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Haslea sigma (Naviculaceae, Bacillariophyta) a new sigmoid benthic species from salt marshes of Southern Brazil

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Abstract

A new sigmoid diatom species *Haslea sigma sp. nov.* was found alive in sediment composed of clay and silt in salt marshes in Southern Brazil. The species is morphologically distinctive by the following combination of characters: 1) sigmoid valve, 2) thickened virgae forming a pseudostauros, 3) central raphe fissures almost straight and 4) terminal raphe fissures slightly curved. It was analyzed in light and electron microscopy and compared with the sigmoid *Haslea nipkowii* and with other spindle-shaped *Haslea* taxa possessing a pseudostauros.

Key words: diatoms, benthic species, brackish water, *Haslea sigma sp. nov.*, taxonomy

Introduction

The genus *Haslea* was described by Simonsen (1974: 46) and interpreted as a link between the section Fusiformes Cleve (1894) of *Navicula* Bory (1822: 128), similar in outline and structure, and some species of *Gyrosigma* Hassall (1845: 435) and *Pleurosigma* W. Smith (1852: 2), which are similar in central nodule morphology. In the protologue the genus was characterized as having "outline spindle-shaped, with acute ends and convex sides rarely parallel in the middle", *Haslea ostrearia* (M. B. Gaillon) Simonsen (1974: 47) was designated as generitype, and eleven species of *Navicula* were transferred to the newly-erected genus and two new species were described.

Subsequently, Poulin *et al.* (2004) transferred *Gyrosigma nipkowii* Meister (1932: 43) to *Haslea* under the name *Haslea nipkowii* (Meister) Poulin & Massé (*in* Poulin *et al.* 2004: 184) based on ultrastructural features and molecular analysis. With this transfer, the genus began to include species with a sigmoid outline.

On the basis of criteria given by Simonsen (1974), Round *et al*. (1990), Massé *et al.* (2001), Poulin *et al.* (2004), and Cox & Williams (2006) *Haslea* can be characterized by presenting: two chloroplasts per cell with more than one pyrenoid per chloroplast (axially located); areolae square to rectangular occluded by hymenes internally and externally overlain by longitudinal strip usually continuous from pole to pole and an accessory rib along the raphe-sternum. Only some species of the genus have a pseudostauros (thickening of the central virgae).

So far, twenty-six taxa of *Haslea* are known, most of which are marine planktonic, with fusiform cells (Guiry & Guiry 2014, Simonsen 1974). *Haslea ostrearia* and *Haslea karadagensis* Davidovich, Gastineau & Mouget *in* Gastineau *et al.* (2012: 472) produces marennine (a water soluble blue pigment), while the other known species do not have this ability.

In the South Atlantic, four species of *Haslea* are known: *Haslea crucigera* (W. Smith) Simonsen (1974: 47), *Haslea wawrikae* (Hustedt) Simonsen (1974: 48) and *Haslea* cf. *trompii* (Cleve) Simonsen (1974: 47) were cited from the Brazilian coast (Torgan *et al.* 1999, Procopiak *et al.* 2006, Tremarin *et al.* 2009, Villac & Tenenbaum 2010, Eskinazi-Leça *et al.* 2013) and *Haslea spicula* (Hickie) Lange-Bertalot (1997: 75) was cited from the Uruguayan coast (Metzeltin *et al.* 2005).

This paper describes a new sigmoid and benthic diatom belonging to *Haslea* from salt marshes of South America and compare it with related taxa.

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Materials and methods

Samples were collected from surficial sediments in May 2013 from the salt marsh of Pólvora Island (32º01'14.424''S–52º05'59.095''W), located in the Patos Lagoon estuary (31º57'S–52º06'W) in Southern Brazil (Fig. 1).

