Acta Botanica Brasilica 26(4): 836-841. 2012.

Morphology and distribution of two epizoic diatoms (Bacillariophyta) in Brazil

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Recebido em 20/12/2011. Aceito em 4/07/2012

RESUMO

(Morfologia e distribuição de duas diatomáceas (Bacillariophyta) epizóicas no Brasil). As diatomáceas epizóicas em copépodos *Pseudohimantidium pacificum* e *Falcula hyalina* foram investigadas em microscopias óptica e eletrônica de varredura, com base em amostras coletadas em diferentes ambientes marinhos da costa brasileira. *Pseudohimantidium pacificum* é reportada pela primeira vez no Oceano Atlântico Sul Ocidental, ampliando significativamente sua faixa de distribuição geográfica. A espécie ocorreu sobre os copépodos *Corycaeus amazonicus* e *Euterpina acutifrons*, e em larvas cypris de Cirripedia. *Falcula hyalina* utilizou um número maior de hospedeiros, particularmente os copépodos *Oithona oswaldocruzii*, *Pseudodiaptomus richardii* e *Acartia* spp. A morfologia e dados biométricos das valvas de ambas as diatomáceas estiveram dentro dos limites registrados na literatura, incluindo as publicações originais. As duas espécies ocorreram em todas as estações de coleta ao longo da região costeira entre as latitudes 12°S e 28°S. *F. hyalina* havia sido registrada anteriormente no Oceano Atlântico Sul Ocidental na latitude 31°S.

Palavras-chave: epibionte, *Pseudohimantidium, Falcula,* copépoda, biogeografia

ABSTRACT

(Morphology and distribution of two epizoic diatoms (Bacillariophyta) in Brazil). The epizoic diatoms *Pseudohimantidium pacificum* and *Falcula hyalina*, which live on copepods, were investigated using light and electron microscopes, based on material gathered from different marine environments along the Brazilian coast. *Pseudohimantidium pacificum* is reported for the first time for the Southwestern Atlantic Ocean, significantly enlarging its range of geographic distribution. This species usually covers the entire body surface of the copepods *Corycaeus amazonicus* and *Euterpina acutifrons*, and of cypris larvae of Cirripedia. *Falcula hyalina* uses a higher number of copepod hosts, particularly *Oithona oswaldocruzii*, *Pseudodiaptomus richardii* and *Acartia* spp. The valve morphology and biometrical data of both diatoms were within the range limits recorded in the literature, including the original publications. Both species occurred in all the sampling stations along the Brazilian coastline stretching from 12°S down to 28°S. *Falcula hyalina* had already been found as far as latitude 31°S in the Southwestern Atlantic Ocean.

Key words: epibiont, *Pseudohimantidium, Falcula,* copepods, biogeography

Introduction

The exoskeleton of crustacean copepods constitutes a convenient habitat for a variety of epibiont microorganisms such as bacteria, microalgae and protozoans (Carman & Dobbs 1997; Walkusz & Rolbiecki 2007). Among these organisms, pennate diatoms have been commonly reported growing on different parts of the body of copepods (Carman & Dobbs 1997). Originally revealed by Giesbrecht (1892) in the Adriatic Sea, epizoic diatoms have since then been recorded in different regions around the world. They are widespread in the Pacific Ocean along the Sea of Japan to the coasts of South America (Hiromi *et al.* 1985; Rivera *et al.* 1986). Several sparse records were made from both margins of the United States and Mexico, Caribbean, Mediterranean Sea and East Africa (Voigt 1960; 1961; Gibson 1979a; 1979b; Navarro 1982; Prasad *et al.* 1989; Garate-Lizarraga & Muñeton-Gomez 2009).

