals with polygonal morphology with six to twelve internal projections, however they did not illustrate specimens with these characteristics. Such internal projections are consistent with the sessile rimoportulae present in the valve mantle. Rimoportulae may also be found in the central region of the valve mantle (Bahls 2012).

The population here studied exhibit greater diameter than the one recorded by Houk (2003), 16–80 μm , and Bahls (2012), 30–81 μm . However, the other features of the taxon are coincident.

Melosira undulata var. *undulata* is an epilithic species occurring in circumneutral, oligohalobous (Foged 1976), and oligotrophic (Carter et al. 2006) environments. The species is frequently found in small arctic lakes and lagoons and with freshwater diatoms (Hustedt 1937, Mahood et al. 1984, McIntire et al. 1994, Stoermer & Julius 2003). It has also been detected in sediments, as well as in the plankton of tropical areas (Manguin 1949, Sovereign 1958, Ferguson Wood et al. 1959, Germain 1981, Huang, 1982, Krammer & Lange-Bertalot 1991, Li et al. 2010). In addition to those, Takano (1967) found *M. undulata* var. *undulata* in brackish water in Abashiri, Japan. This is the first record of *M. undulata* var. *undulata* in Brazilian rivers.

Occurrence in samples: (UNOP-Algae, Herbarium accession numbers) 3156.

Melosira undulata var. *normanii* Arnott. In Van Heurck, Synopsis, p. 90, f. 7. 1882.

LM observations. Cylindrical frustules, united in pairs or short chains. Rounded valve face with rounded internal wall, ornamented with areolate striae in radial rows, sometimes ramified and with a spiral arrangement in the central region. Diameter: 42.3–89.4 μm ; 10–11 striae in 10 μm on the valve face; 12–14 areolae in 10 μm on the valve face (Figures 21–24).

Comments. *Melosira undulata* var. *normanii* differs from the typical variety by the spiral arrangement of central striae on the valve face as also the different internal margin the valve face described above (Krammer & Lange-Bertalot 1991, Brassac et al. 1999). Our study also found a smaller number of areolae (12–14 in 10 μm) in the var. *normanii* compared to the typical variety (17–18 in 10 μm).

Krammer & Lange-Bertalot (1991) comment that the species is taxonomically closer to the genus *Orthoseira* Thwaites due the structure of valve surface, requiring a more detailed taxonomic study. However, Garcia (2009) did a taxonomic comparison of *Melosira undulata* var. *normanii* with the genera *Orthoseira*, *Paralia* Heiberg, *Ellerbeckia* Crawford, *Podosira* Ehrenberg, and *Hyalodiscus* Ehrenberg and concluded that the species features are in accordance with the genus *Melosira*.

