ISSN 0373-580 X
Bol. Soc. Argent. Bot. 49 (1): 21-28. 2014

Analysis of type material of *Pleurosigma formosum* and *P. decorum* (Pleurosigmataceae, Bacillariophyta)

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Summary: The type materials of *Pleurosigma formosum* W. Smith and *P. decorum* W. Smith were examined. Both species were separated hitherto by size range, valve shape and stria density based on light microscopy (LM) analysis. However, the type materials show some overlap in the size and stria density ranges, with subtle differences in the oblique stria intersection angle, which is somewhat smaller for *P. decorum*. Scanning electron microscopy (SEM) analysis shows that both species share several ultrastructural features such as a thick saddle-shaped central raphe nodule, very long overlapping central raphe fissures, short hook-shaped terminal raphe fissures, general morphology of the internal hymen-occluded pores crossed by a recessed bar and presence of some scattered pairs of more deeply recessed pores lacking a bar. However, the type specimens differ in the internal morphology of the hymen-occluded areolar pores, circular, crossed by a recessed bar and rimmed in *P. formosum* and subcircular to elliptical, crossed by a less recessed bar, not rimmed in *P. decorum*. The SEM findings thus support Hendey's view that *P. decorum* and *P. formosum* are separate but closely allied species.

Key words: Fine morphology, *P. decorum*, *P. formosum*, taxonomy, type materials.

Resumen: Análisis de los materiales tipo de *Pleurosigma formosum* y *P. decorum* (Pleurosigmataceae, Bacillariophyta). Los materiales tipo de *Pleurosigma formosum* W. Smith y *P. decorum* W. Smith fueron examinados. Ambas especies fueron separadas hasta ahora por el rango de tamaño, la forma de la valva y la densidad de las estrías sobre la base de su análisis con microscopio óptico (MO). Sin embargo, los materiales tipo muestran alguna superposición en los rangos de tamaño y densidad de estrías, con sutiles diferencias en el ángulo de intersección de las estrías oblicuas, el cual es algo más pequeño en *P. decorum*. El análisis con microscopio electrónico de barrido (MEB) muestra que ambas especies comparten algunos caracteres ultraestructurales tales como el engrosamiento en forma de silla de montar del nódulo central del rafe, la amplia superposición de las fisuras centrales del rafe, la forma de las fisuras terminales del rafe, en gancho corto, la morfología general de los poros internos ocluidos por un himen cruzado por una barra hundida y la presencia de algunos pares esparcidos de poros más profundamente excavados, carentes de barra. Sin embargo, los especímenes tipo difieren en la morfología interna de los poros areolares ocluidos por un himen. Estos son circulares, cruzados por una barra hundida y con bordes engrosados en *P. formosum* y sub-circulares a elípticos, cruzados por una barra menos hundida y sin bordes engrosados en *P. decorum*. Los resultados del análisis con MEB apoyan el punto de vista de Hendey acerca de que *P. decorum* y *P. formosum* son especies separadas pero cercanamente relacionadas.

Palabras clave: *P. decorum*, *P. formosum*, taxonomía, materiales tipo, ultraestructura.

Introduction

Pleurosigma W. Smith (Smith, 1852) is a diatom genus first characterized by having valves convex, commonly sigmoid and striated, with striae visible as dots. *Pleurosigma sensu lato* was split by Cleve (1894) into *Pleurosigma sensu stricto* for those taxa with transverse and oblique striae, and *Gyrosigma sensu stricto* for those with transverse and longitudinal striae. The name *Pleurosigma* W. Smith was conserved against *Gyrosigma* Hassall, *Scalprum* Corda and *Endosigma* Brébisson, which were designated as taxonomic synonyms and rejected (Lanjouw *et al.*, 1956). More details about the taxonomic history of this genus were presented in Sar *et al.* (2012).

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According to VanLandingham (1978), *Pleurosigma sensu stricto* contains 90 validly published taxa and according to Reid (2012) it contains 306 species. Two decades ago Round *et al.* (1990) and Sterrenburg (1991a) pointed out that the genus *Pleurosigma* urgently needed revision and in the same sense recently Reid (2012) stated that species are imprecisely defined and the indiscriminate use of the names renders the reliability of identification highly problematic. Thus, critical investigations of original material are necessary to clarify some taxonomical problems and to establish the specific limits previously confused.

