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southern Brazil**

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## Morphology and ecology of the planktonic diatom *Palmerina hardmaniana* (Greville) Hasle in southern Brazil

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**Abstract:** The diatom *Palmerina hardmaniana* (Greville) Hasle presents a wide geographical distribution in neritic tropical and subtropical regions. In the present work we analyzed plankton samples collected monthly between 1995 and 2007 at the surf zone of Cassino Beach, RS (32° 12' S and 52° 10' W), and in winter 2005 and summer 2007 at the continental shelf and slope in southern Brazil, Santa Marta Grande Cape, SC and Albardão-Chuí, RS regions (28° 23'–33° 07' S and 48° 41'–52° 26' W). We present the detailed morphological description of *P. hardmaniana*, and the first study including electron scanning microscope observations for material from the southwestern Atlantic Ocean. The morphometric data confirm the identity of the species in all its ultra-structural details. *Palmerina hardmaniana* was only observed in summer-autumn months with low cell density (< 500 cells.L<sup>-1</sup>) at both Cassino Beach surf zone and coastal shelf stations. The warm water temperature (18–29 °C) indicates the most probable origin of its inoculum are tropical/subtropical regions. Salinities of 23–36 and the relatively high silicate content indicate the importance of the terrestrial discharge during occasions when *P. hardmaniana* was observed, probably with influence on the nutrient availability. We emphasize that the species was not cited previously for Argentinean and Uruguayan waters and suggest that the southern Brazilian region is close to the southern geographical distribution limit of *Palmerina hardmaniana* in the southwestern Atlantic Ocean.

**Keywords:** phytoplankton, seasonal variation, biogeography, ultra-structure.

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**Resumo:** A diatomácea *Palmerina hardmaniana* (Greville) Hasle apresenta ampla distribuição geográfica em águas neríticas tropicais e subtropicais. No presente trabalho foram analisadas amostras de plâncton, coletadas mensalmente entre 1995 e 2007 na zona de rebentação da Praia do Cassino, RS (32° 12' S e 52° 10' W), e no inverno de 2005 e verão de 2007 na plataforma continental e talude do sul do Brasil, na região de Cabo de Santa Marta Grande, SC e Albardão-Chuí, RS (28° 23'–33° 07' S e 48° 41'–52° 26' W). Apresentamos a descrição detalhada de *P. hardmaniana*, como primeiro estudo com observações de microscopia eletrônica de varredura para material coletado em águas do Oceano Atlântico Sul Ocidental. Os dados morfológicos analisados confirmam a identificação da espécie em todos os seus detalhes estruturais. *Palmerina hardmaniana* somente foi observada nos meses de verão/outono, em baixa densidade (< 500 células.L<sup>-1</sup>) na zona de arrebentação da Praia do Cassino bem como em estações costeiras da plataforma continental. A temperatura quente da água (18–29 °C), indica as águas tropicais/subtropicais como possível origem do inóculo de *P. hardmaniana* no verão-outono. A salinidade entre 23 e 36 e o teor relativamente alto de sílica também indicam a importância da descarga terrestre nas ocasiões de presença de *P. hardmaniana*, exercendo importante papel no suprimento de nutrientes. Salienta-se que a espécie não é citada em águas argentinas e uruguaias e assim, sugerimos que o extremo sul do Brasil representa aproximadamente o limite sul da distribuição geográfica de *Palmerina hardmaniana* no Oceano Atlântico Sul Ocidental.

**Palavras-chave:** fitoplâncton, *Palmerina*, ultraestrutura variação sazonal, biogeografia, indicador biológico.

## Introduction

*Palmerina* is a large marine planktonic diatom (300-650  $\mu\text{m}$ ) with the characteristic form of a sphere segment (hemi-discoid) and numerous plastids. Round et al. (1990) consider that the species is probably limited to the tropics and according to Simonsen (1974) it is neritic. It belongs to the family Coscinodiscaceae, characterized by the presence of several small, stalked rimoportulae around the valve face edge and two macrorimoportulae per valve. The main difference between *Palmerina* and *Coscinodiscus* is the morphology of the macrorimoportula with a smooth aperture in *Coscinodiscus* and crenulated in *Palmerina*. It is important to distinguish *Palmerina* from the also large and hemi-discoid genus *Hemidiscus*, which presents a pseudonodule characteristic of the family Hemidiscaceae.

