

# Valve morphology of some species of *Haslea* Simonsen (Bacillariophyta) from southern Brazil

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**ABSTRACT** – In Brazil, only five species of *Haslea* Simonsen have been found, probably due to their rarity and extremely hyaline valve, though presenting relatively large dimensions. In this study, three species from different localities in southern Brazil were investigated using transmission electron microscopy. *Haslea* cf. *karadagensis* Davidovich, Gastineau & Mouget has several discrepant features compared to the original description of *H. karadagensis*, such as fewer longitudinal striae. The specimens found did not completely conform to the diagnosis of *H. ostrearia* (Gaillon) Simonsen, a very close species recorded previously in the region, since there are differences regarding transapical and apical axes. Our material of *Haslea wawrikae* (Hustedt) Simonsen fit the dimensions of valves found elsewhere, except for a slightly higher number of striae. The newly reported species *Haslea nautica* (Cholnoky) Giffen resembled the valve structure of *H. wawrikae* regarding longitudinal strips and areolae.

**Key Words:** diatom, marennine, southern Brazil, taxonomy

**RESUMO** – **Morfologia da valva de algumas espécies de *Haslea* Simonsen (Bacillariophyta) do Sul do Brasil.** No Brasil, apenas cinco espécies de *Haslea* Simonsen foram encontradas, provavelmente devido à raridade das espécies e valva extremamente hialina, embora apresentando dimensões relativamente grandes. Neste trabalho, três espécies foram investigadas em microscopia eletrônica de transmissão, provindas de diferentes localidades do sudeste e sul do Brasil. *Haslea* cf. *karadagensis* Davidovich, Gastineau & Mouget em nosso material possui algumas características distintas de *H. karadagensis* como um número menor de estrias longitudinais. Estes espécimes certamente não se encaixam bem em *H. ostrearia* (Gaillon) Simonsen, previamente citada para a região, devido à diferenças de medidas de eixos apical e transapical. *Haslea wawrikae* (Hustedt) Simonsen encaixou-se nas dimensões de valvas encontradas em outras regiões, exceto por um número ligeiramente maior de estrias. *Haslea nautica* (Cholnoky) Giffen é um novo registro para a América do Sul, e mostrou estrutura da valva semelhante à *H. wawrikae* em relação à arquitetura das faixas longitudinais e aréolas.

**Palavras-chave:** diatomácea, marenina, Sul do Brasil, taxonomia

## INTRODUCTION

The genus *Haslea* Simonsen was described by Simonsen (1974) to accommodate species of diatoms historically placed in *Navicula* Bory de Saint-Vincent. Moreover, other species belonging to the genera, such as *Gyrosigma* Hassal (Poulin *et al.* 2004), *Navicula* (Giffen 1980, Witkowski *et al.* 2000) and *Stauroneis* Ehrenberg (Bukhtiyarova 1995) have been transferred to *Haslea*. The diagnostic

morphological feature of this genus is the presence of an external roof above the hymenate poroids composed of longitudinal silica strips anchored to the vimines, leaving narrow fissures between the strips (Simonsen 1974, Massé *et al.* 2001, Gastineau *et al.* 2014). In addition, the raphe system bears two straight branches, usually having the primary side more developed than the secondary one, forming the “accessory rib” (Cox 1999).

Species identification is based mainly on the

number of transverse and longitudinal striae, the presence of a short accessory rib in the secondary side and in the orientation of both central and polar raphe fissures. Several species have a pseudostauros; a structure characterized by the thickening of some virgae in the central region of the valve, like *H. crucigera* (Wm. Smith) Simonsen, *H. crucigeroides* (Hustedt) Simonsen, *H. quarnerensoides* (Hustedt) Navarro *et al.*, *H. salstonica* Massé, Rincé & Cox, *H. sigma* Talgatti *et al.* and *H. spicula* (Hickie) Lange-Bertalot.

*Haslea* is a cosmopolitan genus, with roughly 28 species and varieties (Guiry & Guiry 2015); either planktonic, such as *H. gigantea* Hustedt (Simonsen) and *H. wawrikan* (Hustedt) Simonsen or benthic, as *H. ostrearia* (Gaillon) Simonsen, *H. pseudostrearia* Massé, Rincé & Cox, *H. karadagensis* Davidovich, Gastineau & Mouget and *H. sigma*.