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The main epizoic diatom genera are *Falcula* Voigt, *Licmophora* C. Agardh, *Protoraphis* Simonsen, *Pseudohimantidium* Hustedt & Krasske and *Sceptronema* H. Takano (Voigt 1960; Simonsen 1970; Gibson 1979a; 1979b; Takano 1983; Hiromi *et al.* 1985; Hallegraeff & McWilliam 1990). Some diatoms seem to present a degree of specificity regarding the copepod host. Ikeda (1977) was the first to suggest an association between the two organisms. For instance, *Pseudohimantidium pacificum* has been recorded associated to the genus *Corycaeus* Dana (Gibson 1978; 1979a; Rivera *et al*. 1986), though Garate-Lizárraga & Muñeton-Gomez (2009) have recently detected this diatom living on *Farranulla gibbula* Giesbrecht in the Gulf of California, Mexico. Coincidentally, the two copepods have a very similar morphology. On the other hand, *Falcula hyalina* shows noticeable preference by hosts of the genus *Acartia* Dana (Hiromi *et al*. 1985) and, secondarily, by other copepods (Takano 1983; Hiromi *et al*. 1985; Prasad *et al.* 1989).

In this report, we describe two species of epizoic diatoms and their occurrence on the surface of the body of copepods, which were detected in samples from different environments along the Brazilian coast. *Pseudohimantidium pacificum* and *Falcula hyalina* were examined using light and electron microscopes to detect possible morphological and metric differences compared to other specimens described in the literature.

Material and methods

The sample collection (n=30) used in this study was gathered in different localities along the Brazilian coastline (12°S to 28°S), between 1994 and 2011 (Tab.1). Only the material sampled in Paranaguá Bay, Paraná State, had the host copepods identified to the species level. Zooplankton was collected using plankton nets with a 300µm mesh size. Each sample was screened using a stereomicroscope to detect copepods with epizoic diatoms. Individuals were picked up with a micropipette and placed in 15 ml centrifuge tubes for further processing of the diatom frustules.

The diatom cells and the copepods were washed in distilled water followed by cleaning of frustules from the organic matter according to the technique of Hasle & Fryxell (1970). Permanent slides were mounted using Naphrax (refractive index = 1.74) as a mounting medium. For light microscopy (LM), specimens were measured and photographed using an Olympus BX-51 microscope. Samples for scanning electron microscopy (SEM) were prepared by adding a drop of sample onto aluminum stubs, which were air-dried and sputter-coated with gold. Samples were examined using a Jeol-JSM 6360LV scanning electron microscope at an accelerating voltage of 20 kV. Terminology followed Ross *et al*. (1979), with the additions of Round *et al.* (1990). A literature search was facilitated by consulting Gaul *et al.* (1993) and Henderson & Reimer (2003).

Results

Pseudohimantidium pacificum Hustedt & Krasske in Krasske, Archiv fur Hydrobiologie, v. 38, p. 272, pl. 5, Fig. 8, 1941.
Fig. 1-17

Basionym: *Hormophora zavodnikia* Jurilj, Acta Botanica Croatia, v. 16, p. 98, Fig. 3, 1957.

References for identification: Krasske (1941), Simonsen (1970), Gibson (1978; 1979a), Hiromi *et al*. (1985), Rivera *et al*. (1986), Lee *et al*. (1993), Garate-Lizárraga & Muñeton-Gomez (2009).

Cells adhered to copepods by means of mucilage stalks at the apices of the frustules (Fig. 1-5). Plastids are numerous, elliptical (Fig. 6). Valves are arcuate with subrostrate apices (Fig. 7-11). Apical axis 39-46 µm, transapical axis 9-13 µm. Striae are uniseriate, 30-40 in 10 µm, parallel at the valve center (Fig. 10), becoming radiate toward the apices (Fig. 11-13); poroids elongate, 2-3 in 1µm. Each apex has several striae composed of smaller poroids (Fig. 13, 16). Sternum is narrow, strongly curved at the apices to form a hook-like shape (Fig. 13-14, 16) aligned with the rimoportulae at one apex, and bend in the other (Fig. 12). Rimoportulae in number of 3-10 juxtaposed to each other (Fig. 14-15), placed parallel (sometimes almost orthogonal, Fig. 16-17) to the ventral side. The external openings are fused to each other in a common groove (Fig. 16). In the same valve, there are different numbers of rimoportulae at each of the apices.

