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Pleurosigma hinzianum sp. nov. and *P. frenguellianum sp. nov.* (Pleurosigmataceae, Bacillariophyta) from Argentinean coastal waters, in comparison with *P. amara* Stidolph and *P. elongatum* W. Smith

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Two new marine diatom species from Argentinean coastal waters, *Pleurosigma hinzianum* Sterrenburg, Sunesen & Sar, *sp. nov.* and *P. frenguellianum* Sunesen, Sterrenburg & Sar, *sp. nov.*, are described. The characters permitting their identification are specified, based on comparison with type material of the morphologically similar species *P. amara* Stidolph and *P. elongatum* W. Smith in the light (LM) and scanning electron microscope (SEM). New information on the type material of *P. elongatum* is presented and its taxonomic concept is emended. The main criteria for separation of the species discussed here are: valve outline, path of the raphe-sternum, raphe angle and angle of intersection of the oblique striae in LM; and external central and terminal raphe fissures, internal details of the central area, external and internal apical structure, and morphology of the hymen-occluded internal pores in SEM. The occurrence of these (and probably other) species in the genus *Pleurosigma* in net samples is adventitious and not indicative of a true planktonic mode of life.

Key words: fine morphology, *Pleurosigma amara*, *P. elongatum*, *P. hinzianum sp. nov.*, *P. frenguellianum sp. nov.*, taxonomy, type material

Introduction

The genus *Pleurosigma* W. Smith has a complex taxonomic history (Sar *et al.*, 2012) and now includes more than 306 accepted species (Reid, 2012). It is characterized by more or less flattened valves that are sigmoid to straight in outline, a raphe-sternum that is sigmoid and more or less eccentric or straight and central, three stria systems (one transverse and two oblique) and loculate areolae with apically elongated foramina externally and hymen-occluded pores internally.

Diagnostic features for separating species of *Pleurosigma* with the LM were summarized and discussed by Sterrenburg (1991*a*) and include valve shape, raphe-sternum path, raphe angle, intersection angle of the oblique striae, apical structure and stria density. Additional criteria for species characterization with SEM proposed by Cardinal *et al.* (1989) and Sterrenburg (1991*b*) are: morphology of the central bars, central area and external central raphe fissures, and the organization of the areolae around the central nodule. Stidolph (1992), Reid (2002) and Sar *et al.* (2012) included additional ultrastructural features that were shown to be stable with a small intraspecific but a marked interspecific variation: morphology of the internal occluded pores of the areolae and the external terminal raphe fissures and fine external and internal apical details.

As part of a study of diatom populations from coastal waters of the Provinces of Buenos Aires and Río Negro, Argentina, numerous species and one variety of the genus *Pleurosigma* collected in several net samples were described (Sar *et al.*, 2013; Sunesen *et al.*, 2013). Here we describe two new taxonomic entities which at first sight are similar in general appearance to *Pleurosigma amara* Stidolph and *P. elongatum* W. Smith respectively.

Pleurosigma amara was described by Stidolph (1992) from a salt marsh near Napier, New Zealand. *Pleurosigma elongatum* was described by Smith (1852: 6, pl. 1, fig. 4) from marine and brackish material from Poole Bay, UK, without designation of a holotype. The species was characterized by having 'valves linear-lanceolate, flexure slight, extremities acute, median line central …'. Dimensions given (converted to metric values) were: length 330 µm,

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width 25 μm and stria separation of 0.56 μm (*c.* 18 striae in 10 μm). Additional morphometric data were given in Smith (1853: 64). Subsequently, Hendey (1964) examined the slide BM23651 from the W. Smith collection and designated this as the type slide of *P. elongatum.* Stidolph (1992) morphologically separated *P. elongatum* from *P. amara* and Reid (2002) showed a few ultrastructural details of *P. elongatum*, based on SEM study of the type material.

The aims of this study are to describe two new taxa – which are rather similar in LM but clearly different in SEM – and to compare them with two morphologically similar species, *P. amara* and *P. elongatum*. The taxonomic concept of *P. elongatum* is emended, based on examination of its type material.

Materials and methods

The material from Argentina was collected in three areas (Fig. 1), as follows. (1) At several stations along the northern coast of Buenos Aires Province, San Clemente del Tuyú, Santa Teresita, Mar del Tuyú, La Lucila del Mar, Mar de Ajó, Nueva Atlantis, Pinamar, Villa Gesell and Mar Azul, from November 1994 to September 2000 and from March 2008 to March 2013. (2) At several stations along the southern coast of Buenos Aires Province, Los Pocitos, Ría del Jabalí and Bahía San Blas, from May 2008 to March 2013. (3) At several stations in the northern area of San Matías Gulf (Río Negro Province): Punta Orengo, Las Garzas, Banco Reparo, San Antonio Oeste, Los Álamos, Las Grutas, El Sótano and El Fuerte, from April 1998 to May 2000 and from March 2006 to April 2007.

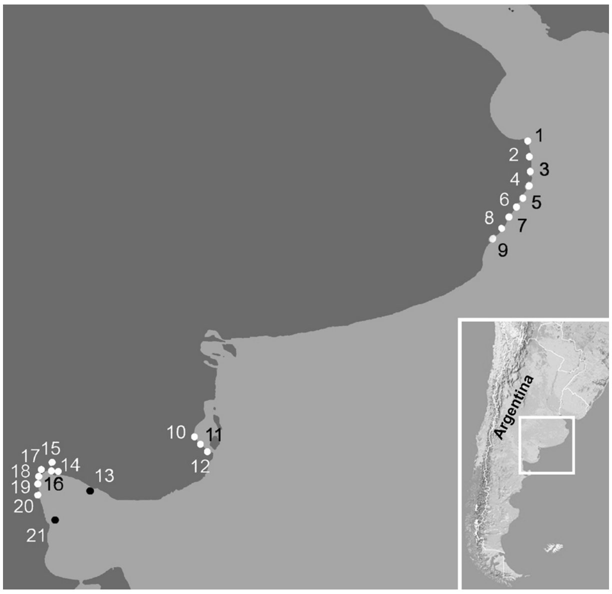


Fig. 1. Map of the marine littoral of Buenos Aires Province and North of Río Negro Province, showing sampling stations and location of the area in Argentina. 1: San Clemente del Tuyú, 2: Santa Teresita, 3: Mar del Tuyú, 4: La Lucila del Mar; 5: Mar de Ajó, 6: Nueva Atlantis, 7: Pinamar, 8: Villa Gesell, 9: Mar Azul, 10: Los Pocitos, 11: Ría del Jabalí, 12: Bahía San Blas, 13: Punta Orengo, 14: Las Garzas, 15: San Antonio Oeste, 16: Banco Reparo, 17: Los Álamos, 18: Las Grutas, 19: Piedras Coloradas, 20: El Sótano, 21: El Fuerte.

Qualitative samples were taken from the surface layer of the water column (between 0 and 5 m) with 30 μm net hauls and fixed with 4% formalin. In the laboratory, the preserved samples were rinsed with distilled water to remove salt and preservatives, and then the organic matter was oxidized according to Hasle & Fryxell (1970) and Prygiel & Coste (2000). The cleaned material was mounted for light and scanning electron microscopy after Ferrario *et al.* (1995). Permanent mounts were made with Naphrax (Brunel Microscopes, Chippenham, UK).

The materials have been 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 4250–4495, LPC 4550–4643, LPC 11001–11353, LPC 11601–11908, LPC 13648–13685.

Observations were made with Leica DM 2500 (phase contrast and differential interference contrast; Leica Microsystems, Wetzlar, Germany), Zeiss Axiovert 40 CFL (phase contrast and Nomarski interference contrast; Zeiss Microimaging, Göttingen, Germany) and Nikon Microphot-FX microscopes. The photomicrographs were made with Leica DM 2500 and Zeiss Axiovert 40 CFL microscopes and Jeol JSMT 100 and Jeol JSM 6360 LV (JEOL, Tokyo, Japan) scanning electron microscopes.

The type slide of *Pleurosigma elongatum*, BM 23651, 'Poole Bay, June 1849', from the Natural History Museum, London, was examined with LM and photomicrographs were made with apochromatic objectives on Kodak TP 2415.

The type material of *P. elongatum*, from 'Poole Bay, June 1849', kept in the Van Heurck Collection, National Botanic Garden of Belgium, Brussels, was cleaned by conventional methods and photomicrographs were made with the Jeol JSM 6360LV. The photomicrographs are mostly reproduced at the same scale, to facilitate comparisons.