The generic name *Palmerina* was suggested by Hasle (1995) as a substitute to *Palmeria* used by Greville in 1865 to describe the diatom, because the homonym *Palmeria* F. von Mueller was used in 1864 to describe an angiosperm of the family Monimiaceae. The study of the ultrastructure details of *Palmerina hardmaniana* Greville and *P. ostenfeldii* (Ostenfeld) Von Stosch revealed that both species are distinguished basically by characteristics such as the presence of a deep fold parallel to the dorsal edge of the valve face (which may be settled by a loricate hypotrich ciliate), the position of the hyaline central area and of the macrorimoportulae in relation to the ventral margin (Von Stosch 1986). *Palmerina hardmaniana* is considered a warm water species, while *P. ostenfeldii* was only observed in Australia and the Gulf of Thailand (Hasle & Syvertsen 1996). In the Atlantic Ocean, *Palmerina* is widely distributed in the north, next to the Orinoco River estuary and the Gulf of Mexico (Von Stosch 1986) and in the South Atlantic. In Northeastern Brazil, *P. hardmaniana* was registered in Ipojuca River (08° 25' S and 34° 58' W; Koening et al. 2002) and Piauí River, Sergipe State (11° 26' S and 37° 20' W; Franco 1991). Along the southern Brazilian coast, *P. hardmaniana* was cited in Paraná, Santa Catarina and Rio Grande do Sul states as *Palmeria hardmaniana* (Rosa, 1982, Dohms 1983, Souza Mosimann et al. 1989, Moreira-Filho et al. 1990, Procopiak et al. 2006). The most austral finding of *P. hardmaniana* was at the coastal area near the limit between Brazil and Uruguay (34° S and 53° W) Dohms (1983), and it has not been recorded further south in Uruguayan (Ferrando et al. 1964) and Argentinean waters (Vouiloud 2003). In the present study, we provide light and electron microscope illustrations of *P. hardmaniana* and report its presence in southern Brazilian coastal waters as a regular summer-fall species. We suggest this would be a good indicator species of climate changes in the Southwest Atlantic based on its geographical distribution limit at southern Brazilian waters.

## Material and Methods

Monthly phytoplankton samples were obtained since 1995 in the framework of the Ecological Long Term Program of Laboratory of Phytoplankton and Microorganisms Ecology at the surf zone of Cassino Beach in front of the Aquaculture Marine Station (EMA) of the Federal University of Rio Grande – FURG (32° 12' 21" S and 52° 10' 28" W). Surface phytoplankton was sampled with a bucket and preserved in 1% lugol solution, and net samples (20  $\mu\text{m}$  mesh aperture) were preserved in 4% buffered formaldehyde. Water samples ( $n = 156$ ) were counted with the use of an inverted microscope (Nikon or Zeiss Axiovert 135) and the Utermöhl technique (10 mL chambers) as described by Hasle (1978). The presence and morphology of *Palmerina hardmaniana* was investigated in 98 net samples from the period 1995 to 2007. The total number of analyzed net samples each season ranged between 27 from austral summer; 25 from autumn; 22 from winter and 24 from spring (see Table 1). In addition, 64 net samples from the continental shelf and slope off Santa Marta

Grande Cape (SC) and Albardão-Chuí (RS) (28° 23'-33° 07' S and 48° 41'-52° 26' W) were obtained during the oceanographic cruises conducted in winter 2005 and summer 2007 on board R.V. Atlântico Sul (PRONEX Project). Morphological analysis and measurements were performed on permanent slides made with aliquots of clean oxidized material (Simonsen 1974), dried on cover slips and mounted on glass slides with Hyrax, using Zeiss Axiovert 135 optical microscope equipped with Nomarski interference contrast and the scanning electron microscopy (SEM). For SEM, cleaned valves and frustules were dried on 16 mm diameter cover slip previously fixed on an aluminum stub, coated with gold at 1 kV for 4 minute, and examined with JEOL JSM6060 at an accelerating voltage of 15-20 kV. The working distance was 10 mm.

