



## Revision of the type material of *Diploneis zannii* Frenguelli (Bacillariophyceae)

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### Abstract

*Diploneis zannii* was first described from the Esteros del Iberá, a subtropical wetland from Argentina. Type material of the species was studied with light and electron microscopy and compared to some similar taxa: *D. fusca*, *D. insolitus*, *D. lenzii*, *D. smithii* and *D. finnica* and other material collected from Brazil and Uruguay. We confirm the validity of *Diploneis zannii* as a species and present an emended diagnosis derived from new information on its morphology. A lectotype and isolectotype are designated.

**Keywords:** *Diploneis zannii*, type material, Frenguelli Collection, Argentina

### Introduction

*Diploneis* Ehrenberg *ex* Cleve (1894: 76) is a large and diverse genus, with species predominantly from marine and brackish habitats and few freshwater representatives. Frustules of *Diploneis* specimens are typically strongly silicified, elliptical to panduriform, with bluntly rounded apices (Round *et al.* 1990). Each valve possesses two longitudinal canals, one on either side of the raphe. The canals open to the exterior through areolae variably arranged, but lack openings to the interior of the cell (Spaulding & Metzeltin 2011). Alveolate striae are composed of complex areolae (Round *et al.* 1990, Jovanovska *et al.* 2015). Externally, the areolae are occluded by volae or cribra, internally the alveoli are covered by a delicate silica membrane. The fine morphology of the areolae, the internal and external raphe ends, the alveoli between the longitudinal canal and the valve margin, are diagnostic features used to differentiate and identify species in the genus (e.g., Idei & Kobayasi 1989, Jovanovska *et al.* 2015).

This study was undertaken within the framework of a research project held to review type materials of freshwater diatoms described by Dr. Joaquín Frenguelli (1883–1958), deposited in his Diatom Collection housed at the División Ficología del Museo de La Plata, Argentina. The revision of type material with scanning electron microscopy (SEM) allows a more accurate assessment of the identity of at least the most common taxa and will improve the accuracy of ecological diagnostic tools that rely heavily on the most inclusive taxonomic categories as the basic units expressing environmental change.

In a study on the diatom flora of the Esteros del Iberá, a subtropical wetland from Corrientes Province, Argentina, Frenguelli described *Diploneis zannii* Frenguelli (1933: 415, pl. 4, fig. 10) as a new species. This species was not reported afterwards from other regions of the country (Vouilloud, 2003, Sar *et al.* 2009) but has been recorded from southern Brazil (Bigunas 2005, Moraes Pacheco *et al.* 2016). The aims of this study were to document the morphological structure of the valves of this species using original material and light (LM) and SEM and compare it with similar species to clarify taxonomic problems.

## Materials and Methods

The specimens studied were found in sample 268 of Frenguelli's Collection, housed at the División Ficología del Museo de La Plata:

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

Raw material was treated and mounted to prepare additional slides (slides 9–16) that were incorporated to the Frenguelli Collection.

All the slides of the series were analysed using a Leica light microscope (LM) equipped with DM 2500 phase contrast and a Leica DM 2500 with DIC (differential interference contrast). Type specimens were referenced using an England Finder™ Graticule.

Raw material was treated to eliminate organic matter following the method described in CENT/TC 230 (2002). A portion was mounted on glass stubs and then coated with gold for observations with SEM using a Carl Zeiss NTS SUPRA 40 SEM in the Centro de Microscopías Avanzadas (CMA) of the Universidad de Buenos Aires.

Terminology follows Idei & Kobayasi (1989), Round *et al.* (1990) and Jovanovska *et al.* (2013, 2015).

## Results

All the specimens found in the studied materials were analyzed and photographed under LM or SEM.

*Diploneis zannii* Frenguelli (1933: 415, pl. IV, fig. 10) (Figs 1–23)

Description: **LM** (Figs 1–16). Valves elliptic-lanceolate (length 37.5–57.5  $\mu\text{m}$ , breadth 27–36  $\mu\text{m}$ , L/B: 1.7–2.2). Longitudinal canals occupying 1/3 of valve width, together forming a narrow lanceolate area. Striae uniseriate, slightly radial at valve center (8–10/10  $\mu\text{m}$ ), strongly radial at poles (10–11/10  $\mu\text{m}$ ), interrupted at canals.