Interestingly, *H. ostrearia* and *H. karadagensis* are able to produce marennine, a blue pigment accumulated at the apices of the cells (Gastineau 2011, Gastineau *et al.* 2012). This pigment increases the commercial value of cultivated oysters in France, since their gills turn green after filter-feeding on phytoplankton containing a large amount of *Haslea*. Marennine has been credited in improving the taste of oysters, leading to higher consumer satisfaction (Gastineau *et al.* 2014).

In Brazil, five species of *Haslea* have been reported so far: *Haslea crucigera*, *H. cf. trompii*, *H. ostrearia*, *H. sigma* and *H. wawrikan* (Torgan *et al.* 1999, Villac *et al.* 2008, Eskinazi-Leça *et al.* 2010, Villac & Tenenbaum 2010, Proença *et al.* 2011, Talgatti *et al.* 2014), all found in coastal shallow waters, stretching from the state of São Paulo to the state of Rio Grande do Sul. Unfortunately, none of these papers provided any description, measurements or photographs of the species, except Talgatti *et al.* (2014, for *H. sigma*), hindering species confirmation or further morphological comparisons.

In this paper, we investigated the valve structure of three *Haslea* species from several coastal locations along the southern Brazilian coast. We also provide a morphological comparison between our material and similar ones recorded worldwide.

## MATERIAL AND METHODS

The samples were collected in different coastal waters along the southeast - southern Brazilian shoreline, during different time periods (Tab. 1). Sampling was carried out using plankton nets with a

20 or 25 µm mesh size, usually through vertical hauls from bottom to surface. Samples were preserved in formaldehyde 1-2% final concentration. Aliquots of a few samples from the Santa Catarina State were examined alive to isolate and cultivate cells in F/2 plus silicate medium (Guillard & Ryther 1962). Cultures were grown at constant light intensity under 12:12 light:dark photoperiod, and salinity of 32 at 25°C.

For electron microscopy, samples were processed according to the technique described by Hasle & Fryxell (1970). A few drops of clean material were added onto transmission electron microscope (TEM) nickel grids (mesh 200), and coated with Formvar/Carbon pellicle (Electron Microscope Supplies). The grids were screened using a Jeol model JM1200 EXII TEM at 80kV acceleration voltage. Samples were deposited in the UPCB herbarium, housed at the Federal University of Paraná, Brazil. Terminology of valve structures followed Cox (1999) and Ross *et al.* (1979).

## RESULTS

Three species were found in our material: *Haslea cf. karadagensis*, *Haslea wawrikan* and *Haslea nautica*, and are described below. Valve morphometrics for each species are shown in Table 2, as well as data on closely related species.

***Haslea cf. karadagensis*** Davidovich, Gastineau & Mouget.

(Figs. 1-5)

Cells solitary; two elongated parietal chloroplasts were observed in the central region of the cell (not illustrated). Marennine bodies readily visible at the apices. Valves lanceolate with subacute apices (Fig. 1); apical axis 70 - 80 µm and transapical axis 6-10 µm. Raphe system runs centrally and straight; central and polar ends straight on both the inner and outer sides of the valve (Figs. 2-4). A conspicuous helictoglossa is visible in the internal side of the polar ending (Fig. 4). The accessory rib runs along the raphe system in the primary side of the valve (Fig. 3). The valvar surface is striate; striae composed of square to rectangular poroids with rounded angles (Figs. 4, 5). Poroids are occluded by hymenes with minute perforations (Fig. 5). There are 37-39 longitudinal striae in 10 µm and 28-32 transverse striae in 10 µm. The longitudinal silica strips are sustained onto vertical walls, which in turn are placed on the longitudinal vimines (Fig. 5). The silica strips consist of a roof over the poroids and

along the extension of the valve surface. The margins of the longitudinal strips are thickened, bordering a thin slit left by the strips (Fig. 5). The slits and the silica strips are variable in width. At each side of the valve there are 16 to 17 silica strips. Valvocopula and the second bands are open, lacking ornamentation (not illustrated).

**Geographic Distribution:** Ukraine (Black Sea, Gastineau *et al.* 2012).

**Occurrence in the samples:** Paraná (Paranaguá bay, UPCB47389), Santa Catarina (Florianópolis, Armação de Itapocoroí, Balneário Camboriú, Itajaí).