At the Cassino Beach surf-zone, the water temperature and salinity were measured with a mercury thermometer and Yellow Spring conductivity meter, respectively, and the dissolved inorganic nutrients (nitrate, nitrite, ammonia, phosphate, and silicate) were measured following standard methods (Strickland & Parsons 1972). Water samples for chlorophyll *a* analysis from all stations were filtered with Whatman GF/F filters and measured with a calibrated Turner fluorometer following the method of Welschmeyer (1994). Temperature and salinity data from the oceanographic cruises were kindly provided by Dr. Osmar Möller Jr. and corresponding nutrient data were obtained from Attisano (2007).

## Results

Morfology of *Palmerina hardmaniana* (Figures 1-16) *Palmerina hardmaniana* (Greville) Hasle, *Diatom Research*, 10: 357. 1995.

*Palmeria hardmaniana* Greville, *Annals and Magazine of Natural History*, ser. 3, 16: 2, Figures 1-4. 1865.

In this study, 16 and 12 valves were analyzed at light and scanning electron microscopy, respectively. The size of the studied frustules varied from 369 to 388  $\mu\text{m}$  in length and from 195 to 207  $\mu\text{m}$  in width. The frustule of *Palmerina* is similar in shape to a sector of a sphere (Figure 1), and its valve is semicircular, with the ventral margin rectilinear and the dorsal margin concave (Figure 2). The apices are broadly rounded (Figure 3). The valve face is almost plain and the central hyaline area is slightly elevated above the general level of the valve (Figure 5). The mantle is narrow along the rectilinear ventral margin and apices, and it is very wide along the convex margin (Figures 5, 9). The striae, formed by loculate areolae (Figure 8) easily observable in light microscopy (Figure 4), are radiated from a central hyaline area as well as hyaline lines (Figure 2). These lines are linear, except next to the apices when they curve slightly (Figures 3, 10, 12) and run all the valve face until the proximity of the valve face edge, where the microrimoportulae are located (Figures 3, 4). Several microrimoportulae are located on the valve face, close to and along the dorsal and ventral margins (7-10 areolae away) (Figures 4, 6, 7, 10-12, 14). The microrimoportulae along the ventral margin are in number of 7 to 13 in 10  $\mu\text{m}$  (Figures 4, 10, 12, 14). Internally, the microrimoportulae have an internal tube, 1 to 1.4  $\mu\text{m}$  long (Figures 15, 16) and externally, the microrimoportulae have apertures slightly elevated on the valve face (Figures 6, 7). Each valve presents two macrorimoportulae located in line and in opposite sides of the concave margin (Figures 2, 12, 13). The macrorimoportulae have crenulated slit (Figures 12, 13).

## Environmental Characteristics

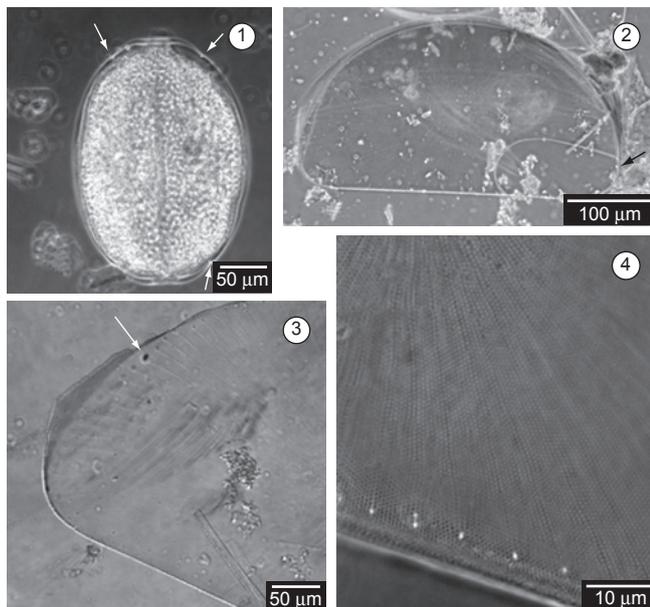
*Palmerina hardmaniana* was observed in low numbers in summer/fall samples from the Cassino Beach surf-zone. In quantitative beach samples ( $n = 156$ ), it was detected only twice (February 1995 and March 2007), in low density (200-400 cells.L<sup>-1</sup>). However, in qualitative beach

**Table 1.** Total number of net samples analyzed each month at Cassino Beach surf zone and corresponding years when *Palmerina hardmaniana* was present.