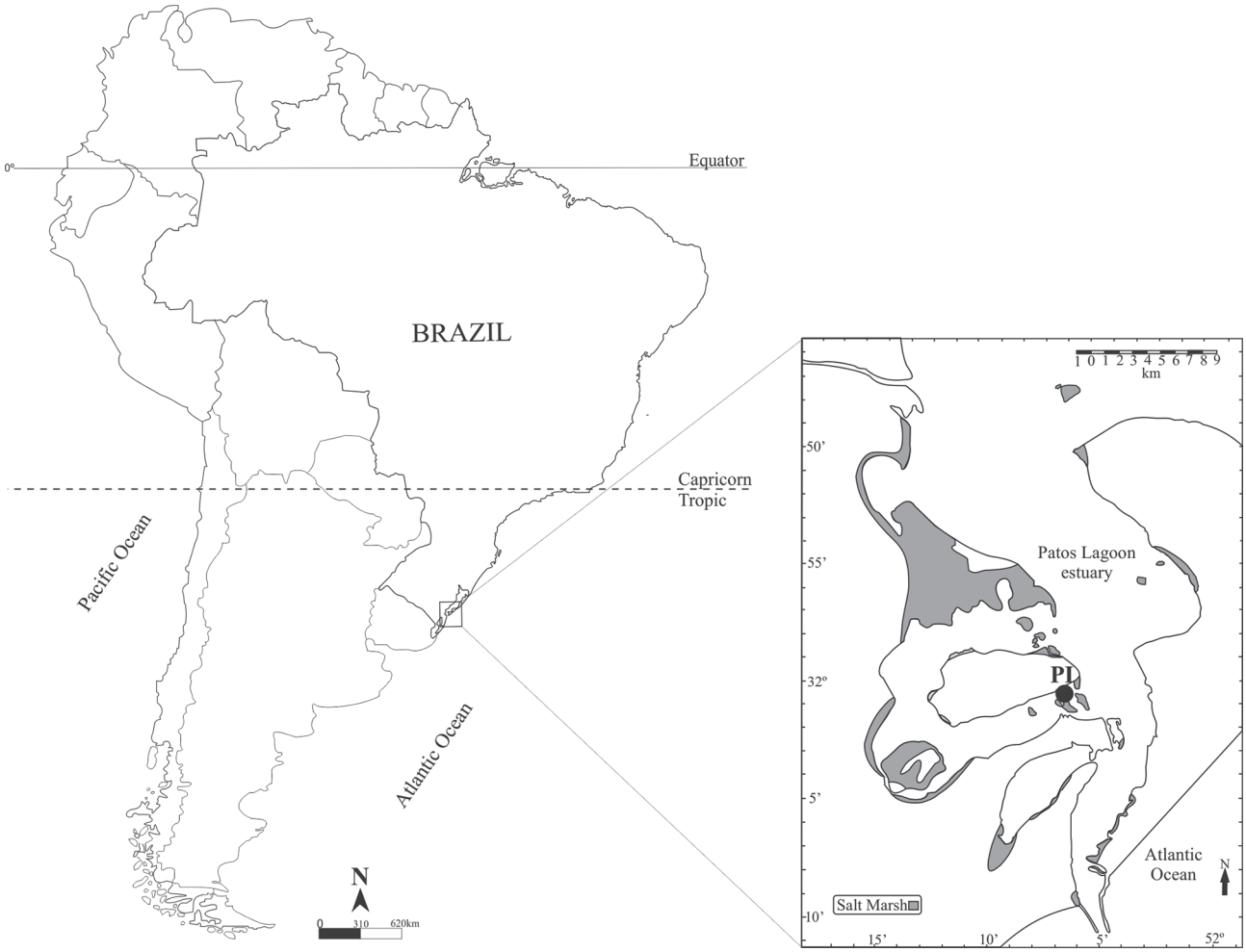


FIGURE 1. Location of Pólvora Island (PI) in salt marshes at Patos Lagoon estuary, Southern Brazil. Modified from Costa (1998).

The surficial sediment was collected by a core (10 cm diameter; 2 cm depth) and at the same time pH, salinity and temperature of interstitial water were measured using a pH meter (PHTEK®), a salinometer (YSI® 30) and a thermometer (Incoterm®). In the laboratory, live motile diatoms were isolated from sediment using the "Trapping method" (Eaton & Moss 1966, adapted by Laudares-Silva & Cimardi 1989).

