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Valve morphology of *Diploneis sudamericana* nov. sp. and *Diploneis elliptica* (Kützing) Cleve

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With 41 figures and 1 table

Abstract: *Diploneis* Ehrenberg *ex* Cleve is a diverse genus, with species predominantly from epipelic, marine habitats; there are a few freshwater representatives. In 1933 Frenguelli reported four taxa of this genus at the Esteros del Iberá, a vast subtropical wetland from Argentina. Two of the four taxa reported were new for science. One of the reported species was *Diploneis elliptica* (Kützing) Cleve (Basionym: *Navicula elliptica* Kützing) a species cited from very diverse environments of every continent.

In this study the type material of *Navicula elliptica* Kützing, deposited at the Natural History Museum in London, was analyzed with scanning electron microscopy and its valve fine structure described. Comparison of this type material with others studied under SEM from America, Europe, Africa and Asia indicate that *D. elliptica* has been widely misidentified. The materials from Iberá, Argentina, deposited under this name in the Frenguelli´s Collection at the Museo de Ciencias Naturales de La Plata, belong to a new species, described here as *Diploneis sudamericana*. These two taxa are very similar when analyzed with LM but clearly differ in the striae and areolae structure. *D. sudamericana* has uniseriate striae at valve center and biseriate near the valve margin; the alveoli are externally occluded by a unique complex cribrate occlusion. This species also differs from others in the areolae of the longitudinal canals and the external proximal raphe ends.

Key words: diatoms, freshwater, *Diploneis elliptica*; *Diploneis sudamericana*, type material.

Introduction

In recent years, a number of publications appeared concentrating on the study of type material to clarify the identity of several diatom taxa, especially of those that are frequently reported in the ecological literature. With increased use of diatoms as indicators of ecosystem health, establishing the identity of at least the most common taxa could improve the accuracy of ecological diagnostic tools relying heavily on the most inclusive taxonomic categories as the basic units expressing environmental change.

Diploneis Ehrenberg *ex* Cleve (1894) is a large and diverse genus, with species predominantly from epipelic, marine habitats and a few freshwater representatives. Frustules of *Diploneis* are typically elliptical to panduriform, with bluntly rounded apices. Each valve possesses two longitudinal canals, one on each side of the raphe. The canals are positioned within the silica cell wall and open to the exterior through areolae, but lack openings to the interior of the cell. The frustules are heavily silicified, with complex loculate areolae. Externally the areolae are occluded by volae or cribra, finely punctate, and internally the alveoli are covered by a delicate silica membrane. The areolae, internal and external raphe ends, alveoli between the longitudinal

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canal, and the valve margin, are diagnostic features to identify species: (Idei & Kobayasi 1989), (Jovanovska et al. 2015).

In a study about the diatom flora of the Esteros del Iberá, a subtropical wetland from Argentina, Frenguelli (1933) reported *Diploneis elliptica* Kützing; *Diploneis elliptica* var. *tropica* Zimmerman, *Diploneis subovalis* var. *argentina* Frenguelli and *Diploneis zannii* Frenguelli. When analyzing with electron microscope raw materials from the Esteros del Iberá deposited in the Frenguelli´s Collection, we found that the specimens that fitted with the materials that Frenguelli assigned to *Diploneis elliptica* have a combination of characters not described in the literature.

Diploneis elliptica (Kützing) Cleve (Basionym: *Navicula elliptica* Kützing) is a species cited from very diverse environments of every continent (Guiry & Guiry 2017), but the only reference to the type material was with light microscopy by Lange-Bertalot & Reichardt (2000). In Argentina, the species was widely mentioned – nearly 30 records – in lakes and rivers of different ecoregions, from Puna to Patagonia, with different environmental characteristics: (Luchini & Verona 1972), (Tell 1985), (Vouilloud 2003). Only a few records are accompanied by LM illustrations, but there are none with SEM.

The fine morphology of the materials from Iberá differed from that described for materials mentioned in the literature as *D. elliptica* from other regions of the world in Krammer (1981), Krammer & Lange Bertalot (1986) and Jovanosvka et al. (2015). To confirm the identity of this species we examined with SEM the type material of *Navicula elliptica* deposited at the Natural History Museum in London.

The aim of this study is to describe the valve morphology of the type material of *Diploneis elliptica* (Kützing) Cleve and to clarify the identity of the materials from the Esteros del Iberá described by Frenguelli as *Diploneis elliptica.*

Materials and methods

Study area: Esteros of Iberá (Corrientes Province, Argentina) is a vast wetland that comprises 13,000 km^2 (1,300,000 has). This is a rain-fed, subtropical ecosystem (1200–1500 mm/year). It is a heterogeneous system of interconnected lagoons, marshes, streams and sandy banks. The drainage has a NE-SW reduced flow to the median basin of the Parana River. The mean annual temperature is 21º C, with minimum of -2º C and maximum of 44º C. Waters are poorly mineralized with electric conductivities ranging from 15 to 45 µS.cm^{-1}, pH ranging from 5 to 7, and high levels of organic matter: Ramsar (2015).