**Reference:** Gastineau *et al.* (2012):469-479, figs. 1, 3, 5-9.

***Haslea wawrikan*** (Hustedt) Simonsen  
(Figs. 6-8)

Valves narrowly lanceolate with acute apices (Fig. 6); 80 – 110 µm apical axis and 4 - 4.5 µm transapical axis. Raphe system straight, lacking a central area (Fig. 7). Central and polar fissures straight, both internally and externally (Figs. 7, 8). Valve surface has two patterns of striae composed of rectangular poroids running parallel to the apical axis (Fig. 8). Transverse striae 22-25 in 10 µm; longitudinal striae 20-22 in 10 µm. No occlusions were observed covering the poroid openings, possibly due to the poor silicification of the valve. At each side of the valve there are three wide longitudinal silica strips; i.e. 20-22 in 10 µm, raised from the valve framework by lateral walls.

**Geographic Distribution:** Southwest Atlantic: Brazil, São Paulo (Villac *et al.* 2008, Eskinazi-Leça *et al.* 2010); North Atlantic: Gulf of Mexico - Florida (Saunders & Glenn 1969), Caribbean Sea - Puerto Rico (Navarro 1983); Mediterranean Sea: Gulf of Naples (Hustedt 1961-66); Indian Ocean: Australia (Von Stosch 1985), Madagascar (Sournia 1968), India and Arabian Sea (Simonsen 1974).

**Occurrence in the samples:** Paraná (Paranaguá Bay, UPCB47389; coastal waters, UPCB63317); São Paulo (coastal waters of Ubatuba).

**References:** Hustedt (1961-66):51, fig. 1204 as *Navicula wawrikan*; Simonsen (1974):48; Von Stosch (1985):339-343, figs. 23, 24

***Haslea nautica*** (Cholnoky) Giffen  
(Figs. 9-17)

Valves lanceolate with subacute apices (Fig. 9); apical axis 27.9 - 32.7 µm and transapical axis 5.0 - 6.0 µm. Raphe system is straight, lacking a central area and/or short accessory rib. The central fissures are curved towards the primary side (Figs. 11, 15), while the polar ones are strongly hooked externally (Figs. 10, 12, 13-14). Valve surface striate (Figs. 9, 13), composed of rectangular poroids, widely separate from each other by hyaline areas in a longitudinal direction (Figs. 11, 15). There are 30 longitudinal striae in 10 µm and 20-26 transverse striae in 10 µm. Poroid perforated by hymenes in a radiate pattern; the ones closest to the poroid margins are larger than the remaining hymenes (Fig. 16). Longitudinal strips are thickened (Fig. 17), about 10-16 strips distributed along the transapical axis; i.e., 20-28 in 10 µm. Valvocopula has one row of poroids (Fig. 10).

**Geographic Distribution:** Southeast Atlantic: Republic of South Africa, Gordon's Bay (Cholnoky 1963); Indian Ocean: Seychelles Islands (Giffen 1980); Pacific Ocean: Baja California (Almeida 2005, Plate 7, Fig. 7).

**Occurrence in the samples:** Paraná (Paranaguá bay, UPCB47389).

**References:** Cholnoky (1963):62, fig. 64; Giffen (1980):146, fig. 21; Witkowski *et al.* (2000):224, Pl.148, figs. 9-11

## DISCUSSION

*Haslea karadagensis* is the second species found to be marennine-producing, besides *Haslea ostrearia* (Gastineau *et al.* 2014). According to literature, these species are closely related, sharing the same frustule morphology, with minor differences concerning in both valve morphometrics and phylogenetic data (Gastineau *et al.* 2012; Tab. 2). For instance, raphe fissures are straight in both species, and there is an accessory rib running alongside the raphe (Robert & Pratt 1973a, Masse *et al.* 2001). On the other hand, they have a different number of longitudinal striae; 57 in 10 µm in *H. karadagensis* and 53 in 10 µm in *H. ostrearia* (Robert & Pratt 1973a, 1973b, Masse *et al.* 2001, Gastineau *et al.* 2012). The valves of our material, identified as *H. cf. karadagensis*,

have a lower number of longitudinal striae (37.5-39 in 10  $\mu\text{m}$ ) than the typical *H. karadagensis* and *H. ostrearia*, justifying our identification in *conferatum*. On the other hand, the number of transverse striae in the Brazilian specimens (28-32.5 striae in 10  $\mu\text{m}$ ) corresponds to the original description of *H. karadagensis*, which has 31 transverse striae in 10  $\mu\text{m}$ , and was relatively different from *H. ostrearia*, which is more finely striated (35-36 striae in 10  $\mu\text{m}$ ; Neuville *et al.* 1971, 1974, Massé *et al.* 2001, Gastineau *et al.* 2012).

