

***Planothidium scrobiculatum* sp. nov. (Bacillariophyta), a new monoraphid diatom from freshwater Pleistocene deposits of South America**

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Abstract: A new monoraphid diatom species appeared successively in paleolimnological records from a Quaternary sediment core (1.5±0.1 Ma) retrieved from Colônia basin (Brazilian Coastal Plain, Atlantic Forest, São Paulo Metropolitan Region), and is formally described as *Planothidium scrobiculatum* sp. nov. We studied the new species through light and scanning electron microscopy and discussed its similarity to other members of the genus. The new species has a unique combination of morphological features that allow its separation from all other species in the genus: (1) lanceolate valve outline with cuneate apices, (2) multiserial striae composed of two to four rows of irregular sized areolae on the rapheless sternum valve (SV), not interrupted at the valve mantle junction, and (3) arrangement of areolae on the valve mantle, grouped in 3 or 4 rows, each composed of 2 to 3 areolae on the SV. Nevertheless, the new species main diagnostic feature is the occurrence of numerous, unusual, small shallow depressions externally located on the SV along the axial area, cavum, and virgae. As a result, *P. scrobiculatum* is different from similar species under LM and SEM by some evident characters or a combination of characters. This study contributes to the knowledge of the so far understudied biodiversity of microorganisms of tropical regions. In addition, the above–mentioned diversity patterns and the wealth of new diatom taxa inside and around the Colônia basin (many yet to be described) suggest that further samplings from other localities may provide an insight into its habitat preferences and range, since this area is an exceptional biological entity, worthy of further study.

Key words: Colônia basin, fossil diatoms, paleolimnology, *Planothidium*, Pleistocene, South America, taxonomy

INTRODUCTION

Planothidium was published by ROUND & BUKHTIYAROVA (1996) to accommodate some diatom specimens related to *Achnanthes lanceolata* (Brébisson ex Kützing) Grunow (CLEVE & GRUNOW 1880), which was formerly considered the type of the genus. The transfer of *A. lanceolata* to *Planothidium* was considered. However, it proved invalid based on the lack of indication of the basionym, which was later on corrected by Lange–Bertalot (LANGE–BERTALOT 1999; VAN DE VIJVER & BOSAK 2019). VAN DE VIJVER et al. (2013) have designated a formal lectotype for this species.

Planothidium currently comprises 62 named species and varieties accepted (KOCIOLEK et al. 2020). According to WETZEL et al. (2019), representatives of the genus *Planothidium* are predominantly epilithic or

epipsammic. STANCHEVA (2019) reported that they can also live epiphytic on aquatic plants and algae, seeming to be more diverse in alkaline environments (WETZEL et al. 2019). Most species are from freshwater environments, although some of them may be found in brackish and marine environments (e.g. RIAUX–GOBIN et al. 2012, 2018). *Planothidium* is characterized by a convex rapheless sternum valve (SV) and a slightly concave raphe valve (RV) (STANCHEVA 2019), both valves having multiserial striae (ROUND & BUKHTIYAROVA 1996). In the SV, striae may be continuous (*‘delicatulum’* type) or interrupted on one side showing a clear space in the central area. Such interruption may be not associated with any internal depression (*‘minutissimum’* type), or may be accompanied by a *‘hoof–mark’* (*‘frequentissimum’* type, when the depression is an internal capped structure, the cavum, or *‘lanceolatum’* type where the depression

is not capped, the sinus) (ROVIRA et al. 2011; VAN DE VIJVER et al. 2013). It was recently suggested that the cavum might act as an optical system that will diffuse or focus light, depending on the refractive index of the matter inside the cavity (BUKHTIYAROVA & LYAKH 2014).

Recent molecular investigations on *Planothidium* identified two clear parallel clades, one of the specimens with either a sinus or a cavum and the other lacking both features. Striae ultrastructure and the merged and/or offset areolae on the valve mantle are also considered of particular taxonomic significance (JAHN et al. 2017). Some authors divided *Planothidium* specimens without terminal raphe fissures curved to the secondary side (SPAULDING et al. 2008; JAHN et al. 2017). Furthermore, type materials reinvestigation and the use of scanning electron microscopy (SEM) associated with a more precise definition of the new freshwater species from around the world considerably improved the genus taxonomy (STANCHEVA 2019).