Analysed material:

Diploneis elliptica sensu Frenguelli 1933

The analysed material belongs to the Series 260–266 and 268, Frenguelli Collection deposited at the División Ficología of the Museo de Ciencias Naturales de La Plata.

Series 260: aquatic macrophytes, Concepción marsh, Corrientes Province, Argentina. December 6th, 1923. Coll. J. Frenguelli. Slides 1–6 and raw material.

Series 261: aquatic macrophytes, Carambola marsh, Corrientes Province, Argentina. December 8th, 1923. Coll. J. Frenguelli. Slides 1–6 and raw material.

Series 263: aquatic macrophytes, Cambá marsh, near Estancia Guayaibí, Corrientes Province, Argentina. December 12th, 1923. Coll. J. Frenguelli. Slides 1–5 and raw material.

Series 264: aquatic macrophytes, Carambola marsh, near San Miguel town, Corrientes Province, Argentina, December 12 th, 1923. Coll. J. Frenguelli. Slides 1–5 and raw material.

Series 268: bottom slime, Itatí-Rincón Lagoon, Corrientes Province, Argentina. November 1922. Coll. T. Zanni. Slides 1–8 and raw material.

Navicula elliptica Kützing

BM 001231786, Natural History Museum of London, UK. Mica fragment, Kützing Collection Nº 1774 b. Locality: Falaise, France.

The slides of the Frenguelli Collection were observed with a light microscope (LM) Leica equipped with DM 2500 phase contrast and a Leica DM 2500 with DIC (differential interference contrast). Type specimens were referenced with an England Finder TM Graticule.

Raw material was treated to eliminate organic matter following the method described in CENT/TC 230 (2002). It was mounted on alumni stubs and then coated with gold for observation with scanning electron microscopy (SEM).

Material mounted on mica was treated with glass cleaner liquid to separate the specimens from mica and then was filtered through a 5 µm polycarbonate filter. The dried filter was sputter coated with gold.

Observations with SEM were carried out in a Jeol JSM-T100 SEM at the Servicio de Microscopía Electrónica del Museo de La Plata and a Carl Zeiss NTS SUPRA 40 SEM in the Centro de Microscopías Avanzadas (CMA) de la Universidad de Buenos Aires.

Terminology used is that suggested in Idei & Kobayasi (1989), Round et al. (1990) and Jovanovska et al. (2015).

Results

Navicula elliptica Kützing

Figs 1–19

Basionym of *Diploneis elliptica* (Kützing) Cléve

Fifteen specimens of the type material of *N. elliptica* Kützing were found under SEM.

Description: SEM. Valves are widely lanceolate. Longitudinal canals H-shaped, occupy 1/4–1/3 of the valve width at the valve center, narrowing to the poles ending in acute ends (Figs 1–4, 13–15). Externally, the raphe is filiform with the shape of the proximal raphe ends being variable, the variability is attributed to different degrees of erosion of the analyzed specimens (Figs 5–6) and distal ends curved to the same side (Figs 1–3, 7). Central area is elliptic and axial area is straight. The longitudinal canals open in two rows of areolae at valve center (Figs 5–6) and one row to the poles (Fig. 1). Areolae have cribrate occlusions slightly sunken (Fig. 9 black arrows). At the central area the canals are sunken and elevated to the poles (Fig. 8). Striae are uniseriate, radial all along the valve, more pronounced at the poles (Figs 1–4, 13–15). Areolae have external cribra slightly sunken (Fig. 9, white arrows); they are simple near the canals and complex to the valve margin (Figs 10, 12 arrow). The external valve margin has a zipper-shaped structure (Fig. 12, arrowheads). Internally, the raphe is straight and runs in a longitudinal depression demarcated by the canals (Figs 13–14). Transapical costae delimit the alveoli that extend from the canals to the valve margin (Figs 13–17). The alveoli are covered by a thin silica membrane (Figs 16, 17 white arrows) and the areolae open in round foramina (Fig. 16 black arrows). The valvocopula is wide and smooth, without areolae (Fig. 11); its pars interior is fimbriate and each fimbria overlaps the costae (Figs 15, 17, 19, arrowheads).

The analyses with LM of the slides deposited in Frenguelli Collection showed 16 specimens that coincide with *Diploneis elliptica* sensu Frenguelli and 12 exemplars were found in the SEM. These materials clearly differ from *Navicula elliptica* in striae structure. They also differ from other species described in the literature so we propose it as a new species for science.