The species occurred in all the samples examined (Tab.1). In the samples collected in Paranaguá Bay, the cells of *P. pacificum* appeared in high abundance on the copepods *Corycaeus amazonicus* Dahl F. and *Euterpina acutifrons* Dana; a few cells were also detected living on a cypris larva of Cirripedia. Diatoms occupied almost all the available copepod exoskeleton, especially the locomotory appendages and antennae, though a few cells were attached on the dorsal surface.

Falcula hyalina Takano, Bulletin of Tokai Regional Fisheries Research Laboratory, n. 111, p. 24-25 Fig. 1, 1983.
Fig. 18-27

References for identification: Voigt (1960), Gibson (1978), Takano (1983), Hiromi *et al.* (1985), Prasad *et al*. (1989), Souza-Mosimann *et al.* (1989).

Living cells have two elongated plastids and 2-3 lipid droplets (Fig. 18). Valves are arcuate with obtuse apices (Fig. 19-22). Ventral side slightly undulated in some valves. Apical axis 24-30 µm, transapical axis 4-5 µm. Valvar surface is striated; 20-24 striae in 10 µm. Poroids are elongated in relation to the apical axis (Fig. 23). Striae are short and uniseriate, parallel in the center (Fig. 25), turning radiate in the

Table 1. Data of plankton samples (n = 30) containing epizoic diatoms, collected in the Brazilian coastline (12°S to 28°S).

Station Locations	Station Labels	Coordinates	Depth at Station	Dates of Samplings
Todos os Santos Bay, Bahia	--	12°58'30"S 38°31'25"W	6.0 m	23/May/1994
Arraial do Cabo, Rio de Janeiro	--	22°58'18"S 42°02'05"W	5.0 m	03/Mar/2010
Santos Bay, São Paulo	--	23°59'20"S 46°19'39"W	4.0 m	15/Jan/2006
Shelf Waters of Paraná	E1	25°42'60"S 48°27'60"W	10.0 m	Oct/1998, Dec/1998 UPCB 63354 and 63359*
Paranaguá Bay, Buoy 12	E1	25°33'63"S 48°20'53"W	13.0 m	Dec/2002, Feb/2003 (8 samples)
Paranaguá Bay, Maciel River	E2	25°33'69"S 48°25'48"W	3.0 m	Jul/2003
Paranaguá Bay, Port of Paranaguá	E3	25°29'05"S 48°31'39"W	8.2 m	Sep/2002, Feb/2003 (6 samples)
Paranaguá Bay, Itiberê River	E4	25°30'93"S 48°29'88"W	2.3 m	Jul/2003
Paranaguá Bay, Europinha	E5	25°33'96"S 48°38'02"W	1.2 m	Jan/2003, Jul/2003
Paranaguá Bay, Antonina city	E6	25°24'18"S 48°42'24"W	3.0 m	Jun/2003, Jul/2003
Itajaí, Santa Catarina	SM-I-1	26°55'00'S 48°34'00"W	20.0 m	10/Nov/2005
Ratones River estuary, Florianópolis, Santa Catarina	--	27°27´45"S 48°32´11"W	1.4 m	20/Jan/2001, 23/Jan/2007
Ribeirão da Ilha, Florianópolis, Santa Catarina	--	27°47'50"S 48°34'03"W	3.0 m	26/Jan/2011
Laguna, Santa Catarina	--	28°29'33"S 48°45'31"W	5.0 m	04/Feb/ 2011

*: Samples deposited at the UPCB herbarium.