Indeed, several populations of one unknown *Planothidium* species were found during an ongoing paleolimnological collection of a sedimentary core of Pleistocene deposits in Colônia basin (located in the Brazilian Coastal Plain, Atlantic Forest, São Paulo Metropolitan Region). A remarkable *Planothidium* diversity was documented for the tropical region of South America (e.g. METZELTIN & LANGE–BERTALOT 1998, 2007; RUMRICH et al. 2000; METZELTIN et al. 2005). Still, a wide geographical distribution was comprehensively demonstrated for the genus, ranging from North America (e.g. POTAPOVA 2012; STANCHEVA 2019; WETZEL et al. 2019), Africa (e.g. GASSE 1986; COCQUYT et al. 1993; COCQUYT 1998; COMPÈRE & RIAUX–GOBIN 2009; COMPÈRE & VAN DE VIJVER 2009; N’GUESSAN et al. 2014), Asia (e.g. KULIKOVSKIY et al. 2015; WETZEL et al. 2019), Oceania (e.g. NOVIS et al. 2012), Europe (e.g. ÁLVAREZ–BLANCO & BLANCO 2013; BLANCO et al. 2013; BAĞ & LANGE–BERTALOT 2014) to the Antarctic region (e.g. VAN DE VIJVER et al. 2013, 2018).

Several studies that includes diatoms have been developed for the Pleistocene period, whether in a paleoenvironmental (e.g. KORSAKOVA et al. 2016; PANAGIOTOPOULOS et al. 2020; SPAULDING et al. 2020) or in a taxonomic context (e.g. KACZMARSKA 1981; McLAUGHLIN & STONE 1986; TANAKA & KOBAYASHI 1996). Also, a large number of new fossil species have been recorded for China (GONG et al. 2013), Japan (TANAKA & NAGUMO 2000; JULIUS et al. 2006; NAYA 2012), Nepal (HAYASHI & TANIMURA 2015), Kenya (BRINDLE et al. 2018), as well as in studies carried out on Baikal Lake (KHURSEVICH et al. 2003) and Black sea (OLSHTYNSKA & TYMCHENKO 2018). However, only a few studies include monoraphid diatoms, most of which are represented by *Achnanthes* Bory (some of them currently transferred to *Achnantheidium* Kützing, *Psammothidium* Bukhtiyarova et Round and *Platessa* Lange–Bertalot) and *Cocconeis* Ehrenberg, rarely accompanied by illustration (e.g. AUER 1959; WORNARDT 1964; O’BRIEN 1968; GASSE 1980;

VANLANDINGHAM 1990; YASUI & KOBAYASHI 2001). Also, a few studies were found to include specifically *Planothidium* representatives. GASSE (1980) illustrated *Planothidium* species during a diatom analysis of a continuous and dated Plio–Pleistocene lacustrine sequence from Africa.

Finally, YASUI & KOBAYASHI (2001) recorded *Planothidium* representatives in the fossil diatom flora composition of Shiotsugata Lagoon in the Echigo Plain, Japan. In addition to other monoraphid diatom genera, the authors illustrated *Achnanthes delicatula* (Kützing) Grunow (\equiv *Planothidium delicatulum* (Kützing) Round et Bukhtiyarova), *A. delicatula* spp. *hauckiana* (\equiv *P. hauckianum* (Grunow) Bukhtiyarova) and *A. lanceolata* (\equiv *P. lanceolatum* (Brébisson ex Kützing) Lange–Bertalot).