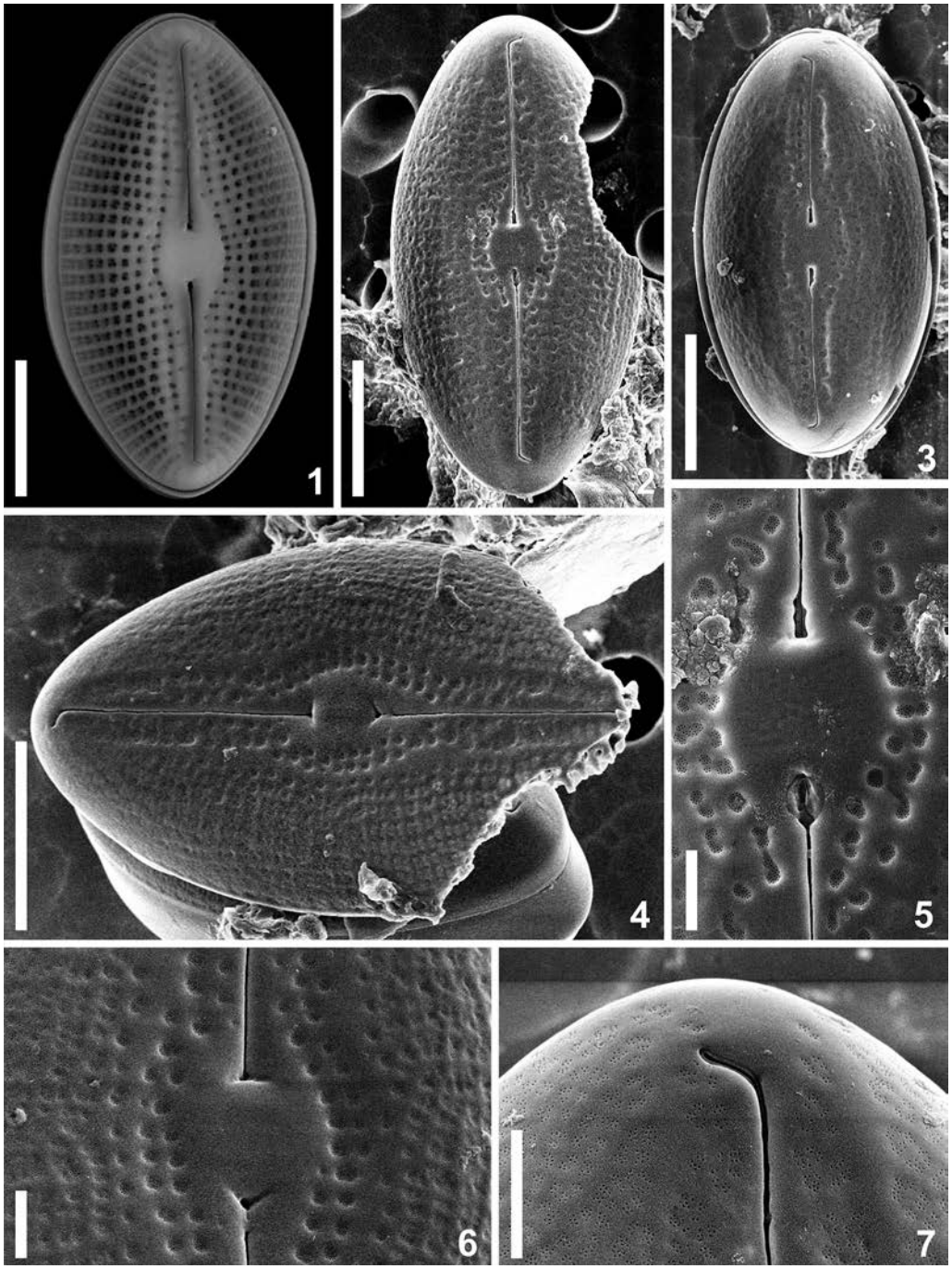

Plate 1. Figs 1–7. *Diploneis elliptica*. SEM. Specimens of type population. External valve views. Figs 1–3. Whole valve (Fig.1 was obtained with backscattered electrons). Fig. 4. Tilted view of a broken valve. Figs 5–6. Details of the valve center, note the prominent central nodule and the areola on the sunken canal. Fig. 5. Note the eroded proximal raphe ends. Fig. 7. Detail of an apex in external view, see the terminal raphe fissure. Scale bars: Figs 1–4: 10 µm; Figs 5–7: 2 µm.