Pleurosigma formosum W. Smith was described by Smith (1852) from marine material of Shoreham Harbour, United Kingdom, without designation of the holotype. The author characterized *P. formosum* as showing "valves linear-lanceolate, gradually attenuated to the somewhat obtuse ends, twisted; median line broad, not central..." and commented in the protologue that "this species is well distinguished by the position of its median line, which, owing to a twist in the valves, appears nearly to coincide with the edge for a considerable distance at either end, and then crosses the valve in a diagonal direction...". Subsequently, Smith (1853) added further morphometric information on *P. formosum* and described a brackish water species as *Pleurosigma decorum* W. Smith, without designation of the holotype, characterizing it as "valve lanceolate, acute, angular, twisted, flexure considerable; median line not central....", with the same stria density but smaller size in comparison with *P. formosum*.

Hendey (1964) analyzed the material from the W. Smith Collection kept in the British Museum under the numbers BM23640 and BM23642 with light microscopy, designated them as type slides of *P. formosum* and *P. decorum* respectively, and discussed the differences between these taxa considering them as separate but closely allied species. According to the ICN (McNeill *et al.*, 2012) the slides chosen by Hendey (1964) should be interpreted as lectotypes.

As far as we can determine, type material of *P. decorum* has not been ultrastructurally analyzed and that of *P. formosum* was briefly analyzed by Reid (2002), who provided some ultrastructural details and enlarged the range of length and width of the species, so that it overlapped that of *P. decorum*. The aim of this paper is to clarify the taxonomy of *P. formosum* and *P. decorum* based on the comparison of type materials with light and scanning electron microscope analysis.

Material and Methods

Comparative studies were made on lectotype slide BM 23640 and material of *Pleurosigma formosum*, from Shoreham Harbour, collected August, 1850, by W. Smith, and on lectotype slide BM 23642 and material of *Pleurosigma decorum*, from Felixstow, Essex, collected without date by C. Topping.

Three specimens of each species were illustrated in the present paper. The LM photomicrographs are scans of Kodak TP2415 negatives taken in brightfield with apochromatic objectives, and the SEM photomicrographs are scans of negatives taken with an ISI SS-40.

Very small samples of the materials corresponding to that of the lectotype slides were cleaned with hot peroxide and yielded a few valves (some intact) for SEM.

Terminology follows Ross *et al.* (1979), Round *et al.* (1990), Sterrenburg (1991a, b) and Reid (2012). The procedure for the measurement of striae, intersection angle of the oblique striae and raphe angle was detailed in Sterrenburg (1991a).

Results

Pleurosigma formosum W. Smith (Figs 1 A-H, 3 A)

Smith 1852, p. 5, pl. 1, figs 1, 2; Smith 1853, p. 63, pl. 20, fig. 195; Hendey 1964, p. 242; Reid 2002, p. 89, figs 54-58.

Light microscopy

Valve linear-lanceolate, sigmoid, with margins parallel centrally, becoming curved in opposite directions toward the ends, vaulted, and with sub-obtuse apices (Fig. 1, A-B), 300-530 µm long and (20) 30-50 µm wide. Raphe-sternum sigmoid, crossing the valve diagonally in the middle part and approaching the opposite convex sides toward the last third of the valve (Fig. 1, A-B). Raphe angle +12 to +14º (Fig. 1, A-B). Central area circular, small, with bilateral dilatation around the central nodule (Fig. 1, C). Terminal areas unilaterally dilated, funnel shaped, in apical position. Striation pattern (Fig. 1, C-D) dominated by the oblique

striae, 10-17 in 10 µm, intersecting at 86-88 (90)º, crossed by transverse striae, 14-20 in 10 µm (Fig. 1, B).

