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Nanofrustulum shiloi (Bacillariophyceae) from the Gulf of San Matías (Argentina): Morphology, distribution and comments about nomenclature

by

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With 26 figures

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Abstract: This paper is devoted to analyse the morphological variability of *Nanofrustulum shiloi* in the Gulf of San Matías, to provide a comparison using our material, European and type materials, and to discuss related nomenclatural problems of this little known species. The material was collected in the Northern area of the Gulf of San Matías, at several locations along the coast. Field and cultured material was studied by means of light and scanning electron microscopy. The morphological variation within *Nanofrustulum shiloi*, a nanoplanktonic species reported for the first time from South Western Atlantic waters, is discussed.

Running title: *Nanofrustulum shiloi* from Gulf of San Matías

Introduction

Most studies on diatoms from the coastal waters of the Gulf of San Matías have been devoted to the microplanktonic size fraction. Small centric and fragilarioid diatoms (2-10 µm) are an important component of the marine nanoplankton in this area but as they are difficult to resolve with the light and even electron microscope they remain almost unknown. Recently we have focused our attention on small diatoms in the framework of a project devoted to isolation and cultivation of microalgae for

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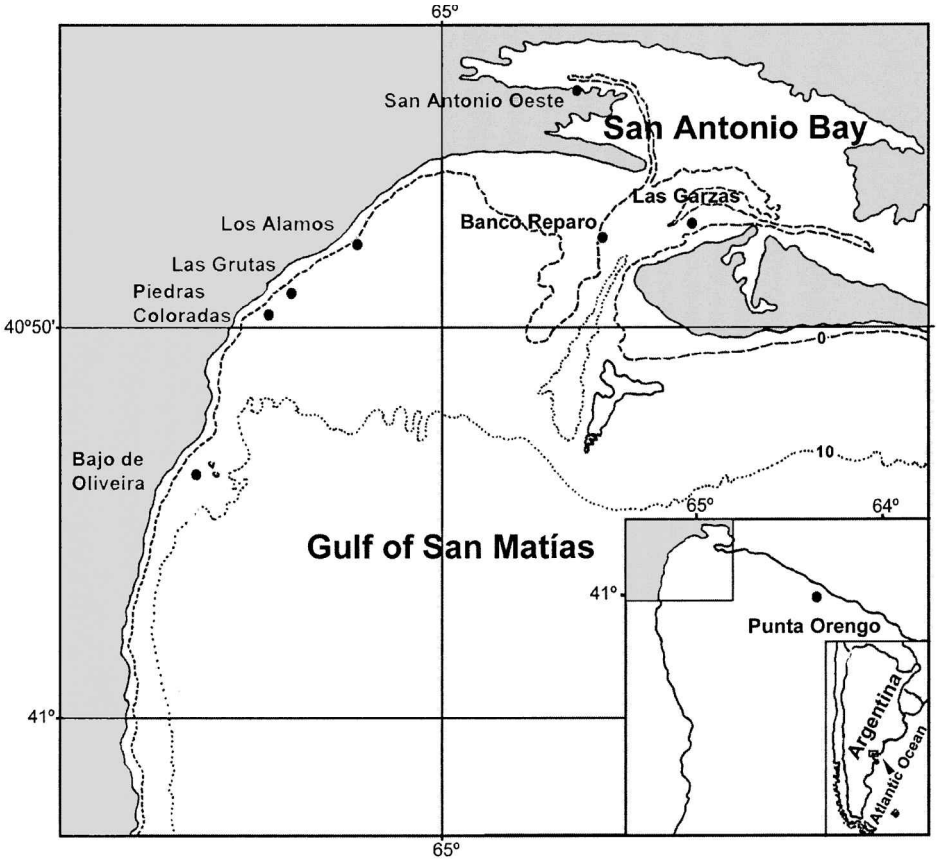


Fig. 1: Map of the Northern region of the Gulf of San Matías showing sampling stations and location of the study area in Argentina.

feeding larval stages of oysters at IBMyP "Alte. Storni" (Río Negro, Argentina). To this end we studied field and cultured material of a fragilarioid diatom belonging to the genus *Nanofrustulum* Round, Hallsteinsen & Paasche.