**Tabela 1.** Número total de amostras de rede analisadas em cada mês e os anos correspondentes nos quais foi observada a presença de *Palmerina hardmaniana* na zona de rebentação da Praia do Cassino.

Month	Number of years analyzed*	Years with <i>P. hardmaniana</i> presence
January	7	2000
February	11	1995, 1996, 1997, 2001, 2003
March	9	1997, 2006, 2007
April	8	2004
May	9	2002
June to December	54	-

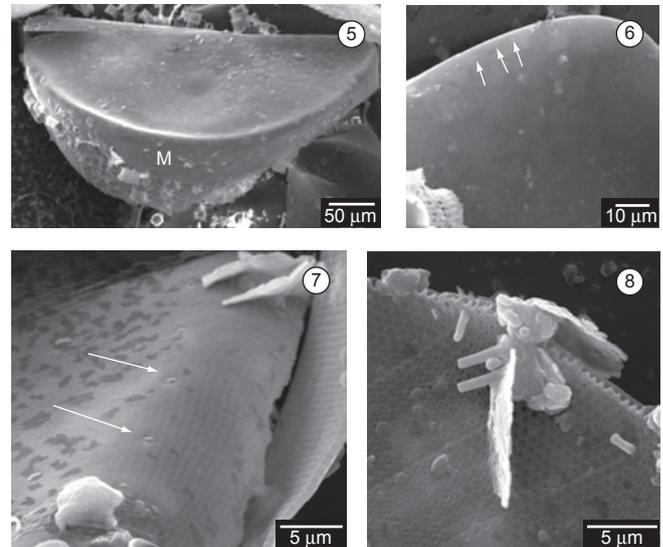
\*Number of samples investigated: Summer = 28; autumn = 25; winter = 22; spring = 29.



**Figures 1-4.** Light microscopy of *Palmerina hardmaniana*. 1) General view of a frustule (fresh material) showing several discoid plastids and macrorimoportulae on the margin (arrows). 2) General view of a frustule (acid clean material). Note the macrorimoportulae (arrow). 3) Partial valve view showing striae and hyaline lines in radial pattern on the valve face. The macrorimoportula is indicated with an arrow. and 4) Ventral margin with the microrimoportulae in line.

**Figura 1-4.** Microscopia ótica de *Palmerina hardmaniana*. 1) Visão geral de uma frústula (célula viva) com diversos cloroplastos e macrorimoportulae na margem (setas). 2) Visão geral de uma frústula (material limpo com ácido). Notar as macrorimoportulae (seta). 3) Vista valvar parcial mostrando estrias e linhas halinas em padrão radial na face da valva. A macrorimoportula está indicada com uma seta. e 4) Margem ventral com uma linha de microrimoportulae.

net samples ( $n = 98$ ), *P. hardmaniana* occurred more frequently, nine times in summer (January to March) and twice in fall months (April, May) of different years (Table 2). The presence of *P. hardmaniana* coincided with high water temperature (19-29 °C; mean 24.0 °C), and variable salinity (22-35; mean 30.6). Chlorophyll *a* concentration was



**Figures 5-8.** Scanning electron microscopy of *Palmerina hardmaniana*. 5) External view of a valve. Note the wide mantle (M) on the dorsal margin. 6) Detail of the valve face at its margin showing the microrimoportulae in line (arrows). 7) Detail of microrimoportulae externally (arrows). and 8) Detail of the wall.