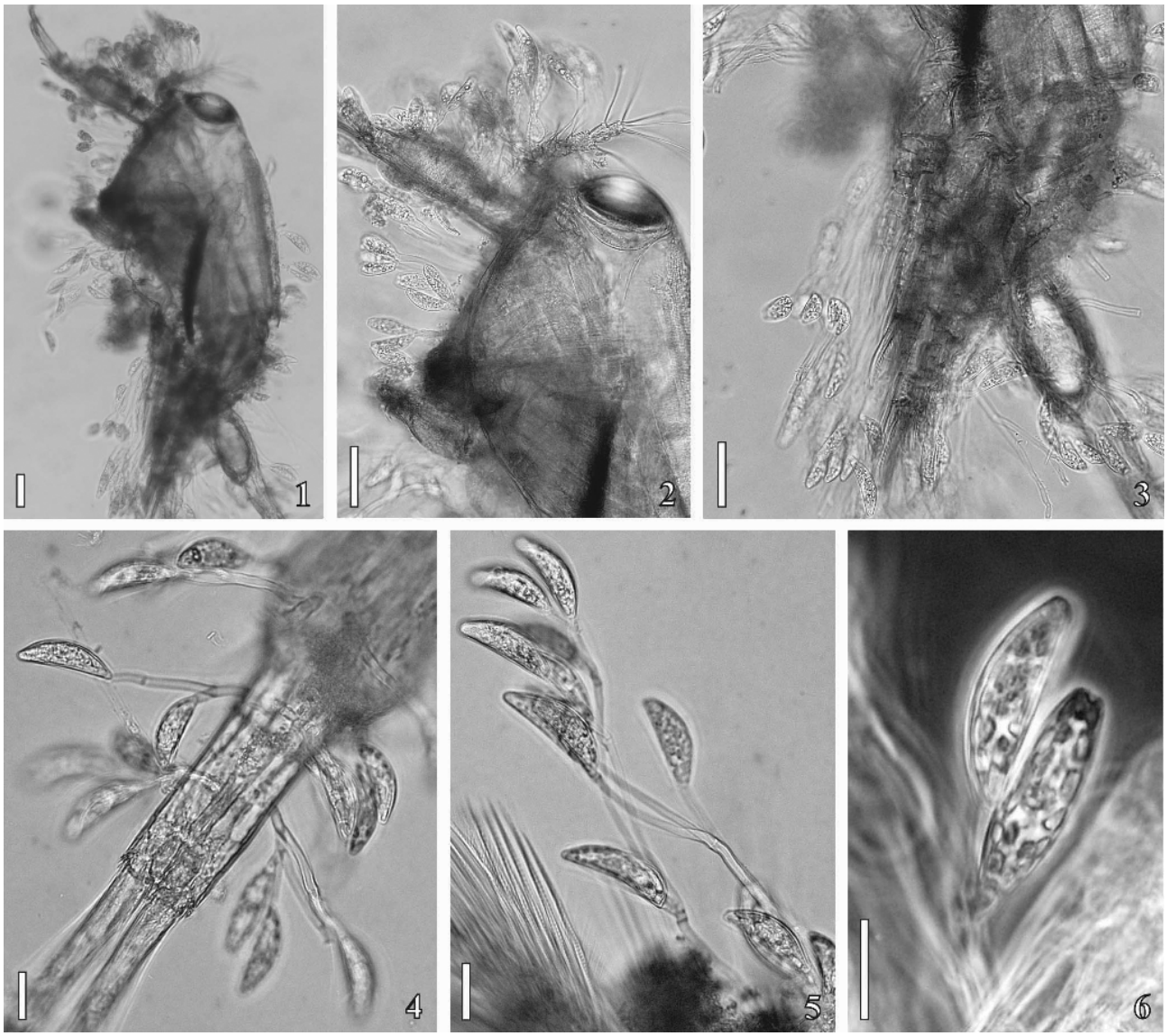
remaining valve (Fig. 24). At the apices, striae are strongly radiate (Fig. 23-24). Sternum is highly enlarged and arcuate, separating the striae into two sectors (ventral and dorsal). Ocellulimbus is small, located at the tip of each of the apices, eccentric (Fig. 26-27). One single rimoportula is placed in one of the apices, usually after the third or fourth dorsal stria. Internally, rimoportula has a labiate fissure (Fig. 26); in the outer side, there is a sessile elongated aperture (Fig. 27).