**SEM** (Figs 17–23). Raphe filiform (Figs 17–19) with external proximal ends straight located at bottom of a depression (Fig. 18); distal ends curved in obtuse angle (Fig. 19) to the same side. Longitudinal canals with one row of areolae separated from striae by conspicuous hyaline area (Figs. 17–19). Externally, each alveolus opens as a uniseriate stria composed of equidistant areolae (10–12/10  $\mu\text{m}$ ) (Figs 18–19). Internally, raphe straight, running through sunken area between longitudinal canals (Figs 20–22); proximal and distal ends simple, helictoglossae slightly developed (Figs 21–22). Transapical ribs straight delimiting alveoli (Figs 20–23). Transapical ribs fungiform in transverse section (Fig. 23, arrows). Areolae open in single foramina (Figs 21, 23, arrowheads).

**Type:**—ARGENTINA. Corrientes: Itatí Rincón Lagoon, Esteros del Iberá, 28°42'00"S; 58°06'00"W, *J. Frenguelli*, 24 February 1941 (**lectotype designated here**, Frenguelli Collection! 268, slide 268 (3), England Finder: N 47 (4), here illustrated as Figs 14–16; **Isolectotype designated here:** Frenguelli Collection! 268, Slides 268 (1–3, 6, 8, 15, 16).

## Discussion

In this study, we compared *Diploneis zannii* with *Diploneis fusca* (Gregory) Cleve (1894: 93), *D. insolitus* Hohn & Hellerman (1966: 123), *D. lenzii* Krasske (1939: 555, as in Lange-Bertalot *et al.* 1996: 67), *D. smithii* (Bréb.) Cleve (1894: 96; basionym: *Navicula smithii* Brébisson in W. Smith 1856: 92) and *D. finnica* (Ehrenb.) Cleve (1891: 43; basionym: *Cocconeis finnica* Ehrenberg 1838: 194) (details summarized in Table 1).

In his original description, Frenguelli suggested that *Diploneis zannii* was similar to *D. fusca* (Frenguelli 1933), which was originally described by Gregory under the name *Navicula smithii* var. *fusca* Gregory (1857: 486). Gregory noted that *N. smithii* var. *fusca* has ovoid valves with round ends and longitudinal canals broader at valve center narrowing to the ends, forming a hyaline rhomboidal narrow area that differs from *D. zannii*, which has a narrow lanceolate area. In addition, *D. fusca* has a broad central nodule.

*Diploneis smithii* is similar to *D. zanni* in its valve outline and measurements but clearly differ in the presence of biseriate striae in the former (Droop 1994).

*Diploneis zannii* is also similar to *D. insolitus*, described by Hohn & Hellerman (1966) from Middle River (Maryland, USA). The original paper has no micrographs, only a drawing of a specimen from the type material (length: 50.9 µm, breadth: 27 µm) (Hohn & Hellerman, 1966, pl. I, fig. 17) that has many similarities to specimens of *D. zannii* in Frenguelli's original illustration and the specimens studied here (Figs 1–16). Desianti *et al.* (2015) revised the type material of *D. insolitus* and the measurements of the valve of *D. insolitus* are similar to those of *D. zannii*. The LM photographs show that details of the central nodule, proximal external raphe ends and raphe fissure coincide and suggest that these two species are conspecific. In many cases *Diploneis* species cannot be differentiated with LM e.g. *Diploneis elliptica* (type material analysed with LM in Lange-Bertalot & Reichardt, 2000) vs. *D. sudamericana* Vouilloud & Sala (Sala *et al.* in press). As there are no SEM illustrations, and no unmounted material of *D. insolitus* to make those comparisons, we have not placed this as a synonymous.