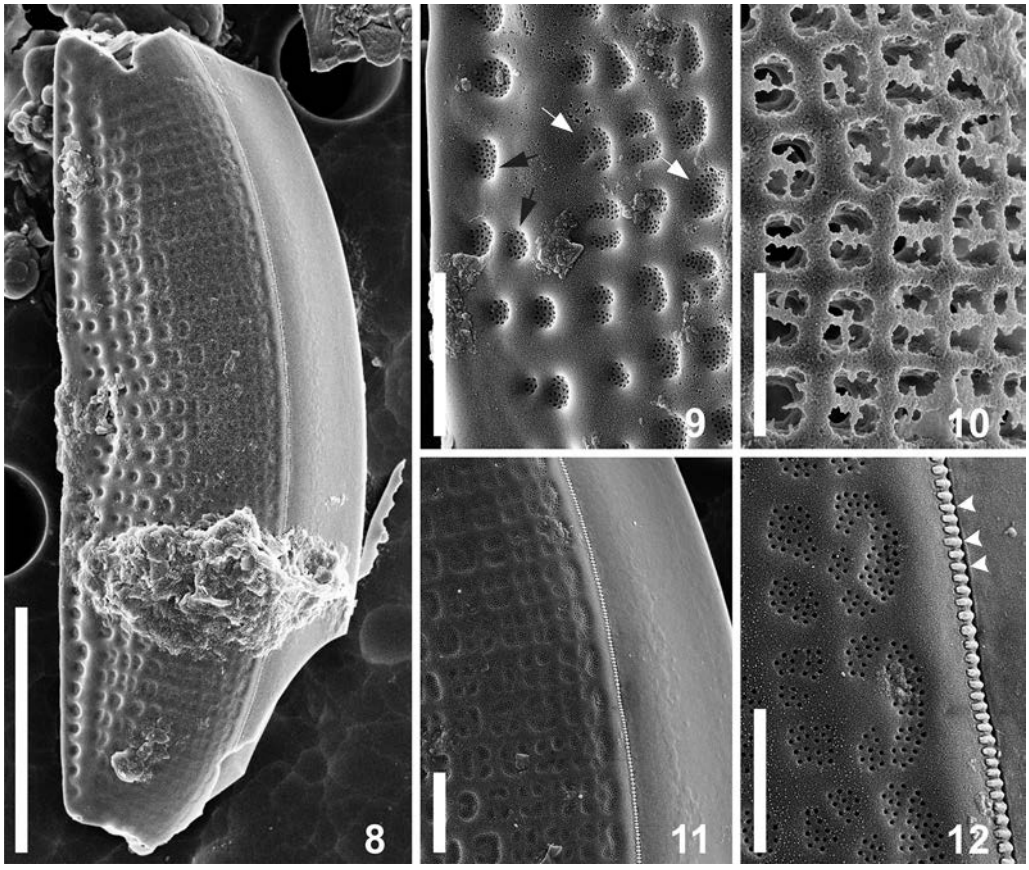

Plate 2. Figs 8–12. *Diploneis elliptica.* SEM. Specimens of type population. External valve views. Fig. 8. Tilted valve showing valve mantle and the valvocopula. Fig. 9. Detail of the areola on the canal (black arrows), the hyaline longitudinal line and the areolae on the alveoli (white arrows). Fig. 10. Details of the areola (eroded valve surface). Fig. 11. Detail of the mantle and smooth valvocopulae. Fig. 12. Detail showing the complex cribrate openings of areolae and the zipper-shaped structure (arrowheads) at valve margin. Scale bars: Fig. 8: 10 µm; Figs 9–11: 2 µm; Fig. 12: 1 µm.

