

Morphology and distribution of three araphid diatoms (*Fragilariophyceae*, *Bacillariophyta*) from marine coastal waters of Argentina

Inés Sunesen^{1,2}, Sofía Romero¹, Ernesto Toubes¹ & Eugenia Alicia Sar^{1,2}

¹ Universidad Nacional de La Plata, Facultad de Ciencias Naturales y Museo, División Ficología Dr. Sebastián A. Guarrera, Paseo del Bosque s/n, 1900 La Plata, Argentina. isunesen@fcnym.unlp.edu.ar

² Consejo Nacional de Investigaciones Científicas y Técnicas.

Recebido em 01.X.2014. Aceito em 18.X.2015.

ABSTRACT – This study deals with the morphology, taxonomy and distribution of three species of araphid marine pennate diatoms belonging to the Class *Fragilariophyceae*, Subclass *Fragilariophycidae*. Material was collected in marine coastal waters from the provinces of Buenos Aires and Río Negro (Argentina) and analyzed using light and scanning electron microscopy. *Pravifusus inane* (Giffen) Garcia and *Pteroncola inane* (Giffen) Round, were found in the water column, and *Pseudohimantidium pacificum* Hustedt & Krasske was recorded as epizoic on planktonic copepods. The three genera are cited for the first time in Argentina, and *Pteroncola inane* for first time in the South Atlantic Ocean. The distribution of *Pravifusus inane* and *Pseudohimantidium pacificum* is extended southward in the Southwest Atlantic Ocean, and the distribution of *Pteroncola inane* is extended to the Southwest Atlantic Ocean. The order *Protoraphidales* Round was validated by providing a taxonomic characterization, as well as indicating the generitype.

Key-words: araphid diatoms, *Pravifusus*, *Pseudohimantidium*, *Pteroncola*

RESUMO – **Morfologia e distribuição de três diatomáceas arrafídeas (*Fragilariophyceae*) de águas costeiras marinhas da Argentina.** O objetivo deste estudo está focado em análises morfológicas, taxonômicas e de distribuição de espécies de três gêneros de diatomáceas pertencentes à Classe *Fragilariophyceae*, Subclasse *Fragilariophycidae*. O material foi coletado em águas costeiras marinhas das províncias de Buenos Aires e Río Negro (Argentina) e analisados por meio de microscopia óptica e eletrônica de varredura. *Pravifusus inane* (Giffen) Garcia e *Pteroncola inane* (Giffen) Round foram encontradas na coluna d'água, enquanto *Pseudohimantidium pacificum* Hustedt & Krasske, foi registrada como epizoica em copépodos planctônicos. Os três gêneros são citados pela primeira vez para a Argentina e *Pteroncola inane* pela primeira vez para o Oceano Atlântico Sul Ocidental. A distribuição de *Pravifusus inane* e *Pseudohimantidium pacificum* é estendida para ao sul do Oceano Atlântico Sul Ocidental e a distribuição de *Pteroncola inane* é estendida para o Oceano Atlântico Sul Ocidental. A Ordem *Protoraphidales* Round foi validada fornecendo uma caracterização taxonômica, bem como indicando o gênero tipo.

Palavras-chaves: diatomáceas arrafídeas, *Pravifusus*, *Pseudohimantidium*, *Pteroncola*

INTRODUCTION

The *Fragilariophyceae* Round is characterized by possessing a sternum and a pennate arrangement of striae, and commonly benthonic living as epizoic, epiphytic, epilithic, epipsammic, etc. (Round *et al.* 1990). Kooistra *et al.* (2008) included two striking features for characterizing the group, the presence

of fields of pores (or slits) and of rimoportulae near the valve apices. Kooistra *et al.* (2003), Medlin & Kaczmarek (2004) and Williams & Kociolek (2007), among other authors, established that araphid diatoms do not constitute a monophyletic group. Medlin & Sato (2009) recognized two groups into the araphid diatoms, the 'core araphid' and the 'basal araphid', which were not formally described

and have been comprehensively questioned by Williams & Kociolek (2010). Taking into account the uncertainties of ongoing phylogenetic evidence we follow Round *et al.* (1990) for practical reasons. Nevertheless, we consider the boundaries of the Genera, Families and Orders should be more precisely circumscribed.

In Argentina there are a few ultrastructural studies dealing with araphid diatoms, usually limited to taxa occasionally found in the phytoplankton of shallow coastal waters from Provinces of Buenos Aires and Río Negro (Sar & Ferrario 1990, Sar 1996, Sar & Sunesen 2003, 2014, Sar *et al.* 2007, Sunesen & Sar 2007).

This work presents the study of three araphid diatom species belonging to genera not previously recorded in Argentina: *Pravifusus* Witkowski, Lange-Bertalot & Metzeltin, *Pteroncola* Holmes & Croll and *Pseudohimantidium* Hustedt & Krasske. The three genera contain a few species each, and are restricted to certain environments.

Pravifusus was described by Witkowski *et al.* (2000) based on *P. hyalinus* Witkowski, Lange-Bertalot & Metzeltin. Later on, *P. inane* (Giffen) Garcia (2005) and *P. brasiliensis* Garcia (2011) were added to the genus.

Pteroncola was erected by Holmes & Croll (1984) based on *P. marina* Holmes & Croll. Subsequently, Round *et al.* (1990) based on the analysis of the type material of *Dimerogramma inane* Giffen (1970) established it is conspecific with *P. marina* and proposed Giffen's epithet as the correct name of the taxon. Recently, *P. carlinii* Almandoz & Ferrario in Almandoz *et al.* (2014) was added to the genus.

Pseudohimantidium is a monotypic genus erected by Hustedt & Krasske in Krasske (1941) based on *P. pacificum* Hustedt & Krasske.

MATERIAL AND METHODS

The material from Argentina was collected bimonthly at several stations along the Northern coast of the Province of Buenos Aires from November 1994 to September 2000 and from March 2008 to March 2013 and at several stations in the Northern area of San Matías Gulf (Province of Río Negro) from April 1998 to April 2000 and from March 2006 to April 2007 (Fig. 1).

Samples were taken from the surface layer of the water column (between 0 and 5 m) with a plankton net 30 µm mesh size, and fixed with 4% formalin.

In the laboratory, copepods gathered in the plankton samples were analyzed with light microscopy (LM) and those with epizoic species were picked with micropipette using a Zeiss Axiovert 40 CFL inverted microscope. All the preserved samples were rinsed with distilled water using a centrifuge to remove salt and preservatives, and then the organic matter was oxidized according to Prygiel & Coste (2000). The cleaned material was mounted for light and scanning electron microscopy according to Ferrario *et al.* (1995). Permanent mounts were made with Naphrax resin (Brunel Microscopes, Chippenham, U.K.). Observations and photomicrographs were made with light microscopes Leica DM 2500 (phase contrast and differential interference contrast) and Zeiss Axiovert 40 CFL (phase contrast and Nomarski interference contrast) and scanning electron microscope Jeol JSM 6360 LV.

The 731 samples studied were deposited in the Herbarium of the División Ficología Dr. Sebastián A. Guarrera, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata under the numbers LPC 4250 to 4495, LPC 4550 to 4645, LPC 11001 to 11353 and LPC 13648 to 13685. Terminology follows Ross *et al.* (1979), Round *et al.* (1990), with some additions proposed by Gibson (1979).

RESULTS

Pravifusus inane (Giffen) Garcia, Diatom Research, 20: 277, figs. 1-15. 2005.

(Figs. 2–10)

References: Giffen (1975): 76, figs. 33–35 (as *Campylosira inane* Giffen).