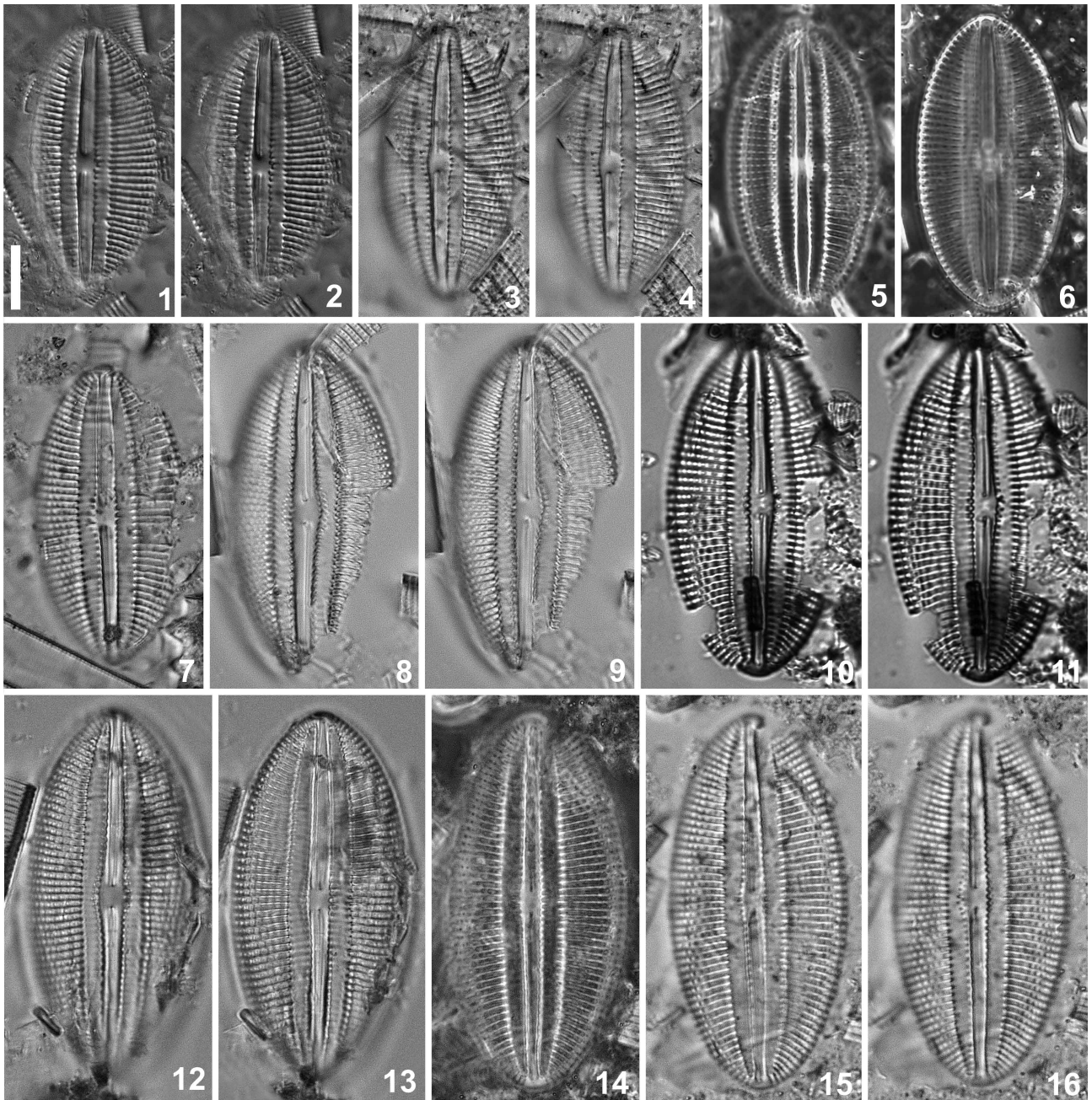
**TABLE 1.** Comparison of *D. zannii* with similar taxa

Taxon	Reference	Length (µm)	Width (µm)	Striae/10 µm (center)	Striae/10 µm (ends)	Areolae/10 µm
<i>Diploneis zannii</i> Frenguelli	This study (n=17)	37–57.5	20–28.5	8–10 uniseriate	10–12	10–12
	Frenguelli (1933)	51–76	27–36	7–8 uniseriate	-	-
<i>Navicula smithii</i> var. <i>fusca</i> Gregory	Gregory (1857)	30–63	14–28	10 --	-	-
<i>Diploneis lenzii</i> Krasske	Lange-Bertalot <i>et al.</i> (1996)	33–50	20–30	8 uniseriate	-	14
<i>Diploneis insolitus</i> Hohn & Hellerman	Hohn & Hellerman (1966)	32.6–50.9	20.5–27	8–10 uniseriate	10–11*	12*
<i>Diploneis smithii</i> morph. 1 <i>sensu</i> Droop	Droop (1994)	41.3–73.3	20.5–31.5	6.1–7.3 biseriate alternate	-	-
	Metzeltin <i>et al.</i> (2005)	49.5–59.5*	24.5–29.5*	7–8* uniseriate*	10*	2/5*
<i>Diploneis finnica</i> (Ehrenberg) Cleve	Idei & Kobayasi 1989	35–120	21–45	7–8 biseriate	-	12–14
<i>Diploneis</i> sp.	Metzeltin & Lange-Bertalot 2007*	41.5–46.5*	21.5–23.5*	9–11* uniseriate	10*	12*