Scanning electron microscopy

Raphe sternum narrow slightly thickened internally, closely bordered by a row of small areolae (Fig. 1, E). Central area conspicuous, saddle shaped, internally short and raised, with central bars surrounding the circular central nodule (Fig. 1, E). Central internal raphe endings coaxial, slightly dilated (Fig. 1, E). External central raphe fissures markedly projecting into the central area, undulated toward the same side and overlapping for some distance in parallel then curved in opposite directions (Fig. 1, F). Terminal endings finishing in helictoglossae turned toward the concave side of the valve (Fig. 1, G). External terminal raphe fissures hook-shaped (Fig. 1, H), oppositely bent toward the concave sides of the valve. Areolae arranged in decussate rows, opening by an external slit-like foramen (Fig. 1, F, H) and by a circular, rimmed, hymen-occluded internal pore crossed by a recessed bar (Fig. 1, E; Fig. 3, A arrows). Scattered on the internal valve surface there are some paired rimmed pores slightly larger and more deeply recessed in the siliceous layer than the others, lacking the central bar (Fig. 1, E; Fig. 3, A arrowheads).

Remarks: It should be noted that the range of valve width of the specimens examined by Reid (2002) (Table 1) is broader than that found by Hendey (1964) and in this study on the type material (Table 1). Nevertheless, the specimen pertaining to the type material illustrated by Reid (2002, Fig. 54), perfectly agrees in width (35 µm) with those found by us in the type material (Table 1). The extension of the size ranges described by Reid (2002) can be explained by the inclusion of additional populations in the analysis.

Pleurosigma decorum W. Smith (Figs 2 A-F, 3 B-C)

Smith 1853, p. 63, pl. 21, fig. 196; Hendey 1964, p. 242.

Light microscopy

Valve lanceolate, sigmoid, with sides between the middle and the end one convex and the opposite barely concave, vaulted, and with sub-acute apices, 220-317 µm long and 24-33 µm wide (Fig. 2, A). Raphe-sternum sigmoid, eccentric, crossing the valve diagonally and approaching opposite sides toward the ends. Raphe angle + 15º. Central area circular, with bilateral dilatation around the central nodule. Terminal areas unilaterally dilated, funnel shaped, in apical position. Raphe fissure not clearly visible in LM. Striation pattern dominated by the oblique striae, 13-17 in 10 µm, intersecting at 81-83 (90)º, crossed by transverse striae, 18-20 in 10 µm (Fig. 2, B).

Scanning electron microscopy

Valve vaulted at the centre (Fig. 2, C) and toward the poles (Fig. 2, D) with both sides of the valve roof-shaped. Raphe sternum narrow, thickened internally (Fig. 2, C-D). Central area conspicuous, saddle shaped, internally short and raised, with central bars surrounding the central nodule (Fig. 2, C). Internal central raphe endings coaxial, slightly dilated (Fig. 2, C). External central raphe fissures markedly projecting into the central area, undulated toward the same side and overlapping in parallel for some distance (Fig. 2, D). External terminal raphe fissures hook-shaped (Fig. 2, F), oppositely bent. Terminal endings finishing in helictoglossae turned toward the concave margin of the valve, bordered by a siliceous ridge with a large apical pore (Fig. 2, D). Areolae arranged in decussate rows opening by an external slit-like foramen (Fig. 2, D-E) and by a sub-circular to elliptical, hymen-occluded internal pore crossed by a subtle recessed bar (Fig. 2, C-D arrows; Fig. 3, B-C arrows). Scattered on the internal valve surface there are some paired internal pores slightly larger, circular, and more deeply recessed on the siliceous layer than the others, lacking the central bar (Fig. 2, C-D arrowheads; Fig. 3, B-C arrowheads).

Remarks: It should be noted that the valve width of the specimens examined by Hendey (1964) in the lectotype slide is rather narrow. Smith (1853) illustrated a specimen of comparable length but greater width (31 µm) and the specimen from the type material we illustrate in LM is also of comparable length but 33 µm wide. A further extension of the size ranges of this species may be expected in additional populations.

Hendey designed the BM 23642 as type slide of *P. decorum* and Ipswich as type locality, this

Table 1. Morphometric data of *P. formosum* and *P. decorum*. Descriptions and measurements follow the literature included in the column reference. Abbreviations: nd, no data; *, measured from pictures given in referred literature.