In Brazil, WETZEL et al. (2013), who described *Planothidium incuriatum* C.E. Wetzel, Van de Vijver et Ector, found that the species is widely distributed worldwide when carrying out taxonomic studies on *Planothidium* using samples from southern Brazil (rivers of the Rio Pardo hydrographic basin). Furthermore, WETZEL & ECTOR (2014a) described the new species *P. bagualensis* C.E. Wetzel et Ector, and highlighted the need of a precise definition on the identity of the most common taxa to improve the accuracy of diagnostic tools that rely on diatom taxonomy and ecology. Finally, WETZEL & ECTOR (2014b) proposed the new combination *Planothidium lagerheimii* (Cleve) C.E. Wetzel et Ector (\equiv *Cocconeis lagerheimii* Cleve), a common species often reported in freshwater and aerial environments identified in previous studies either as *Cocconeis rudis* Frenguelli or *Planothidium salvadorianum* (Hustedt) Lange–Bertalot (\equiv *Achnanthes salvadoriana* Hustedt). Regarding material from Southeastern Brazil, WETZEL et al. (2019) described *P. brasiliense* C.E. Wetzel et S. Blanco when reviewing the types of material from historical collections. Some other authors focused on ecological and floristic surveys (e.g. FERRARI & LUDWIG 2007; FARIA et al. 2010; BARTOZEK et al. 2018), whereas RUWER & RODRIGUES (2018) represents the only paleolimnological study using diatoms, in which two *Planothidium* species (*P. bagualensis* and *P. aff. rostratum* (Østrup) Lange–Bertalot) from the upper Paraná river floodplain over the last ~1000 years are mentioned and illustrated using both light and scanning electron microscopy.

This study describes a new monoraphid diatom species *Planothidium scrobiculatum* sp. nov. from the Colônia basin. Detailed examination of LM and SEM microscopy examination of the valve ultrastructure is presented. Morphological comparison is conducted with the most similar taxa based on literature information.

MATERIAL AND METHODS

We sampled the material used in this study from a 5200 cm long sedimentary core (COL17–3) retrieved from Colônia basin (23°52’ S, 46°42’20” W, 900 m a.s.l.) in August 2017 using a built-in pushing corer with rotary tubing mounted on a 6T

Caterpillar drilling rig, and posteriorly sub-sampled every 3 cm for diatom analysis. The age model based on radiocarbon dates, OSL authigenic $^{10}\text{Be}/^9\text{Be}$ and paleomagnetism provided an age of 1.5 Ma at the base of the core (SIMON et al. 2020). Samples corresponding to depth of 4376–77 cm and 4910–11 cm were selected for this study for including *Planothidium* material, which were respectively dated at 1.3 and 1.41 Ma, which matches with the Pleistocene period. See LEDRU et al. (2015) for detailed information on the Colônia basin geology and history.

A slice of sediment (about 0.5 g) was oxidized for 2–6 h in hot H_2O_2 and HCl according to BATTARBEE (1986). Afterwards, the cleaned material was repeatedly rinsed with distilled water to neutrality, and the oxidized material was mounted using Naphrax (R.I. = 1.74). Diatoms were examined under LM at $1000\times$ magnification using a Zeiss Axio Imager A2 light microscope equipped with DIC and a digital camera model AxioCamMR5. Relative abundance was estimated by counting at least 400 valves in total at an efficiency count of at least 90% (PAPPAS & STOERMER 1996). For SEM, analysis were performed at the LIST, Luxembourg Institute of Science and Technology and at the Instituto de Química – USP, Universidade de São Paulo. For the first one, subsamples of the oxidized suspensions were filtered and rinsed with additional deionized water through a 3 μm isopore polycarbonate membrane filter (Merck Millipore), subsequently attached to aluminum stubs and coated with platinum using a BAL-TEC MED 020 Modular High Vacuum Coating System for 30 s at 100 mA. An ultra-high-resolution analytical field emission (FE) scanning electron microscope Hitachi SU-70 (Hitachi High-Technologies Corporation, Tokyo, Japan) operated at 5 kV and 10 mm distance was used for the analysis. SEM images were taken using the lower (SE-L) detector signal. Also, parts of the oxidized material were placed on aluminum stubs and coated with gold at 1 kV for 5 min in a Balzers Sputtering/SDC030 sputter coater at the Instituto de Botânica, and SEM observations were made with a Jeol – JSM 7401F (FEG) operated at 5 kV at the Instituto de Química – USP. Photomicrographs were digitally edited and plates containing light and scanning electron microscopy images were created using CorelDraw X8.

