

***Staurosirella acidophila* sp. nov., a new araphid diatom (Bacillariophyta) from southeastern Brazil: ultrastructure, distribution and autecology**

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Abstract – A new diatom species, *Staurosirella acidophila*, is described from surface sediments and phytoplankton samples from tropical reservoirs in São Paulo, Brazil. Description of the new taxon is based on morphological characteristics observed using light (LM) and scanning electron microscopy (SEM). The new species is characterized by having rhombic-lanceolate valves with acuminate to rostrate ends, striae uniseriate, uninterrupted from valve face to valve mantle and areolae elongated, slit-like. Double line of incipient spines is for the first time reported for the genus *Staurosirella*. It belongs to the group of species morphologically related to *Staurosirella pinnata* sensu auct. non null. and shares some resemblance with species currently belonging to the genus *Punctastriata*. Comparison with similar species currently ascribed to the genera *Punctastriata*, *Staurosira* and *Staurosirella* are provided. The new species is described from sites that represent good ecological status (low nutrient concentrations and low pH) for the Metropolitan Region of São Paulo. Relative abundances and autecological information complement the present study, which is a contribution to the knowledge of diatoms thriving in environments with good ecological conditions in Brazil.

Araphid diatoms / morphology / new species / São Paulo / taxonomy / tropical reservoirs

Résumé – Une nouvelle espèce de diatomée, *Staurosirella acidophila*, est décrite à partir de sédiments de surface et d'échantillons de phytoplancton de réservoirs tropicaux de São Paulo, Brésil. La description du nouveau taxon est basée sur les caractéristiques morphologiques observées à l'aide de la microscopie optique (MO) et électronique à balayage (MEB). La nouvelle espèce est caractérisée par des valves rhombiques-lancéolées avec des extrémités acuminées à rostrées, des stries unisériées, sans interruption entre la face valvaire et le manteau, et des aréoles allongées, en forme de fente. Une double ligne d'épines marginales est pour la première fois signalée pour le genre *Staurosirella*. Elle appartient au groupe

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d'espèces morphologiquement liées à *Staurosirella pinnata* sensu auct. non null. et partage une certaine ressemblance avec des espèces appartenant au genre *Punctastriata*. Des comparaisons avec des espèces similaires actuellement attribuées aux genres *Punctastriata*, *Staurosira* et *Staurosirella* sont fournies. La nouvelle espèce est décrite à partir de sites qui représentent le bon état écologique (faibles concentrations en nutriments et pH bas) pour la Région Métropolitaine de São Paulo. Des informations sur les abondances relatives et l'autécologie viennent compléter cette étude, qui est une contribution à la connaissance des diatomées dans un environnement avec de bonnes conditions écologiques au Brésil.

Diatomées araphidées / morphologie / nouvelle espèce / São Paulo / réservoirs tropicaux / taxonomie

INTRODUCTION

The genus *Staurosirella* D.M. Williams & Round *emend.* E. Morales (Williams & Round, 1987; Morales & Manoylov, 2006a) was created to accommodate a group of species characterized by uniseriate striae composed of lineolae bearing developed volae, a wide sternum, and apical pore fields of variable size composed of several rows of round areolae. The genus also has wide valvocopulae with well-developed fimbriae and girdle bands that can be open or closed, as well as an abvalvar edge of the mantle only slightly convex or parallel to the valve face-mantle junction.

The taxonomy and circumscription of this genus have long been problematic, especially in reference to species within *Staurosirella*, *Staurosira* Ehrenb. and *Punctastriata* D.M. Williams & Round, among which there are apparent morphological and molecular intergradations (Morales, 2001; Medlin *et al.*, 2008). In addition, there are taxonomic drift and nomenclatural problems, as well as a considerable trail of publications containing few or no ultrastructural analysis (Morales *et al.*, 2014a).

A recent revision of type material of *Staurosirella pinnata* (Ehrenb.) D.M. Williams & Round concluded that this material did not correspond to *Staurosirella*, but a species related to *Denticula* Kützing (Morales *et al.*, 2013a). Several illustrated reports of *S. pinnata* in the literature show at least a dozen morphological variants, some of which could be related to known taxa, but still need to be reassessed in the light of detailed data on the type material (Gaul *et al.*, 1993; Henderson & Reimer, 2003). In spite of their common occurrence, the taxonomy of many small fragilarioid species remains largely unresolved (Morales, 2005; Morales *et al.*, 2013a).