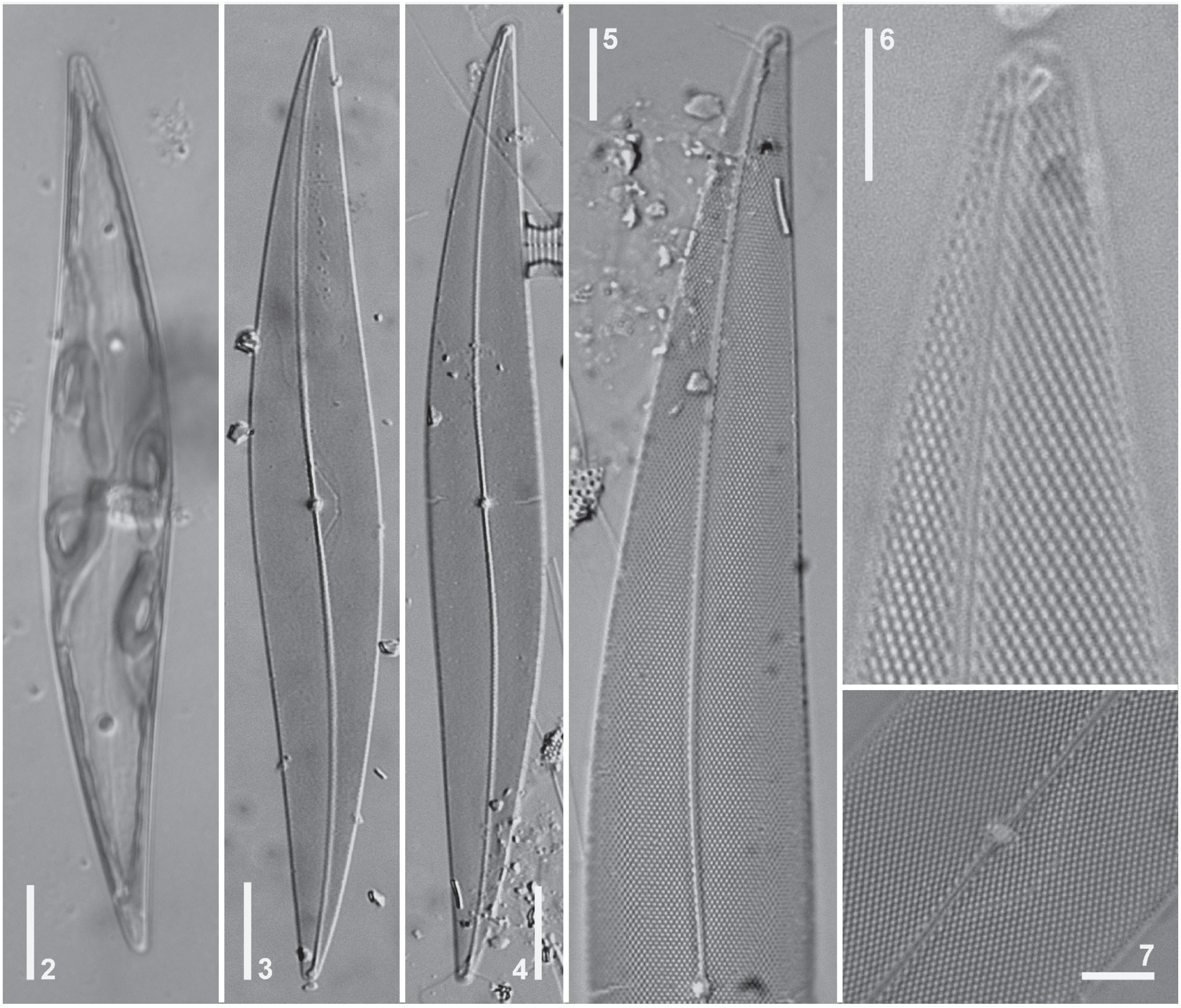
Terminology follows Ross *et al.* (1979), Round *et al.* (1990), Sterrenburg (1991*a*, 1991*b*), Stidolph (1992), Sterrenburg *et al.* (2003) and Reid (2012). The procedure for measurement of the intersection angle of the oblique striae and the raphe angle was described in Sterrenburg (1991*a*); the term 'calcar' – a spur-like detail at the apex seen in LM and represented in SEM by an external fissure in the valve surface and/or particular internal details – was introduced by Sterrenburg in Stidolph (1992).

Results

Pleurosigma hinzianum **Sterrenburg, Sunesen & Sar, *sp. nov.***

(Figs 2–24, Table 1)

Description in LM: Cell with one or two ribbon-shaped chloroplasts (Fig. 2) sometimes convoluted at the valve centre and lying appressed to the girdle towards the poles. Valve lanceolate, slightly sigmoid, with acute apices (Figs 3–4), 181–206 μm long and 20–28 μm wide. Ratio length/width 7.0–8.7. Raphe-sternum eccentric, with single sigmoid curvature, closer to the convex sides towards the apices (Figs 2–4). Raphe angle +7° to +8° (Figs 3–4). Central area circular,

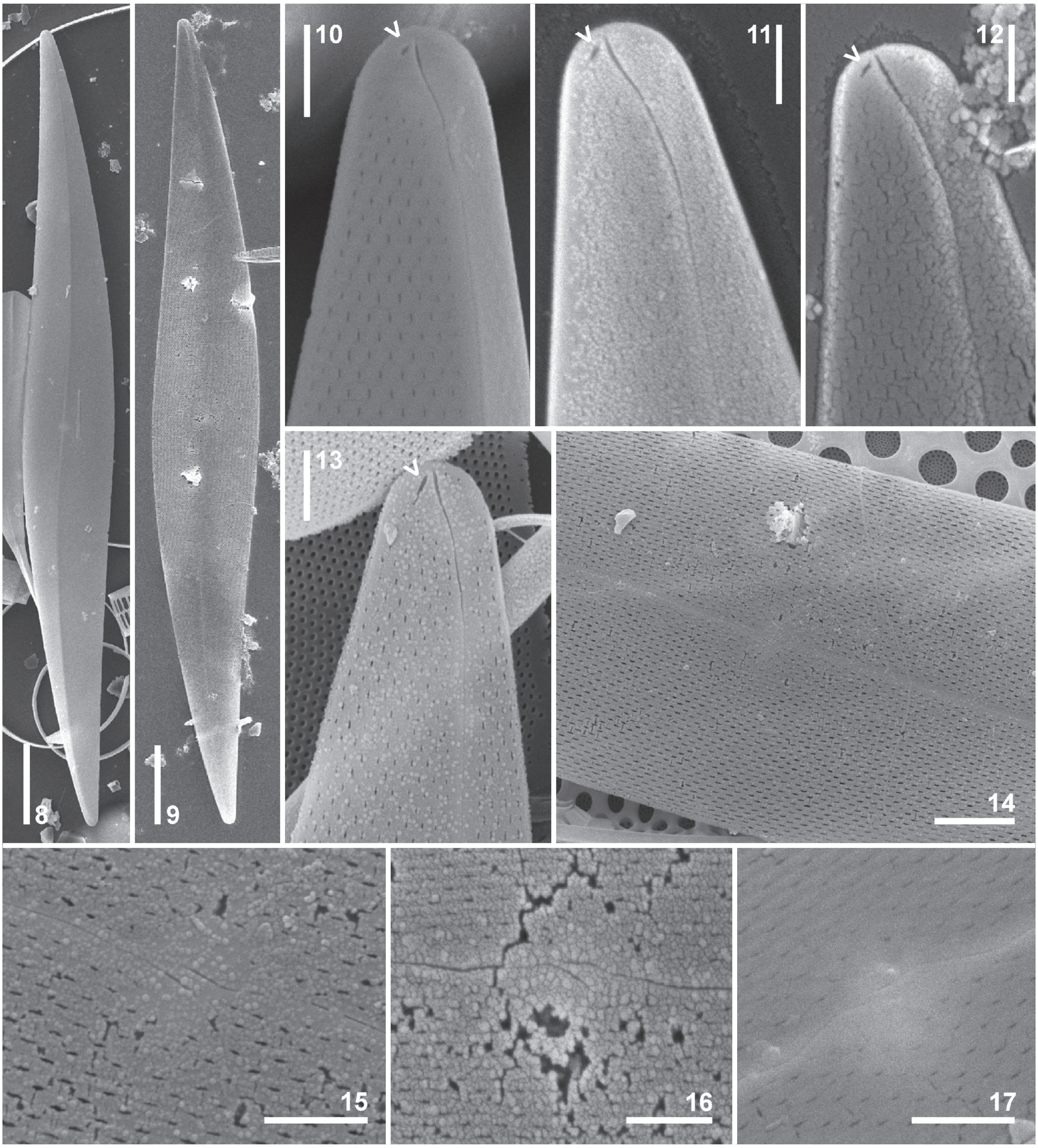


Figs 2–7. *Pleurosigma hinzianum*. Holotype (Figs 4–5); Paratype (Figs 3, 6–7). LM. **Fig. 2**, Cell showing ribbon-shaped chloroplast. **Figs 3–4**, General appearance of valve. **Figs 5–6**, Detail of valve apex. **Fig. 5**, Note terminal area unilaterally dilated. **Fig. 6**, Note terminal helictoglossa. **Fig. 7**, Detail of the central area of the valve. Note central nodule showing internal raphe ends. Scale bars: Figs 2–4 = 20 µm, Fig. 5 = 10 µm, Figs 6–7 = 5 µm.