We performed a non-metric multi-dimensional scaling (nMDS) ordination analysis based on Bray–Curtis dissimilarities to compare morphological differences between similar species. The total of 62 specimens were examined based in the new species type material ($n = 36$) and literature information ($n = 26$). Basic morphometric values were taken into account: (i) valve width, (ii) length, (iii) number of striae in 10 μm , and (iv) valve width and length ratio. Permutational multivariate analysis of variance (PERMANOVA; 999 permutations) was used to examine the statistical significance of the differences. Data were analyzed using the ‘vegan’ package (OKSANEN et al. 2016) in R version 3.1.2 (R DEVELOPMENT CORE TEAM 2015). Holotype permanent slides and cleaned samples were deposited

at the “Herbário Científico do Estado Maria Eneyda P. Kauffmann Fidalgo” (SP), São Paulo State Department of Infrastructure and Environment, Brazil. Isotope slides are deposited at the Botanic Garden Meise (BR), Belgium.

OBSERVATIONS

Planothidium scrobiculatum, Marquardt et C.E. Wetzel sp. nov. (Figs 1–36 LM; 37–48 SEM)

LM description: (RV: Figs 1–18; SV: Figs 1–36) – Valves lanceolate to elliptic–lanceolate with convex margins and cuneate apices, smaller valves sometimes with rounded apices (Fig. 34). Valve dimensions ($n = 36$): 9.2–28.2 μm length, 5.5–9 μm width. (Figs 1–18) – Axial area linear to narrowly lanceolate. Central area with 1 unilateral large horseshoe-shaped hyaline area, with a relatively large cavum. Striae radiating throughout the entire valve, 10–13 in 10 μm . Areolae not discernible in LM. (Figs 19–36) – Axial area very narrow, linear, only slightly widening towards the central area. Central area is small, rounded, sometimes slightly asymmetric (Fig. 3) to almost absent in the smallest valves (Figs 17, 18), bordered on each side by usually two irregularly shortened radiate striae. Raphe branches are straight, with expanded, drop-like proximal raphe endings. Terminal raphe fissures are not discernible in LM. Striae radiate throughout the entire valve, never convergent near the apices, 12–13 in 10 μm . Areolae are not discernible in LM.

SEM observations: RV (Figs 37–40) – Externally, striae are broader than the virgae, composed of 3–5 (6 near apices) rows of small rounded areolae (Figs 37, 38). Proximal external raphe endings expanded, relatively close to each other (Figs 37, 38). Distal raphe fissures are bent to the same direction, continuing shortly onto the valve mantle (Figs 37, 38). Internally, central raphe endings deflected into opposite directions near the large, rounded central nodule (Figs 39, 40). Distal raphe ends terminate internally in small helictoglossae (Figs 39, 40). SV (Figs 41–48) – Externally striae composed of 2–4 rows of nearly rounded irregular sized areolae (usually 3), the middle row being slightly smaller than the parallel outer rows (Figs 41–45). Striae portion near the axial area often composed of 2 rows of areolae (Figs 41–45). Near the valve mantle, striae reach up to 4 areolae and are not interrupted near the valve mantle junction (Figs 41–45). Mantle areolae are grouped in 3 or 4 rows, each composed of 2 to 3 areolae (Figs 41–45).

Table 1. PERMANOVA test performed on morphological differences resulting groups in the nMDS using the Bray–Curtis distance measure.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(> F)
Species	1	0.075	0.075	16.453	0.215	0.001
Residuals	60	0.275	0.005		0.785	
Total	61	0.350			1	