The diatoms trapped in the Whatman® 105 paper (2x2 cm) were processed using nitric acid (1:1), heated for 15 min over fire using a Bunsen burner, rinsed to neutral pH with distilled water, and mounted in Naphrax. To perform the analysis of samples with scanning electron microscopy (SEM) oxidized material was dried on coverslips that were glued to aluminum stubs with adhesive double-sided tape and metalized with platinum (16 nm) using a BAL-TEC SCD 005 coating equipment. The material was examined with light microscopy (LM) using a Leica DM 2500 equipped with differential interference contrast (DIC) and a Leica DFC 420 C camera and a Zeiss Axioplan with an Axiocam ERc 5s camera. Observations with SEM were made using a JEOL JSM-5200 (20 mm working distance, 15 kV) and a JEOL JSM-6060 (10 mm working distance, 20 kV). Permanent slides are deposited at the Prof. Dr. Alarich Rudolf Holger Schultz Herbarium (HAS) under the numbers 6698 and 6699, Museu de Ciências Naturais-Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre, Brazil and at the Herbarium of the División Ficologia "Dr. Sebastián A. Guarrera" under the number 13001, Facultad de Ciencias Naturales y Museo, Argentina.

The abundance of taxa was obtained through the counting of approximately 200 valves in permanent slides seeking to reach 80% of the sampling efficiency, according to Pappas & Stoermer (1996).

The granulometric analyses of sediment were performed in the Centro de Estudo e Geologia Costeira of Universidade Federal do Rio Grande do Sul (CECO-IG-UFRGS) and the classification followed the Shepard (1954) model.

Morphometric measurements were made on 26 valves. Morphological terminology follows Anonymous (1975), Ross *et al.* (1979), Barber & Haworth (1981) and Round *et al.* (1990). All LM and SEM images were assembled using Corel Designer X6®.

Taxonomic treatment

Division Bacillariophyta

Class Bacillariophyceae Haeckel 1878 emend. D.G. Mann *in* Round *et al.* 1990

Order Naviculales Bessey 1907 emend. D.G. Mann *in* Round *et al.* 1990

Family Naviculaceae Kützing 1844

Genus *Haslea* Simonsen 1974

Haslea sigma Talgatti, Sar & Torgan *sp. nov.* (Figs. 2–35)

Description in LM:—The cells have two apically elongated chloroplasts (in valvar view) with pyrenoids axially located (Figs. 2–8). The frustule is delicate, the striation is inconspicuous and it is possible to visualize only the raphe sternum, the pseudostauros and the valve sides (Figs. 9–23). The valves are sigmoid, lanceolate, with almost parallel sides in the middle (Figs. 2–23). The ends are curved towards opposite sides with cuneate apices. Raphe and raphe sternum are sigmoid.

Description in SEM:—*External view*: The central raphe fissures are almost straight, slightly bent towards primary side of the valve and drop-shaped (Figs. 32, 33). The terminal raphe fissures are slightly curved to opposite sides towards the concave side of the valve and expanded slightly (Figs. 26-28). Valve surface has straight, parallel and longitudinal strips of silica (Figs. 24, 27, 32, 33), which cover the striae and are separated by narrow slits (arrows in Fig. 27). Two of these slits go to the end of valve and come together at the far tip of the pole (Fig. 26). The cingulum appears to be composed by two open, plain bands: valvocopula and copula (Figs. 25, 28).

Internal view: The central raphe endings are coaxial, simple, straight and not expanded (Figs. 29, 31), whereas each polar raphe ending is expanded and terminates in a raised and straight helictoglossa (Figs. 34, 35). On the primary side there is an accessory rib that accompanies the raphe close to the helictoglossa, which is raised and overlaps the raphe sternum (black arrow in Fig. 30, black arrow-head in Fig. 34). In the center, this rib merges with one thickened virga that almost reaches to the valve margin (Figs. 25, 29–31). On the secondary side there is also an accessory rib, however it is shorter than that of the primary side, it is raised, but does not overlap the raphe sternum, and in the center this rib merges with one or two thickened virgae (Figs. 29–31). These thickened virgae form a pseudostauros that is visible in LM (Figs. 9–23). Striae uniseriate, parallel, formed by quadrate areolae occluded by hymenes (Figs. 31, white arrow in 34) and crossed at a right angle by a longitudinal pattern.