small, with a thick barely bilaterally dilated central raphe nodule (Figs 3–5, 7). Central internal raphe endings coaxial (Fig. 7). Terminal areas small, unilaterally dilated, funnel-shaped, in apical position (Figs 5–6), with helictoglossa twisted towards the concave side of the valve (Fig. 6). Striation dominated by the oblique striae, 19–22 in 10 µm, intersecting at an angle between 64° and 69° in the central part of the valve, and crossed by transverse striae, 22–24 in 10 µm (Figs 5–7).

Description in SEM: External view: Valve vaulted towards the apices, angled at the raphe (Figs 8–9). Raphe bordered by a narrow, inconspicuous, hyaline area (Figs 10–17). Central external raphe fissures deeply projecting into the central area, variable in undulation, either overlapping for some distance (Figs 15–16) or not overlapping (Fig. 17), and curving in opposite directions. Terminal raphe fissures straight, obliquely bent towards the concave side of the valve (Figs 10–13). Apices with a short, buttonhole-shaped opening, near the terminal part of the raphe fissure, on the concave side of the valve (Figs 10–13, arrowheads). Areolae arranged in decussate striae, opening by apically elongated slit-like foramina (Figs 10–17). Foramina around the central area shorter than in the remainder of the valve (Figs 15, 17).

Internal view: Raphe-sternum narrow, thickened (Figs 18–23). Central area short and raised, with slender central bars of approximately equal length, surrounding the oval raphe nodule (Figs 22–23). Central raphe endings coaxial, dilated in the proximal part (Figs 22–23). Polar raphe endings terminated in a well-developed, looped helictoglossae, turned towards the concave side of the valve (Figs 20–21). Apices with a buttonhole-shaped opening located near the helictoglossa (Figs 20–21, arrowheads). Areolae opening by elliptical, hymen-occluded pores crossed by siliceous bars except at the periphery of the central area (Figs 22–23).



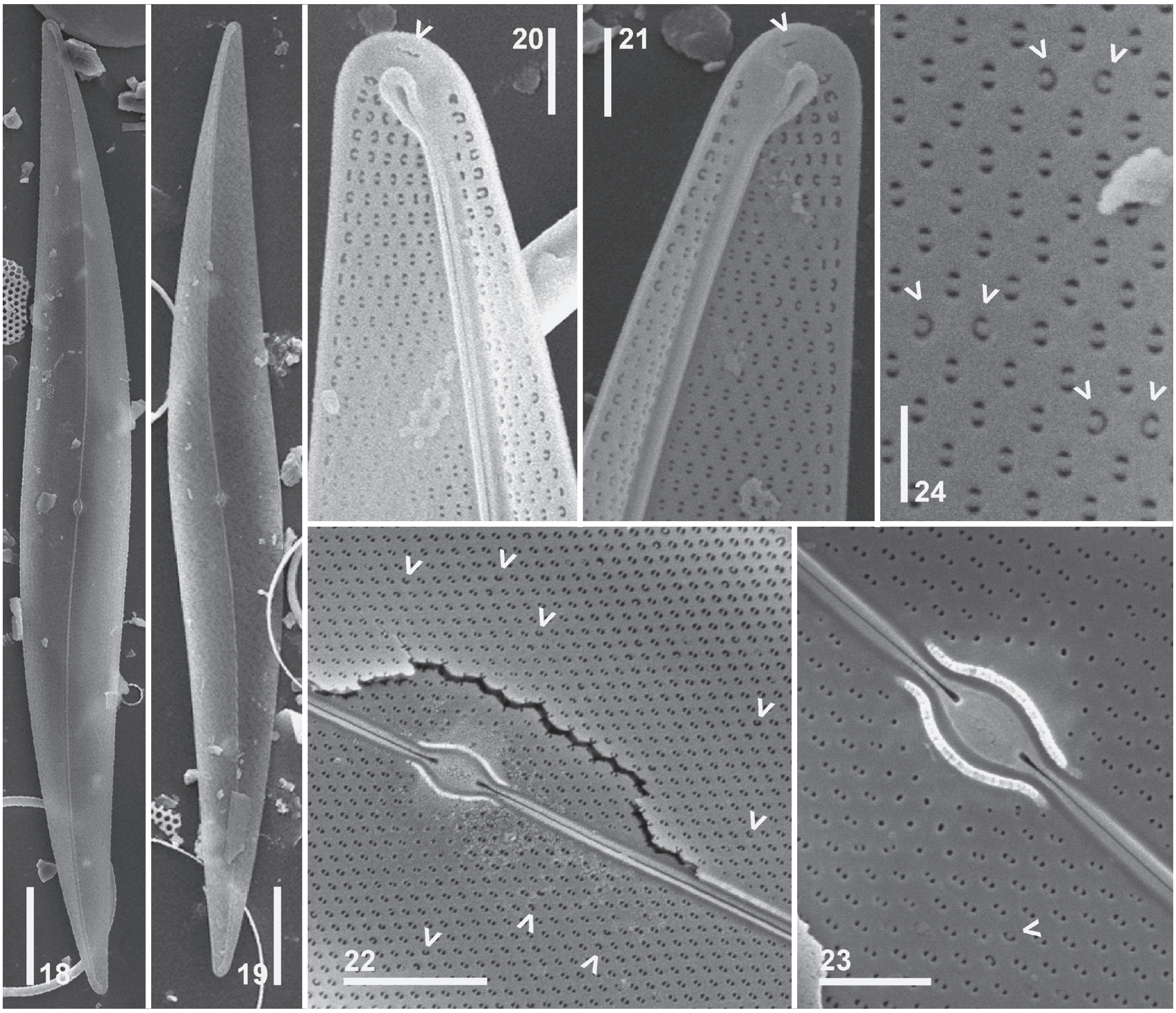
Figs 8–17. *Pleurosigma hinzianum*. Type material from sample used to prepare the holotype slide. SEM. External views. **Figs 8–9**, General appearance of the valve. **Figs 10–13**, Valve apex showing straight terminal fissure straight, bent towards the concave side of the valve and the adjacent buttonhole-shaped opening (arrowhead). **Fig. 14**, Central part of the valve. Note apically elongated slit-like foramina. **Figs 15–17**, Detail of the central area showing raphe fissures. Scale bars: Figs 8–9 = 20 µm, Fig. 14 = 5 µm, Figs 10–13, 15–17 = 2 µm.

Pores paired, C-shaped, appearing scattered over the valve surface (Figs 22–24, arrowheads). Areolae bordering the raphe-sternum and the non-perforated valve margin not completely aligned (Figs 20–21).

This new species is named after Friedel Hinz of the Alfred Wegener Institute for Polar and Marine Research in Bremerhaven, Germany in recognition of her long-term assistance to the diatom research community.

HOLOTYPE: Slide LPC 4262 (1) here designated, labelled ‘holotipo de *Pleurosigma hinzianum*, Nueva Atlantis, 27/03/1995’ (deposited in the Herbarium of the División Ficología ‘Dr. Sebastián A. Guarrera’).

ISOTYPE: Slide LPC 4262 (2) here designated, labelled ‘isotipo de *Pleurosigma hinzianum*, Nueva Atlantis, 27/03/1995’ (deposited in the Herbarium of the División Ficología ‘Dr. Sebastián A. Guarrera’).