Falcula hyalina occurred in all the samples examined (Tab. 1). The host copepods of *F. hyalina* from Paranaguá Bay were *Oithona oswaldocruzii* Oliveira*, Pseudodiaptomus richardi* Dahl F.*, Acartia lilljeborgii* Giesbrecht and *Acartia tonsa* Dana.

Discussion

Epizoic diatoms have been recorded from different oceans around the world, despite the peculiar habitat they grow in. Surprisingly, only a few publications have reported these interesting diatoms for the South Atlantic Ocean. This is the first report of *Pseudohimantidium pacificum* from the Southwestern Atlantic Ocean, found along the Brazilian coast (12°S to 28°S). The species was recorded in the North Atlantic from the Caribbean Sea to the east coast of North America, the Pacific Ocean and the Mediterranean Sea (Gibson 1979a; Hiromi *et al*. 1985; Garate-Lizarraga & Muñeton-Gomez 2009).

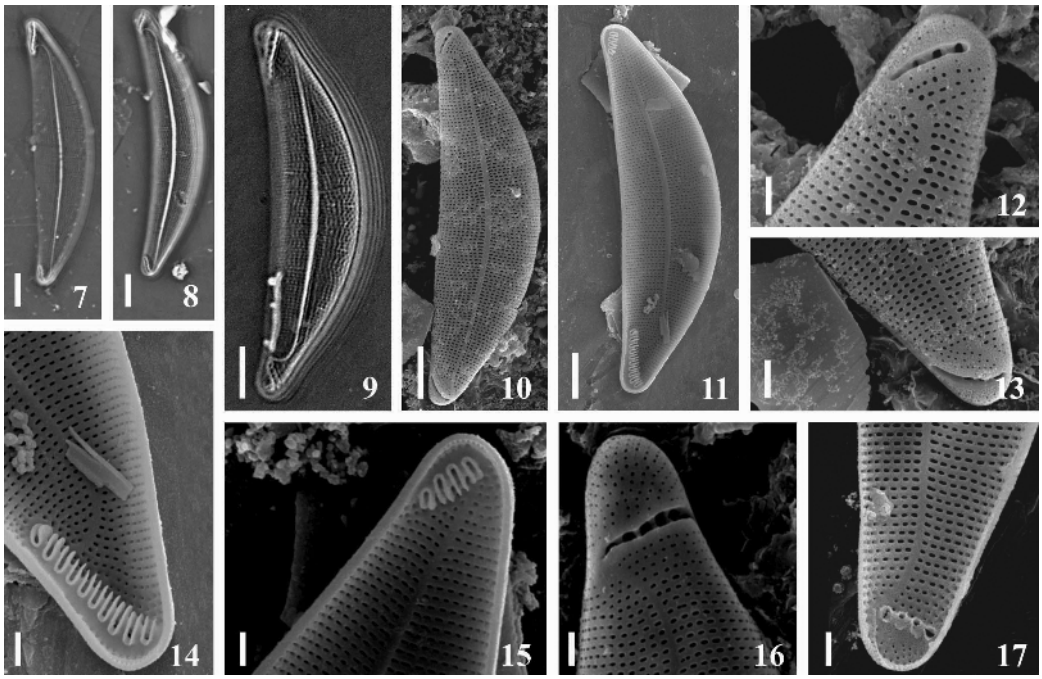
The other species found in this study, *Falcula hyalina*, had been reported only for the Pacific Ocean (Japan and Western Australia) and the North Atlantic Ocean (Gulf of Maine, Florida) (Takano 1983; Hiromi *et al*. 1985; Prasad *et al*. 1989). Regarding its distribution in Brazil, *F. hyalina* was previously known only from the Bay of Tijucas in the state of Santa Catarina (Souza-Mosimann *et al*. 1989) and the Lagoon of Peixe (31°00'46"S, 51°09'51"W) in the state of Rio Grande do Sul (Donadel & Torgan 2010), the latter corresponding to the most austral report of *F. hyalina* in the Atlantic Ocean. In both of these places, the authors reported *F. hyalina* living on the copepod *Acartia lilljeborgii* Giesbrecht. In our material, both species were found in all the samples examined from the Brazilian coastline, encompassing a wide variety of environments, such as estuaries, bays and coastal waters, thus suggesting that their geographic distribution might be wider than previously recorded.