Valve dimensions (n = 26): 55.8–70 µm long, 5.8–7.2 µm wide, 30–40 transapical striae in 10 µm, and 35–40 longitudinal striae in 10 µm.

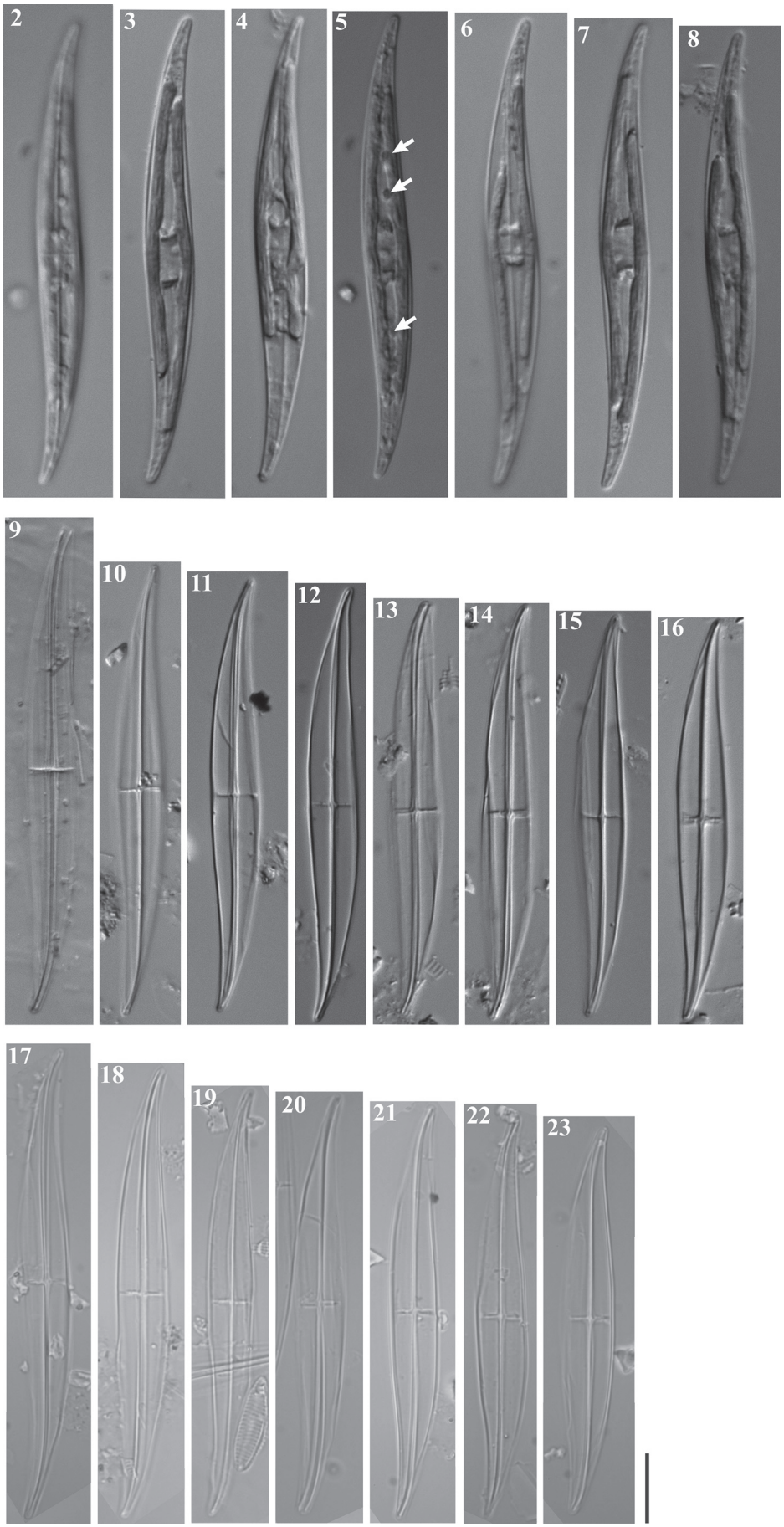
Holotype:—BRAZIL. Rio Grande do Sul State, Rio Grande city, Pólvora Island (32º01'14.424''S–52º05'59.095''W), benthic sample, collected by D. Talgatti and L. Bertolli, May 17, 2013. Circled specimen on slide *HAS 6699 (Museu de Ciências Naturais-Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre, Brazil)*, here designated. Figure 12 corresponds to the holotype specimen.