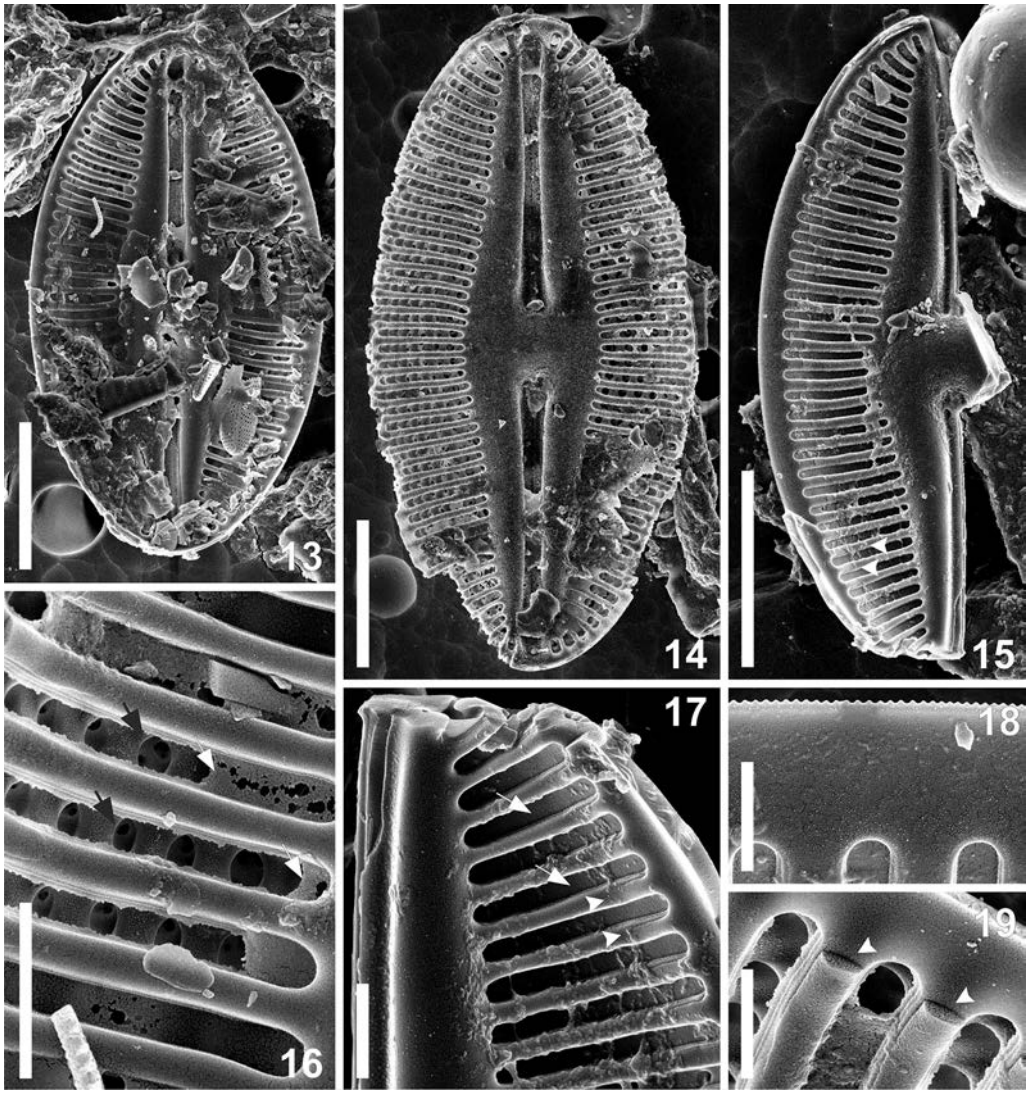

Plate 3. Figs 13–19. *Diploneis elliptica.* SEM. Specimens of type population. Internal valve views. Figs 13–14. Whole valves. Fig. 15: Broken valve. Figs 16–17. Details showing alveoli covered with thin layer of silica (white arrows) and internal foramina (black arrows). Fig. 18. Details of the valve margin in showing scalloped border corresponding to zipper-shaped structure (arrowheads). Fig. 19. Detail of costae showing fragments of fimbriae of valvocopula (arrowheads). Scale bars: Figs 13–15: 10 µm; Figs 16–17: 2 µm; Figs 18–19: 1 µm.

Diploneis sudamericana Vouilloud & Sala nov. sp.

Figs 20–41

Synonyms: *Diploneis elliptica* Kützing *sensu* Frenguelli 1933

Diploneis subovalis Cleve *sensu* Metzeltin et al. 2005

Holotype: Slide 268-2, Finder N42(4) (Figs 26–27)

Type locality: Itatí Rincón Lagoon, Esteros del Iberá, Corrientes Province, Argentina.

Etymology: the specific epithet refers to the region where the species was found.

Description: LM. (Figs 20–30). Valves are elliptic. Longitudinal canals are wide, they occupy 1/4–1/3 of the valve width at the valve center and narrow to the poles ending in acute ends. Externally, the canals are delimited by a hyaline longitudinal line and have round areolae irregularly arranged. Central area is round to ovoid and axial area is slightly wide at valve center, narrowing to the valve ends. Striae, uniseriate to the axial area and biseriate to the valve margin, are slightly radial at valve center and strongly radial to the ends.

SEM. (Figs 31–41). Externally, the raphe is filiform (Figs 31–34) with proximal ends curved to the same side in a round depression (Fig. 33); distal ends are curved at obtuse angles (Fig. 34). The longitudinal canals have 1–2 rows of areolae occluded by volae (Figs 32–34 arrowheads), separated from the striae by a conspicuous hyaline area (Fig. 33). At the valve exterior each alveolus has a single opening covered by a complex cribrate occlusion (Figs 32–33). Internally,

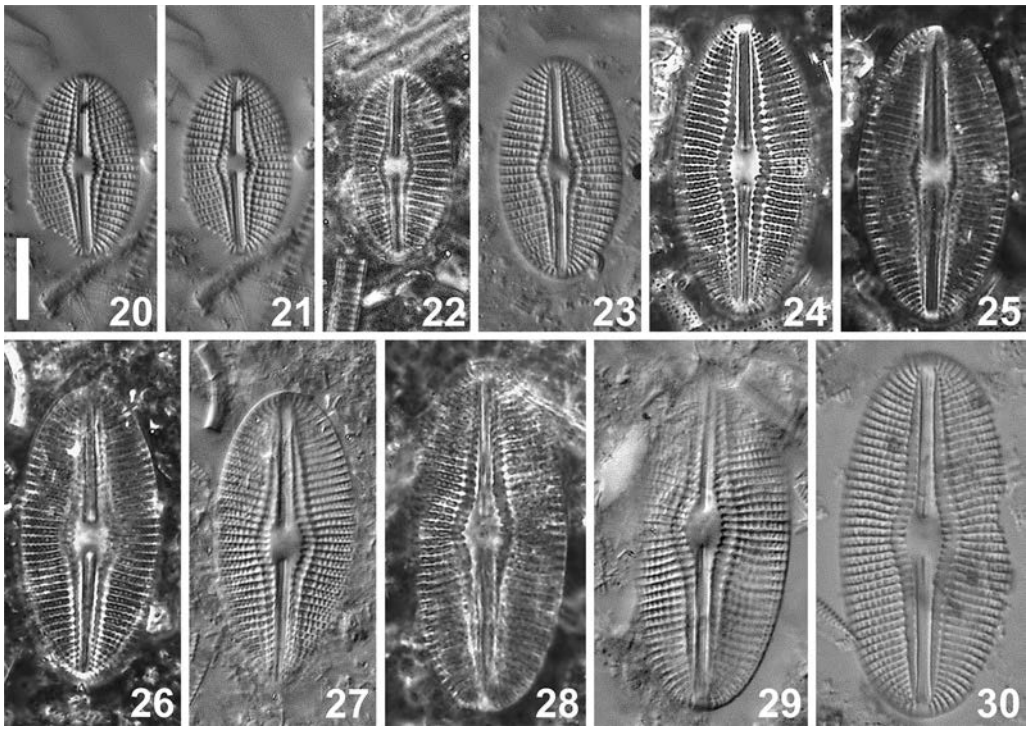

Plate 4. Figs 20–30. *Diploneis sudamericana* sp. nov. LM. Valve views, showing population variability. Figs 26–27. Holotypus. Scale bar: 10 µm.