Figs 18–24. *Pleurosigma hinzianum*. Type material from sample used to prepare the holotype slide. SEM. Internal views. **Figs 18–19**, Valve showing eccentric raphe-sternum. **Figs 20–21**, Valve apex showing polar endings finishing in loop-shaped helictoglossa. Note a buttonhole-shaped opening adjacent to the helictoglossa (arrowhead). **Fig. 22**, Central part of the valve showing central area short and raised central area with central bars surrounding the oval raphe nodule. Note pairs of C-shaped pores (arrowheads). **Fig. 23**, Detail of the central area. Note central coaxial raphe endings coaxial and pairs of C-shaped pores (arrowhead). **Fig. 24**, Elliptical, hymen-occluded pores crossed by a bar. Detail of paired C-shaped pores (arrowheads). Scale bars: Figs 18–19 = 20 µm, Fig. 22 = 5 µm, Figs 20–21, 23 = 2 µm.

PARATYPE: Slide LPC 4263 (2) here designated, labelled ‘paratipo de *Pleurosigma hinzianum*, Pinamar, 27/03/1995’ (deposited in the Herbarium of the División Ficología ‘Dr. Sebastián A. Guarrera’).

TYPE LOCALITY: Nueva Atlantis, 36°45′81″S-56°40′04″ W, Buenos Aires Province, Argentina.

DISTRIBUTION IN THE STUDY AREA: *Pleurosigma hinzianum* occurred in samples collected at Nueva Atlantis 27 March 1995, Pinamar 27 March 1995, Villa Gesell 21 March 2011 and Santa Teresita 8 March 2013, in the Province of Buenos Aires. *Pleurosigma hinzianum* only appeared in samples collected in the late summer and early autumn.

REMARKS: The combination of characters observed in the type material of the marine species *Pleurosigma hinzianum* can be summarized as follows: (1) valve lanceolate, slightly sigmoid with acute apices, (2) raphe-sternum eccentric, (3) raphe angle +7° to +8°, (4) angle of intersection of the oblique striae 64° to 69°, (5) terminal external raphe fissures straight, bent towards the concave side of the valve, (6) buttonhole-shaped opening adjacent to the raphe fissure, obliquely oriented towards the concave side of the valve, (7) apices with a buttonhole-shaped opening adjacent to the looped helictoglossa, (8) hymen-occluded pores crossed by a siliceous bar.

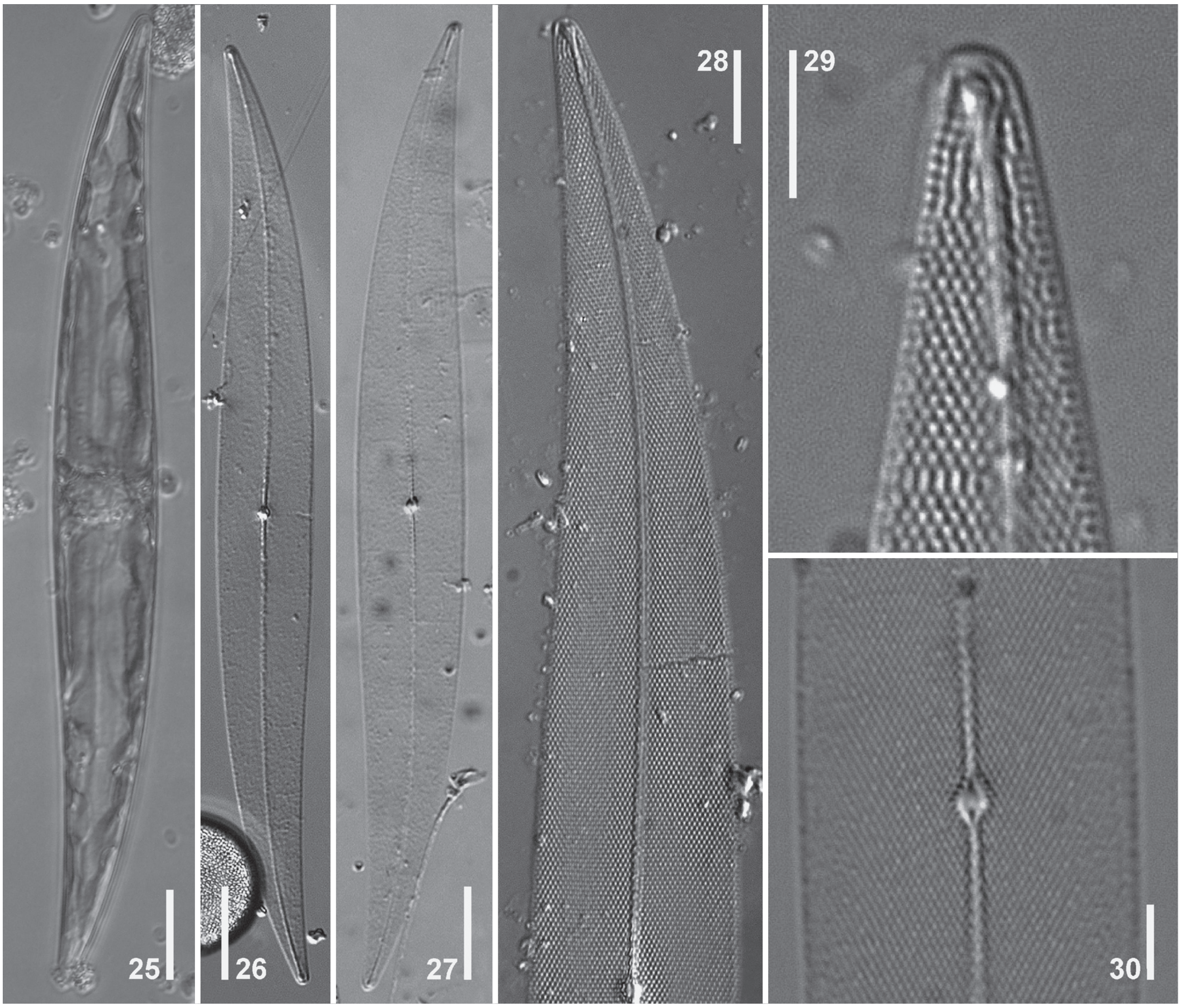
Table 1. Morphometric data obtained from specimens of *Pleurosigma hinzianum* and *P. frenguellianum* and from type material of *P. amara* and *P. elongatum*. Abbreviations: nd, no data; *, measured or observed from pictures in the literature.

Taxon	Reference	Length in µm	Width in µm	Length/ width	Transverse striae in 10 µm	Oblique striae in 10 µm	Stria angle	Characteristics of raphe sternum	Morphology of the apices	Valve outline
P. hinzianum sp. nov.	This study (Figs 2–23) (n = 32)	181–206	20–28	7.0–8.7	22–24	19–22	64°–69°	Raphe sigmoid. Raphe angle +7/+8°	Acute	Lanceolate, slightly sigmoid
P. frenguellianum sp. nov.	This study (Figs 24–45) (n = 56)	161–289	20–29	8.2–11.2	20–22	18–20	53°–58°	Raphe almost central, slightly sigmoid towards the apices. Raphe angle +6/ +7°	Acute	Linear lanceolate, slightly sigmoid
P. amara Stidolph	Stidolph (1992) Type material (Figs 44–51, 64–76)	172–360	28–32	*8.9	20–21	20–21	61°–64°	Raphe almost central, slightly sigmoid towards the apices. Raphe angle +5°/ +7°	Acute	Lanceolate, slightly sigmoid
P. elongatum W. Smith	Smith (1852)	330	25	*13.2	nd	18	nd	Median line central.	Acute	Linear lanceolate, flexure slight
	Smith (1853)	210–386	nd	nd	nd	19	nd	*Raphe central, slightly sigmoid towards the apices	Acute	Lanceolate, flexure moderate
		*362	*31	*11.6				*Raphe angle ca +4°		
	Hendey (1964)	130–380	24–30	nd	nd	16–20	60°–70°	Central	Acute	Linear lanceolate, gently sigmoid
	Reid (2002)	130–380	20–30	*10.5	18–20	16–19	68°	Raphe central, slightly sigmoid *Raphe angle *c.* +5°	Acute	Linear lanceolate, slightly sigmoid
	This study Figs (46–52) (n = 3)	200–225	20–23	10.0	20–22	18–20	63°–65°	Raphe central, slightly sigmoid towards the apices. Raphe angle +5°	Acute	Linear lanceolate, slightly sigmoid