Overall, the Brazilian material was morphologically closer to *H. karadagensis* than to *H. ostrearia*, despite the metric deviation of the longitudinal striae. Therefore, it is essential to revise previous identifications of *H. ostrearia* in Brazil, a frequently cited species for coastal waters, based exclusively on light microscopy (Moreira Filho *et al.* 1990, Procopiak *et al.* 2006, Proença *et al.* 2011). There should be an effort to reliably discriminate the local species based on phylogenetic data, using the rDNA ITS1, 5.8s and ITS2 markers, since these have been proved appropriate for diatom systematics (Lim *et al.* 2012, Fernandes *et al.* 2013). Comparing our material of *H. karadagensis* with other species, it readily differs from other close species by the direction of the terminal raphe fissures. *Haslea salstonica* Massé, Rincé et Cox and *H. crucigera* (Wm. Smith) Simonsen have deflected central and polar fissures, while in *H. karadagensis* they are straight. The shorter accessory rib is lacking in this species and in *H. ostrearia*, but is present in *H. salstonica* and *H. crucigera*. Moreover, the central virgae in these latter species are thickened, forming the “pseudostauros” (Cox 1980, Cardinal *et al.* 1984, Lobban 1984, Massé *et al.* 2001); however, this structure is absent in *H. karadagensis* and *H. ostrearia* (Robert & Pratt 1973a, 1973b, Ricard 1987, Gastineau *et al.* 2012). Regarding the number of striae on the valve surface, the latter two species - and our material - are more densely striate (see above) than *H. crucigera* (17 transapical and 20 longitudinal striae in 10  $\mu\text{m}$ ) and *H. salstonica* (15 transapical and 25 longitudinal striae in 10  $\mu\text{m}$ ) (Massé *et al.* 2001).

In *Haslea pseudostrearia* Massé, Rincé et Cox, another related species, there is typically a shorter accessory rib and the polar terminal fissures are hooked (Massé *et al.* 2001), while in *H. karadagensis* and *H. ostrearia* they are straight. On the other hand, the central fissures are almost straight in all the three species. The number of transapical striae in *H. pseudostrearia* (34-36 in 10  $\mu\text{m}$ ) is closer to *H.*

*ostrearia* (35-36 in 10  $\mu\text{m}$ ) than to *H. karadagensis* (Massé *et al.* 2001, Gastineau *et al.* 2012) and *H. cf. karadagensis* from our specimens (28-32 in 10  $\mu\text{m}$ ). However, *H. pseudostrearia* has a lower number of longitudinal striae (42 in 10  $\mu\text{m}$ ) when compared to *H. karadagensis* and *H. ostrearia*, these having 58 and 53 longitudinal striae in 10  $\mu\text{m}$ , respectively. In this regard, our material is broader striate (37-39 in 10  $\mu\text{m}$ ) than *H. pseudostrearia*. *Haslea trompii* (Cleve) Simonsen is another species with similar features to *H. cf. karadagensis*, such as straight central and polar fissures both externally and internally (Semina 2003, Scott & Thomas 2005). Nevertheless, *H. trompii* has more acute apices, its central raphe endings are closer than in *H. cf. karadagensis*, and it lacks a thickness on one side of the axial area (Semina 2003).

In this study, we confirmed the poroid occlusion in electron microscopy for *H. cf. karadagensis*, showing that the structure of the hymenes is quite similar to those of other species previously investigated, such as *H. ostrearia* (Neuville *et al.* 1971, Pl. I, fig. 3), and the not formally proposed new species “*Haslea silbo*” (Gastineau 2011, fig. 4F, Gastineau *et al.* 2014).