Isotype:—BRAZIL. Rio Grande do Sul State, Rio Grande city, Pólvora Island (32º01'14.424''S–52º05'59.095''W), benthic sample, collected by D. Talgatti and L. Bertolli, May 17, 2013. Circled specimen on slide *LPC 13001 (Herbarium of the División Ficologia "Dr. Sebastián A. Guarrera", Faculdad de Ciencias Naturales y Museo, Argentina)*, here designated.

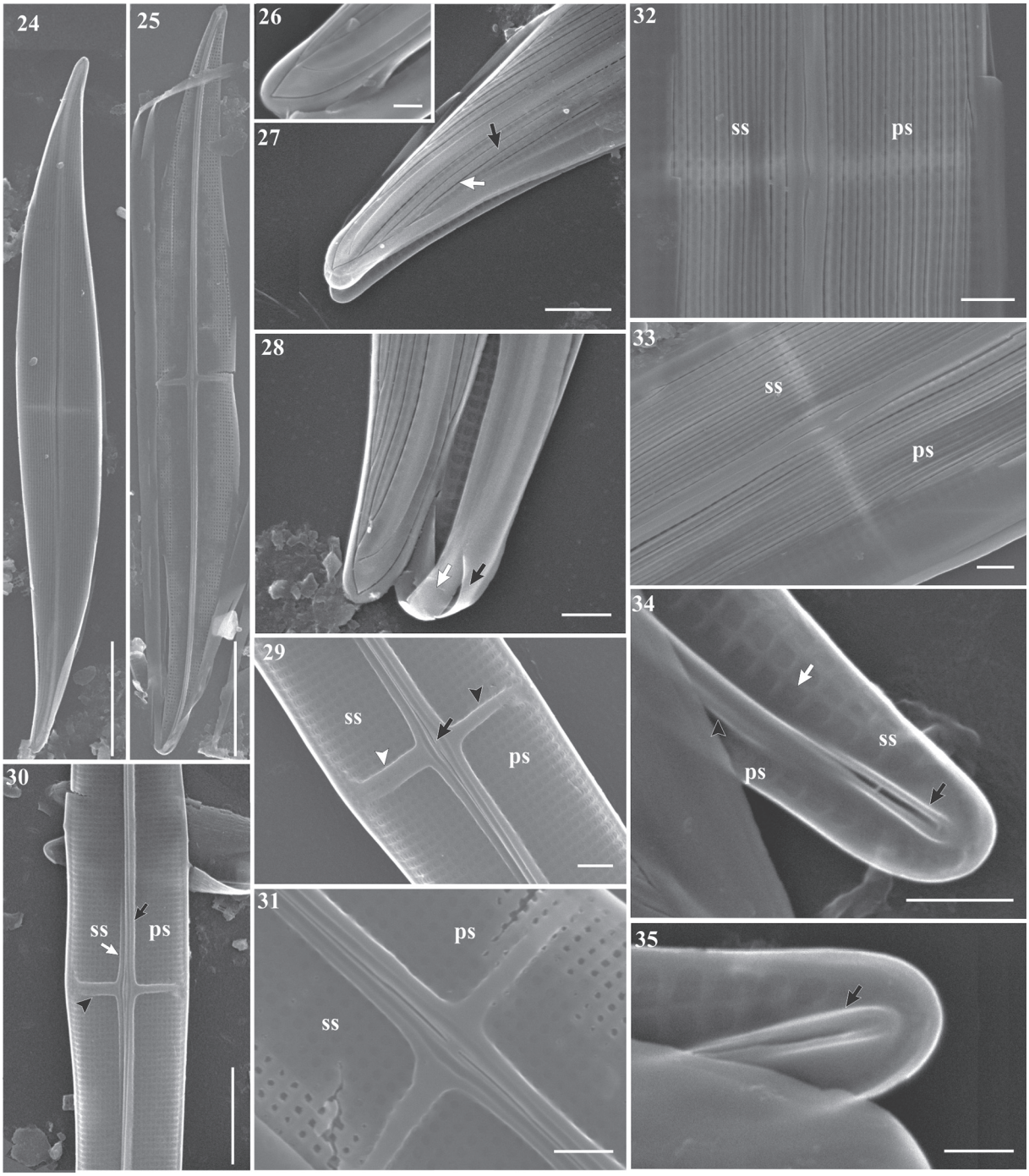
Etymology:—The specific epithet refers to the valve shape that resembles an "S" (σίγμα in Greek).