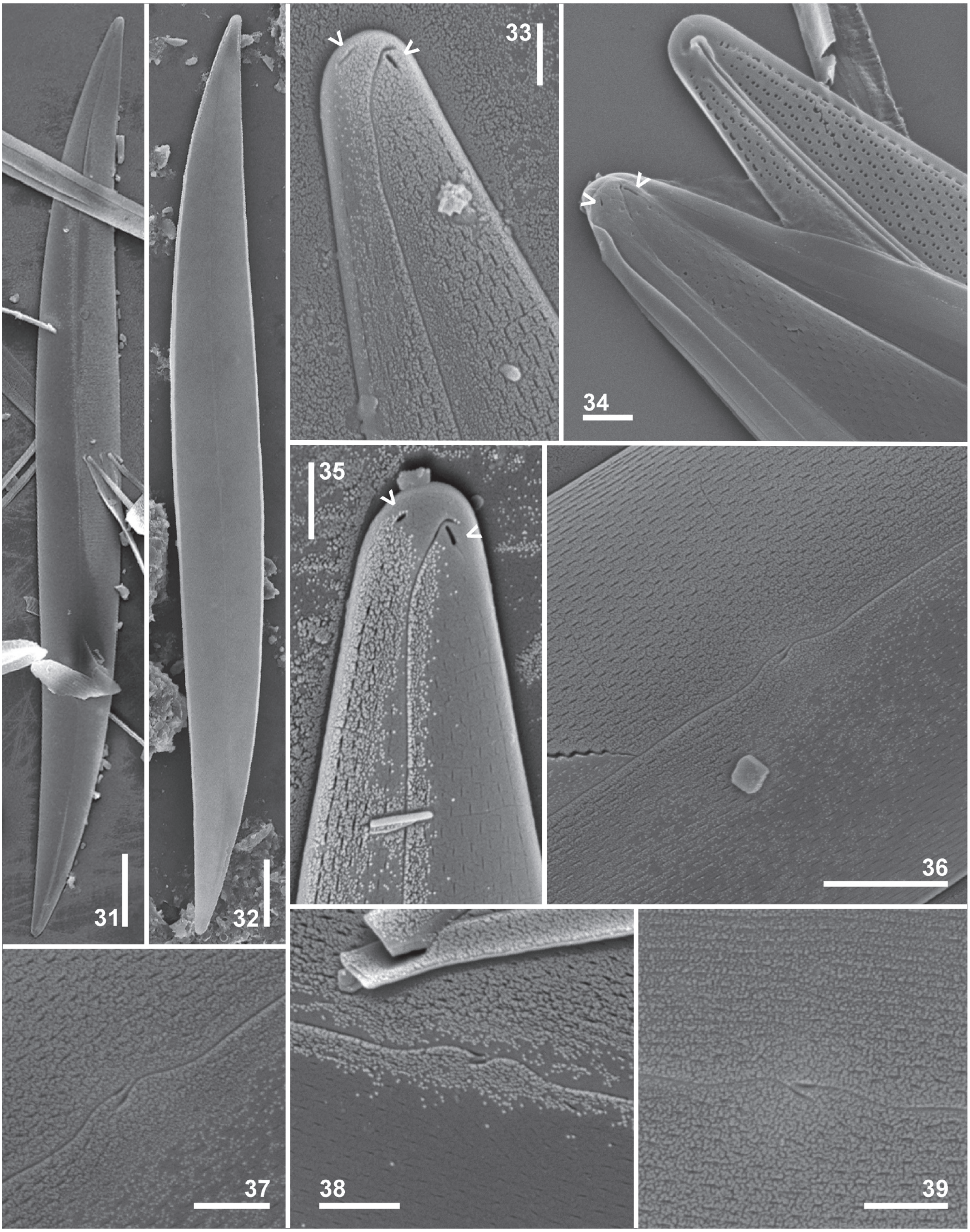
Pleurosigma frenguellianum* Sunesen, Sterrenburg & Sar, *sp. nov.
(Figs 25–47, Table 1).

Description in LM: Cell with one or two ribbon-shaped chloroplasts (Fig. 25) sometimes convoluted at the valve centre and lying appressed to the girdle towards the poles. Valve linear-lanceolate, slightly sigmoid, with acute apices (Figs 26–27), 161–289 µm long and 20–29 µm wide. Ratio length/width 8.2–11.2. Raphe-sternum almost central, sigmoid towards the apices approaching the convex side of the valve. Raphe angle +6° to +7° (Figs 26–27). Central area oval, small, with a thick barely bilaterally dilated central raphe nodule (Figs 26–28, 30). Central internal raphe endings dilated, coaxial (Fig. 29). Terminal areas small, unilaterally dilated, funnel-shaped, in apical position (Figs 28–29), with helictoglossa slightly twisted towards the concave side of the valve (Fig. 29). Striation pattern dominated by the oblique striae, 18–20 in 10 µm, intersecting at an angle between 53° and 58° in the central part of the valve, and crossed by transverse striae, 20–22 in 10 µm (Figs 28, 30). At the valve apex the stria orientation changing from the 3-system (*sensu Pleurosigma*) to the 2-system (*sensu Gyrosigma*) (Figs 28–29) as in *P. angulatum*.

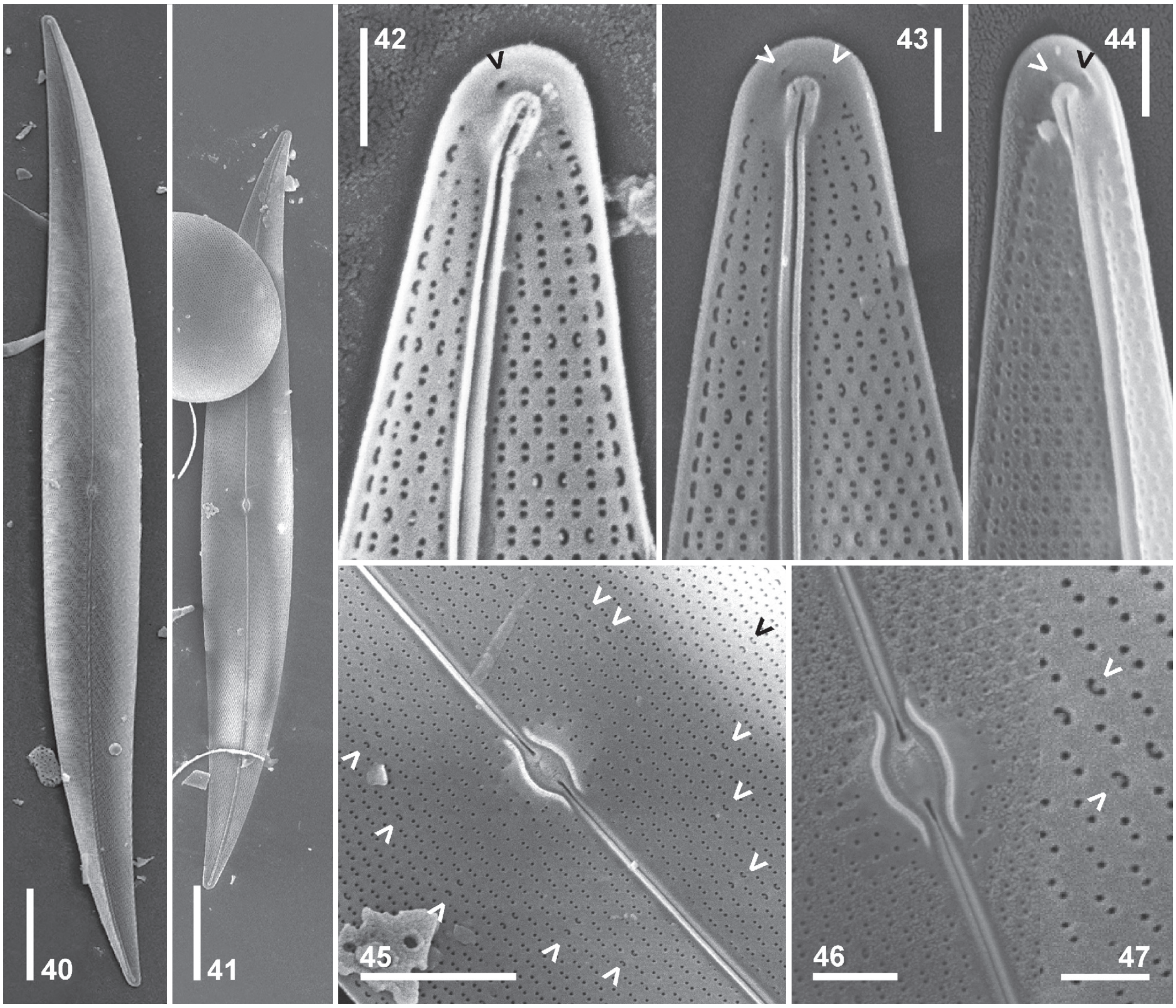
Description in SEM: External view: Valves slightly vaulted near the apices (Figs 31–32). Raphe bordered by a narrow, inconspicuous, hyaline area (Fig. 36). Central external raphe fissures deeply projecting into the central area, undulating in the same direction, with the ends more or less parallel, overlapping, and spoon-shaped dilated (Figs 36–39). Terminal raphe fissures hook-shaped, obliquely bent towards the concave side of the valve. Apices with two short, diagonally