During our study, we found a peculiar species that we identified as *Haslea nautica*, which has not been previously studied using electron microscopy, and is a new record for South America. Its valves have a surface composed of rectangular areolae - perforated by a specific pattern of hymenes - lying upon wide longitudinal strips. Consequently, the number of longitudinal striae is much lower than in the remaining species, like *H. ostrearia*, *H. karadagensis*, *H. gigantea* and others studied so far (see Tab. 2); such a feature readily differentiates *H. nautica* from those species. On the other hand, two species morphologically more similar to *H. nautica* were found in the literature, *Haslea howeana* (Hagelstein) Giffen and *Haslea britannica* (Hustedt) Witkowski. *H. howeana* shares several features with *H. nautica*, for instance, both are coarsely striated with 15-18 transverse striae in 10  $\mu\text{m}$  and 15 longitudinal striae in 10  $\mu\text{m}$ , with curved apical raphe endings (Cholnoky 1963, Giffen 1980, Witkowski *et al.* 2000, Lobban *et al.* 2012). The differences between *H. howeana* and *H. nautica* are with the central fissures, shape of the valve and direction of the transverse striae (Tab. 2). *Haslea nautica* has central fissures slightly curved to the same side, while in *H. howeana* they are strongly hooked (Lobban *et al.* 2012). In addition, the valve shape of *H. howeana* is linear-lanceolate with slightly produced apices (Witkowski *et al.*

2000, Lobban *et al.* 2012). In *H. nautica*, the valves are lanceolate with subacute apices. Moreover, the transverse striae of *H. howeana* are radiate in the middle of the valve, and slightly convergent at the apices; instead, the striae of *H. nautica* are slightly radiate, becoming parallel only toward the apices (our material, Witkowski *et al.* 2000). The overall appearance of *H. britannica*, another related species, somewhat resembles *H. nautica*, especially the nature of the striation and the type of raphe fissures (see the Hustedt's type illustrated in Simonsen 1987, Witkowski *et al.* 2000). However, *H. britannica* has a parallel striae pattern in the middle region and radiate near the apices, with linear-lanceolate valves (Hustedt & Aleem 1951, Giffen 1980, Simonsen 1987, Witkowski *et al.* 2000).

The last, somewhat similar, species to *H. nautica* that was formerly studied using electron microscopy is *Haslea wawrikan* (Hustedt) Simonsen, with the same arrangement of the longitudinal strips and areolae as in *H. nautica* (described above). Moreover, the species share other similarities. There are about the same number of transverse striae; 20-26 in 10 µm in *H. nautica* and 22-23 in 10 µm in *H. wawrikan* (Von Stosch 1985, our material, Tab. 2). The longitudinal striae observed in this study and in other literature (Tab. 2) are similar in *H. wawrikan*. These ranges of striae for *H. wawrikan* and *H. nautica* are in contrast with those of other species discussed here, such as *H. ostrearia*, *H. karadagensis*, *H. pseudostrearia*, *H. gigantea* and others, which are more densely striate, usually having more than 30 transapical striae and 33 longitudinal striae in 10 µm (Tab. 2). On the other hand, the main differences between *H. wawrikan* and *H. nautica* found in our material lie on the length of the valve and the morphology of raphe system. *Haslea wawrikan* is a very large and narrow species, its length varying from 280 to 560 µm (Hustedt 1961, Simonsen 1974, Von Stosch 1985), while the valve length of *H. nautica* is much smaller, spanning from 27.9 to 32.7 µm in our material and 45 to 60 µm elsewhere (Cholnoky 1963). The transapical axis in both the species is about the same; 4.4-6.5 µm in *H. wawrikan* and 5.0-6.0 µm in *H. nautica* from Brazil. Regarding the raphe system, in *H. nautica* both the polar and central fissures are curved. Instead, *H. wawrikan* has straight fissures at both the raphe branches (Von Stosch 1985; our results).

The specimens of *H. wawrikan* recorded in our material have the same morphometrics of other material found elsewhere, except for valve length, which were much longer, up to 560 µm, in

the material of Von Stosch (1985; our Tab. 2). Our findings expand the geographic distribution of *H. wawrikan* in Brazilian waters, since it had previously been recorded only for São Paulo State (Villac *et al.* 2008, Eskinazi-Leça *et al.* 2010), though it should be kept in mind that these studies did not provide further data, such as description or pictures.

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**Table 1.** Samples examined in this study, as well their respective locations and sampling dates.