\*measured in the publication

Another taxon worth considering is *Diploneis (zannii* Frenguelli var.?) *lenzii* Krasske (1939: 555, Fig. 15, collected in Brazil from mud from Ramalho, Estado de Bahia (type locality), Bodocongó, Estado de Paraíba, Borburema and Chemada, Estado de San Paulo). In the protologue, Krasske points out that *D. lenzii* differs from *D. zannii* in the structure of the canals, but it is not possible to differentiate certainly these two taxa even with the more recent LM micrographs of type material in Lange-Bertalot *et al.* (1996: Taf. 31, figs 5–7). These two taxa appear conspecific but the lack of SEM photographs of the type material of *D. lenzii* makes it impossible to establish synonymy as in the case of *D. insolitus*.

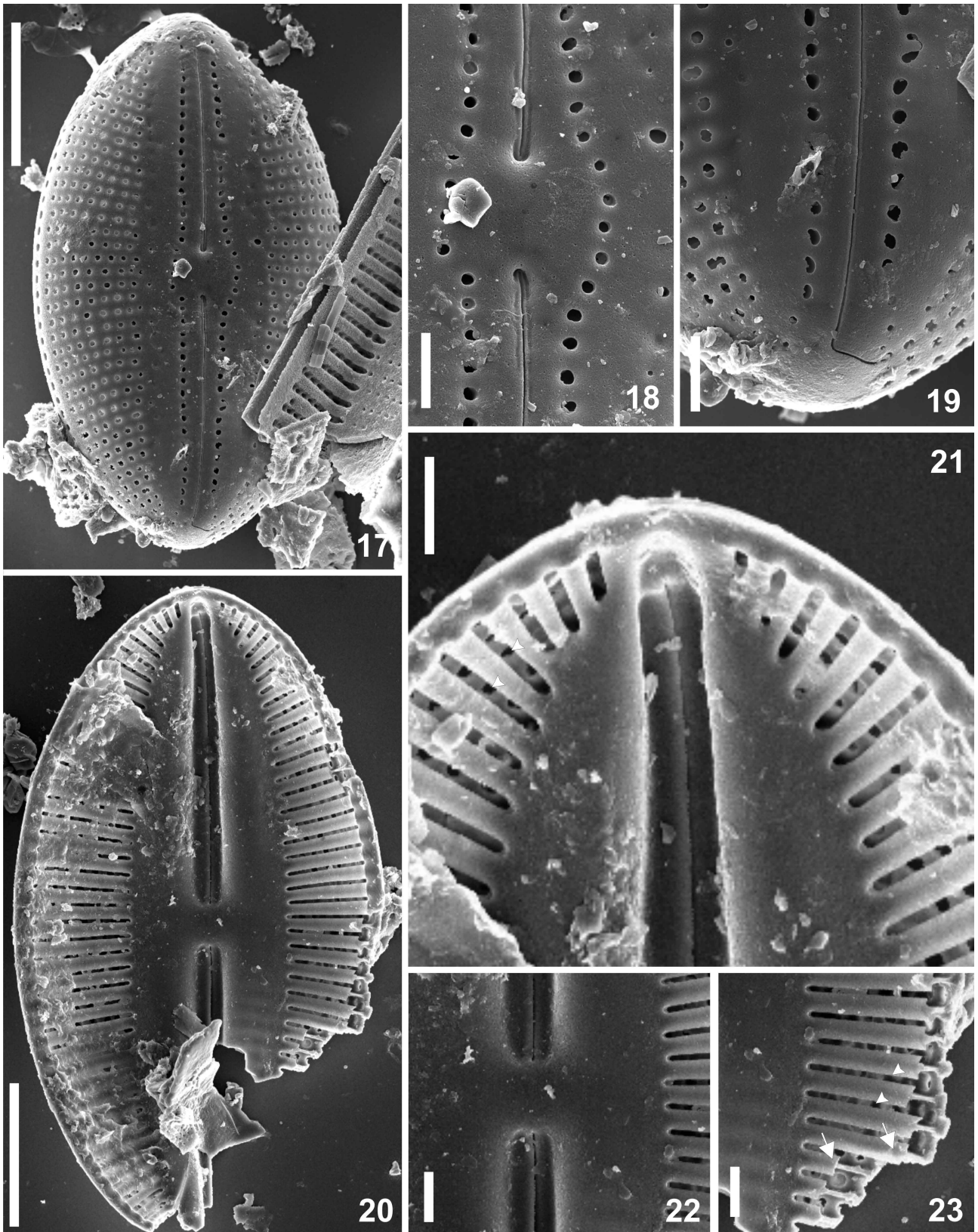
Metzeltin *et al.* (2005), studying the diatom flora of Uruguay, illustrated three specimens that they assigned to *Diploneis smithii* (Brébisson) Cleve Morphotype 1 *sensu* Droop (1994, pl. 114, Figs 1–3). Droop, however, notes that *D. smithii* Morphotype 1 is elliptic with broadly rounded ends and having the typical structure of *D. smithii* with biseriate striae with alternating pores (Droop 1994). Comparison of the morphometric data and LM illustrations in Droop (1994) allow us to conclude that the specimens from Uruguay were misidentified and correspond to *D. zannii*.