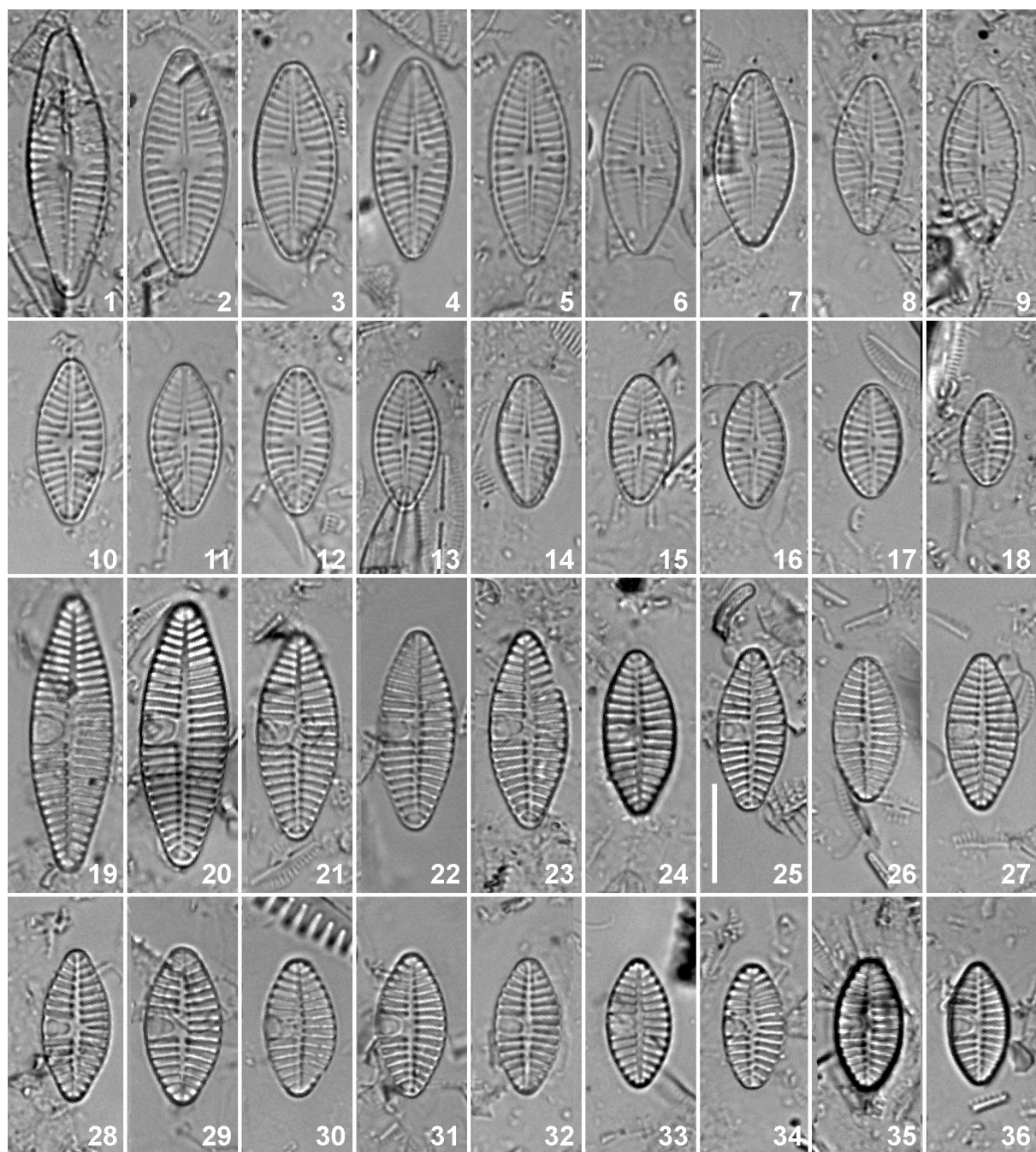
Numerous small superficial depressions present along the axial area, cavum and virgae (Figs 41–45). Sharp straight depression lines irregularly occurring on the axial area, but also transapically oriented on the virgae (Figs 41–43). Internally, a well-developed cavum is present with borders joined to the neighbour interstriae (Figs 46, 48). Striae internally sunken between raised virgae (Figs 46, 48).

Etymology: The specific epithet “*scrobiculatum*”, Latin for ‘pitted’, refers to the rapheless valve pattern surface quite unusual in other *Planothidium* species.

Type locality: Brazil. São Paulo, Parelheiros District: Colônia basin (23°52'S, 46°42'20"W, 900 m a.s.l.), sediment core, present at the 4376–77 and 4910–11 cm depth, respectively dated at ~1.3 and 1.41 Ma., leg. M.–P. Ledru & A.O. Sawakuchi, Aug. 2017.

Holotype: SP!, slide SP365549 (Herbário Científico do Estado “Maria Eneyda P. Kauffmann Fidalgo”, São Paulo, from 4910–11 cm depth (~1.41 Ma) in the COL17–3 core, Brazil, here depicted in Figs 1–36). The holotype specimen is shown in Fig. 5 (RV) and Fig. 21 (SV).

Isotype: BR! slide BR 4595 (Meise Botanic Garden, Meise, Belgium).



Figs 1–36. LM micrographs of *Planothidium scrobiculatum* Marquardt et C.E.Wetzel sp. nov. from holotype slide SP365549 sampled from Colônia basin, São Paulo, Brazil: (1–18) raphe valve view; (19–36) rapheless sternum valve view. Scale bar 10 μ m.