Figs 25–30. *Pleurosigma frenguellianum*. Holotype (Figs 26, 28–29). Material from Bahía San Blas 07/05/11 (Fig. 25) and from Piedras Coloradas 05/26/06 (Figs 27, 30). LM. **Fig. 25**, Cell showing ribbon-shaped chloroplast. **Figs 26–27**, General appearance of the valve. **Figs 28–29**, Detail of valve showing striation pattern. **Fig. 29**, Note terminal unilaterally dilated area and change of striation pattern at valve apex. **Fig. 30**, Detail of the central area of the valve. Note central nodule showing internal raphe ends. Scale bars: Figs 25–27 = 20 µm, Fig. 28 = 10 µm, Figs 29–30 = 5 µm.



Figs 31–39. *Pleurosigma frenguellianum*. Type material from sample used to prepare the holotype slide (Figs 31, 35, 38) and the paratype slide (Figs 33, 36–37, 39). Material from Ría del Jabalí 09/29/08 (Fig. 32) and from Nueva Atlantis 03/27/95 (Fig. 34). SEM. External views. **Figs 31–32**, General appearance of the valve. **Figs 33–35**, Valve apex showing hook-shaped terminal fissure, bent towards the concave side of the valve and two adjacent buttonhole-shaped openings (arrowheads). **Fig. 36**, Central part of the valve. Note apically elongated slit-like foramina. **Figs 37–39**, Detail of the central area showing raphe fissures. Note that ends are more or less parallel, overlapping, spoon-shaped dilated. Scale bars: Figs 31–32 = 20 µm, Fig. 36 = 5 µm, Figs 33–35, 37–39 = 2 µm.



Figs 40–47. *Pleurosigma frenguellianum sp. nov.* Type material from sample used to prepare the holotype slide (Fig. 40) and the paratype slide (Figs 44, 46). Material from Nueva Atlantis 03/27/95 (Figs 41–43, 45, 47). SEM. Internal views. **Figs 40–41**, Valve showing almost central raphe-sternum, sigmoid towards the apices. **Figs 42–44**, Valve apex showing change of striation pattern and polar endings in helictoglossa. Note two isolated pores adjacent to the helictoglossa (arrowheads). **Fig. 45**, Central part of the valve showing short and raised central area with central bars surrounding the oval raphe nodule. Note pairs of C-shaped pores (arrowheads). **Fig. 46**, Detail of the central area. Note coaxial central raphe endings. **Fig. 47**, Elliptical, hymen-occluded pores crossed by a bar. Detail of paired C-shaped pores (arrowheads). Scale bars: Figs 40–41 = 20 µm, Fig. 45 = 5 µm, Figs 42–44, 46 = 2 µm, Fig. 47 = 1 µm.

arranged buttonhole-shaped openings near the terminal part of the raphe fissure (Figs 33–35, arrowheads). Areolae arranged in decussate striae, opening by apically elongated slit-like foramina (Figs 33–39). Areolae bordering the non-perforated valve margin perfectly aligned (Figs 33–34, 36).

Internal view: Raphe-sternum narrow, thickened (Figs 40–47). Central area short and raised, with slender central bars of similar length, surrounding the oval raphe nodule (Figs 45–46). Central raphe endings coaxial, dilated in the proximal part (Figs 45–46). Polar endings terminated in a well-developed helictoglossa with parallel lips, slightly turned towards the concave side of the valve (Figs 42–44). Apices with two isolated pores, one on each side of the helictoglossa (Figs 42–44, arrowheads). Areolae opening by elliptical, hymen-occluded pores crossed by siliceous bars (Figs 42–44, 46–47) except at the periphery of the central area (Figs 45–46). Pores paired, C-shaped, appearing scattered over the valve surface (Figs 45, 47, arrowheads). Areolae bordering the non-perforated valve margin completely aligned and C-shaped (Figs 42–44).

This new species is named after Dr Joaquín Frenguelli of the Museo de La Plata, Argentina, who started the study of diatoms in Argentina in the early 20th century.

Holotype: Slide LPC 11220 (1) here designated, labelled 'holotipo de *Pleurosigma frenguellianum*, Villa Gesell, 02/07/2010' (deposited in the

Herbarium of the División Ficología 'Dr. Sebastián A. Guarrera').

Isotype: Slide LPC 11220 (2) here designated, labelled 'isotipo de *Pleurosigma frenguellianum*, Villa Gesell, 02/07/2010' (deposited in the Herbarium of the División Ficología 'Dr. Sebastián A. Guarrera').

Paratype: Slide LPC 11221 (1) here designated, labelled 'paratipo de *Pleurosigma frenguellianum*, Mar Azul, 02/07/2010' (deposited in the Herbarium of the División Ficología 'Dr. Sebastián A. Guarrera').

Type locality: Villa Gesell, 37°16′50,70″S–56°58′53″W, Buenos Aires Province, Argentina.

Distribution in the study area: *Pleurosigma frenguellianum* occurred in samples collected in Santa Teresita 27 March 1995; Nueva Atlantis 27 March 1998; Villa Gesell 2 February 2010, 22 March 2011; Mar Azul 26 March 2010, 2 July 2010, 8 March 2013; Ría del Jabalí 29 September 2008 and Bahía San Blas 29 September 2010, 5 July 2011, 11 October 2011, 18 March 2013 in the Buenos Aires Province and in Piedras Coloradas 26 March 2006, Gulf of San Matías, Río Negro Province. *Pleurosigma frenguellianum* shows a wider distribution in space and time than *P. hinzianum* appearing between late summer and spring.

Remarks: The combination of characters observed in the type material of the marine species *Pleurosigma frenguellianum* can be summarized as follows: (1) valve linear-lanceolate, slightly sigmoid, with acute apices, (2) raphe-sternum almost central, sigmoid towards the apices, (3) raphe angle +6° to +7°, (4) angle of intersection of the oblique striae 53° to 58°, (5) stria pattern changing in orientation from 3-system to 2-system near the valve apex, (6) ends of the central external raphe fissures more or less parallel, overlapping, spoon-shaped dilated, (7) terminal external raphe fissures hook-shaped, (8) two diagonally arranged buttonhole-shaped openings adjacent to the raphe fissure, (9) two isolated pores adjacent to the helictoglossa, which has parallel lips, (10) areolae bordering the non-perforated valve margin perfectly aligned, internally C-shaped, (11) hymen-occluded pores crossed by a siliceous bar.