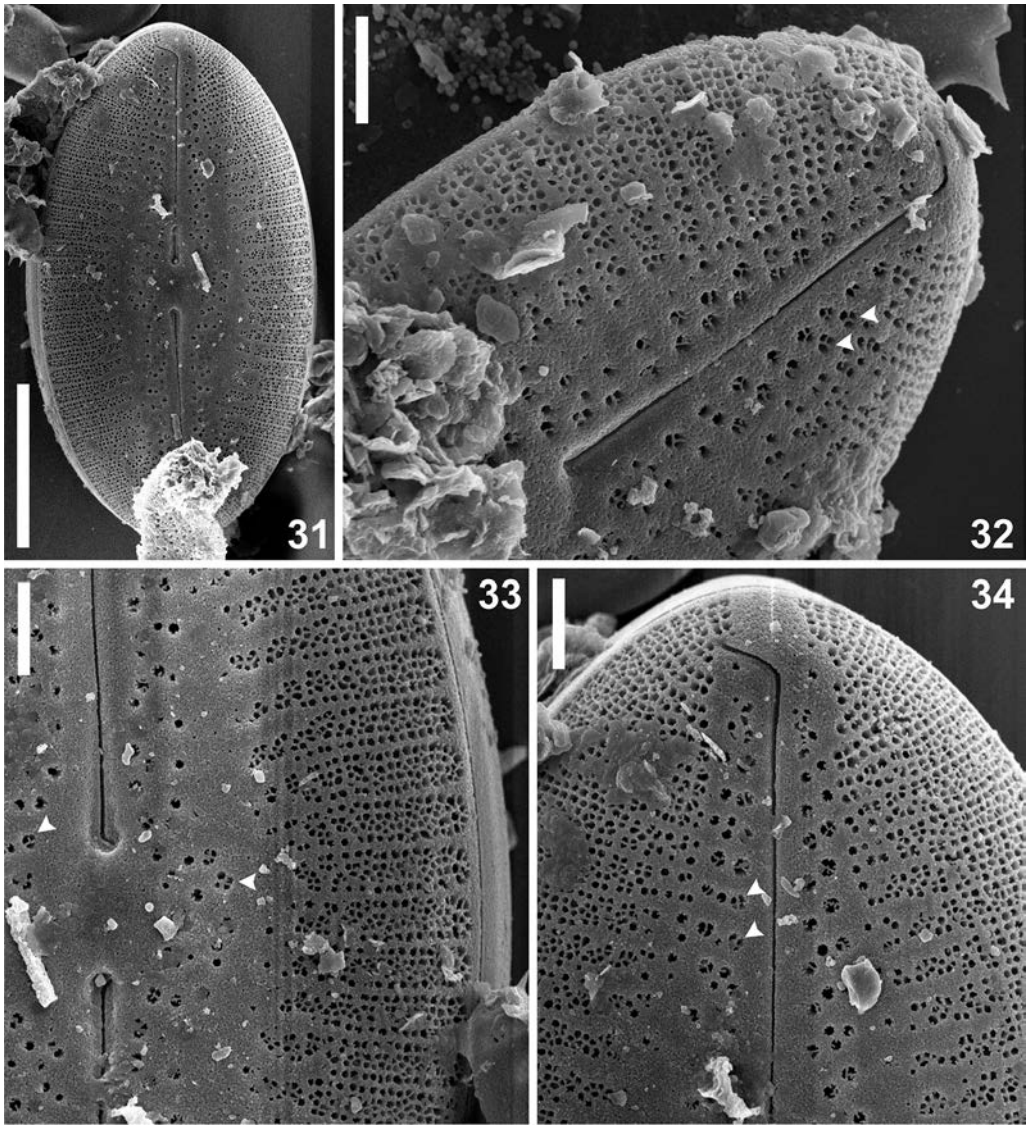

Plate 5. Figs 31–34. *Diploneis sudamericana* sp. nov. SEM. External valve views. Fig. 31. Whole valve. Fig. 32. Tilted valve showing valve surface and mantle, white arrowheads show details of the areolae of the longitudinal canals. Fig. 33. Detail of the valve center, note the central area, the proximal raphe ends, the areolae covered with volae on the canal (arrowhead) and the complex cribrate occlusion over the alveoli. Fig. 34. Detail of an apex in external view, see the terminal raphe fissure and the areolae covered with volae on the canal (arrowheads). Scale bars: Fig 31: 10 µm; Figs 32–34: 2 µm.