Cells are asymmetric, dorsiventral and isopolar, with more or less produced slightly rostrate ends (Fig. 2). The valve is lunate, with almost straight to slightly convex ventral margin, 19–28 µm long, 3.2–3.5 µm wide (Figs. 3, 4), limited by a regular row of marginal spines, 18–28 in 10 µm (Figs. 2–4). Ratio length / width: 5.4–8.7. The valve surface is flat, lacking areolae throughout, with subtle, small submarginal granules (Figs. 5, 6 arrowheads), limited by a regular row of marginal spines in dorsal and ventral side. The valve mantle is almost straight, with a single ring of simple unoccluded pores irregularly distributed, 4–5 in 10 µm (Figs. 7–10 arrowheads). On each pole there is one small pore field with two poroids placed on a circular and flat platform (Figs. 5, 6, 8, 10, arrows).

Examined material: ARGENTINA, Province of

Río Negro, Banco Reparó, 23.III.1999 (LPC 4605); Las Grutas, 4.V.1999 (LPC 4609); El Sótano, 1.VI.1999 (LPC 4618); Las Grutas, 4.X.1999 (LPC 4626); El Sótano, 24.XI.1999 (LPC 4627); Las Grutas, 26.V.2006 (LPC 13664); Piedras Coloradas, 26.V.2006 (LPC 13665); Piedras Coloradas, 5.VIII.2006 (LPC 13671); Las Grutas, 1.IX.2006 (LPC 13674); Las Grutas, 19.IX.2006 (LPC 13676); Piedras Coloradas, 3.II.2007 (LPC 1368).

Remarks: According to Garcia (2011) the genus *Pravifusus* includes three species *P. inane*, *P. hyalinus* and *P. brasiliensis* Garcia. Specimens of *P. inane* from Argentina analyzed in this study agree with those analyzed by Garcia (2005) from Southern Brazil in lunate valve outline, flat and structureless valve surface, marginal row of spines, one ring of unoccluded pores at the valve mantle, and a small pore field with two poroids at each pole. The morphometric data of specimens from Argentina show subtle differences with those from Brazil analyzed by Garcia (2005) in spine density, higher in the former than in the latter, and length/width ratio, smaller in specimens in the former than in the latter. *Pravifusus inane* can be distinguished from *P. hyalinus* and *P. brasiliensis* by the morphology and position of the apical pore field, with two poroids and placed in a small flat platform between the valve surface and the valve mantle in the former, and with higher number of poroids placed in the valve mantle in the other species.

Distribution in Argentina: This species has been found sporadically, scarce to common, in phytoplankton samples from Banco Reparó, Las Grutas, Piedras Coloradas and El Sótano, Province of Río Negro, all year round.

Pteroncola inane (Giffen) Round in Round *et al.* The Diatoms, Cambridge University Press, London, p. 390, figs. a–i. 1990.

(Figs. 11–22)

References: Holmes & Croll (1982): 268, figs. 2–15 (as *Pteroncola marina* Holmes & Croll); Giffen (1970): 91, figs. 20–22 (as *Dimerogramma inane* Giffen); Álvarez-Blanco & Blanco (2014): 112, pl. 9, figs. 1–6, pl. 51, figs. 1–5.

Frustules are rectangular (Figs. 11, 12), 6.8–20.5 μm long, and 3.8–8 μm height, without resolvable striation in LM. The valve outline is elliptic in small specimens to linear-elliptical in large specimens (Figs. 13, 14, 17–20), 1.5–3.8 μm wide. The valve

surface is flat and curved in a deep mantle (Figs. 17–20), with a subtle, central, linear sternum extending from pole to pole externally. Transapical ribs are perpendicular to the sternum, evenly spaced, 70–78 in 10 μm , extending to the valve mantle edge (Figs. 17–19) except at the poles where a field of pores is placed (Figs. 16–19, 21, 22, arrows). Apical pore fields composed of three to five horizontal rows of aligned pores. Some transapical ribs are slightly wider at the valvar margin (Figs. 16, 19, white arrowheads). Striae are alveolate, alveoli open externally by a row of small areolae, 11–12 in 1 μm , sunken between the ribs (Figs. 16, 19, 21). Internally alveolus opens by small, elliptical foramina aligned in a row placed between the plain valve surface and the valve mantle (Fig. 22, double arrowhead). There is one rimoportula near each pole, inserted in alveoli close to the sternum, opening by a sub-circular to elliptical pore without external projection (Figs. 17, 19, 21, black arrowheads). Internally rimoportulae are placed in the plain area of the valve surface and are small and sessile (Figs. 20, 22, arrowheads). Cingulum with several, plain, open bands, with splits nearly 180° apart at poles. Valvocopula narrower than first and second copulae (Figs. 15, 16). First copula shows an advalvar row of pores (Fig. 16, black arrowheads) and a developed flange (pars interior) (Figs. 20–22).

Examined material: ARGENTINA, Province of Río Negro, Las Grutas, 5.IV.2006 (LPC 13651); Las Grutas, 25.V.2006 (LPC 13653); Las Grutas, 26.V.2006 (LPC 13664); Las Grutas, 31.VII.2006 (LPC 13668); Las Grutas, 19.IX.2006 (LPC 13678); Piedras Coloradas, 3.II.2007 (LPC 13681).

Remarks: Specimens of *Pteroncola inane* analyzed in this study are smaller in size and present higher striae density than those described by Holmes & Croll (1984, as *P. marina*); nevertheless both perfectly agree in fine morphology. *Pteroncola inane* clearly differs from *P. carlinii* (Almandoz *et al.* 2014) by: the valve outline (arcuate to linear-elliptical in the latter) and the external extension of the alveoli (they are long, from the central, subtle sternum to the valve margin in *P. inane* and short, from the central, broad, arcuate to elliptical sternum, not reaching the valve mantle in *P. carlinii*). In addition, both species present rimoportulae with similar external and internal morphology; however, there is one rimoportula near each apex in *P. inane* and only one near one of apex in *P. carlinii*. The apical pore fields of both species are similar in fine morphology; they are composed

by three to five rows of aligned porelli in *P. inane*, and by only three rows in *P. carlinii*.

Distribution in Argentina: This species has been found sporadically, scarce, in phytoplankton samples from Las Grutas and Piedras Coloradas, Province of Río Negro, all year round.

Pseudohimantidium pacificum Hustedt & Krasske in Krasske, Archiv für Hydrobiologie, 38: 272, pl. 5, fig. 8. 1941.

(Figs. 23–40)

References: Gibson (1979): 149, figs. 1–13; Rivera *et al.* (1986): 21, figs. 1–35; Round *et al.* (1990): 446, figs. a–g; Fernandes & Calixto-Feres (2012): 837, figs. 1–17.