Ecology:—*Haslea sigma* was found as the second most abundant species (14.79%) and the only unique representative of the genus on the sediment surface on Pólvora Island. Other abundant taxa were *Nitzschia rautenbachiae* Cholnoky (1957: 76), *Nitzschia* spp. and *Navicula* spp. This sediment was composed mainly by clay (79%) and silt (13%). The taxon was found alive in oligohaline zone (salinity 3.0 ‰), at temperature 20 ºC and pH 7.1.

Diagnosis:—*Haslea sigma* differs from *Haslea nipkowii* (Meister) Poulin & Massé by having thickened virgae forming a pseudostauros, central raphe fissures almost straight (not overlapping), and terminal raphe fissures slightly curved (not T-shaped).



FIGURES 2–23. LM: *Haslea sigma sp. nov.*. Figs. 2–8. Live material under DIC. Cells in valve view showing two apically elongated chloroplasts (in valvar view) with pyrenoids axially located (arrow). Figs. 9–16. Acid cleaned valves in DIC. Figs. 17–23. Acid cleaned valves in brightfield. Fig. 12. Holotype. Scale bar = 10 μm.



FIGURES 24–35. SEM: *Haslea sigma sp. nov.* Figs. 24, 26–28, 32, 33. *External view*. Fig. 24. General view showing the outline of the valve. Fig. 26. Detail of two slits forming a wedge-like structure and detail of slightly curved terminal raphe. Fig. 27. Apice of valve showing the valve surface with straight, parallel and longitudinal strips (black arrow) separated by narrow slits (white arrow). Fig. 28. Detail of open valve showing cingulum composed by valvocopula (black arrow) and one copula (white arrow). Figs. 25, 29–31, 34, 35. *Internal view*. Fig. 25. General view showing the outline of the valve. Fig. 29. Valve center presenting raphe ending (black arrow), and a pseudostauros formed on primary side (ps) by one thickened virga (black arrow-head) and on secondary side (ss) by two thickened virgae (white arrow-head). Fig. 30. Center of valve showing on primary side part of accessory rib (black arrow), on secondary side the short accessory rib (white arrow) and the pseudostauros (black arrow-head). Fig. 31. Center of valve with pseudostauros, note that on primary and secondary sides the pseudostauros is formed by only one thickened virga. Figs. 32, 33. Longitudinal strips and slits, and central raphe fissures drop-like, bent to primary side. Figs. 34, 35. Apices showing helictoglossa (black arrow), quadrate areolae (white arrow) and end of accessory rib (arrow-head) on the primary side. Scale bars = 10 µm (Figs. 24, 25), 5 µm (Fig. 30), 2 µm (Fig. 27), 1 µm (Figs. 28, 29, 31–34), 0.5 µm (Figs. 26, 35).

TABLE 1. Morphological and morphometric features of *Haslea sigma sp. nov.* in comparison with similar taxa of *Haslea*. nd= no data.