Taxon	Reference	Length (µm)	Width (µm)	Transverse striae in 10 µm	Oblique striae in 10 µm	Stria angle	Characteristics of raphe sternum	Morphology of the apices	Valve outline
P. formosum Smith	Smith (1852)	383	30	nd	0.846 µm apart	nd	Not central	Obtuse	Linear-lanceolate, twisted
	Smith (1853)	358-452	nd	nd	14	nd	Not central *Raphe angle + 14º	Obtuse	Linear-lanceolate, twisted
	Hendey (1964)	360-500	34-46	14-16	10-14	of about 90º	Strongly sigmoid, eccentric toward the ends of the valve	Sub-acute	Linear to linear-lanceolate, sigmoid
	Reid (2002) Type material (Figs 54-58)	300-530	20-50	18-20	14-17	90º	Eccentric, running along the edge of the valve for last quarter. *Raphe angle + 12º	nd *Sub-obtuse	Linear-lanceolate slightly sigmoid
	Lectotype This study	352-444	32-36	19-20	14-16	86-88º	Sigmoid, approaching opposite sides toward the ends. Raphe angle + 14º	Sub-obtuse	Linear-lanceolate, sigmoid
P. decorum Smith	Smith (1853)	248-317 *279	nd *31	nd	14	nd	Not central *Raphe angle + 15	Acute	Lanceolate, twisted
	Hendey (1964)	220-260	24-28	19	15-17	of about 90º	Sigmoid and strongly eccentric toward the ends of the valves	Sub-acute	Lanceolate to linear-lanceolate
	Lectotype This study	254-260	27-33	18-20	13-15	81-83º	Sigmoid and strongly eccentric toward the ends of the valves Raphe angle + 15º	Sub-acute	Sigmoid-lanceolate