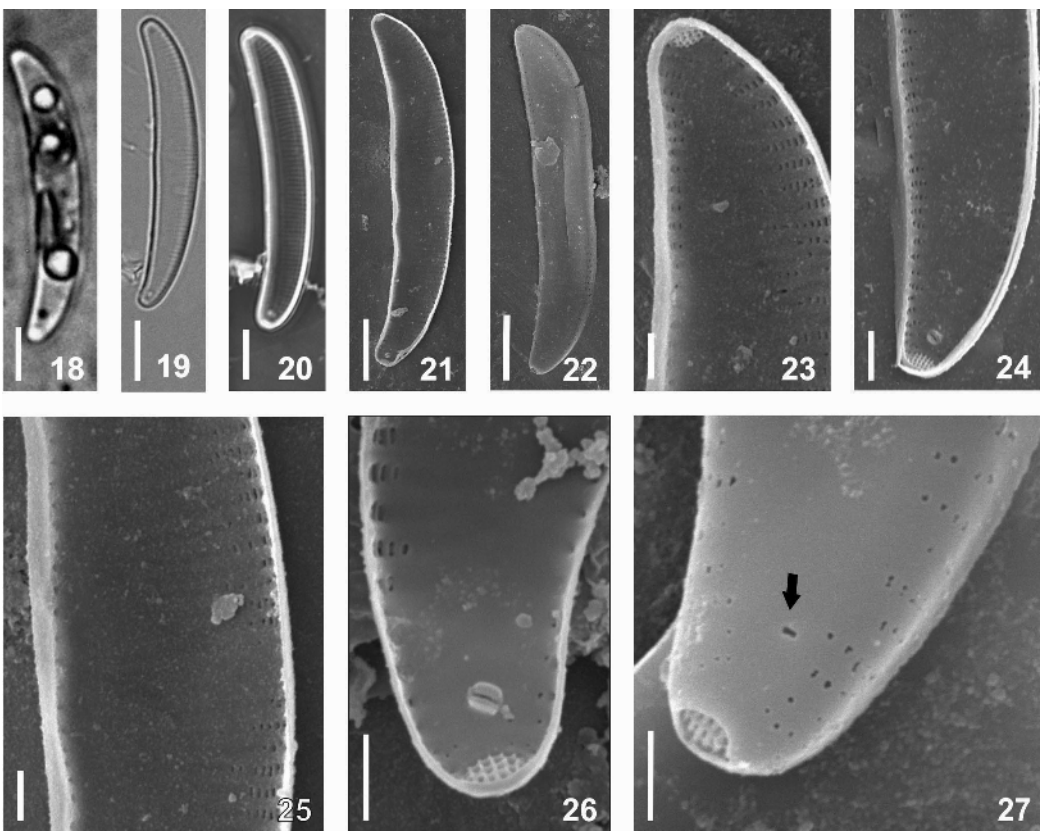
Figures 1-6. *Pseudohimantidium pacificum* Hustedt & Krasske, living cells; 1-3. Colonies virtually covering the exoskeleton of the copepod *Corycaeus* sp., LM; 4-5. Detail of cells with long mucilage stalks; 6. Arcuate cells with plastids. Scale bar: Fig. 1-3, 5: 50 µm; Fig. 4, 6: 20 µm.