**Figura 5-8.** Microscopia eletrônica de varredura de *Palmerina hardmaniana*. 5) Vista externa de uma valva. Notar o amplo manto (M) na margem dorsal. 6) Detalhe da face da valva mostrando em sua margem as microrimoportulae formando uma linha (setas). 7) Detalhe externo das microrimoportulae (setas). e 8) Detalhe da parede.

high ( $> 4 \mu\text{g.L}^{-1}$ ) when *P. hardmaniana* was present, except in February 2001 ( $2.1 \mu\text{g.L}^{-1}$ ). Nitrate ( $0.35\text{-}2.35 \mu\text{M}$ ) and phosphate concentrations ( $0.01\text{-}1.98 \mu\text{M}$ ) were usually low, but ammonium ( $0.36\text{-}9.40 \mu\text{M}$ ) and silicate concentrations ( $0.84\text{-}35.15 \mu\text{M}$ ) were more variable. The atomic ratios showed low values of N:P ( $< 10:1$ ) in six occasions, and extremely high values were observed twice (February 1997 and 2003) due to lowest phosphate concentration. The resulting N:Si values showed that silicate exceeded nitrogen concentration (N:Si  $< 1$ ) on eight occasions and that nitrogen exceeded the silicate concentration on three (Table 2).

In the southern Brazilian continental shelf, *Palmerina hardmaniana* was not detected in the winter cruise (August/September 2005) samples. In the summer cruise (February 2007), the diatom was observed in net samples at coastal stations off Santa Marta Grande Cape (SMGC) (stations 1, 11, 12) and the region Albardão-Chuí (A-C) (stations 20, 22, 23, 32) with water depth ranging between 20 and 54 m (Table 3). Mean water temperature was lower off SMGC ( $17.92\text{-}20.09$  °C) than in the southernmost area ( $20.32\text{-}22.16$  °C) while mean salinity was lower in the latter ( $33.15\text{-}33.98$ ) compared to SMGC ( $35.48\text{-}35.95$ ). Except ammonia, the concentration of nutrients was higher at A-C, and the N:P ratio was lower. The N:Si values showed that silicate always exceeded nitrogen concentration (N:Si  $< 1$ ). Chlorophyll concentration was rather similar in both areas (Table 3).

## Discussion

The dimension and general morphology of *Palmerina hardmaniana* frustules in LM and SEM are in agreement with the original description and details presented by von Stosch (1986) and Round et al. (1990). The plain valve face, hyaline central area located next to the valve centre, microrimoportulae positioned in line and close to the ventral margin and the macrorimoportulae with crenulate slit correspond to *P. hardmaniana* features. The external microrimoportulae

**Table 2.** Environmental parameters in the surf-zone of Cassino Beach at sampling days with presence of *Palmerina hardmaniana*. Mean and standard deviation in the last line.**Tabela 2.** Parâmetros ambientais na zona de rebentação da Praia do Cassino nos dias de coleta com presença de *Palmerina hardmaniana*. Média e desvio padrão nas últimas duas linhas.

Year/month	Temperature °C	Salinity	Chlorophyll µg.L <sup>-1</sup>	Nitrate µM	Ammonia µM	Phosphate µM	Silicate µM	N:P	N:Si
1995/02	24.0	28.0	6.5	0.35	0.36	0.52	35.15	1.37	0.02
1996/02	25.0	35.0	1.6	1.29	6.07	0.72	32.90	10.22	0.22
1997/02	24.0	31.0	12.7	1.02	9.40	0.01	7.00	> 100	1.49
1997/03	23.0	28.0		1.19	0.72	1.98	28.81	0.96	0.07
2000/01	26.0	31.0	4.3	0.75	5.28	0.42	1.53	14.36	3.94
2001/02	23.0	30.0	1.2	0.80	7.48	0.99	19.41	8.36	0.43
2002/05	19.0	22.0	4.3	2.35	1.19	0.44	11.02	8.05	0.32
2003/02	29.0	32.0	6.3	2.32	5.94	0.03	32.40	> 100	0.25
2004/04	24.0	35.0	21.2	0.46	2.12	0.16	0.84	16.13	3.07
2006/03	22.0	32.0	17.8	1.57	2.00	0.58	9.89	6.16	0.36
2007/03	25.0	33.0	5.0	1.53	1.10	0.73	10.21	2.61	0.28
Mean	24.0	30.6	8.1	1.24	3.79	0.60	17.20	7.58	0.95
(sd)	(2.5)	(3.7)	(6.8)	(0.67)	(3.14)	(0.55)	(13.03)	(5.43)	(1.34)