This genus was erected by Round et al. (1999) on the basis of *Nanofrustulum shiloi* (Lee, Reimer & McEnery) Round, Hallsteinsen & Paasche. The protologue includes a single description (descriptio generico-specifica), as the Art. 42.1 of the ICBN accepts for the monotypic genera, and illustrations of several strains and samples, which reveal subtle morphological differences.

The purposes of this contribution are: to analyse the morphological variability of *Nanofrustulum shiloi* in the Gulf of San Matías, to provide a comparison among our materials, the material described and illustrated by Round et al. (1999), and the type material published by Lee et al. (1980), and to discuss related nomenclatural problems of this little known genus.

Materials and methods

This investigation is based on field and culture samples from the Northern area of the Gulf of San Matías, Argentina, at several locations along the coast (Fig. 1). Phytoplankton samples were taken between April 1998 and April 2000 in the superficial layer of the water column with 30 µm net hauls. The clonal cultures were isolated in February 1999, when the water temperature was 21°C and salinity 34 psu, by picking out cells with a micropipette under a Wild M 20 microscope. Individual cells were washed several times in filtered seawater and when free of contaminants transferred into tissue culture wells containing 2 ml of f/2 medium (Guillard 1975) prepared with Los Álamos water (34 psu) diluted to 27 psu with freshwater and autoclaved. The culture wells were maintained at room temperature ≅ 22°C and in continuous light supplied by cool-white fluorescent tubes and regularly checked for algal growth. When color was detected, cultures were scaled up to 20 ml tubes and later 250 ml flasks.

Qualitative samples and aliquots of the cultures were fixed with 4% formalin and deposited in the "Colección de Diatomeas Argentinas, Departamento Cientifico Ficología, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata".

Samples were rinsed with distilled water to remove salt and preservatives, and the organic matter was oxidized according to the method described by Hasle & Fryxell (1970). Material was mounted for light (LM) and scanning electron microscope (SEM) according to Ferrario et al. (1995). Permanent slides were made with Hyrax. Observations and microphotographs were made using Nikon Microphot-FX microscope equipped with phase contrast and Jeol JSMT 100 scanning electron microscope.

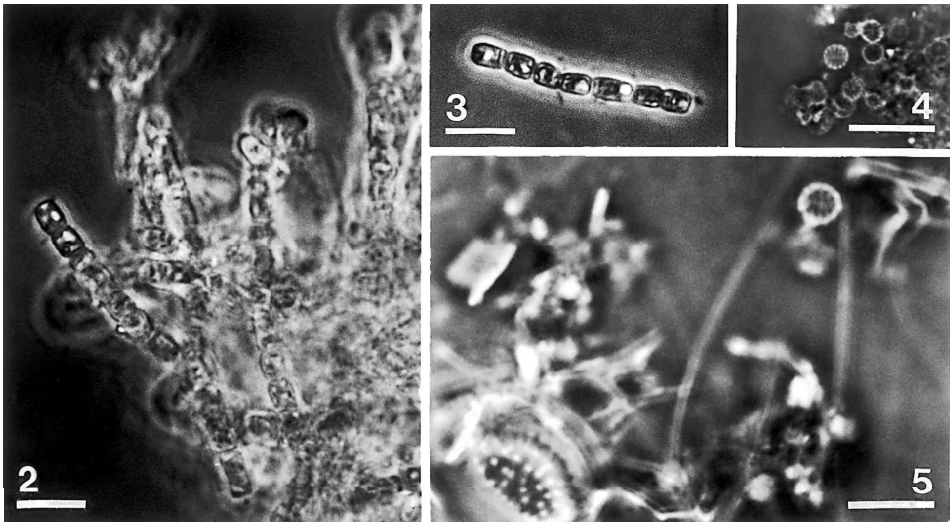
The general terminology follows Ross et al. (1979) and Round et al. (1990).