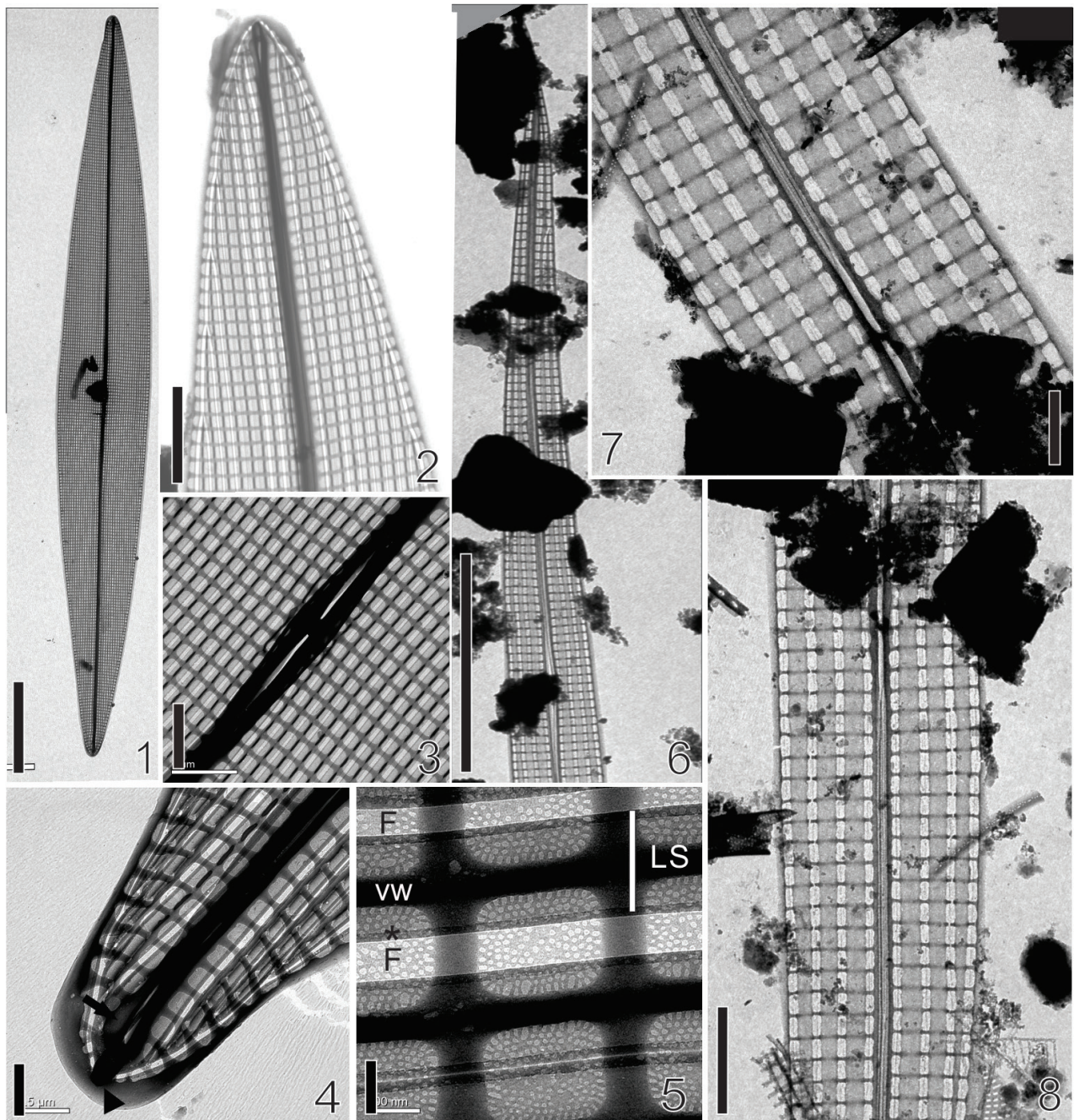
Station Locations	Station Labels	Coordinates	Depth at station	Sampling Date
Beira-mar, Florianópolis, SC	n. 24	27°35'12" S/48°33'26" W	4 m	29/Dec./2012
Armação Itapocoroí, SC	---		3 m	15/Dec/2012
Balneário Camboriú, SC	--		1 m	28/Jul/2012
Coastal waters, Itajaí, SC	SM-I-1	26°55'00"S/48°34'00"W	20 m	10/Nov/2005
Paranaguá Bay, PR	--	25°29'05"S/48°31'39"W	2.0 m	2011 (07/Jan, 03/May, 13/May, 10/Jun)
Paranaguá Bay, PR	E4 Alarme	25°30'93"S/48°29'88"W	2.3 m	Jul/2003
Coastal waters, Paranaguá, PR	E1 (RAM)	25°42'60" S/48°27'60" W	10 m	Jan/1998
Coastal waters, Ubatuba, SP	UBA 093	23°45'00" S/044°31'41" W	37 m	13/Nov/2012
Coastal waters, Ubatuba, SP	27	23°30'45"S/45°05'30"W	8 m	09/Apr/2013