Cell with numerous, elliptical chloroplasts (Fig. 24). The frustule is wedge-shaped, markedly dorsiventral and attached to marine copepods by mucilaginous stalks (Figs. 23–24). The valve outline is crescent shaped, 42–47 μm long, 8.5–10.5 μm wide, with the dorsal margin very curved and the ventral margin slightly curved to almost straight with subcuneate apices (Figs. 25–27). The valve surface is more or less convex and curves gently into the mantles; dorsal mantle is deeper than ventral mantle (Figs. 28, 30–32). The sternum at the one apex is hook-like shaped almost perpendicular to the row of rimoportulae (Figs. 31–33, 40), reaching the other apex nearly straight and almost parallel to the row of rimoportulae (Figs. 31, 32, 34–36, 39). The valve has striae punctate, sternum curved, asymmetric at poles and large pore rows at apices (Figs. 25–27, arrowheads). The striae are uniseriate, parallel at the centre and becoming radiate towards the poles, 30–35 in 10 μm . Areolae subrectangular throughout the valve surface, circa 3 in 1 μm , circular at the poles around the apical labiate grooves (Figs. 33–35). There is one field of slits at the margin in each pole (Figs. 34–37, arrowheads). The rimoportulae are aligned in an oblique row at each pole, internally open lipped, grouped to 4–8. The number of rimoportulae is either the same or different at both poles (Figs. 31, 32, 35, 36). Rimoportulae open externally by pores aligned in the apical labiate grooves (Figs. 28, 29, 33–35). Girdle is wider on the dorsal side (Figs. 37, 38) than in the ventral side (not showed), it is composed by several open bands (Fig. 30, arrowhead). The cingulum in dorsal side has a wide valvocopula and copulae decreasing in width in abvalvar direction (Fig. 38). All the bands present similar structure: one advalvar row of subcircular pores, a plain pars media and one abvalvar row of slit-like pores. The pars

media and the row of slit-like pores become shorter towards the distal side of the cingulum (Fig. 37).

Examined material: ARGENTINA, Province of Buenos Aires, Claromec , 11.III.2009 (LPC 11116); Mar Azul, 7.V.2010 (LPC 11209).

Remarks: The specimens examined during this study are similar in fine morphology and fit into the range of measurement presented by Fernandes & Calixto-Feres (2012), although the range of stria density is considerably wider in the material from the Brazil. Specimens showed by Rivera *et al.* (1986) from Chilean coastal waters are also similar to those presented here; however they are generally larger and less densely striate than those from Argentina and Brazil.

Distribution in Argentina: This species has been found sporadically, scarce, epizoic on the harpacticoid copepod *Euterpina acutifrons* collected with phytoplankton net from Mar Azul and Claromec , Province of Buenos Aires, in summer and early autumn.

DISCUSSION

The generic description of *Pravifusus* was emended by Garcia (2005) when the author transferred *Campylosira inane* to the genus *Pravifusus* as *P. inane*. The recent inclusion of a new species into the genus, *P. brasiliensis* Garcia (2011), enlarge the generic limit in reference to the morphology of the apical pore field placed at both poles between the valve surface and the mantle. It is formed by: two poroids on a platform as in *P. inane*, two slits with few poroids as in *P. hyalinus*, or four to five rows of poroids covered by external small plate-like projection as in *P. brasiliensis*. Specimens with linear-lanceolate valves of *Pravifusus* resembles three monotypic genera, *Hyaloneis* (Hustedt) Amspoker (2008), *Hyalinella* Witkowski, Lange-Bertalot & Metzeltin in Witkowski *et al.* (2000), and *Porannulus* Hamilton & Poulin in Hamilton *et al.* (1997) in having a structureless valve surface, and several open girdle bands. Nevertheless, *Pravifusus* has a marginal row of spines, which is absent in the other three genera. Garcia (2005) assigned *Pravifusus* to the Family *Fragilariaceae* Greville, Orden *Fragilariales* Silva emend. Round in Round *et al.* (1990).

The distribution of *Pravifusus inane* is currently limited to a few areas of the South Atlantic Ocean.

It was previously reported from Bahía Saldanha, South Africa, under the name *Campylosira inane* by Giffen (1975), related to brown masses of *Melosira nummuloides* filaments, and sandy beaches of Santa Catarina, Southern Brazil, by Garcia (2005) as a common psammic diatom. In this study we report the genus *Pravifusus* and *P. inane* for first time for Argentina and extends its distribution Southward in the Southwest Atlantic Ocean.

The generic description of *Pteroncola* was emended by Ferrario & Almandoz in Almandoz *et al.* (2014) when the authors included *P. carlinii* into the genus. *Pteroncola inane* was misidentified as *Fragilaria hyalina* (Kützing) Grunow by Sullivan (1979) and Poulin *et al.* (1984). The latter species was subsequently analyzed in a comprehensive paper (Hasle & Syvertsen 1981), in which the specimen showed by Sullivan (1979: fig. 2) in outside view was explicitly and erroneously included as previous SEM analysis. *Fragilaria hyalina* differs from *Pteroncola* species by presenting: weakly silicified marginal spines (absent in *Pteroncola*), uniseriate striae formed by a row of poroid areolae visible in internal and external views (alveolate striae opening internally by a small foramen in *Pteroncola*), apical pore fields with closely packed porelli arranged in a more or less fan shaped pattern (few horizontal rows of aligned pores in *Pteroncola*), and girdle bands areolate, with two rows of elongate areolae separated by a plain pars media (unperforated except in the case of the first copula which present an advalvar row of pores). Guslyakov *et al.* (1992) repeated the confusion transferring *Diatoma hyalinum* Kützing, better known as *Fragilaria hyalina*, to the genus *Pteroncola* as *P. hyalina* (Kützing) Guslyakov based on two different taxa. One of them, showed in SEM (Guslyakov *et al.* 1992: plate 23, figures 1–6) is conspecific with *Fragilaria hyalina* (Hasle & Syvertsen 1981, SEM figs. 30–35, TEM 36–44). The other taxon showed in TEM (Guslyakov *et al.* 1992: plate 24, figures 1–6) agrees with small specimens of *Pteroncola inane* in having alveolate striae, apical pore fields formed by a few horizontal rows of aligned pores. Guslyakov *et al.* (1992) in the remarks of *P. hyalina* mentioned that species often was confused with *Striatella delicatula* (Kützing) Grunow because of fine structure. According to Navarro & Williams (1991) *Hyalosira delicatula* Kützing is the correct name of *S. delicatula* (homotypic synonym) and the generitype of the resurrected genus *Hyalosira* Kützing. The Family *Pteroncolaceae* erected by Guslyakov in Guslyakov *et al.* (1992) includes

features of *Diatoma hyalinum*, taxon that clearly is not correctly assigned to *Pteroncola*. We followed Round *et al.* (1990) in including the genus in the Family *Fragilariaceae*, Order *Fragilariales*.

Considering that *Pteroncola inane* was previously misidentified, for practical reasons we only included illustrated references for analyzing its distribution. *P. inane* was considered as cosmopolitan by Witkowski *et al.* (2000), it was reported from Sea Point, near Cape Town, South Africa, under the name *Dimerogramma inane* by Giffen (1970) as epifitic on *Polysiphonia virgata* (C. Agardh) Sprengel; Mississippi Sound, Gulf of México, under the name *Fragilaria hyalina* by Sullivan (1979) as epiphytic on sea grasses; Monterrey Bay, U.S.A., under the name *Pteroncola marina* by Holmes & Croll (1984) as epizoic on diving seabirds; the Caspian Sea by Karayeva *et al.* (1991) on various type of substracts; Baltic Sea of Sweden by Snoeijs (1993) as epiphytic; coastal waters of Hokkaido, Japan by Nagumo & Tanaka (1994) as epiphytic on *Ptilota flicina* J. Agardh; the Mediterranean Sea, widespread by Witkowski *et al.* (2000), as epiphytic, epizoic and on the sediments; coastal waters of Hachijo Island, Izu Islands, Japan by Suzuki & Nagumo (2004) as epiphytic on *Asparagopsis taxiformis* (Delile) Trevisan de Saint-Léon; coastal waters of Trejin-jima, Japan by Fukuda *et al.* (2011) as epiphytic on *Zostera marina* Linnaeus; and the Mediterranean coast by Álvarez-Blanco & Blanco (2014) as benthonic. In this study we report the genus *Pteroncola* for first time for Argentina and *P. inane* for first time for South Atlantic Ocean extending its distribution to the Southwest Atlantic Ocean.