Results

Nanofrustulum shiloi (Lee, Reimer & McEnery) Round, Hallsteinsen & Paasche Round, Hallsteinsen & Paasche, 1999: 343-356, figs 1-33. Lee, Reimer & McEnery, 1980: 43-47, figs 2, 12-13. Figs 2-26

Frustules rectangular, forming chains linked by interlocking marginal spines. Valves circular to subcircular, flat or slightly domed, 2.0-3.5 µm in diameter. Striae uniseriate, 15-25 (30) in 10 µm, consisting of a few large elliptical, radially elongated areolae or a higher number of subcircular to subrectangular ones. Areola occluded by rotae on the inside of the valve, 2-4 (5-7) in 1 µm. Sternum ill-defined, wide or narrow. Valve surface plain or with warts. Valve mantle deep, sloping or vertical, with 1 or 2 (3) areolae beneath the spines. One apical pore at each end of the sternum, sometimes 2, 3 or more per pole, eventually a small apical pore field on both poles. Marginal spines placed on the striae along the junction of the valve and mantle, variable in form, thickness and length, more or less complex. Valvocopulae plain consisting of two segments and copulae composed by series of scale-like segments, without areolae.

Distribution: This marine diatom is worldwide in distribution in temperate and tropical coastal waters (Round et al. 1999). Lee et al. (1980) isolated the species from Red Sea foraminiferans, Hallegraeff & Burford (1996) obtained several strains from the Gulf of Carpentaria, Coral Sea and Port Smith in Australian waters, and Round et al. (1999) found it in the Oslofjord, Wurzburg, River Ribble, south coast of England and Woods Hole USA as free living or symbiotic. In this study we report *Nanofrustulum shiloi* for the first time in South Western Atlantic waters, present all year round.



Figs 2-5. *Nanofrustulum shiloi.* LM. Figs 2-4. Las Grutas clones. Figs 2-3. Colonies in girdle view. Fig. 4. Valve views. Fig. 5. Field material. Figs 2-4: scale bars = 10 µm; Fig. 5: scale bar = 5 µm.