**Table 2.** Morphological features of select *Haslea* species compared to the material found in southern Brazil.

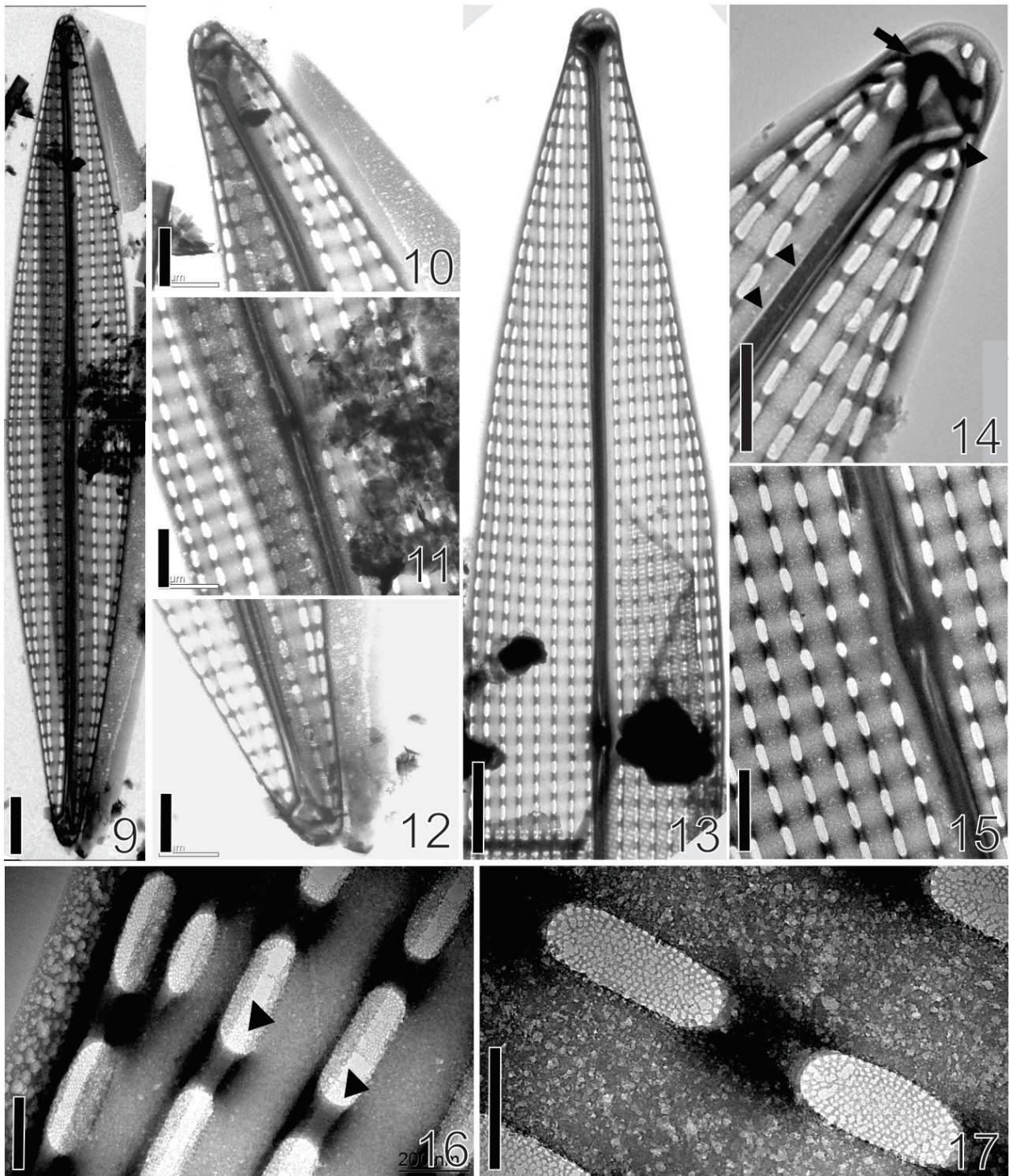
	Length (µm)	Width (µm)	Transapical striae in 10 µm	Longitudinal striae in 10 µm	Pseudo-stauros	Accessory rib	Shorter accessory rib	Central raphe endings	Polar raphe endings
<i>Haslea britannica</i> <sup>1</sup>	39	8	20	24	absent	nd	nd	nd	nd
<i>Haslea crucigera</i> <sup>2,3,4</sup>	80-184	10-13	10-17	20-25	present	present	present	curved strongly hooked	curved
<i>Haslea howeana</i> <sup>5,6</sup>	30-62	7-10	17-18	nd	absent	nd	nd	straight	curved
<i>Haslea karadagensis</i> <sup>7</sup>	26.5-84.0	4.5-11.2	31.4	57.8	absent	present	absent	straight	straight
<i>Haslea nautica</i> <sup>5</sup>	45-60	9-12	15	15	absent	nd	nd	straight	curved
<i>Haslea ostrearia</i> <sup>2,7,8</sup>	28.6-123.5	6.1-12	36	53	absent	present	almost indistinct	straight	straight
<i>Haslea pseudostrearia</i> <sup>2</sup>	37-43	6-7	34-36	42	absent	present	present	straight	curved
<i>Haslea salstonica</i> <sup>2</sup>	60-65	17	15	25	present	present	present	curved	curved
" <i>Haslea silbo</i> " <sup>9</sup>	72.7	4.5-10.4	33	33	absent	present	present	straight	straight
<i>Haslea wawrikae</i> <sup>10,11,12</sup>	280-560	4.4-6.5	18-23	10-40	absent	absent	absent	straight	straight
<i>Haslea cf. karadagensis</i> (this study)	70-80	6-10	28-32.5	37,5-39	absent	present	absent	straight	straight
<i>Haslea wawrikae</i> (this study)	80-110	4.0-4.5	22-25	20-22	absent	absent	absent	straight	straight
<i>Haslea nautica</i> (this study)	27.8-32.7	5.0-6.0	20-26	30	absent	present	absent	straight	hooked