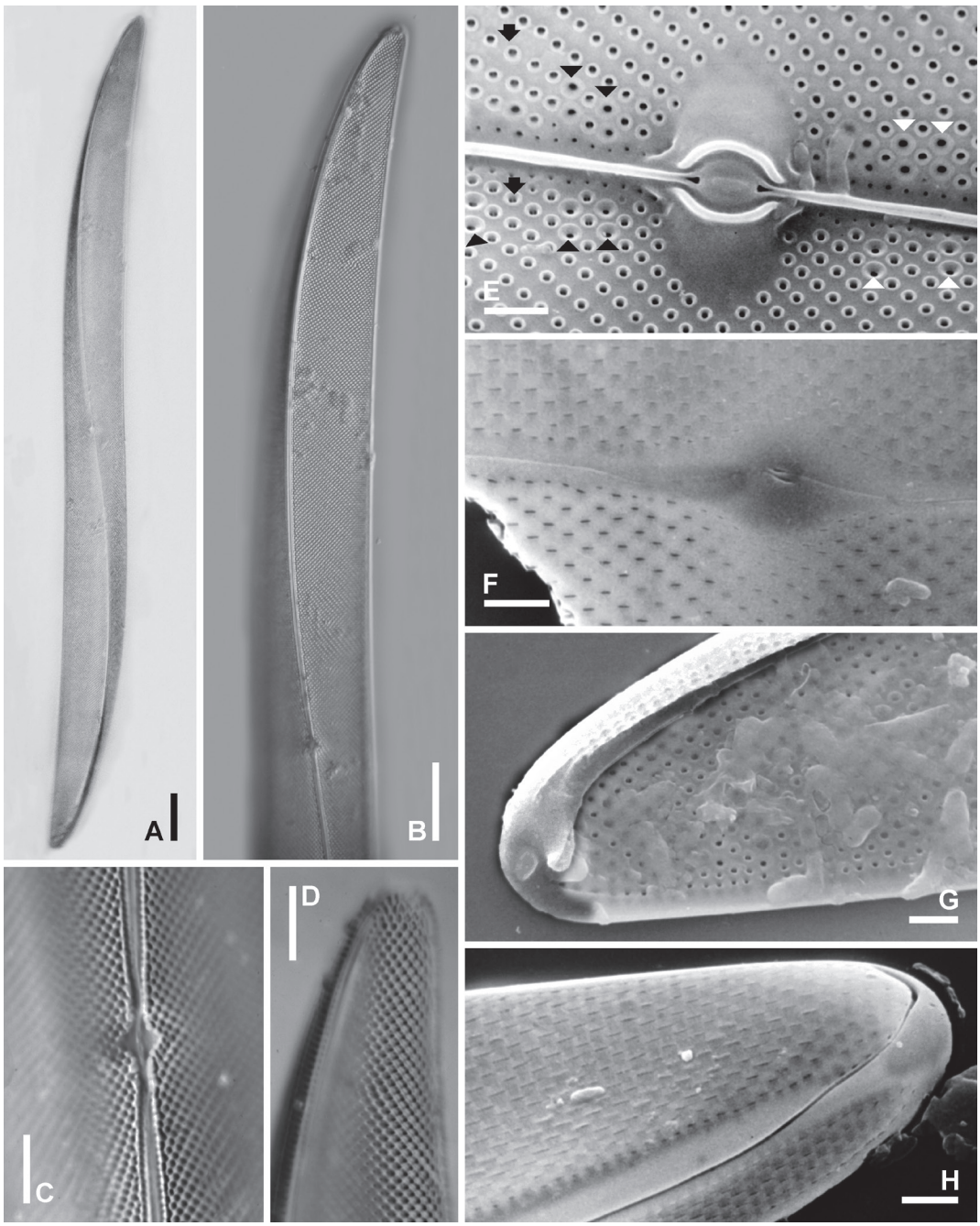

Fig. 1. *Pleurosigma formosum*, type material. LM: A-D, SEM: E-H. **A**: Valve linear, sigmoid. **B**: Detail of A showing central area and terminal areas unilaterally dilated, funnel shaped, in apical position. **C**: Central area circular. **D**: Detail of an apex showing striation pattern. **E, G:** Internal views. **F, H**: External views. **E**: Central area saddle shaped with central bars surrounding the circular, central nodule. Note circular, rimmed, hymen-occluded pore, crossed by a recessed bar (arrows) and pairs of pores more deeply recessed in the siliceous layer and lacking the central bar (arrowheads). **F**: Detail of a valve showing central raphe fissures. **G**: Polar ending terminating in helictoglossa. **H**: Apex showing terminal raphe fissure hook-shaped. Scale bars: A-B, 25 µm; C-D, 5 µm; E-H, 2 µm.