Simonsen (1970, 1974) included several taxa described after Krasske (1941) as heterotypic synonyms of *Pseudohimantidium pacificum* and pointed out that the species shows a great variability of frustular morphology. Simonsen (1970) included *P. pacificum* in his Family *Protoraphidaceae*, characterized by S-shaped sternum and a row of rimoportulae opening externally by pores aligned in the apical groove at each pole. Round *et al.* (1990) assigned this Family to the Order *Protoraphidales*; nevertheless, considering the Art. 38.2 Ex1 of the ICB (McNeill *et al.* 2012) the taxon has not been validly published because it was published without a description or a diagnosis or a reference to a former one (hence, a *nomen nudum*). We provided a description (see below) to validate Round's Order based on the description of the Family *Protoraphidaceae* Simonsen (1970) taking into account it is the only

Family included in the Order.

Pseudohimantidium pacificum was reported from Pacific Ocean in front of Chile (20° 39' S, 70° 20' W) by Krasske (1841) and from oceanic and neritic zones of the Southeastern Pacific Ocean at least as far as 38°S as epizoic on cyclopoid copepods *Corycaeus* sp. by González & Vergara (1984) and Rivera *et al.* (1986). Russel & Norris (1971) reported it under the name *Sameioneis carinaes* Russel & Norris as epizoic diatom living on planktonic copepods from Puget Sound, Washington State. Simonsen (1974) found the species on *Corycaeus* spp. from Atlantic Ocean, Pacific Ocean, Indian Ocean, Gulf of Guinea, Persian Gulf and the Mediterranean Sea. Gibson (1978) reported *P. pacificum* on cyclopoid copepods as *Corycaeus* spp. and *Farranula gracilis* Dana as well as on harpacticoid copepod *Euterpina acutifrons* Dana from the Florida Current waters of the Western North Atlantic Ocean. Skovgaard & Saiz (2006) found it on *Corycaeus* spp. from coastal waters of the Northwest Mediterranean Sea. Gárate-Lizárraga & Muñetón-Gómez (2009) on *Farranula gibbula* Giesbrecht from La Paz Bay, Gulf of California, Mexican Pacific and Fernandes & Calixto-Feres (2012) on *Corycaeus amazonicus* F. Dahl and *Euterpina acutifrons* from Brazilian coastline between 12°S to 28°S. Navarro (1982) quoted it from Cayo Enrique, Southwestern coast of Puerto Rico and Lee *et al.* (1993) from the Yellow Sea. In this study we report the genus *Pseudohimantidium* and *P. pacificum* for first time for Argentina and extend its distribution southward in the Southwest Atlantic Ocean.

Validation of the Order *Protoraphidales* Round ex Sar & Sunesen

Description

Cells solitary or grouped in colonies, attached to the substrate through mucilage stalks. Frustules rectangular, straight or curved in girdle view, or wedge-shaped. Valves linear, clavate or lunate, with punctate striae and sternum S-shaped. One row of rimoportulae at each apex, externally opened in a curved slit. Apical slit field placed at the polar valve mantle. Girdle composed by several open and punctate bands. Members epizoic on marine copepods.

The order accommodates one family (*Protoraphidaceae* Simonsen) and two genera (*Pseudohimantidium* Hustedt & Krasske and

Protoraphis Simonsen). Type of Order is Genus *Protoraphis* Simonsen (1970).

ACKNOWLEDGEMENTS

The authors wish to thank two anonymous reviewers for useful suggestions that help us to improve our manuscript. The research was supported by grants from the Universidad Nacional de La Plata 11/N640 and from the Consejo Nacional de Investigaciones Científicas y Técnicas, PIP0067.

REFERENCES

- Almandoz, G.O., Ferrario, M.E., Sullivan, M.J., Ector, L. & Schloss, I.R. 2014. A new *Pteroncola* species (*Bacillariophyceae*) from the South Shetland Islands, Antarctica. *Phycologia* 53:188-194.
- Álvarez-Blanco, I. & Blanco, S. 2014. Benthic diatoms from Mediterranean coast. *Bibliotheca Diatomologica* 60:1-409.
- Amspoker, M.C. 2008. Transfer of the marine diatom *Dimerogramma hyalinum* Hustedt to the new araphid genus *Hyaloneis*. *Diatom Research* 23:11-18.
- Fernandes, M.F. & Calixto-Feres, L. 2012. Morphology and distribution of two epizoic diatoms (*Bacillariophyta*) in Brazil. *Acta Botanica Brasilica* 26:836-841.
- Ferrario, M.E., Sar, E.A. & Sala, S.E. 1995. Metodología básica para el estudio del fitoplancton con especial referencia a las diatomeas. In *Manual de Métodos Ficológicos* (K. Alveal, M. E. Ferrario, E. C. Oliveira & E. Sar, eds.). Universidad de Concepción, Concepción, p. 1-23.
- Fukuda, K., Suzuki, H., Tanaka, J. & Nagumo, T. 2011. Epiphytic diatoms on leaves of seagrass *Zostera marina* Linnaeus Tenjin-jima, Kanaguawa Prefecture, Japan. *Bulletin of the Nippon Dental University General Education* 40:57-63.
- Gárate-Lizárraga, I. & Muñetón-Gómez, M.S. 2009. Primer registro de la diatomea epibionte *Pseudohimantidium pacificum* y de otras asociaciones simbióticas en el Golfo de California. *Acta Botánica Mexicana* 88:31-45.
- García, M. 2005. Araphid psamic diatoms from Brazilian sandy beaches I: an emended description to genus *Pravifusus* Witkowski, Lange-Bertalot & Metzeltin. *Diatom Research* 20:275-280.
- _____. 2011. Morphology and distribution of the diatom *Hyaloneis hyalinum* and a description of *Pravifusus brasiliensis* sp nov. *Diatom Research* 26:5-11.
- Gibson, R.A. 1978. *Pseudohimantidium pacificum*, an epizoic diatom new to the Florida Current (western North Atlantic Ocean). *Journal of Phycology* 14:371-373.