<sup>1</sup>Hustedt & Aleem (1951), <sup>2</sup>Massé *et al.* (2001), <sup>3</sup>Cardinal *et al.* (1984), <sup>4</sup>Lobban (1984), <sup>5</sup>Witkowski *et al.* (2000), <sup>6</sup>Lobban *et al.* (2012), <sup>7</sup>Gastineau (2012a), <sup>8</sup>Robert & Pratt (1973b), <sup>9</sup>Gastineau (2012b), species not formally described, <sup>10</sup>Von Stosch (1985), <sup>11</sup>Hasle & Syvertsen (1996), <sup>12</sup>Hustedt (1961-1966), nd: no data.





**Figs. 1-8.** 1-5. *Haslea cf. karadagensis*. 1. General view of the lanceolate valve; 2. Apical region of the valve illustrating striae pattern. Note the special peripheral slit of the striae at the margin; 3. Central region; proximal fissures straight; 4. Valve apex detailing the structure of distal raphe fissures: internal raphe fissure with helictoglossa (arrow), and external straight fissure (arrowhead); 5. Close-up of valve framework near the center. F: longitudinal fissure; VW: virgae wall; LS: longitudinal silica strips; \* = a thickened margin of longitudinal strip; 6-8. *Haslea wawrikae* (TEM). 6. Broken slender valve showing the acicular shape; 7. Central valve region. Proximal raphe fissures are straight; 8. Valve view near the center, showing the disposition of areolae and longitudinal strips. Bars: **Figs. 1, 6** = 10  $\mu$ m; **Figs. 2, 8** = 2  $\mu$ m; **Figs. 3, 7** = 1  $\mu$ m; **Fig. 4** = 0,5  $\mu$ m; **Fig. 5** = 100 nm.



**Figs. 9-17.** *Haslea nautica* (TEM). **9.** General view of an acicular valve; **10, 12.** Valve apices showing distal curved fissures. Note valvocopula; **11.** Central region of valve; raphe bearing slightly bent proximal fissures; **13-15.** Valve view of specimen having a thin striation; **14.** Detail of apex showing accessory rib (arrowheads), internal raphe fissure with helictoglossa (arrow) and external curved raphe fissure (arrowhead); **15.** Central valve region; **16.** Longitudinal strips (arrowheads); **17.** Two poroids perforated by hymenes arranged in radiate pattern. Note larger hymenes at the margin of the poroids. Bars: **Figs. 9, 13** = 2  $\mu\text{m}$ ; **Figs. 10, 11, 12, 14, 15** = 1  $\mu\text{m}$ . **Figs. 16, 17** = 200 nm.