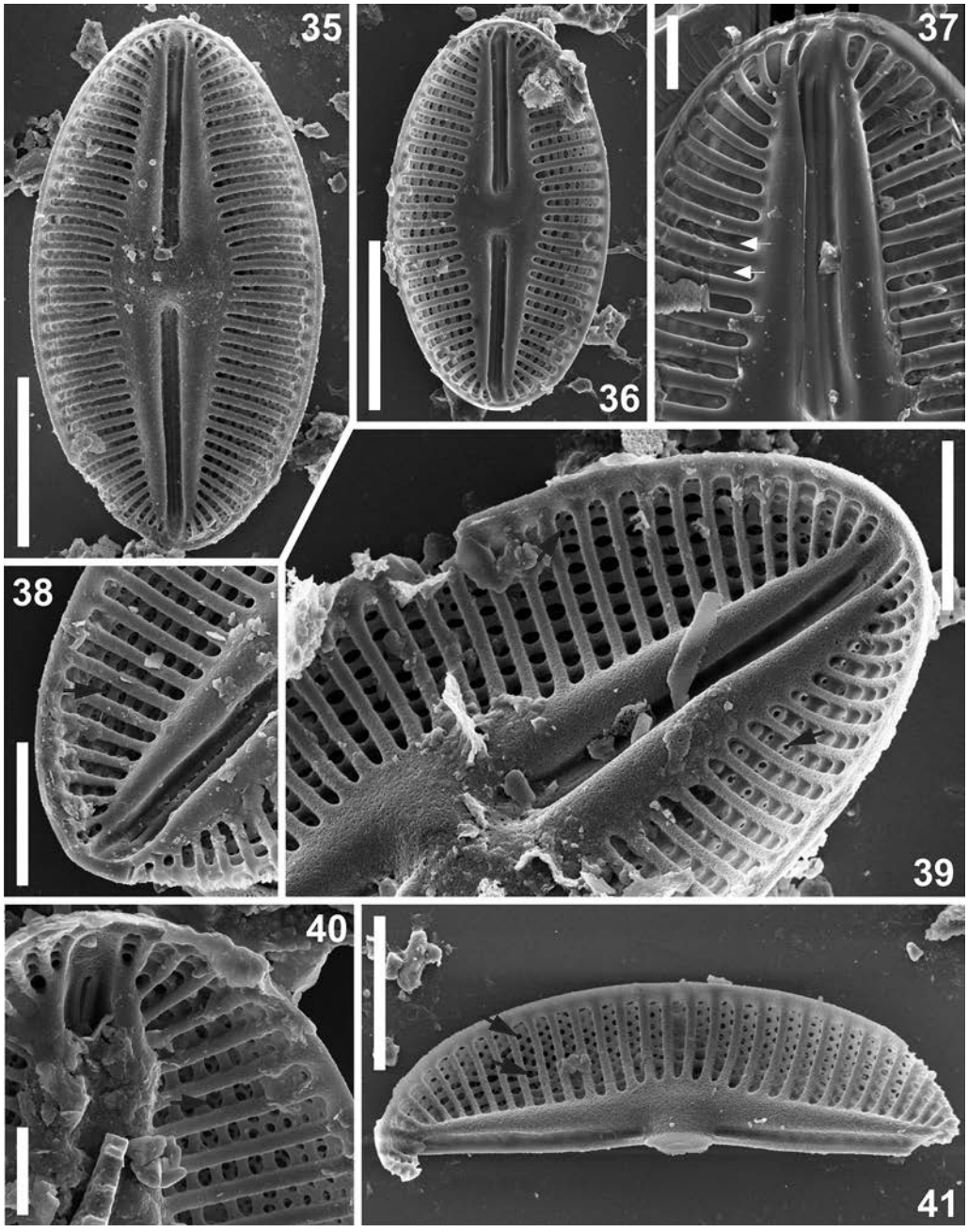

Plate 6. Figs 35–41. *Diploneis sudamericana* sp. nov. SEM. Internal valve views. Figs 35–36 Whole valve. Figs 37–41. Details of internal views showing the simple proximal and distal of raphe ends, helictoglossae slightly developed, alveoli covered with thin layer of silica (white arrows) and internal foramina, single at valve center becoming double to the valve margin (black arrows). Scale bars: Figs 35–36, 41: 10 µm; Figs 38–39: 5 µm; Figs 37, 40: 2 µm.

the raphe is straight and filiform running through a sunken area between the longitudinal canals (Figs 35–37, 39); proximal and distal ends are simple and helictoglossae are slightly developed (Figs 37–39). Costae are straight delimiting the alveoli which are covered by a thin silica membrane (Fig. 37 white arrows). Areolae open in single foramina to the valve center becoming double to the valve margin (Figs 39–41 black arrows). Valve margins smooth (Figs 37–39).

Discussion

The analyses with SEM of the type material of *Diploneis elliptica* allowed us to verify if this broadly distributed species has been properly identified in the past. This European material, among other fine morphological characteristics, has uniseriate striae with slightly sunken cribra becoming more complex to the valve margin; longitudinal canals externally depressed and valve margins with a zipper-like structure. The comparison with other records of the species also studied with SEM demonstrated that there is a great confusion about the identity and distribution of this taxon.