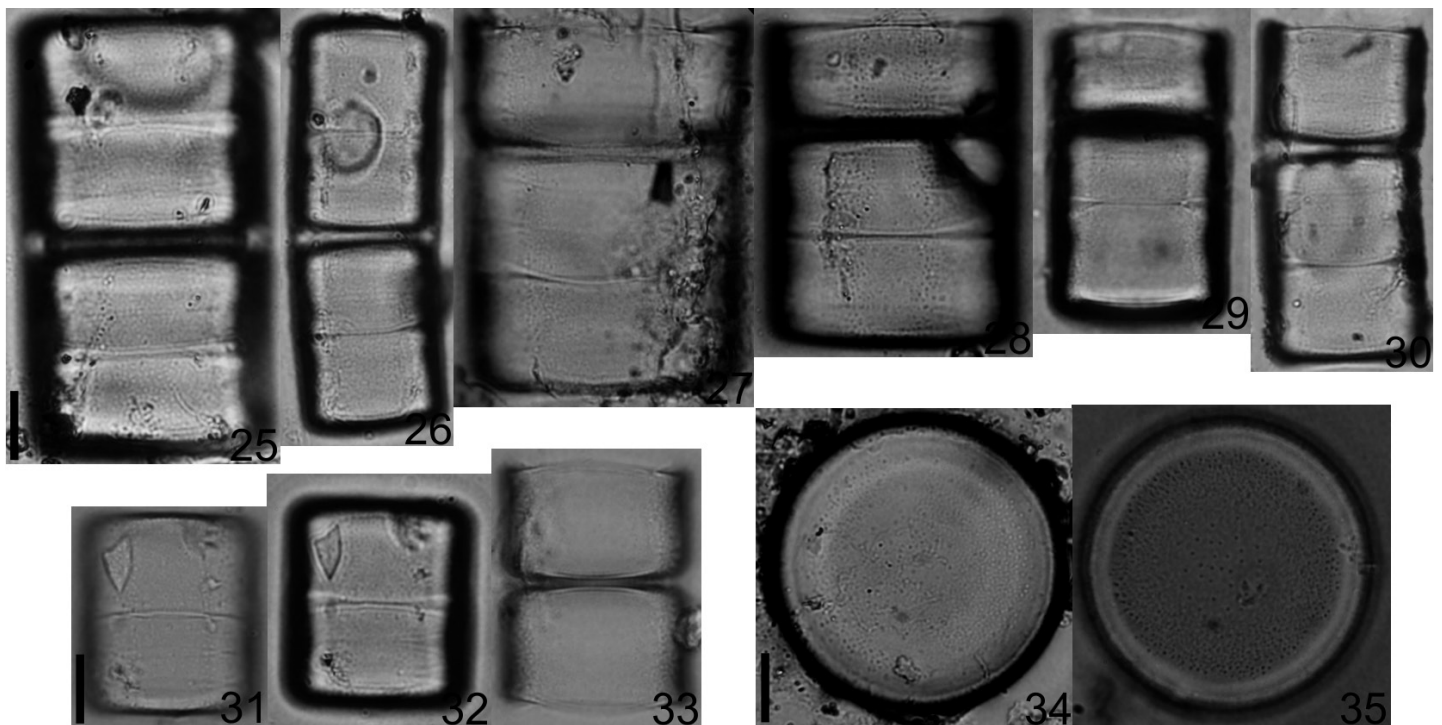
Records of *Melosira undulata* var. *normanii* from Brazil: in Rio Grande do Sul State, plankton from a brackish lagoon (Rosa et al. 1994), sand from Guaíba river, and plankton from Emboaba lagoon (Garcia 2009); in Paraná State, plankton from Iguaçu river (Brassac et al. 1999, Nardelli et al. 2014); and in Pernambuco State, estuarine plankton from Barra da Jangada (Branco 2007).

Occurrence in samples: (UNOP-Algae, Herbarium accession numbers) 3156, 3408, 3471, 3601, 3770.

Melosira varians C. Agardh. Flora oder Botanische Zeitung, 10:628. 1827.

LM observations. Cylindrical frustules forming straight chains connected to the valve face through small marginal spines (Figures 25–33). Circular valve face ornamented by delicate and inconspicuous areolae (Figures 34 and 35). Mantle height: 24.6–40.0 μm ; diameter: 18.9–50.7 μm .

Comments. *Melosira varians* occurs mainly in alkaline conditions (pH 7–8.5), in oligotrophic to heterotrophic environments, with moderate



Figures 25–35. *Melosira varians*, LM. Figures 25–33. Girdle view. Figures 34–35. Valve view. Scales: 10 μm .

oxygen, and requires periodically high levels of nitrogen. Occurs mainly in water bodies, but it is also regularly found in humid environments (Soltanpour-Gargari et al. 2011).

Melosira varians is a very common species in freshwater, occurring in considerable abundance in streams and lakes, naturally eutrophic to polluted, throughout North America (Stoermer & Julius 2003). It is also very common in Brazilian waters, with more than 30 records from the Paraná (Tremarin et al. 2009) as opposed to *M. undulata* and *M. muscigena* Iwahashi, which have few taxonomic records around the world.

Occurrence in samples: (UNOP-Algae, Herbarium accession numbers) 3156, 3161, 3213, 3225, 3338, 3342, 3408, 3412, 3467, 3471, 3530, 3536, 3601, 3606, 3670, 3680, 3709, 3714, 3753, 3758, 3770, 3775, 3779, 3784.

Conclusion

This is an important study about the knowledge of *Melosira* diversity in Brazil and taxonomy of the genus as a whole. *Melosira undulata* var. *normanii* and *M. muscigena* are taxa with restricted distribution, which have few taxonomic records around the world. We did not find enough individuals in the samples to analyze in electron microscopy the taxon *M. undulata* var. *normanni* as also the typical variety, for highlighting the differences between both. There is a need for new studies under scanning electron microscopy, with a larger number of individuals, in order to discuss details of the ultrastructure.

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