**FIGURES 1–16.** *Diploneis zannii*, LM. Specimens from type population. Valve views showing specimen variation. Figs 1–13 = Isolectotypes (same specimens in different focus). Figs 14–16 = Lectotype (same specimen in different focus). Scale bar: 10  $\mu$ m.

*Diploneis finnica* is similar to *D. zannii* in the LM but *D. finnica* is larger (although dimension ranges overlap). SEM micrographs show that *D. finnica* has irregularly arranged pores on the longitudinal canals and biseriata striae (Idei & Kobayasi 1989).

*D. zannii* was reported in Argentina only from the NE of the country (Frenguelli 1933). It has been recorded from southern of Brazil (Bigunas 2005, Moraes Pacheco *et al.* 2016) and from the results of this study it is present in the Brazilian Amazon (Metzeltin & Lange Bertalot 2007) and Uruguay (Metzeltin *et al.* 2005). In conclusion, *Diploneis zannii* has a distribution restricted to subtropical regions of South America. Here we present an emended diagnosis with information about fine morphology based on SEM analyses of the type material and designate a lectotype and isolectotypes.



**FIGURES 17–23.** *Diploneis zannii*, SEM. 17–19: External valve view. 17. Whole valve. 18. Detail showing central area and proximal raphe ends. 19. Detail showing valve end and distal end of raphe. 20–23. Internal valve view. 20. Whole valve. 21. Detail showing valve end; note distal end of raphe, helictoglossa and round areolae of the striae (arrowheads). 22. Detail showing central area. 23. Detail of broken valve, note the fungiform section of transapical ribs (arrows) and the rounded areolae (arrowheads). Scale bars: Figs 17, 20: 10  $\mu$ m; Figs 18–19, 21–23: 2  $\mu$ m.

**Emended diagnosis:** Valves elliptic-lanceolate, 37.5–57.5 µm length, 27–36 µm breadth. Longitudinal canals occupying 1/3 of valve width, together forming a narrow lanceolate area. Striae uniseriate, slightly radial at valve center, 8–10/10 µm, strongly radial at ends, 10–11/10 µm, interrupted at canals. Transapical ribs fungiform in transverse section. Raphe filiform; external proximal ends straight, located at bottom of a depression; distal ends curved in obtuse angle to same side. Internally raphe straight, running through sunken area between longitudinal canals; proximal and distal ends simple, helictoglossae slightly developed. Longitudinal canals with row of areolae separated from striae by conspicuous hyaline area. Externally, each alveolus opens as a uniseriate stria composed of equidistant areolae, 10–12/10 µm.

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