Aiming at solving some of this taxonomic morass, species have been transferred to or described as new within *Staurosira*, *Staurosirella* and *Punctastriata* (e.g. Morales & Edlund, 2003; Morales, 2005; Garcia, 2006; Morales & Manoylov, 2006b; Levkov & Williams, 2011; Rioual *et al.*, 2014), in many cases based on the revision of type materials (e.g. Hamilton & Siver, 2008; Morales *et al.*, 2010, 2013b).

The abundance and distribution of fragilarioids are directly related to water physical and chemical conditions (Morales, 2001; Kingston, 2003; Potapova & Charles, 2003), therefore the correct identification of taxa is associated to a proper determination of their autecology and biogeography (Cox, 1987; Morales *et al.*, 2014b). The association of LM and SEM analysis in diatom investigations, especially for the study of small-sized fragilarioid taxa, is crucial for identification since diagnostic characters are beyond the limit of resolution of the light microscope

(Morales *et al.*, 2010). When taxa sharing similar morphology are analyzed using only LM, their identification results in a combination of different taxa under a common name, with the consequent misinterpretation of their environmental requirements and geographical distributions (Lobo *et al.*, 1990; Morales *et al.*, 2001, 2014b; Potapova & Charles, 2002).

Diatoms are sensitive to water quality and are good indicators of current trophic status. When stored in sediments, they also provide a rather accurate record of past ecological changes (Bennion & Simpson, 2011; Fontana *et al.*, 2014). Nevertheless, diatom knowledge from sites that hold good ecological quality is still needed, given the increasing threats of freshwater ecosystems by human impacts (Dudgeon *et al.*, 2005; Kociolek & Stoermer, 2009).

In the present work, we describe a new species of *Staurosirella* combining the use of LM and SEM, and provide ecological information for it. This is a common taxon in two water supply reservoirs of the Alto Cotia System, which is located in one of the largest forested areas in the Atlantic Plateau (Metzger *et al.*, 2006). The reservoirs are well protected and may be considered reference sites for pre-enrichment conditions for the Metropolitan Region of São Paulo (CETESB, 2011; Almeida & Bicudo, 2014).

MATERIAL AND METHODS

Study area

The Alto Cotia System belongs to the Tietê River basin and has two reservoirs, Pedro Beicht (23°39'S, 46°57'W) and Cachoeira da Graça (23°44'S, 46°58'W), both located within the Biological Reserve of Morro Grande (Cotia, São Paulo State, Brazil, Fig. 1, Table 1). The elevation of this forest area ranges from 860 to 1,075 m a.s.l., the air temperature oscillates between 13 and 21°C, and the annual precipitation average is 1,339 mm. The average precipitation for the driest month (winter) ranges from 30 to 60 mm, and for the wettest month (summer) varies from 150 to 200 mm (Metzger *et al.*, 2006). Pedro Beicht reservoir was built in 1933, has a surface area of 2.9 km², a maximum depth of 10 m and a volume of 14,800 × 10³ m³. Cachoeira da Graça reservoir was built in 1916, has a surface area of 0.2 km², a maximum depth of 3.0 m and a volume of 2 × 10⁶ m³. Pedro Beicht reservoir is located upstream and regulates the flow of the Cotia River, discharging directly into the Cachoeira da Graça reservoir. The Alto Cotia System provides drinking water for ca. 500,000 inhabitants of the Metropolitan Region of São Paulo (Metzger *et al.*, 2006; Almeida & Bicudo, 2014).

Sample preparation and analysis

Seven sampling stations were defined (Fig. 1), distributed along the Pedro Beicht reservoir (5 stations) and Cachoeira da Graça reservoir (2 stations). Sampling was carried out during winter (June 2010) and summer (December 2010). At each sampling site, water samples were collected with a Van Dorn sampler along the reservoir vertical profile (subsurface, mean depth, and 1 m above the sediments) for phytoplankton and limnological analyses. Temperature (°C), pH and conductivity