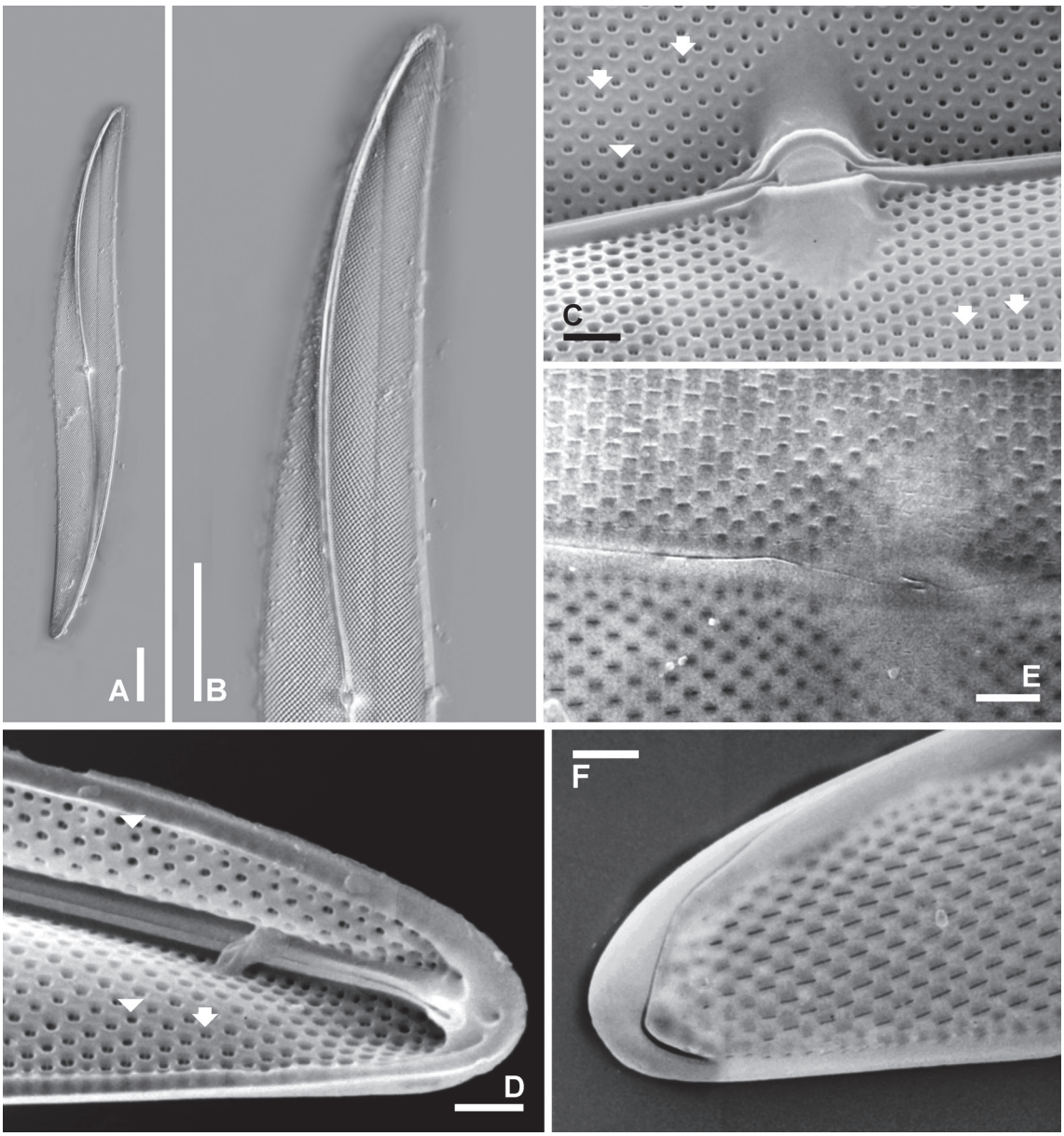

Fig. 2. *Pleurosigma decorum*, type material. LM: A-B, SEM: C-F. **A**: Valve lanceolate, sigmoid. **C-D**: Internal views. Note sub-circular to sub-elliptical, hymen-occluded pore crossed by a recessed bar (arrows) and pairs of pores more deeply recessed in the siliceous layer and lacking the central bar (arrowheads). **C**: Central area saddle-shaped with central bars surrounding the circular, central nodule. **D**: Polar ending terminating in helictoglossa. **E-F**: External views. **E**: Detail of a valve showing central raphe fissures. **F**: Apex showing terminal hook-shaped raphe fissure. Scale bars: A-B, 25 µm; C-F, 2 µm.

locality was quoted by Smith (1853) together with another, Felixstow, Essex, in which the collector was Topping who was mentioned as the collector of the material of the slide 23642 by Reid (2012: 24). Thus, considering that the selection of the slide is clear and the assignment of the locality was erroneous, the type locality should be corrected changing it to Felixstow, Essex.