Regarding the material gathered from Paranaguá Bay, Paraná, where the copepods and other crustaceans were identified to the species level, the epizoic diatoms presented some degree of preference in relation to the copepod hosts. *Pseudohimantidium pacificum* was on *Corycaeus amazonicus* and *Euterpina acutifrons*, confirming what has been reported in the literature already. However, *Falcula hyalina* was growing on a higher number of hosts, such as *Acartia lilljeborgii*, *Acartia tonsa*, *Oithona oswaldocruzii* and *Pseudodyaptomus richardi*. From these copepods, only *A. tonsa* had been recorded as a host for *F. hyalina*; thus, our findings add three new hosts to the list of Hiromi *et al.* (1985). To date, the copepods mentioned above correspond to the most abundant species of the year-round zooplankton community of Paranaguá bay and adjacencies (Lopes *et al.*, 1998).

Biometrical data of *P. pacificum* from the Brazilian material agree well with the original (Krasske 1941) and other later publications (Simonsen 1970; Gibson 1978; Rivera *et al.* 1986). To date, in Krasske's (1941) original material the apical axis varied from 44 µm to 78 µm, while the transapical axis was 9-11 µm. However, the number of striae (30-32 in 10 µm) was lower than in Brazilian specimens (30-40 in 10 µm). Furthermore, Rivera *et al.* (1986) found a larger range for the transapical axis, 9.8 µm to 19.0 µm, and the number of striae as low as 24 in 10 µm. Several discrete morphological differences were found in the Brazilian specimens compared with what has been published in the literature. For instance, there were 4-10 rimoportulae in the valves from Brazil, while other authors recorded a maximum of nine (Russel & Norris 1971; Rivera *et al.* 1986). Moreover, some valves of our material presented the external opening



Figures 7-17. *Pseudohimantidium pacificum* Hustedt & Krasske, cleaned valves. 6-9. General views of valves in LM. 10. External view; 11. Internal view; 12-13. External view of rimoportulae fissures and curved sternum; 14-15. Internal view of valve, illustrating rimoportula lying near the ventral side; 16. External view of valve having rimoportula fissures almost perpendicular to the ventral side; 17. Internal view. Rimoportulae are aligned perpendicular to the ventral side. Microscopy: 7-9: LM; 10-17: SEM. Scale bar: 7-11: 5 μm; 12-17: 1 μm.



Figures 18-27. *Falcula hyalina* Takano. 18-20, LM; 21-27; SEM. 18. Living cell. 19-20. Valves in bright field (19) and phase contrast (20). Striae and rimoportulae are readily visible; 21. Internal view; 22. External view; 23. Internal view of apex showing two sectors of striae separated by enlarged sternum; 24. Internal view. Note rimmed labiate fissure of rimoportula and ocellulimbus; 25. Striae pattern in the middle of the valve; 26. Internal view of valve detailing the rimoportula and ocellulimbus; 27. External view of apex with the ocellulimbus and elongated aperture of rimoportula (arrow). Scale bar: 18-22: 5 μm; 23-27: 1 μm.

of rimoportulae almost orthogonal to the ventral margin instead of being parallel as usually observed elsewhere.

The valves of *Falcula hyalina* examined in this work fit well into the dimensions published for other regions (Gibson 1978; Hiromi *et al*. 1985; Prasad *et al*. 1989). Measurements reported in the original description of Takano (1983) are 20-38 µm apical axis, 3.9-5.5 µm transapical axis and 20-23 striae in 10 µm. Regarding the earlier reports from Brazil, our material has similar dimensions to the specimens investigated by Souza-Mosimann *et al*. (1989) and Donadel & Torgan (2010) from Southern Brazil.

Acknowledgements

The Centre of Electron Microscopy housed at the Federal University of Paraná state made the equipments available. This work was supported by the National Research Council (CNPq/MCT PROTAX, n. 562151/2010-9) and the National Fund for the Environment/Brazilian Environmental Ministry under the contract CVI 008/2002 (ALARME Project).

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