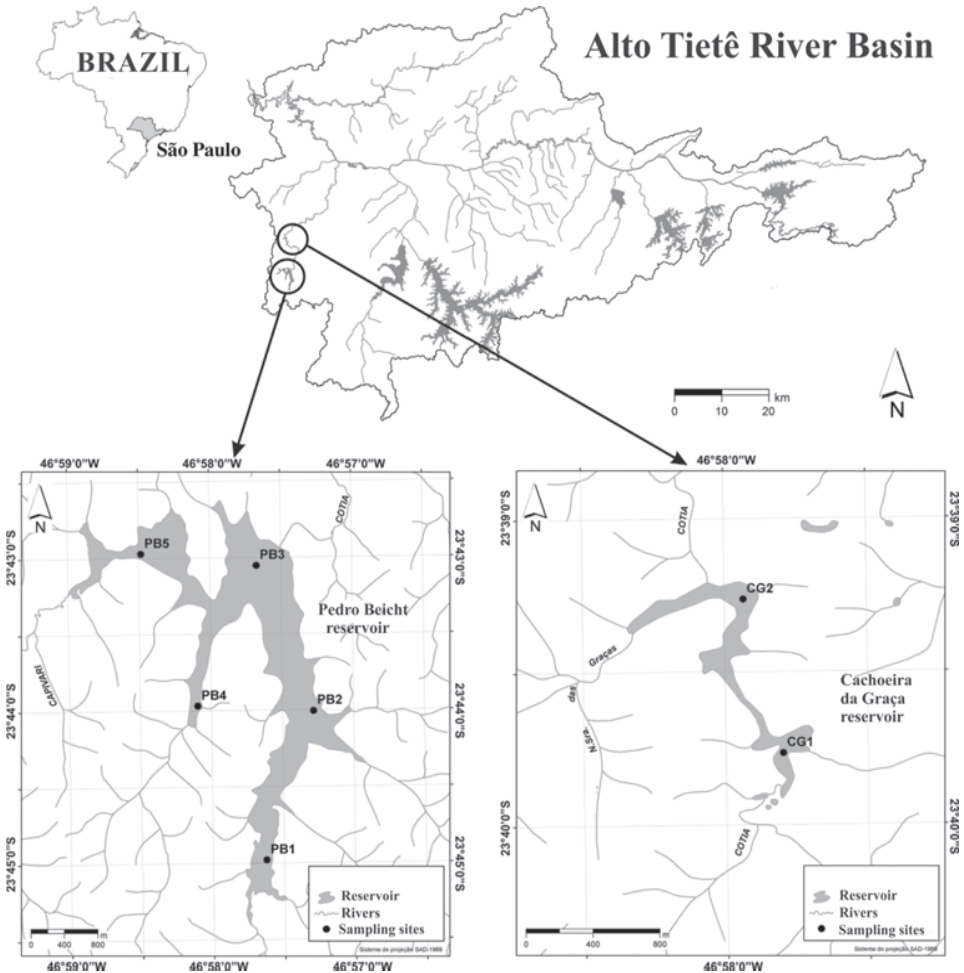


Fig. 1. Location of the Alto Cotia Water Supply System in the Tietê River Basin, Metropolitan Region of São Paulo, São Paulo State, Brazil. The sampling sites in Pedro Beicht (PB) and Cachoeira da Graça (CG) reservoirs appear depicted in the frames at the bottom of the figure.

($\mu\text{S cm}^{-1}$) were measured in the field using standard electrodes (Horiba U-53). Analytical procedure for dissolved oxygen (mg L^{-1}), ammonium ($\mu\text{g L}^{-1}$), nitrate ($\mu\text{g L}^{-1}$), soluble reactive silica (mg L^{-1}), total nitrogen and total phosphorus ($\mu\text{g L}^{-1}$), free carbon dioxide (mg L^{-1}), and bicarbonate ions (mg L^{-1}) followed Standard Methods (APHA, 2005). Chlorophyll *a* ($\mu\text{g L}^{-1}$) corrected for phaeophytin analysis was measured using 90% ethanol (Sartory & Grobbelaar, 1984).

Short sediment cores were collected during winter, with a gravity corer (UWITEC[®] Mondsee, Austria), and the top 2 cm sections were saved for analyses. Analyses included total phosphorus (TP), total organic carbon (TOC), total nitrogen (TN), grain size and diatoms. Methods are described in Wengrat & Bicudo (2011) and Fontana *et al.* (2014).