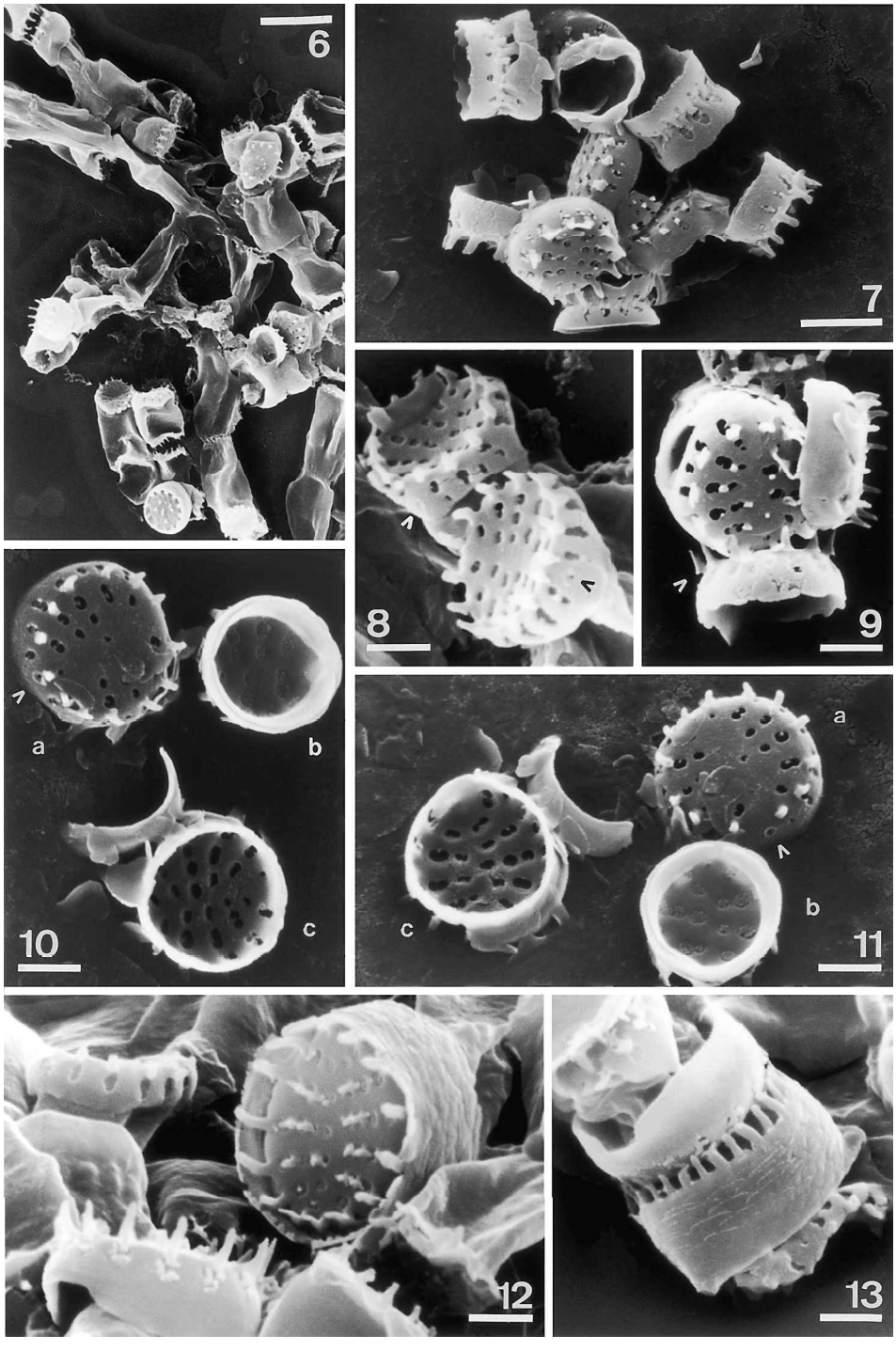
Discussion

Round et al. (1999) erected the genus *Nanofrustulum* establishing that the generitype is *Nanofrustulum shiloi* (Lee, Reimer & McEnery) Round, Hallsteinsen & Paasche, transferred by them to this genus. The basionym of this species is *Fragilaria shiloi* Lee, Reimer & McEnery described by Lee et al. (1980) with the slide A.-G.C. 38063 in Collection of Diatoms, Academy of Natural Sciences of Philadelphia as holotype. Round et al. (1999) designated the slide BM 100180 as holotype of *Nanofrustulum shiloi*, however according with the ICBN art. 7.4 this taxon must be typified by the type of the basionym, slide A.-G.C. 38063.

Materials illustrated by Lee et al. (1980), Hallegraeff & Burford (1996: as *Pseudostaurosira shiloi*), Round et al. (1999) and our own material showed some morphological variability.

Our clones isolated from Las Grutas (Figs 2-4, 6-13) presented less morphological variation than the field material collected in the same and neighbouring areas. The