Analysis of type material of Pleurosigma elongatum *W. Smith*

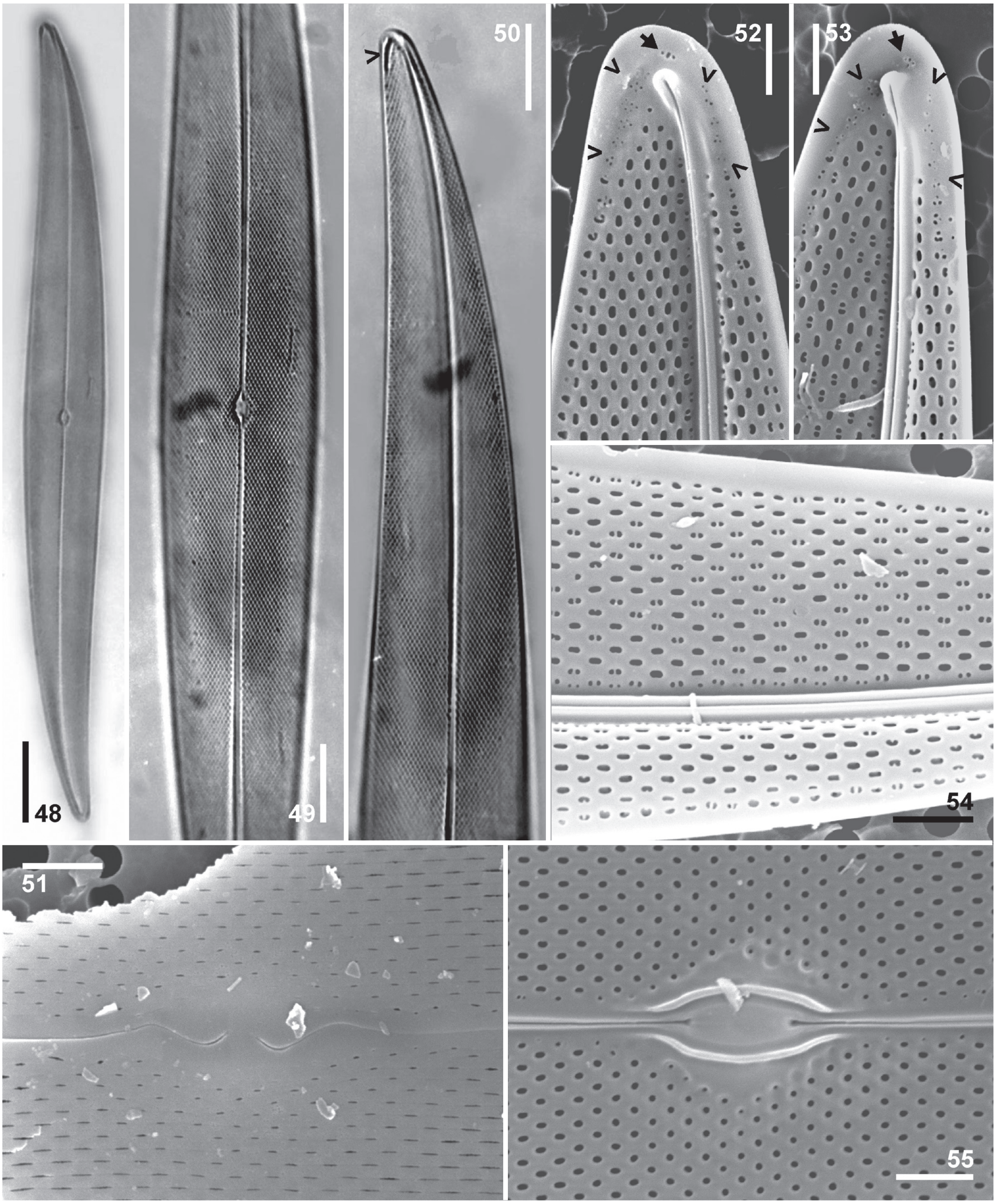
(Figs 48–55, Table 1)

The type material was examined in LM and SEM to obtain more data on the morphology, such as the internal morphology of the areolae and the ultrastructural equivalent of the 'calcar' seen in LM. Specimens of the type material pictured by Reid (2002, figs 24, 26) show the areolar pores adjacent to the raphe-sternum crossed by bars whilst elsewhere they do not show bars. In Sterrenburg *et al.* (2005), it was shown that such bars develop in the last stage of valve formation, but not necessarily in all pores.

A typical specimen in the type material, on slide BM 23651, is shown in Figs 48–50. The valve is slender lanceolate, moderately sigmoid, gradually tapering and vaulted towards the ends, with somewhat acute apices, 200 µm long, 20 µm wide. The raphe-sternum is almost straight for most of its length (Figs 48–49), becoming moderately sigmoid and eccentric near the apices (Fig. 50). The terminal areas are small, unilaterally dilated, funnel-shaped, in apical position (Fig. 50). A calcar is clearly visible in LM as a spur-like shadow at the apex (Fig. 50, arrowhead). Raphe angle +5°. The central area is small, oval and symmetric (Fig. 49). The transverse striae number 20–22 in 10 µm, the oblique 18–20 in 10 µm. The angle of intersection of the oblique striae is 63°–65°.

SEM study of the type material in the Van Heurck Collection yielded three valves, an incomplete one in external view and two in internal view. The central external raphe fissures are twice deflected to the same side, becoming W-shaped, ending at the same level in the central area (Fig. 51). The loculate areolae are arranged in decussate striae, opening by apically elongated slit-like foramina, shorter around the central area than elsewhere (Fig. 51). The raphe-sternum is internally thickened (Figs 52–55). The central area is small and raised, with central bars of approximately equal length surrounding the oval raphe nodule (Fig. 55) and central internal raphe endings coaxial, dilated as small triangles (Fig. 55). The terminal internal raphe ends in helictoglossae turned towards the concave side of the valve. On the concave side, they are bordered by a series of rosette-shaped clustered pores (Figs 52–53, arrowheads), which form the SEM equivalent of the 'calcar' seen in LM. There is a cluster of 2–4 small pores adjacent to the helictoglossa (Figs 52–53, arrows). The areolae open internally by hymen-occluded pores, roundish only in a small area around the central nodule (Fig. 55) and elliptical in the rest of the valve (Figs 52–54). The hymen-occluded pores in the central part are not crossed by a bar, whilst a row of pores adjacent to the raphe-sternum does show such narrow silica bars and the rest of the valve shows pores with, as well as others without, bars (Figs 52–55).

Records from Argentina: Pleurosigma elongatum was recorded from Buenos Aires coastal waters by Müller Melchers (1959) and from continental waters of Argentina by Solari & Claps (1984) (Arroyo San Clemente, Buenos Aires Province), Romero (1993) (Río Colorado, Province of La Pampa), Vouilloud & Leonardi (2001) (in artificial channels, Buenos Aires Province) and Rodríguez *et al.* (2006) (epixylic in a section of the lower river Luján and a tributary,



Figs 48–55. *Pleurosigma elongatum* Smith. Type material from 'Poole Bay, June 1849', kept in Van Heurck Collection (Figs 48–55). Figs 48–50, LM. Figs 51–55, SEM. **Fig. 48**, General appearance of the valve. **Fig. 49**, Detail of the central part of the valve. **Fig. 50**, Detail of valve apex showing calcar (arrowhead). **Fig. 51**, Detail of the central area of the valve in external view showing W-shaped raphe fissures. **Figs 52–53**, Detail of valve apex in internal view showing helictoglossa bordered by a series of rosette-shaped clustered pores and one apical rosette (arrowhead). **Fig. 54**, Detail of the elliptical hymen-occluded internal pores, some of which are bisected by a bar. **Fig. 55**, Detail of the central area of the valve in internal view. Note circular hymen-occluded pores not bisected by a bar. Scale bars: Fig. 48 = 20 µm, Figs 49–50 = 10 µm, Figs 51–55 = 2 µm.

Buenos Aires Province), without morphological analysis or illustrations. Maidana (1994) determined material from the Quaternary sediments of the Salinas del Bebedero as *P. elongatum*, but the dimensions given and the valve outline of the specimens drawn do not match those of *P. elongatum* or of the new species described here. Thus we are not able to confirm the presence of *P. elongatum* in Argentina.

Remarks: The combination of characters observed in the type material of the marine species *Pleurosigma elongatum* can be summarized as follows: (1) valve linear-lanceolate, slightly sigmoid, with acute apices, (2) calcar present, (3) raphe-sternum almost central, sigmoid towards the apices, (4) raphe angle +4° to +5°, (5) angle of intersection of the oblique striae 63°–68° (including data of Reid, 2002), (6) central external raphe fissures W-shaped, (7) helictoglossae turned towards the concave side of the valve and laterally bordered by a series of rosette-shaped clustered pores, (8) hymen-occluded pores of the areolae may be bisected by a bar.

Discussion

In Sterrenburg *et al.* (2003) it was pointed out that *Pleurosigma* species have a fully functional raphe, allowing active movement across and through substrata, which would serve no useful purpose for a planktonic diatom. The presence of *Pleurosigma* species and other raphe-bearing diatoms in the plankton can be attributed to three causes. Firstly, in shallow turbulent waters such as the localities examined here, benthic diatoms are easily dislodged from the substratum and suspended in the water column. Secondly, long-established epiphyton readily detaches from macrophytes to enter the water column. The samples studied here also contain raphe-bearing diatoms of the typically epiphytic genus *Cocconeis*. Thirdly, even in stagnant pools sediments rich in benthic diatoms may become suspended and dispersed as a result of photosynthetic oxygen production. A planktonic occurrence does not necessarily equate to a planktonic mode of life; such an incidental presence has been characterized as tychoplanktonic.