- _____. 1979. An ultrastructure study of *Pseudohimantidium pacificum* Hustedt & Krasske (*Bacillariophyceae*, *Protoraphidaceae*) with special reference to the labiate processes. In Proceedings of the Fourth Symposium on Recent and Fossil Marine Diatoms (R. Simonsen, ed.), Nova Hedwigia, Beiheft 64:147-156.
- Giffen, M.H. 1970. New and interesting marine and littoral diatoms from Sea Point, near Cape Town, South Africa. *Botanica Marina* 13:87-99.
- _____. 1975. An account of the Littoral Diatoms from Langebar, Saldanha Bay, Cape Province, South Africa. *Botanica Marina* 18:71-95.
- González, H. & Vergara, L. 1984. Interacción ecológica entre la diatomea epizoica *Pseudohimantidium pacificum* Hust. & Krasske, 1941 y copépodos del género *Corycaeus*. *Revista de Biología Marina, Valparaíso* 20(1):77-90.
- Guslyakov, N.E., Zakordonets, O.A. & Gerasimiyuk, V.P. 1992. Atlas diatomovykh vodoroslei bentosa severo-zapadnoi chasti Chernogo morya i prilegainschikh vodoemov. [Atlas of benthic diatoms North-Western part of the Black Sea and the adjacent water bodies]. *Naukova Dumka, Kiev*, 112 p.
- Hamilton, P.B., Poulin, M., Yang, J.-R. & Klöser, H. 1997. A new diatom genus, *Porannulus* (*Bacillariophyta*), associated with marine sponges around King George Island, South Shetland Islands, Antarctica. *Diatom Research* 12:229-242.
- Hasle, G.R. & Syvertsen, E.E. 1981. The marine diatoms *Fragilaria striatula* and *F. hyalina*. *Striae* 14:110-118.
- Holmes, R.W. & Croll, D.A. 1984. Initial observations on the composition of dense diatom growths on the body feathers of three species of diving seabirds. In Proceedings of the seventh International Diatom Symposium (D.G. Mann, ed.). Biopress Ltd, Bristol, p. 325-336.
- Karayeva, N.I., Dzhafarova, S.K. & Akhundova, N.A. 1991. Novye dlya SSSR rod i ridy Bacillariophyta iz Kaspiiskogo Morya. [Genera and species of Bacillariophyta from the Caspian Sea new for the USSR]. *Algologia* 1(2):66-70.
- Kooistra, W.H.C.F., De Stefano, M., Mann, D.G. & Medlin, L.K. 2003. The phylogeny of the Diatoms. In *Progress in molecular and subcellular biology* (W.E.G. Müller ed.). Springer, Berlin Heidelberg 33:59-97.
- Kooistra, W.H.C.F., Forlani, G. & De Stefano, M. 2008. Adaptations of araphid pennate diatoms to a planktonic existence. *Marine Ecology* 29:1-15.
- Krasske, G. 1941. Die Kieselalgen des chilenischen Küstenplanktons. *Archiv für Hydrobiologie* 38:260-287.
- Lee, J.H.; Lee, J.Y. & Kim, M.O. 1993. The fine structure of the marine epizoic pennate diatom *Pseudohimantidium pacificum* in Korean coastal waters. *Journal of the Oceanological Society of Korea* 28:202-211.
- McNeill, J., Barrie, F.R., Buck, W.R., Demoulin, V., Greuter, W., Hawksworth, D.L., Herendeen, P.S., Knapp, S., Marhold, K., Prado, J., Prud'homme Van Reine, W.F., Smith, G.F., Wiersema, J.H. & Turland, N.J. 2012. International Code of Nomenclature for algae, fungi, and plants (Melbourne Code). Adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011. *Regnum Vegetabile* 154, 240 p. Koeltz Scientific Books, Königstein.
- Medlin, L.K. & Kaczmarek, I. 2004. Evolution of the diatoms: V. Morphological and cytological support for the major clades and a taxonomic revision. *Phycologia* 43:245-270.
- Medlin, L.K. & Sato, S. 2009. The use of the terms centric and pennate. *Diatom Research* 24:503-508.
- Nagumo, T. & Tanaka, J. 1994. Epiphytic diatoms on a Red Alga, *Ptilota filicina* J. Ag., in Hokkaido, Japan. *Memoirs of the National Science Museum* 27:43-53.
- Navarro, J.N. 1982. A survey of the marine diatoms of Puerto Rico IV. Suborder Araphidineae: Families Diatomaceae and Protoraphidaceae. *Botanica Marina* 25:247-263.
- Navarro, J.N. & Williams, D.M. 1991. Description of *Hyalosira tropicalis* sp. nov. (*Bacillariophyta*) with notes on the status of *Hyalosira* Kützing and *Microtabella* Round. *Diatom Research* 6:327-336.
- Poulin, M., Bérard-Therriault, L. & Cardinal, M. 1984. Les diatomées benthiques de substrats durs des eaux marines et saumâtres du Québec 3. *Fragilarioideae* (*Fragilariales*, *Fragilariaceae*). *Le Naturaliste canadien* 111:349-367.
- Prygiel, J. & Coste, M. 2000. Guide Méthodologique pour la mise en oeuvre de l'Indice Biologique Diatomées. Agences de l'Eau, Ministère de l'Aménagement du Territoire et de l'Environnement, Direction de l'Eau & Centre d'étude du Machinisme Agricole du Génie Rural des Eaux et Forêts, France, 134 p.
- Rivera, P.S., González, H.E. & Barrales, H.L. 1986. Cingulum and valve morphology of *Pseudohimantidium* Hustedt & Krasske (*Bacillariophyceae*). *Phycologia* 25:19-27.
- Ross, R., Cox, E.J., Karayeva, N.I., Mann, D.G., Paddock, T.B.B., R. Simonsen, & Sims, P.A. 1979. An amended terminology for the siliceous components of the diatom cell. In Proceedings of the Fourth Symposium on Recent and Fossil Marine Diatoms (Simonsen, R., ed.), Proceedings of the Fourth Symposium on Recent and Fossil Marine Diatoms. Nova Hedwigia, Beiheft 64:513-533.

- Round, F. E., Crawford, R. & Mann, D. G. 1990. The Diatoms. Morphology and Biology of the Genera. Cambridge University Press, London, 747 p.
- Russell, D.J. & Norris, R.E. 1971. Ecology and taxonomy of an epizoic diatom. *Pacific Science* 25:357-367.
- Sar, E.A. 1996. Flora diatomológica de Bahía San Antonio (Prov. de Río Negro, Argentina). O. *Pennales* I. *Revista Mus. La Plata* (n. s.) 14, Botánica 107:399-432.
- Sar, E.A. & Ferrario, M.E. 1990. *Licmophora flabellata*: ultrastructure and taxonomy I. Implications. *Diatom Research* 5:403-408.
- Sar, E.A. & Sunesen, I. 2003. *Nanofrustulum shiloi* (Bacillariophyceae) from Gulf of San Matías (Argentina): morphology, distribution and comments about nomenclature. *Nova Hedwigia* 77:399-406.
- _____. 2014. *Sceptronema orientale* (Licmophoraceae, Licmophorales) epitypification and emendation of specific and generic descriptions. *Phytotaxa* 177 (5): 269-279.
- Sar, E.A., Sunesen, I. & Fernández, P.V. 2007. Marine Diatoms from Buenos Aires coastal waters (Argentina). II. *Thalassionemataceae* and *Rhaphoneidaceae*. *Revista Chilena de Historia Natural* 80:63-79.
- Simonsen, R. 1970. *Protoraphidaceae*: eine neue Familie der Diatomeen. *Nova Hedwigia*, Beiheft 31:383-394.
- _____. 1974. The diatom plankton of the Indian Ocean Expedition of R/V 'Meteor' 1964-1965. 'Meteor' Forschungsergebnisse Reihe D 19:1-107.
- Skovgaard, A. & Saiz, E. 2006. Seasonal occurrence and role of protistan parasites in coastal marine zooplankton. *Marine Ecology Progress Series* 327:37-49.
- Snoeijs, P. 1993. *Intercalibration and distribution of diatom species in the Baltic Sea*. v. 1. The Baltic Marine Biologists Publication 16 a. Opulus Press, Uppsala, 129 p.
- Sullivan, M.J. 1979. Taxonomic notes on epiphytic diatoms of Mississippi Sound, U.S.A. *Nova Hedwigia*, Beiheft 64:241-249.
- Sunesen, I. & Sar, E.A. 2007. Diatomeas marinas de aguas costeras de la Provincia de Buenos Aires (Argentina). III Géneros potencialmente nocivos *Asterionellopsis*, *Cerataulina*, *Ceratonopsis* y *Leptocylindrus*. *Revista Chilena de Historia Natural* 80:493-507.
- Suzuki, H. & Nagumo, T. 2004. Epiphytic diatoms on a red alga *Asparagopsis taxiformis* (Delile) Trevisan from Hachijo Island, Izu Islands, Japan. *Bulletin of the Nippon Dental University General Education* 33:61-71.
- Williams, D.M. & Kociolek, J.P. 2007. Pursuit of a natural classification of diatoms: History, monophyly and the rejection of paraphyletic taxa. *European Journal of Phycology* 42:313-319.
- _____. 2010. Classifications of convenience: the meaning of names. *Diatom Research* 25:213-216.
- Witkowski, A., Lange-Bertalot, H. & Metzeltin, D. 2000. Diatom flora of marine coasts I. *In* *Iconographia Diatomologica* (H. Lange-Bertalot, ed). Koeltz Scientific Books, Königstein, v.7, 925 p.