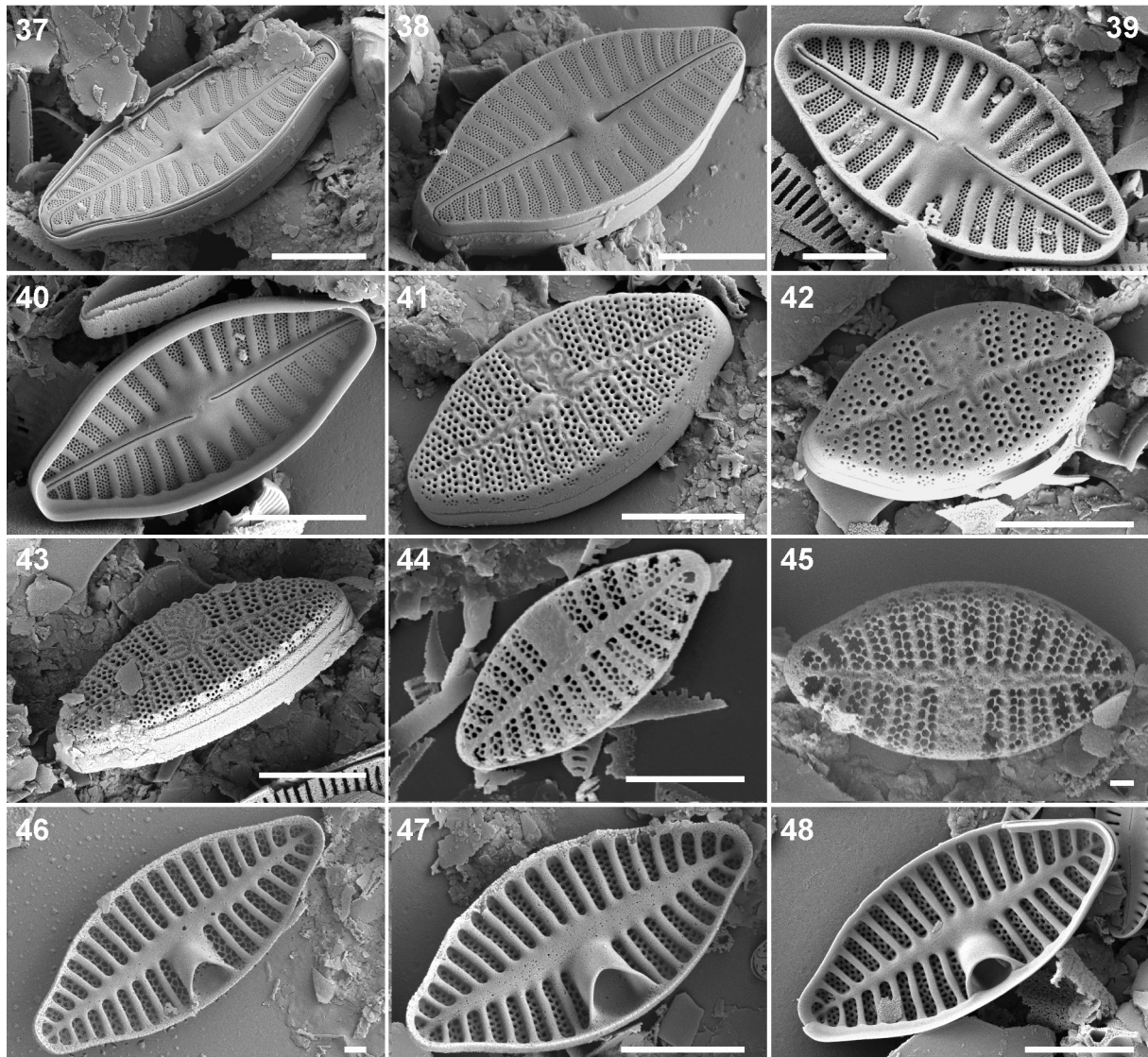
Associated diatom species: Populations of the new species are not very large and their maximum relative abundances in the selected slide is 7.9%. Among the species found in the holotype slide, dominant taxa are *Naviculadicta* sp. (70.8%), *Pseudostaurosira* sp. (3.7%), and *Staurosirella* cf. *crassa* (4.2%). The ongoing paleoenvironmental reconstruction along the retrieved core aims at elucidating the proper identity of observed taxa.