Diploneis elliptica was originally described as *Navicula elliptica* by Kützing in 1844 from samples collected at Falaise, France. In the original description Kützing only mentions that the species is 45 µm long and that it is "distinctively" striated with 12–13 striae in 10 µm. In 1894, Cleve (p. 92) transferred the species to the genus *Diploneis*, and described it as "Valve elliptical, with broad and rounded ends. L. 0.02 to 0.037; B. 0.011 to 0.02 mm. Central nodule of medium size quadrate. Furrows narrow, of the same breadth throughout. Transverse rows of puncta 10 to 13 in 0.01 mm. Alveoli 10 to 14 in 0.01 mm forming irregular longitudinal rows". The author did not illustrate the species but he referred it to Kützing´s drawings. Among others he mentioned illustrations in Van Heurck (1880) Pl. X Fig. 10 (upper figure) and Schmidt's Atlas VII f. 29, 32. It seems that these materials do not belong to the same taxon, as the drawings show an important morphological variation and SEM analyses are necessary to confirm their identity.

Afterwards, Krammer & Lange-Bertalot (1986) gave a wide range of dimensions for *D. elliptica* and showed the areola structure (Pl. 108, fig. 6). Besides, the authors mention it as an epipelic cosmopolitan taxon frequent in temperate oligotrophic waters. Cocquyt (1998) reported *D. elliptica* from Lake Tanganyika (Central Africa) and illustrated one specimen with SEM. Recently, Jovanovska and collaborators (2015) studied with SEM and described specimens collected in Lake Hövsgöl (Mongolia) under this name. All of these specimens differ from the type material here described. These observations demonstrated that *D. elliptica* is a misidentified taxon that has probably been erroneously considered cosmopolitan.

Diploneis sudamericana is similar to *D. elliptica* when analyzed with LM (see Lange-Bertalot & Reichardt 2000: Pl. 7, Figs 1–3). Morphometric parameters of *D. sudamericana* coincide with dimensions given in Cleve (1894) but the type specimens illustrated in Lange-Bertalot & Reichardt (2000) are larger and have fewer striae and areolae in 10 µm (Table 1). These two species clearly differ in the striae structure, uniseriate at valve center and biseriate near the valve margin in the former while in the latter they are completely uniseriate. They also differ in the structure of the alveolus that is externally occluded by a unique complex cribrate occlusion in *D. sudamericana,* while in *D. elliptica* it is occluded by distinctive areolae with external cribra; those cribra are simple near the canals and complex towards the valve margin. Other important differences are the areolae of the longitudinal canals and the external proximal raphe ends.

The specimens described by Metzeltin et al. (2005) as *D. subovalis* Cleve collected in Tacuarí Stream and Río de La Plata (Uruguay), correspond to *D. sudamericana*. *Diploneis subovalis* is similar to *D. sudamericana*, in dimensions and LM valve structure (Cleve 1894) but these two species differ in that *D. subovalis* has narrow longitudinal canals, longer valves and 18 areolae /10 µm (Table 1).

Diploneis kahlii Lange-Bertalot & Rumrich (Rumrich et al. 2000), described from River Las Cruces (Chile), is similar to *D. sudamericana* (Table 1) but it differs in that the canals are narrow the length of the valve and in the areolae of the canals that are continuous with the striae.

Table 1. Comparison of *Diploneis elliptica* and *D. sudamericana* with allied taxa.

Taxa	Reference	Length (µm)	Width (µm)	L/W	Striae / 10 µm (center)	Striae / 10 µm (ends)	Areolae / 10 µm
Diploneis sudamericana nov. spec.	This study	22–46.6	12.5–19.7	1.6–2.2	10–12	10–16	12–15
Diploneis elliptica (Kützing) Cleve	This study	34–44	16.5–22	1.7–2	9–10	9–10	10–13
Navicula elliptica Kützing	Kützing (1844)	45	–	–	12–13	–	–
Diploneis elliptica (Kützing) Cleve	Cleve (1894)	20–37	11–20	–	10–13	–	10–14
	Lange-Bertalot & Reichardt (2000)	38.5–58*	19.5–31.5*	1.8–2*	7–8*	9*	10–12*
	Krammer & Lange-Bertalot (1986)	20–130	10–60	–	8–14	–	12–14
	Cocquyt (1998)	34.5	14.5	–	10	–	10
	Jovanovska et al. (2015)	21–40	13–21	–	8–11	–	8–15
Diploneis subovalis Cleve	Cleve (1894)	39	19	–	10	–	18
	Metzeltin et al. (2005)	18–26.5*	10–11.5*	1.7–2.4*	10–12*	–	–
Diploneis kahlii Lange-Bertalot & Rumrich	Rumrich et al. (2000)	25–55	13–23	–	9–12	–	12–15
Diploneis krammeri Lange-Bertalot & Reichardt	Lange-Bertalot & Reichardt (2000)	(10) 20–65	(7) 10–25	–	10–14	–	10–15

* measured from the illustrations of the publication.

Diploneis krammeri Lange-Bertalot & Reichardt (2000), a species that was described from the Alps in Austria, is also similar to *D. sudamericana* as viewed with LM (Table 1), but clearly differs in the fine structure of the valve. The longitudinal canals at the valve centre are externally depressed and have two rows of areolae, while at the ends they are elevated and have a single row of areolae. Besides, the striae are uniseriate, composed of rounded cribrate areolae.

The results of this study show once more the importance of analyzing the fine morphology of the type materials, particularly of those taxa early described and considered cosmopolitan.

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