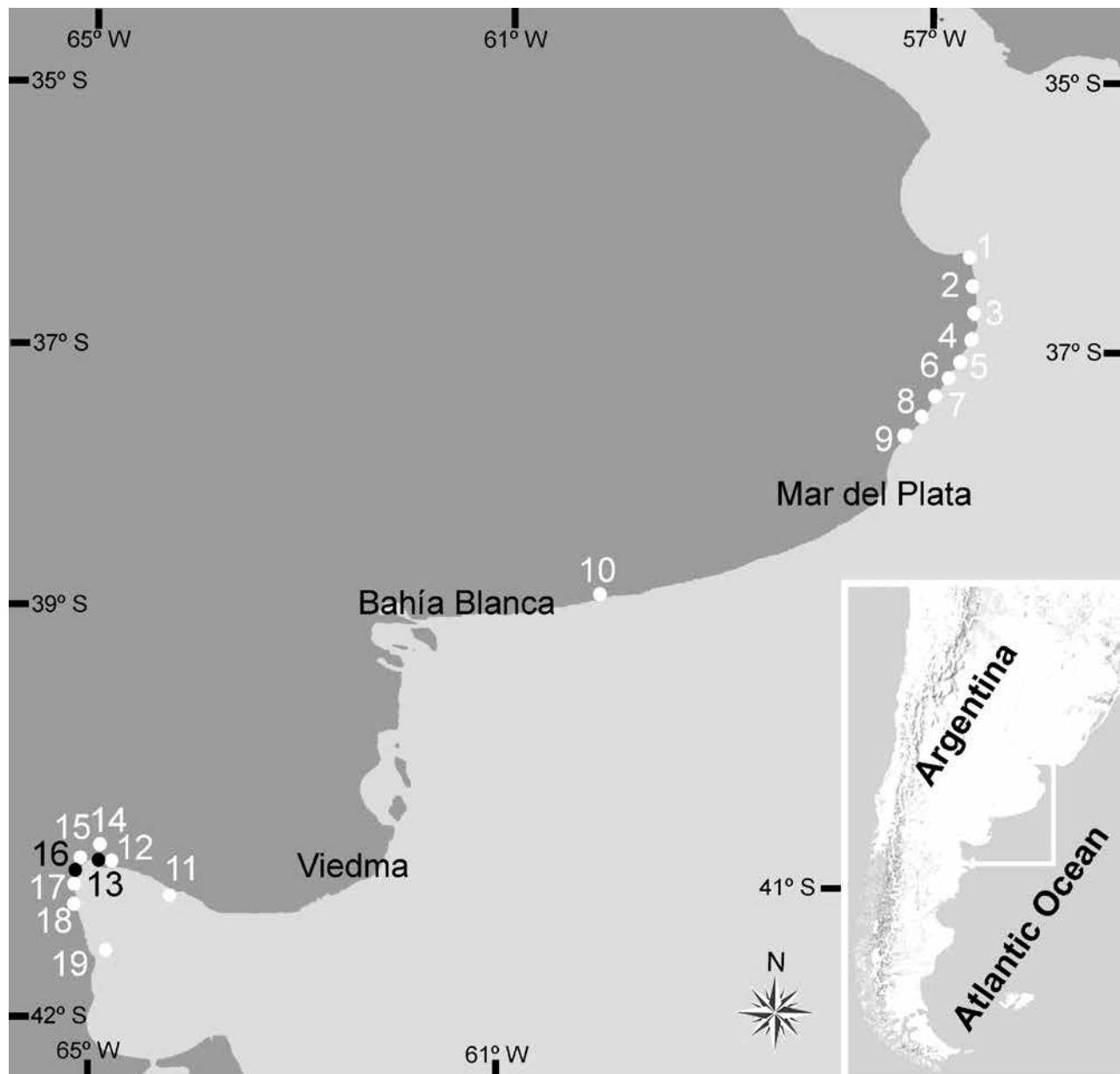
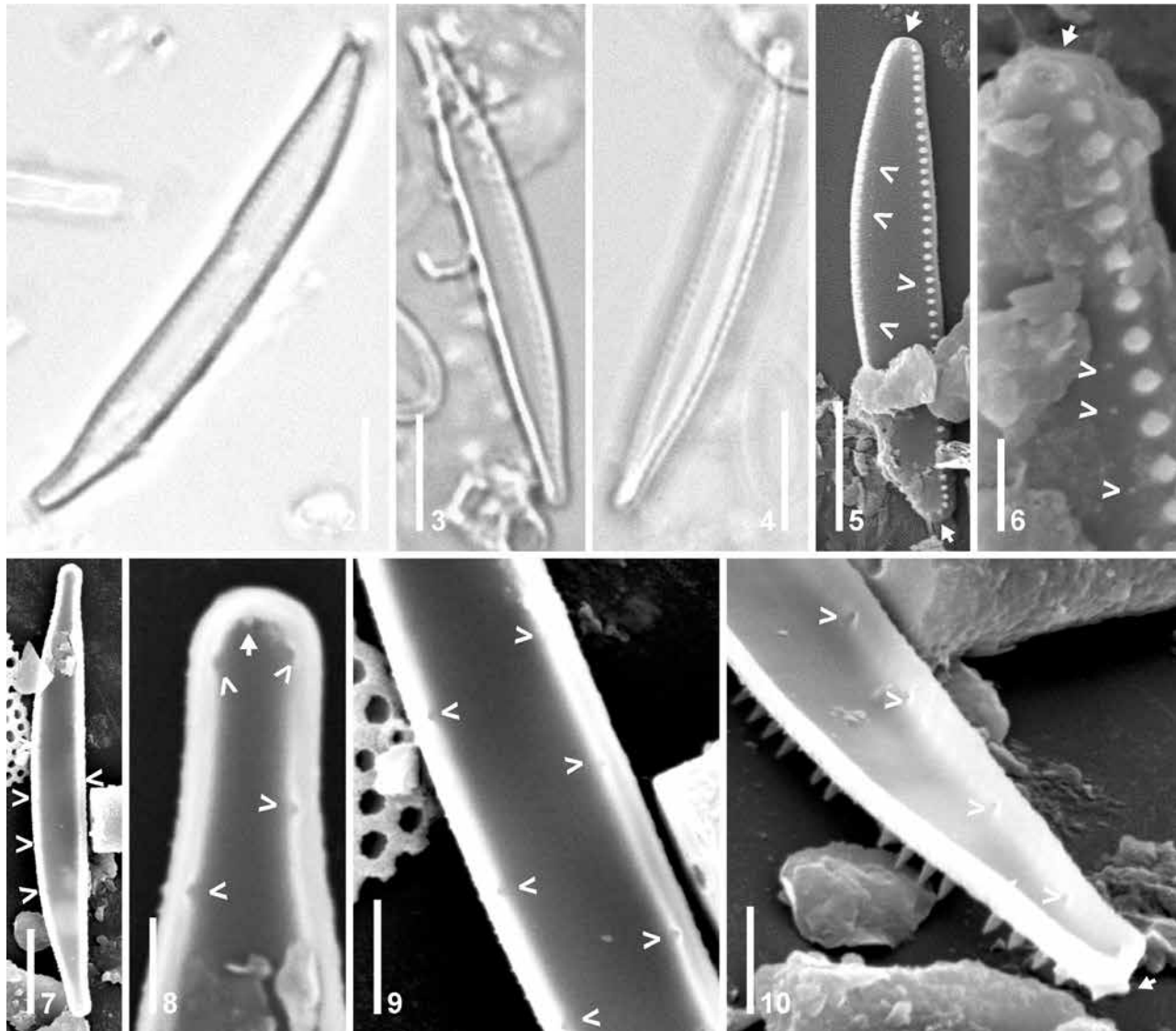
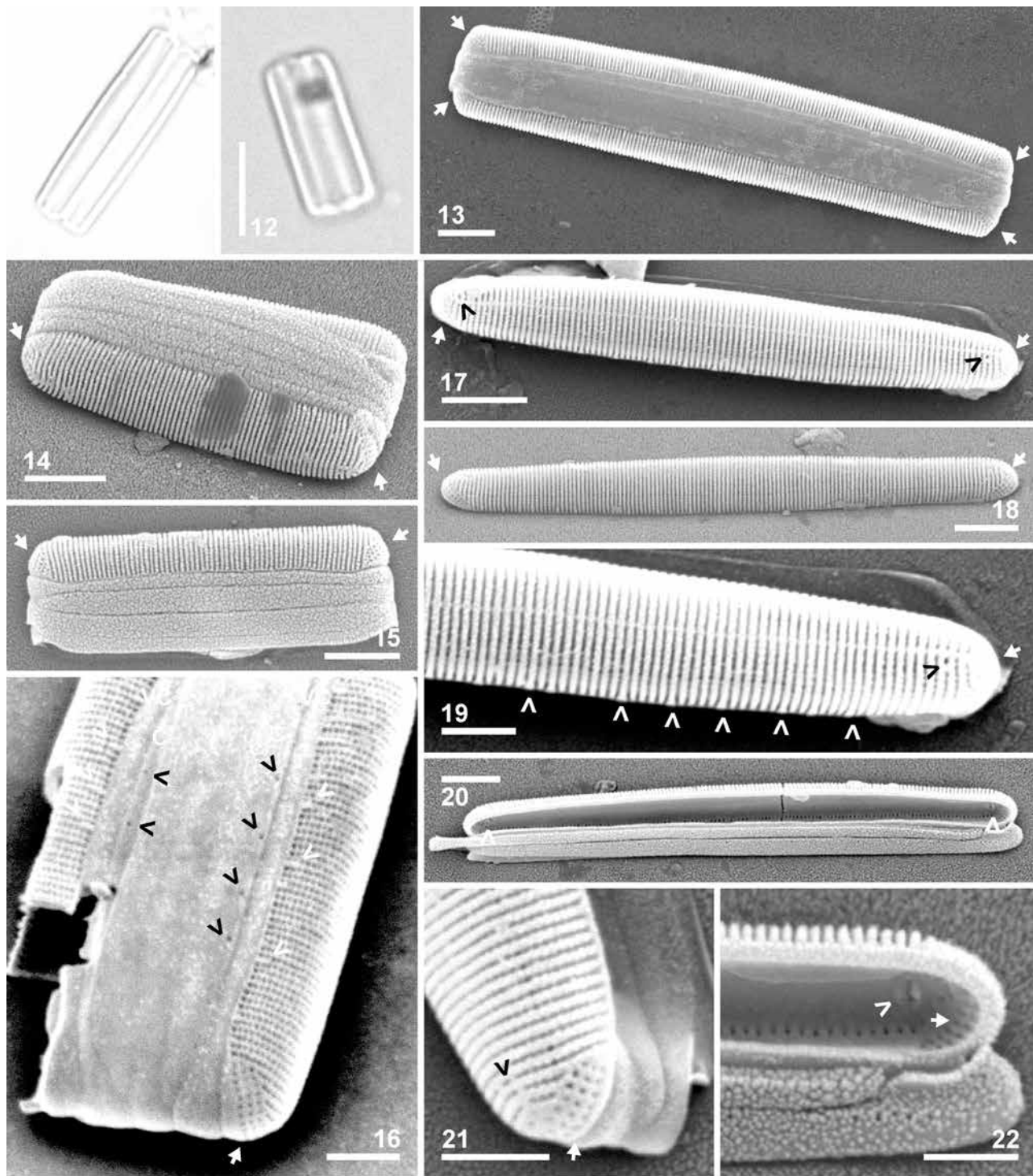