In the LM, *Pleurosigma hinzianum* Sterrenburg, Sunesen & Sar *sp. nov.* (Figs 2–7) resembles *Pleurosigma frenguellianum* Sunesen, Sterrenburg & Sar *sp. nov.* (Figs 25–30) in general appearance and dimensions (see Table 1). Both species show subtle differences in the valve outline (more lanceolate in *P. hinzianum* and more linear in *P. frenguellianum*), and in the raphe-sternum path (more eccentric in the middle part of the valve in *P. hinzianum* and almost central in *P. frenguellianum*). The principal LM features separating *P. hinzianum* and *P. frenguellianum* are the angle of the oblique striae in the central part of the valve (64°–69° in the former and 53°–58° in the latter) and the orientation of the striae at the valve apex, in 3-systems in *P. hinzianum* and in 2-systems in *P. frenguellianum*.

In the SEM, these species show several important differences in the external central raphe fissures and in the external and internal apical structure. The overlapping part of the central external raphe fissures is not dilated in *P. hinzianum* (Figs 14–17) whereas it is dilated and spoon-shaped in *P. frenguellianum* (Figs 36–39). The apices in external view differ in the morphology of the terminal raphe fissures: straight, oblique, with an adjacent buttonhole-shaped opening in *P. hinzianum* (Figs 10–13) and hook-shaped, with two adjacent diagonally arranged buttonhole-shaped openings in *P. frenguellianum* (Figs 33–35). In internal view, the helictoglossa is accompanied by one buttonhole-shaped opening in *P. hinzianum* (Figs 20–21, arrowheads) and by two isolated pores in *P. frenguellianum* (Figs 42–44, arrowheads).

Pleurosigma hinzianum (Figs 2–7) resembles *P. amara* Stidolph (see figs 44–51 in Stidolph 1992) in general appearance, but shows subtle differences in the dimensions of the valves, stria density, angle of intersection of the oblique striae and raphe angle. *Pleurosigma frenguellianum* (Figs 25–30) resembles *P. amara* (Stidolph 1992, figs 44–51) in the path of the raphe-sternum and in the raphe angle but differs in valve outline, in the angle of intersection of the oblique striae (Table 1) and in the orientation of the striae at the valve apex. The ranges of valve dimensions and stria density are not useful for separating *P. hinzianum* and *P. frenguellianum* from *P. amara*. More useful LM features are: the path of the raphe-sternum, which is sigmoid in *P. hinzianum* (Figs 3–4) but almost central in the middle of the valve becoming sigmoid only towards the apices in *P. amara* (Stidolph 1992, fig. 44). The calcar is present in *P. amara* (Stidolph 1992, figs 48–49) but absent in both *P. hinzianum* (Figs 5–6) and *P. frenguellianum* (Figs 28–29). Although some characters separate *P. hinzianum* and *P. frenguellianum* from *P. amara* in the LM, the main characters for differentiation are ultrastructural, principally related to details of the external and internal apical structure. The terminal external raphe fissures are straight, obliquely bent towards the concave side of the valve in *P. hinzianum* (Figs 10–13), hook-shaped and obliquely bent towards the concave side in *P. frenguellianum* (Figs 33–35) and broadly curved over the valve apex in *P. amara* (Stidolph 1992, figs 64–65). Additionally, there is a small buttonhole-shaped opening adjacent to the raphe fissure towards the concave side of the valve in *P. hinzianum*, two diagonally arranged buttonhole-shaped openings adjacent to the raphe fissure in *P. frenguellianum* and two terminal long and slender grooves parallel to the valve margin in *P. amara*.

The internal apical view shows modified slit-like areolae in the proximity of the helictoglossa in *P. amara* (see figs 68–69 in Stidolph 1992) while the areolae adjacent to the helictoglossa are similar to those at the valve margin in *P. hinzianum* (Figs 20–21) and *P. frenguellianum* (Figs 42–44). Another useful feature for separating *P. frenguellianum* and *P. amara* is the morphology of the end of the central external raphe fissures: generally parallel, constantly overlapping and dilated in the former (Figs 36–39) and markedly variable without overlapping or dilatation in the latter (see fig. 76 A–G in Stidolph, 1992).

Another species that looks similar to the new species described here is *P. elongatum*. The type material of *P. elongatum* has been previously analysed and some ultrastructural information was added by Reid (2002, figs 22–26). Our specimens of *P. elongatum* (Figs 48–50) perfectly match those shown by Smith (1852, pl. 1, fig. 4; see also Smith, 1853, pl. 20, fig. 199) and by Reid (2002, figs 22–23, 25) in valve outline, path of the raphe-sternum, angle of intersection of the oblique striae and raphe angle (see Table 1). The transverse striae are slightly denser than the oblique in our specimens and in those shown and described in Reid (2002, figs 23–24, 26) (Table 1).

Ultrastructural details of the internal central area (Fig. 55) match those shown in Reid (2002, fig. 26) as regards outline, extension and morphology of the internal raphe ends, central bars around the nodule and hymen-occluded pores. The central external raphe fissures, which have not been previously shown in the type material, are W-shaped in the specimen retrieved (Fig. 51) and resemble those pictured by Stidolph (1992, fig. 79) for the Sterrenburg Collection sample from Robbenoordbos, North Holland, material. However, as is evident from the various shapes seen in *P. hinzianum*, the external central raphe fissure shape is not always a constant character, as pointed out in Sterrenburg (1990). The apex of the specimen pictured by Reid (2002, fig. 24) in internal view is apparently eroded, with an atypical areola pattern that might be interpreted as an early stage of the valve morphogenesis (Sterrenburg *et al.* 2005). Specimens analysed in the present study are not eroded and show apical details that could be considered consistent with those described by Reid (2002, fig. 24), the helictoglossae are laterally bordered by a series of rosette-shaped clustered pores including a terminal one (Figs 52–53, arrowheads). The type specimens examined and the specimens from Robbenoordbos examined by Stidolph (1992, figs 80–82) showed the same aspect of internal occluded pores: circular, single, without bars, in the middle part of the valve and elliptical, bisected by a bar in the vicinity of the raphe-sternum. Additionally, the material of the present study (Figs 52–54) and that of Stidolph (1992, fig. 81) show bisected pores alternating with elliptical single pores over the valve. It is unlikely that all valves are in the early stages of morphogenesis, thus it is probable that the internal pores are more morphologically variable than claimed for this genus (see Round *et al*., 1990).

The LM comparison between *Pleurosigma frenguellianum* (Figs 25–30) and *P. elongatum* (Figs 48–50) shows some similarities in valve outline, path of the raphe-sternum and stria density. Nevertheless, both species can be separated by the angle of intersection of the oblique striae (53°–58° in the former and 63°–68° in the latter), the raphe angle (+6°/+7° in the former and +4°/+5° in the latter) and the pattern of striation at the apex. *Pleurosigma hinzianum* (Figs 2–7) differs from *P. elongatum* in valve outline, path of the raphe-sternum and raphe angle but it is similar in the angle of intersection of the oblique striae (Table 1). A calcar is present in *P. elongatum* (Fig. 50) and absent in both *P. hinzianum* (Figs 5–6) and *P. frenguellianum* (Figs 28–29). The comparison of these species in SEM reveals differences in details of the internal apical structure: there is a series of rosette-shaped clustered pores laterally to the helictoglossa with a cluster at the apex in *P. elongatum* (Figs 52–53), whereas the areolae adjacent to the helictoglossa are similar to those at the valve margin, with one isolated apical pore in *P. hinzianum* (Figs 20–21) and with two isolated apical pores in *P. frenguellianum* (Figs 42–44). In addition, in the new species, all the internal hymen-occluded pores except for a few surrounding the central area are crossed by a bar, unlike the situation in *P. elongatum*.

The main LM features of the species discussed are the valve outline, the path of the raphe-sternum, the raphe angle, the angle of intersection of the oblique striae and the striation pattern at the apex. In SEM, the external central and terminal raphe fissures, internal details of the central area, external and internal apical details and morphology of the hymen-occluded pores are important characters. The results of the present study, like those of Stidolph (1992) and Sar *et al.* (2012, 2013) for instance, demonstrate that detailed electron microscopic studies are necessary for the description of new species in the genus *Pleurosigma* and re-examination of the type material of similar species is also necessary to avoid potential taxonomic confusion.

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