Figs 6-13. *Nanofrustulum shiloi.* Las Grutas clones. SEM. Fig. 6. Colonies. Fig. 7. Sibling valves showing spatulate linking spines. Fig. 8. Tilted valves in external view. The arrows show apical pores. Fig. 9. Valves in external view. Arrow shows triangular spines with a basal, downwardly projecting spinula. Figs 10-11. Same group of valves seen from different angles. a Valve in external view showing an apical pore at both ends of the sternum. b Valve in internal view showing valvocopula. c Valve in internal view. Figs 12-13. Note the girdle with copulae composed of a series of scale-like segments. Fig. 12. Valve in external view with small rounded areolae and warts. Fig. 13. Sibling valves in girdle view. Fig. 6: scale bar = 5 µm; Fig. 7: scale bar = 2 µm; Figs 8-13: scale bars = 1 µm.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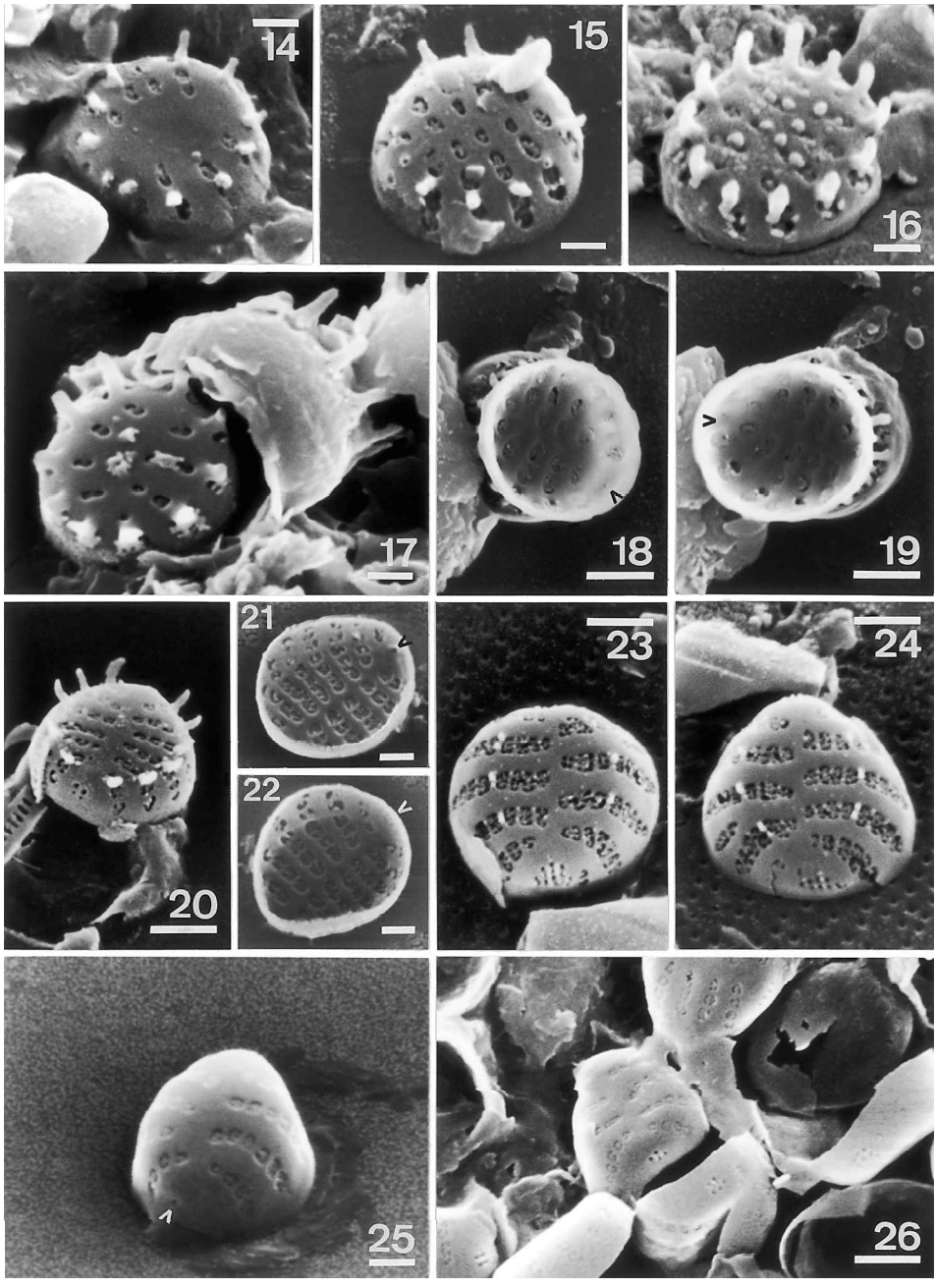