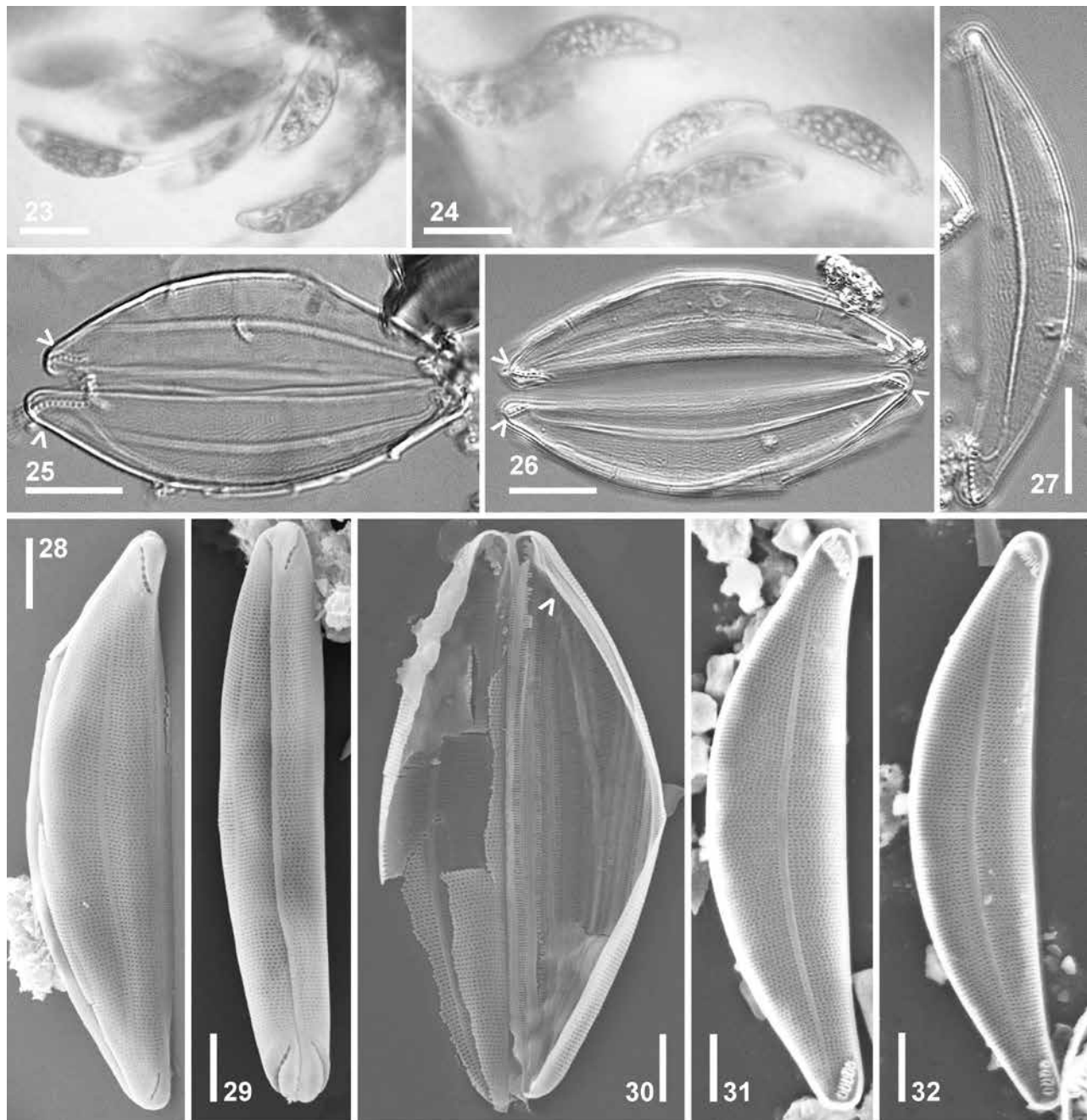
Fig. 1. Map of Province of Buenos Aires, showing sampling stations and location of the area in Argentina. 1. San Clemente del Tuyú; 2. Santa Teresita; 3. Mar del Tuyú; 4. La Lucila del Mar; 5. Mar de Ajó; 6. Nueva Atlantis; 7. Pinamar; 8. Villa Gesell; 9. Mar Azul; 10. Claromecó; 11. Punta Orengo; 12. Las Garzas; 13. San Antonio Oeste; 14. Banco Reparó; 15. Los Alamos; 16. Las Grutas; 17. Piedras Coloradas; 18. El Sótano; 19. El Fuerte.