Table 1. Occurrence of *Staurosirella acidophila* in the Alto Cotia System reservoirs

Reservoir	Station	Geographic coordinates	Date	Habitat	Depth (m)	Register herbarium	Abundance (%)
Pedro Beicht	PB1	23°44'94.6"S 46°57'66.0"W	18.06.10	Surface sediments	2.7	SP427578	0.1
Pedro Beicht	PB3	23°43'04.8"S 46°57'67.6"W	18.06.10	Surface sediments	10.0	SP427580	0.2
Pedro Beicht	PB4	23°44'09.5"S 46°58'16.0"W	18.06.10	Surface sediments	1.0	SP427581	1.4
Pedro Beicht	PB5	23°43'00.0"S 46°58'64.7"W	18.06.10	Surface sediments	3.0	SP427582	1.2
Pedro Beicht	PB4	23°44'09.5"S 46°58'16.0"W	18.06.10	Phytoplankton	0.5	SP427595	0.2
Cachoeira da Graça	CG1	23°39'77.9"S 46°57'92.8"W	18.06.10	Surface sediments	1.6	SP427583	6.3
Cachoeira da Graça	CG2	23°39'29.0"S 46°57'78.4"W	18.06.10	Surface sediments	1.8	SP427584	6.8
Cachoeira da Graça	CG2	23°39'29.0"S 46°57'78.4"W	07.12.10	Phytoplankton	0.5	SP427591	0.2

Diatom samples (surface sediments and phytoplankton) were oxidized according to methods described by the European Committee for Standardization (2003) using concentrated H₂O₂ (37%) and hydrochloric acid. Oxidized subsamples were rinsed with deionized water and permanent slides were prepared using Naphrax[®] mounting medium. For SEM studies, aliquots of processed material were filtered and rinsed with deionized water through a 3 µm Isopore[™] polycarbonate membrane filter (Merck Millipore). Filters were mounted on aluminum stubs and coated with platinum using a BAL-TEC Med 020 Modular High Vacuum Coating System for 30 s at 100 mA. The cleaned samples were examined using an ultra-high resolution analytical field emission (FE) scanning electron microscope Hitachi SU-70 (Hitachi High Technologies) operated at 5 kV and 10 mm distance. LM images were taken with a Leica DFC 45D microscope and images were captured and measured using Leica Application Suite (LAS) program. Micrographs were digitally manipulated and plates containing LM and SEM images were created using CorelDraw X6[®].

Abundance and morphometric analyses

Diatom quantification was made at a magnification of 1,000× using a Zeiss microscope (Axioskop 2 plus) with oil immersion objective. At least 400 valves were counted per slide (Battarbee, 1986), and until reaching a sampling efficiency of 90% (Pappas & Stoermer, 1996). Species abundances were calculated as percentage of the total counts. In total, 21 samples were analyzed for diatoms, 7 from surface sediments and 14 from phytoplankton (7 from winter and 7 from summer). Taxonomic metrics such as length, width (middle of valve), width at apex (at 2 µm before the end the valve), and striae density were taken from the LM digitized images. Terminology of valve morphology was based on Ross *et al.* (1979) and Barber & Haworth (1981).

The form factor was used as an aid in the description of the morphology of valves. The factor is a dimensionless ratio that relates the area of a specimen to its perimeter using the following relationship:

$$\text{Form factor} = (4\pi A)/P^2$$

where A is the area and P is the perimeter. The ratio is normalized, so that the measurements range between 0 (a line) and 1 (a circle). Measurements were done following Siver & Baskette (2004). For this, the mathematical shape of a rhombus was considered. The area was calculated by multiplying the lengths of the diagonals, i.e., multiplying the width at the middle of valve by its length. The perimeter of the valve was represented by the addition of the length of each side of the rhombus, i.e. the distances of the apex to middle of the valve.

RESULTS

Staurosirella acidophila sp. nov.

Figs 2-41

Valvae rhombeae-lanceolatae isopolares vel nonnunquam heteropolares apicibus acuminatisve rostratis. Sternum amplum lineare-lanceolatum intra extraque elevatum. Longitudo: 10.8-19.5 μm ; latitudo: 4.5-6.8 μm ; ratio longitudinis/latitudinis 2.4-3.7; latitudo ad apices 1.5-2.7 μm . Striae alternantes vel leviter radiatae ad extremos, 8-9 in 10 μm . Areolae non discernendae in microscopio photonico, apicaliter elongatae in microscopio electronico, rimiformes. Volae parvae e marginibus interioribus longioribus exorientes. Spinae inceptivae nonnusquam bifurcatae, spinibus linea duplicate in nonnullis valvis observata. Areae pororum apicales effectae ambabus apicibus, Singulae porelli rotundi a margine albido obtecti. Cingulum ex taeniis apertis amplis nonporosis.