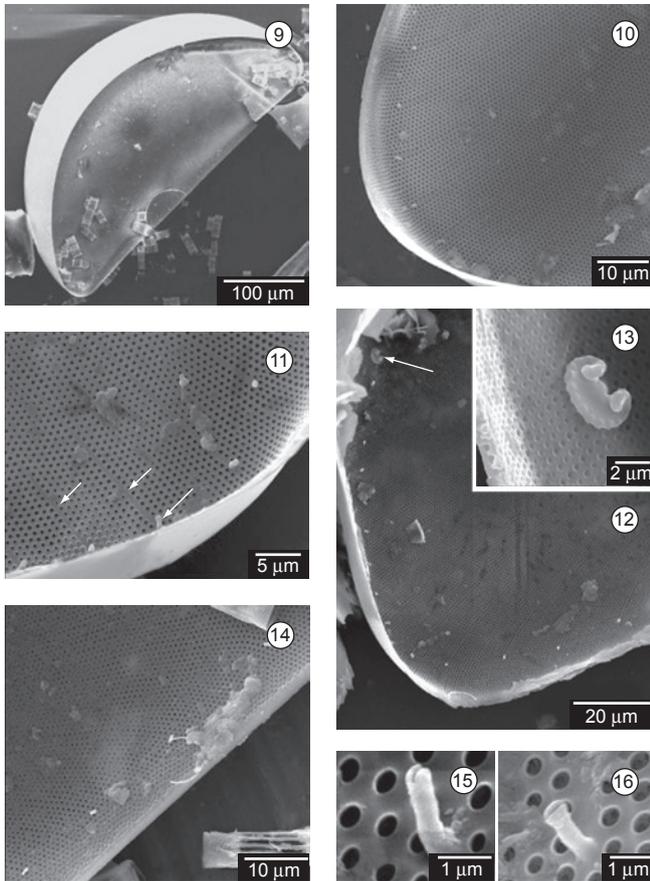
**Table 3.** Environmental parameters (mean values) at coastal stations off Santa Marta Grande Cape, SC (#1, #11, #12) and Albardão-Chuí, RS region (#20, #22, #23, #32), with presence of *Palmerina hardmaniana*. Station depth in parenthesis. Nutrient data from Attisano (2007).**Tabela 3.** Parâmetros ambientais (valores médios) das estações situadas na região do Cabo de Santa Marta Grande, SC (#1, #11, #12) e Albardão-Chuí, RS (#20, #22, #23, #32), com presença de *Palmerina hardmaniana*. Profundidade das estações entre parêntesis. Dados de nutrientes de Attisano (2007).

Station/mean depth	Temperature °C	Salinity	Chlorophyll µg.L <sup>-1</sup>	Nitrate µM	Ammonia µM	Phosphate µM	Silicate µM	N:P	N:Si
#1 (21 m)	17.92	35.95	3.0	1.17	5.64	0.54	11.02	13.00	0.62
#11 (32 m)	20.09	35.81	0.9	0.78	4.10	0.48	36.40	10.74	0.13
#12 (54 m)	17.95	35.48	0.9	1.00	3.83	0.47	17.02	10.94	0.28
#20 (24 m)	21.82	33.15	0.7	4.24	1.99	1.07	35.92	6.37	0.17
#22 (20 m)	22.16	33.98	1.4	2.86	2.23	0.65	51.38	7.36	0.10
#23 (34 m)	20.81	33.83	1.2	2.96	2.40	0.99	23.91	6.04	0.22
#32 (38 m)	20.32	33.81	1.6	4.82	2.46	0.95	34.90	8.49	0.21

opening with slightly elevated apertures on the valve face is a new observation to *P. hardmaniana*.