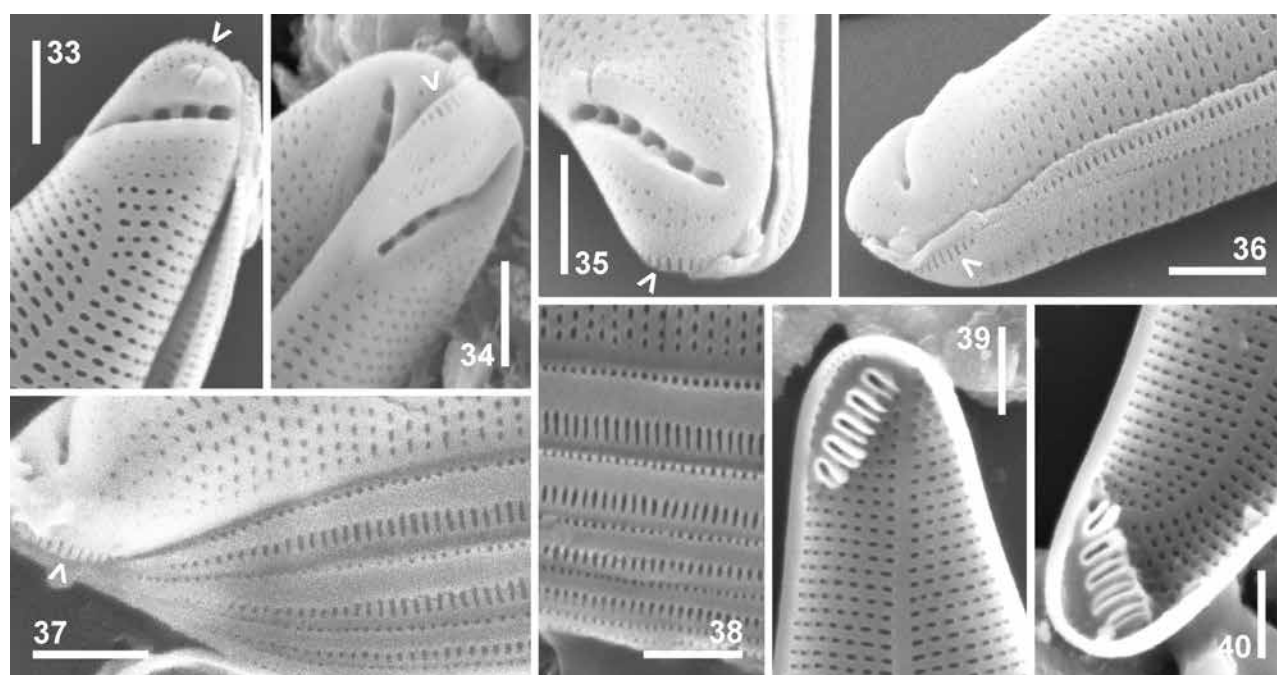
Figs. 2 -10. *Pratifusus inane*. 2 - 4. (LM) and 5 - 10 (SEM); 2 - 4. Frustules in valvar view showing the marginal row of spines; 5, 6. External view. Note valve surface, structureless with marginal spines (arrowheads) and small pore field (arrows); 7 - 10. Internal view showing simple, unoccluded, scattered pores placed on the valve mantle (arrowheads); 7. General aspect of the valve; 8. Valve apex showing two pores corresponding to the apical pore field (arrow); 9. Details of valve; 10. Detail of a tilted valve showing external ring of spine and circular platform of the pore field (arrow). Scale bars: **Figs. 2 - 5, 7 = 5 μm**; **Figs. 9, 10 = 2 μm**, **Figs. 6, 8 = 1 μm**.



Figs. 11-22. *Pteroncola inane*. **11, 12.** (LM) and **13 - 22.** (SEM); **11, 12.** Frustules in girdle view; **13-16.** Frustules in girdle view showing flat valve surface and curved deep mantle, apical pore fields (arrows), numerous girdle bands; **16.** Note row of areolae sunken between ribs, apical pore field, cingulum with the first copula having an advalvar row of pores (black arrowheads), vc: valvocopula, c: copulae; **17.** Valve in external view showing narrow sternum. Note elliptical pores of rimoportulae (black arrowheads) and apical fields of pores (arrows); **18.** Tilted valve. Note apical fields of pores (arrows); **19.** Detail of a valve showing transapical ribs slightly thickened towards the margin (white arrowheads), rimoportula opening (black arrowhead) and apical pore field (arrow); **20.** Valve tilted showing the internal view. Note rimoportulae at poles (arrowheads); **21.** Apex showing pore field (arrow). Note opening of rimoportula (black arrowhead); **22.** Apex in internal view showing a row of elliptical foramina of the alveoli, sessile rimoportula (arrowhead) and pore field (arrow). Scale bars: **Figs. 11, 12** = 5 μm ; **Figs. 13-15, 17, 18, 20** = 2 μm ; **Figs. 16, 19, 21, 22** = 1 μm .



Figs. 23 - 32. *Pseudohimantidium pacificum*. **23 - 27.** (LM) and **28 - 32.** (SEM); **23, 24.** Stalked, epizoic cells. Note numerous, elliptical chloroplasts; **25, 26.** Collapsed frustules showing curved sternum and apical grooves with rows of rimoportulae (arrowheads); **27.** Valve. Note the hooked end of the sternum and row of rimoportulae; **28.** Frustule in valvar view showing curved sternum, asymmetric poles and apical labiate grooves; **29.** Collapsed frustules showing valve mantle and apical grooves; **30.** Broken frustules with numerous girdle bands (arrowhead shows open bands); **31, 32.** Valve in internal view showing curved sternum, asymmetric at poles, and rows of rimoportulae at the apices. Scale bars: **Figs. 23, 24** = 20 μm ; **Figs. 25 - 27** = 10 μm ; **Figs. 28 - 32** = 5 μm .



Figs. 33 - 40. *Pseudohimantidium pacificum* (SEM). **33 - 35.** Valve apices showing grooves and field of slit at the mantle edge (arrowheads); **36.** Apex of a collapsed frustule showing one incomplete girdle band. Note apical field of slits (arrowhead); **37.** Apex of frustule from the dorsal side showing one advalvar row of subcircular pores, a plain pars media and one abvalvar row of slit-like pores; **38.** Girdle bands from the dorsal side; **39 - 40.** Apices of the same valve showing a row of rimoportulae. Note sternum almost straight in **39** and hooked-like in **40**. Scale bars: **Figs. 33 - 40** = 2 μm