	Haslea sigma sp. nov.	*H. crucigera*	*H. crucigeroides*	*H. nipkowii*	*H. salstonica*	*H. sulcata*	*H. spicula*	*H. quarnerensoides*
Features/Source	This study (n= 26)	Massé *et al.* (2001)	Hustedt (1961)	Poulin *et al.* (2004)	Massé *et al.* (2001)	Cleve (1894)	Cleve (1894)	Hustedt (1961)
Chloroplast (shape and location)	Apically elongated with pyrenoids axially located	Band-like lie against the girdle on each side of cell	nd	Apically elongated lie along each margin extending lightly under the valve face	Apically elongated lie against the girdle each side of cell	nd	nd	nd
Valve outline	Sigmoid with almost straight margins in the center of valve	Lanceolate to linear -lanceolate	Lanceolate with rostrate apices	Sigmoid, linear to slightly linear-lanceolate	Naviculoid rhombic	Linear with subacute ends	Narrow lanceolate with subacute ends	Fusiform with acute ends
Length (µm)	55.8–70.0	95–97	94	131–191	60–65	88–109	50–130	66–124
Breadth (µm)	5.8–7.2	11–12	12	15.5–20	17	8–9	4–13	9–13
Transapical striae in 10 µm	30–40	15	22	23–25	17	21	25–29	26 –32
Longitudinal striae in 10 µm	35–40	20	22	31–34	25	13–14	nd	22 (28)
Pseudostauros	Present formed by two or three virgae thickened	Present formed by two or three virgae thickened	Present	Absent	Present formed by two or three virgae thickened	Present	Present	Present
Acessory rib	Present	Present	nd	Present	Present	nd	nd	nd
Shorter accessory rib	Present	Present	nd	Present	Present	nd	nd	nd
Central raphe endings	Straight	Straight	nd	Straight	Straight	nd	nd	nd
Central raphe fissures	Almost straight, slightly bent towards primary side of valve	Slightly expanded and turned to the same side	nd	Slightly deflected toward the same side and gently overlapping	Enlarged and curved, unilaterally deflected	nd	nd	nd
Terminal raphe endings	Straight helictoglossa	Straight helictoglossa	nd	Straight helictoglossa	Straight helictoglossa	nd	nd	nd
Terminal raphe fissures	Slightly curved, bent to opposite sides	Sharply deflected to the same side	nd	T-shaped	Strongly deflected to same side	nd	nd	nd

Discussion

Haslea sigma sp. nov. resembles *H. nipkowii* in outline since both are the only species of the genus that have sigmoid valves. Differences may be summarized as follows: the new species has a pseudostauros, and terminal raphe fissures curved and bent to opposite sides, whereas *H. nipkowii* lacks pseudostauros and has T-shaped terminal raphe fissures (see Poulin *et al.* 2004: 189, Figs. 30, 31). Furthermore, *H. sigma* is shorter and narrower, and has a higher striae density than *H. nipkowii* (Table 1).

Presence of the pseudostauros is an important feature to delimit groups in *Haslea*. The new species has this feature, and a pseudostauros has also been found in *H. crucigera* (Wm. Smith) Simonsen (1974: 47)*, H. crucigeroides* (Hustedt) Simonsen (1974: 47)*, H. salstonica* Massé, Rincé & Cox (Massé *et al.* 2001: 619)*, H. spicula* (Hickie) Lange-Bertalot and *Haslea quarnerensoides* (Hustedt) Navarro, Micheli & Navarro (2000: 113). Massé *et al.* (2001) discussed the structure of the pseudostauros when comparing *H. salstonica* with *H. crucigera,* and they observed that in *H. crucigera* the development of the pseudostauros might be variable. Pseudostauros shape varied in the specimens of *H. sigma* examined during this study, some individuals had one thickened virga on each valve side and in others there was one virga on the primary side and two on the secondary side (white and black arrow-head in Fig. 29, 31). According to Round *et al.* (1990) a pseudostauros is lacking in planktonic species of *Haslea*, and based on this observation we could initially infer that the occurrence of this structure is restricted to the benthic species. However, subsequent studies have shown that some benthic species newly described (e.g. *H. nipkowii* and *H. pseudostrearia* Massé, Rincé & Cox *in* Massé et al. 2001: 622) do not have pseudostauros, either. Thus more study on this subject is needed.

Haslea sigma has an accessory rib along the raphe sternum on the primary side, a shorter rib on the secondary side of the valve and straight helictoglossae, such as is seen in *H. crucigera,* and *H. salstonica.* It cannot yet be compared with *H. crucigeroides, H. sulcata, H. spicula* and *H. quarnerensoides*, which seem to present accessory rib along the raphe sternum but have yet to be studied with SEM.

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