Frustules rectangular in girdle view, usually solitary or forming short chains of up to two cells (Fig. 2). Central area absent. Sternum raised internally and externally (Figs 18-21). Costae raised internally and externally, giving the characteristic "punch-hole" appearance to striae (Figs 33, 34). Abvalvar edge of valve parallel to valve face/mantle edge. Striae uniseriate, uninterrupted from valve face to mantle (Figs 36, 38). First areola very evident (Figs 35, 37). Spines irregularly located on the costae. Rimoportula absent. Valvocopulae wider, bearing short fimbriae (Fig. 41).

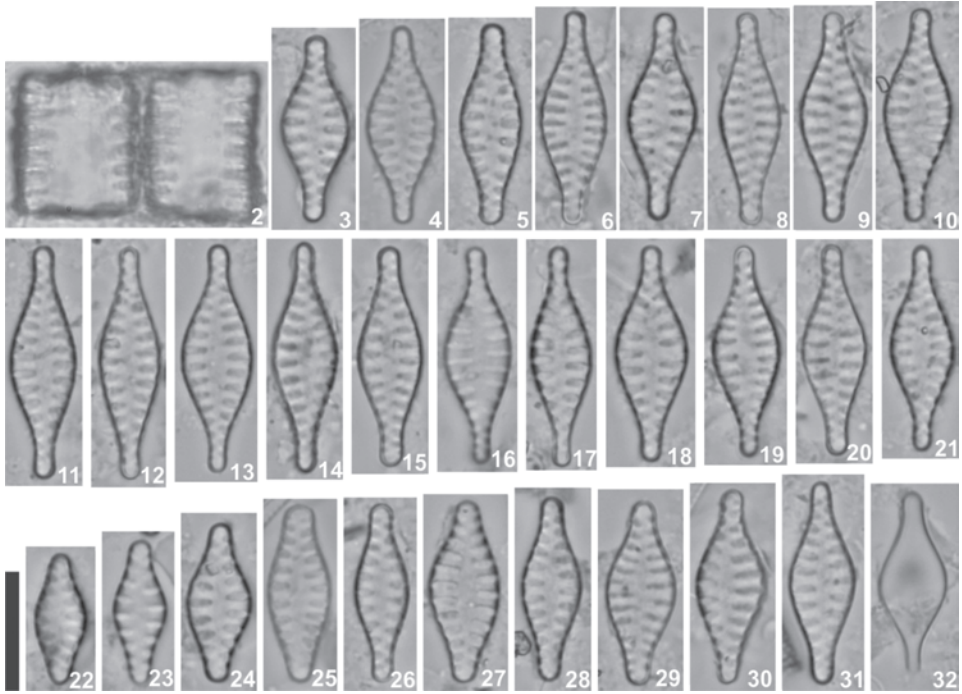
Holotypus: Population on slide SP427584, partially illustrated here in Figs 2-32 (Herbário Científico do Estado Maria Eneyda P. Kauffmann, Instituto de Botânica, São Paulo State, Brazil), collected by P. D. Almeida & D. C. Bicudo, 18-VI-2010 (Table 1).

Isotypus: Population on slide BR-4418 (Botanical Garden Meise).

Type Locality: Cachoeira da Graça reservoir, Cotia, São Paulo, Brazil (23°39'05"S, 46°57'58"W).

Etymology: The specific epithet *acidophila* refers to the slightly acidic waters (5.8-6.9) where this species was found.

Taxonomic remarks: *Staurosirella acidophila* has several morphological features of the genus *Staurosirella* such as uniseriate striae composed by slit-like areolae, punch-hole striae, a wide sternum, well-developed apical pore fields, valvocopulae with well-developed fimbriae and girdle bands and open girdle bands (Morales & Manoylov, 2006b). Therefore, the allocation of the new species in *Staurosirella* is justified.