DISCUSSION

An extensive literature search found a few *Planothidium* species with a morphology similar to *P. scrobiculatum* Marquardt et C.E.Wetzel sp. nov., but none of them shared the combination of features of the present new species.

Indeed, one diagnostic characteristic of the new species is the unusual, numerous small superficial depressions present externally on the SV, along the axial area, cavum and virgae, which was not previously described for any *Planothidium* species. In addition, based on LM and SEM morphology, *P. scrobiculatum* shows a particular combination of features that are sufficient to separate the new species from all others, such as: (1) lanceolate valvar outline with cuneate apices, (2) multiseriolate striae composed of 2–4 rows of irregular sized areolae at the SV, which are not interrupted at the valve mantle junction, and (3) areolae on the valve mantle are grouped in 3 or 4 rows, each composed of 2 to 3 areolae on SV. Nevertheless, a few species are worth of some consideration and a detailed comparison is discussed below.

Planothidium comperei C.E.Wetzel, N'Guessan



Figs 37–48. SEM micrographs of *Planothidium scrobiculatum* Marquardt et C.E.Wetzel sp. nov. from type material SP365549 (4910–11 cm depth; Figs 43–46) and material SP365548 (4376–77 cm depth; Figs 37–42, 47, 48) sampled from Colônia basin, São Paulo, Brazil: (37, 38) external raphe valve view; (39, 40) internal raphe valve view; (41–43) external rapheless sternum valve view. Note the numerous small superficial depressions. (41, 43) note sharp straight depression lines irregularly occurring on the axial area and transapically oriented on the virgae; (44, 45) internal rapheless valve; detail of cavum aperture. Scale bars 5 µm (37–44, 47, 48), 1 µm (45, 46).

et Tison–Rosebery (N’GUESSAN et al. 2014), a tropical species from Ivory Coast, shares some similarity with *P. scrobiculatum* due to its lanceolate valve outline and the rounded central area. Moreover, *P. comperei* has rostrate apiculate apices, elongated when compared to the cuneate apices of the new species. The nMDS ordination space (Fig. 49) revealed significant differences between them (PERMANOVA, $F = 16.453$, $p < 0.001$; Table 1) providing a sufficient basis for distinguishing them as separate species. Regarding the SV, SEM observation reveals differences in terms of surface roughness between both species. Whereas *P. comperei* valve surface has round, shallow irregular depressions along the apical axis, *P. scrobiculatum* not only shows the typical numerous small superficial depressions along the axial area, cavum and virgae, but also with sharp straight depression lines irregularly occurring on the axial area and transapically oriented on the virgae (Figs 41–43). Finally, one row of irregular sized areolae (2–4) is present on the valve mantle of *P. comperei*. However, in the present new species mantle areolae are grouped in 3 or 4 rows, each composed of 2 to 3 areolae (Figs 42–43) with a less evident interruption in the valve mantle junction (Figs 41–45) when compared to the clearly interrupted striae in *P. comperei*.

Planothidium hinzianum C.E. Wetzel, Van de Vijver et Ector (WETZEL et al. 2019) described from northern Germany shows some characteristics similar to those of *P. scrobiculatum*, such as the abruptly interrupted striae on the valve mantle junction, as well as the mantle areolae grouped in small groups of up to 9 (WETZEL et al. 2019). However, *P. hinzianum* has round, acutely apices opposite to the cuneate ones of *P. scrobiculatum*. *Planothidium hinzianum* has also a relatively broad asymmetrical axial area in the SV, and an asymmetrical

wedge-shaped central area in the RV, bordered on each side by 1–2 shortened striae and lacking striae on the other side. In contrast, *P. scrobiculatum* presents from linear to narrowly lanceolate axial area (SV) and a small and rounded central area (RV) (Figs 1–36).