Figs 14-26. *Nanofrustulum shiloi.* Field material. SEM. Fig. 14. Valve in external view showing short striae, wide sternum, and sloping valve mantle with one areolae beneath the spines. Figs 15-17. Valves in external view showing steeper and deeper valve mantle. Figs 18-19. Same sibling valves from different angles. Arrows show the single apical pore. Fig. 20. Valve in external view. Figs 21-

cultured material showed circular valves with striae on the valve surface formed by 2-4 rectangular, radially elongate (Figs 8,10), or circular (Fig. 12) areolae. The central zone of the valve is plain (Figs 8, 11) or with warts (Figs 6, 9, 12). The valve mantle is sloping (Fig. 11) or steeper and deeper (Fig. 8) with one areola beneath the spines and a small apical pore at each end of the sternum (Figs 8-11) surrounded by a few small warts (Fig. 8) or ringed (Fig. 11). The marginal spines are spatulate (Fig. 7) to triangular, simple (Figs 10,11) or with a basal, downwardly projecting spinula (Figs 8, 9).

Clones from Las Grutas resemble the clone 13 figured by Lee et al. (1980) and Oslofjord strain 1, the Wurzburg strain and River Ribble material illustrated by Round et al. (1999), in spite of the presumed environmental variation at the isolation sites. Our clones were isolated from plankton samples at 21°C and 34 psu while Oslofjord strain 1 was isolated from plankton samples at 15°C and 24 psu, and the River Ribble material was obtained near the mouth of the River Ribble.

Examination of field material allowed us to delimit morphological variation in natural populations. Some specimens collected in Banco Reparo, Las Grutas and Piedras Coloradas (Figs 5, 14-19) resemble cultured ones in valve and mantle striation, linking spines and apical pores at the ends of the sternum.

Only a few valves from Banco Reparo (Figs 23, 24) revealed striae composed by densely disposed areolae, a more complex apical pore field at both ends of the sternum and 3 (4) areolae beneath the shorter and thinner spines, similar to those of Oslofjord strain 2 (Round et al. 1999).

Other specimens from Piedras Coloradas (Figs 20-22) had the areolae densely disposed in the striae, valve mantle with 2-3 areolae beneath the spines and a little apical pore field composed of four pores at each end of the sternum or by four pores at one end and a single one at the other (Figs 21, 22). This material is very similar to cells found in a sample from the south coast of England which were figured by Round et al. (1999), but lacks the warts on the valve. Some specimens without marginal spines (Figs 25-26) were found in the same sample from Piedras Coloradas having areolae densely disposed in the striae and a little apical pore field.

Round et al. (1999) pointed out that the morphological variation could be related to the fact that their material was maintained in cultures. However we found a similar variation in field material, with specimens intermediate in fine morphology (Figs 20-22) between the simplest (Figs 6-19) and the more complex (Figs 23-24). Taking into account that there is a continuum of variation in many features within *Nanofrustulum shiloi* we think that a separation at the specific level is not possible, and we conclude that this is a very polymorphic species.

22. Internal view of the same valve in different angles. Arrows show one apical pore in Fig. 21, and four apical pores in Fig. 22. Figs 23-24. External view of the same valve at different angles. Fig. 25. Valve in external view without spines. Arrow shows a single apical pore. Fig. 26. Group of valves in external view without spines. Figs 14-17, 21-22, 25: scale bars = 0.5 μm; Figs 18-20, 23-24, 26: scale bars = 1 μm.

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