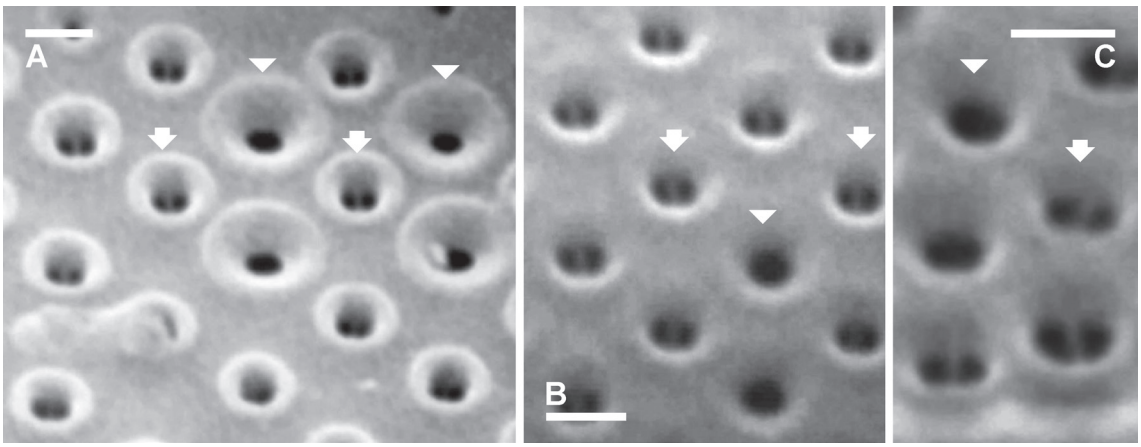

Fig. 3. *Pleurosigma formosum* and *P. decorum*, type materials. SEM. **A**: Internal detail of the areolae of *P. formosum*. Note circular, hymen-occluded rimmed pore, crossed by a recessed bar (arrows) and pairs of pores more deeply recessed in the siliceous layer and lacking the central bar (arrowheads). **B-C**: Internal details of areolae of *P. decorum* from central part and apical part of the valve respectively. Note sub-circular to sub-elliptical, hymen-occluded pore crossed by a recessed bar (arrows) and pairs of pores more deeply recessed in the siliceous layer and lacking the central bar (arrowheads). Scale bars: A-C, 0.5 µm.

Discussion

Pleurosigma decorum was considered a synonym of *P. formosum* by Cleve (1894), whose opinion was followed by some diatomologists of the first half of the last century as Mann (1907) but refuted by others, including Hendey (1964) who stated that both are separate but closely allied species differentiated by size, stria density and valve outline based on the LM analysis of the type materials. However, the comparison of the morphometric data given by Smith (1852, 1853), Hendey (1964) and Reid (2002) based on type material of these species, revealed that the ranges of length, width, transverse and oblique striae density show some overlap (Table 1). Thus, it appears necessary to compare type materials with SEM for determining if *P. formosum* and *P. decorum* are conspecific. SEM analysis of type material of *P. formosum* and *P. decorum* shows that these species share several morphological features, such as a thick and strongly elevated saddle-shaped central raphe nodule, very long overlapping central raphe fissures, short hook-shaped terminal raphe fissures and general morphology of the internal hymen-occluded pores crossed by a recessed bar. In addition, they share a fine detail not yet noticed in other species of the genus examined. Besides the normal internal areolar pores with recessed bars (arrows marked in the Fig. 1, E; Fig. 2, C-D; Fig. 3, A-C) there is a smaller number of pairs of internal areolar pores that are larger, more deeply recessed in the siliceous layer than the others (more markedly so in the case of *P. formosum*) and lack the recessed bar (arrowheads marked Fig. 1, E; Fig. 2, C-D; Fig. 3, A-C). The distribution of these larger internal pores over the valve appears to be irregular in both species. Despite the ultrastructural similarities there are clear differences in some details of the internal hymen-occluded pores of the areolae, *P. formosum* presents circular, hymen-occluded internal pores, crossed by a recessed bar and clearly rimmed (features previously observed by Reid 2002) while *P. decorum* shows sub-circular to elliptical, hymen-occluded internal pores, crossed by a less recessed bar and not rimmed (features confirmed in San Matías Gulf populations of *P. decorum* by Sar *et al.* 2013).

In summary, based on our LM and SEM analysis we agree with Hendey (1964) in considering *P. formosum* and *P. decorum* as two species with different size range (despite some overlap) and clearly different valve shape (*P. formosum* being more linear than *P. decorum*). In addition, SEM shows a difference in the morphology of the internal areolar pores. Minor differences in size between

Smith's data and Hendey's and our findings in the lectotype slides are to be expected. The original slides of Smith are not known to exist, the lectotype slides were made later in the 19th century.

Acknowledgements

We thank María Esther Meave del Castillo for her careful work of revision that helped us to improve the manuscript. The research was supported by grants from CONICET, PIP0067 and from the Universidad Nacional de La Plata, 11/N640.

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Recibido el 10 de mayo de 2013, aceptado el 7 de octubre de 2013.