From the Andean mountains of South America, a similar cavum-bearing taxon worth comparison is *Planothidium infrequens* Lange–Bertalot et Rumrich (RUMRICH et al. 2000: 212, pl. 27, figs 3–9), which share similarities regarding SV that show the same areolae structures and numbers. *Planothidium infrequens*, however, has distinct rostrate apices. In contrast, *Planothidium rhombicuneatum* Lange–Bertalot et Rumrich (RUMRICH et al. 2000: 214, pl. 29, figs 1–12) presents the same valve outline as *P. scrobiculatum*, but does not possess a cavum. Instead, it shows a wide unilateral sinus on the SV. Differences between *P. infrequens* and *P. comperei* are discussed in N’GUESSAN et al. (2014). Based on this study, *P. scrobiculatum* presents larger valve dimensions when compared to *P. infrequens*.

From the Holarctic region, specifically at the Lake Baikal a place known for its high endemism rates, three interesting *Planothidium* that share similar valve outlines with *P. scrobiculatum*, but have distinct ultrastructural elements, such as striae patterns, valve surface, and lack of cavum: *Planothidium baicalorhombicum* Kulikovskiy et Lange–Bertalot (KULIKOVSKIY et al. 2015: 59, pl. 68, figs 1–18), *Planothidium rugosum* Kulikovskiy et Lange–Bertalot (KULIKOVSKIY et al. 2015: 286, pl. 68, figs 19–23), and *Planothidium baicalacutum* Kulikovskiy et Lange–Bertalot (KULIKOVSKIY et al. 2015: 57, pl. 66, figs 1–10). A fourth taxon, *Planothidium makarevichae* Kulikovskiy et Lange–Bertalot (KULIKOVSKIY et al. 2015: 286, pl. 72, figs 1–14) has the same valve outline and a cavum, but with much shorter striae on the SV valve and a distinct (oval) central area on the SV.

WETZEL et al. (2019) has discussed the importance of characteristic features mainly present on the SV during *Planothidium* identification. Among these features, the authors highlighted the pattern of valve surface smoothness (with irregular lines on the axial area and virgae or showing ‘round’ depressions instead), the number of areolae per stria, the striae interrupted or not towards valve mantle, and consequently the number of areolae on the mantle. Therefore, *P. scrobiculatum* is different from all other similar species under LM and SEM by recognizable characters or character combination. In this study, the SV surface roughness showed small differences among some individuals currently analyzed, sometimes less pronounced (Fig. 41) compared with other specimens (Figs 42, 43). Probably, factors such as valve reduced size, as well as the state of deterioration should be considered.

Finally, the Colônia basin Pleistocene diatom flora showed to be quite diverse, and new species are being described. Distribution of *P. scrobiculatum* in Brazil is yet to be known and, so far, the taxa described in this paper has been observed only as fossil from the

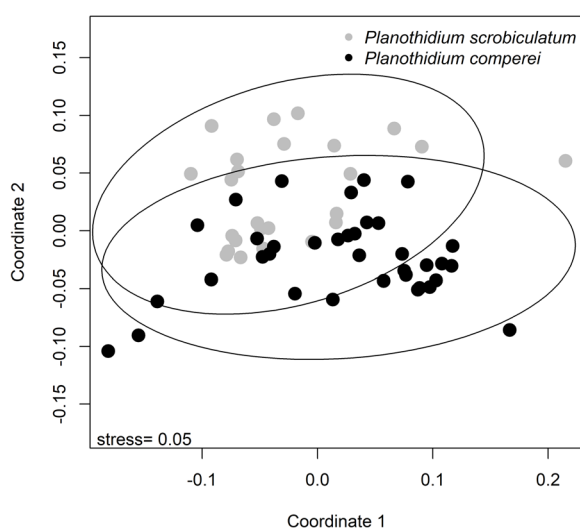


Fig. 49. Non-metric multi-dimensional scaling (nMDS) ordination of *P. scrobiculatum* from type material SP365549 (4910–11 cm depth; black circle) and *P. comperei* from N’GUESSAN et al. (2014; grey circle) based on four morphological characters and distinguished by cluster analysis (symbols).

Pleistocene. This study contributes to the knowledge of the hitherto understudied microorganism biodiversity of tropical regions. In addition, such patterns of diversity and the wealth of new diatom taxa in and around Colônia basin (many yet to be fully described) suggest that further samplings from other fossil localities may provide insight into its habitat preferences and range. In addition, this is an area of unique biological entity, therefore, requires further studies.

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