The presence of *P. hardmaniana* in the southern Brazilian beach and coastal waters was restricted to summer and fall periods, which indicates a strongly seasonal occurrence in this geographical area. Although the occurrence frequency of *P. hardmaniana* was low in the surf zone of Cassino Beach (11.2%; 11 out of 98 analyzed net samples), its presence was quite predictable, as it was only observed in summer/fall samples (January to May) in ten out of the eleven studied years, i.e., in all studied years except 2005. On the continental shelf, it was also observed in summer and not in winter samples. *P. hardmaniana* was previously reported in the neritic region in the fall 1976 (30° 01' S and 50° 05' W; 21 °C; Rosa 1982), and southwards near the Brazil and Uruguay border in summer 1981 (32-34° S and 53° W; 22.5 °C; Dohms 1983). The seasonal cycle of hydrography on the continental shelf and slope waters in southern Brazil is influenced by the geographical positioning (32° S to 34° S) of the oceanographic front between Subantarctic Shelf Water and Subtropical Shelf Water, and the discharges of La Plata River and Patos Lagoon, which generally peak in late winter/spring (Piola et al. 2000). In

summer/fall periods, the influence of tropical/subtropical waters is evident in southern Brazilian neritic waters and warm water phytoplankton species as *P. hardmaniana* extend nearshore (Dohms 1983), together with zooplankton species characteristic of Brazil Current (Montú et al. 1997). The phytoplankton composition at Cassino Beach surf-zone also changes significantly in summer, influenced by the proximity of Subtropical Shelf Water. In our study, *P. hardmaniana* was restricted to coastal stations and tolerant to a relatively large salinity range (22-36), like in the northeastern Brazilian Ipojuca estuary (Koenig et al. 2002). In Vietnamese waters, this species was observed at the thermocline depth with high chlorophyll concentration, and not at surface (Boonyapiwar 2000), indicating it was taking advantage of higher concentration of subsurface nutrients. The low nutrient concentrations at the Cassino Beach surf zone and resulting N:P ratios probably contributed to the low cell numbers of *P. hardmaniana*. In addition, the physical characteristics of the surf-zone, like turbulence generated by waves, may also hamper the growth of this large diatom species and favor high abundances of the rather small-sized cell *Asterionellopsis glacialis* (Odebrecht et al. 1995, Garcia & Gianuca 1997, Rörig et al. 2006).



**Figures 9-16.** Scanning electron microscopy of *Palmerina hardmaniana*. 9) Internal general view of a valve. Note the wide mantle along the convex margin. 10) Detail of the apex. 11) Detail of the apex showing the hyaline lines and microrimoportulae (arrows). 12) Internal view of the apex showing the macrorimoportula on the dorsal margin (arrow). 13) Detail of a macrorimoportula with crenulated aperture. 14) Detail of the ventral margin and microrimoportulae in line. and 15-16) Details of microrimoportulae.

**Figura 9-16.** Microscopia eletrônica de varredura de *Palmerina hardmaniana*. 9) Visão geral de uma valva em seu lado interno. Notar o amplo manto ao longo da margem convexa. 10) Detalhe do ápice. 11) Detalhe do ápice mostrando as linhas hialinas e microrimoportulae (setas). 12) Vista interna do ápice mostrando a macrorimoportula na margem dorsal (seta). 13) Detalhe de uma macrorimoportula com abertura crenulada. 14) Detalhe da margem ventral com as microrimoportulae em linha. e 15-16) Detalhes das microrimoportulae.

*Palmerina hardmaniana* is widely distributed in tropical and subtropical areas. In southern Brazil, the lowest temperature coinciding with the presence of *P. hardmaniana* in fall was 21 °C (Rosa 1982) and 17.92 °C (present study, Santa Marta Grande Cape). The absence of *Palmerina* from Argentinean and Uruguayan waters (Ferrando et al. 1964, Vouiloud 2003, Ferrari & Vidal 2006) indicates the southernmost region of Brazil is close to or even represents the distribution limit of the species in the Southwestern Atlantic Ocean.

We suggest that *P. hardmaniana* would be a good phytoplankton indicator of climatic changes in the southwestern Atlantic, due to the following characteristics: it is a large species, easy to identify in net samples, its presence is seasonal with a high occurrence probability each year, i.e., in the summer/fall and temperature >18 °C, and its southern geographical distribution limit in neritic/coastal water is at present, close to latitude 34 °S.

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