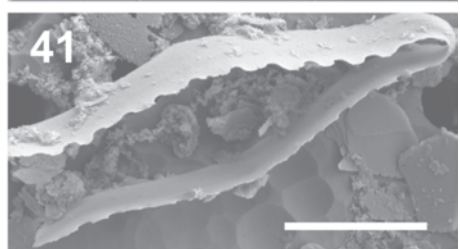
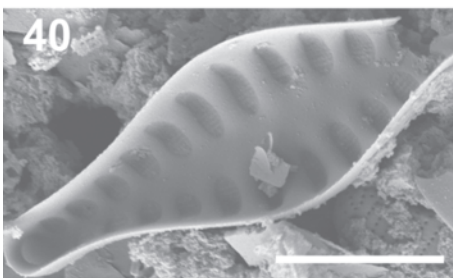
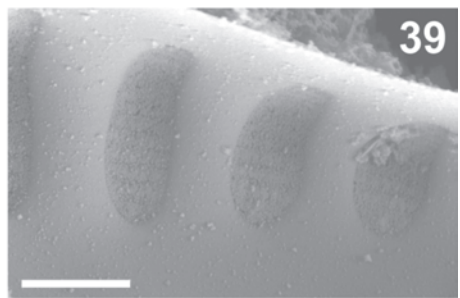
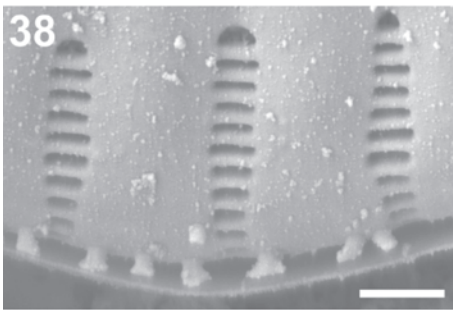
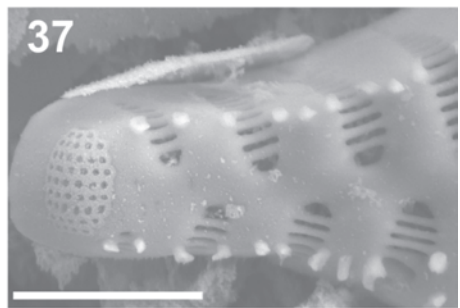
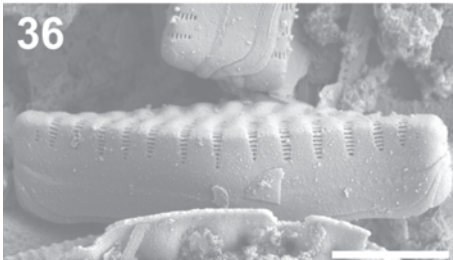
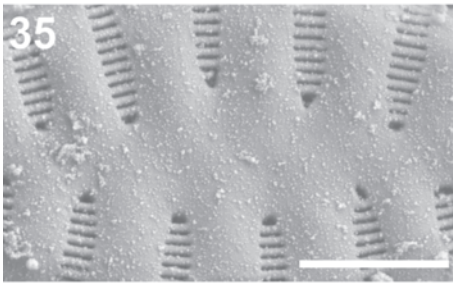
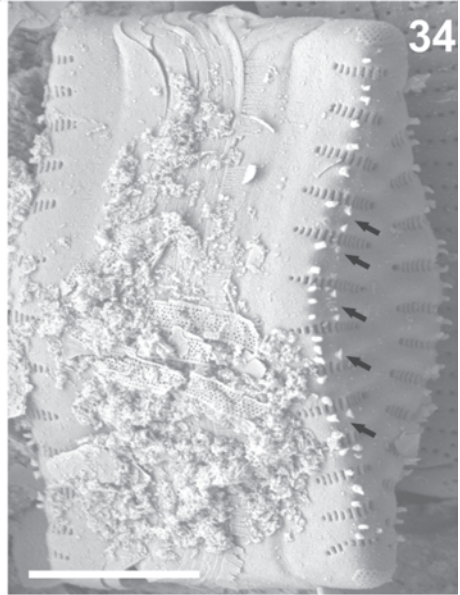
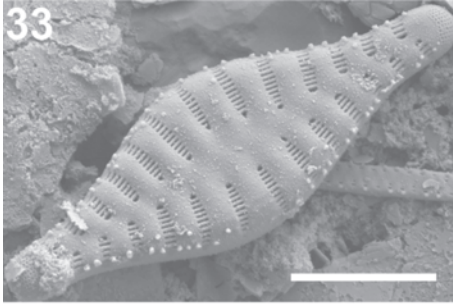


Figs 2-32. LM. *Stausosirella acidophila* from type material (SP427584). 2-32. Individuals showing size diminution series. 2. Two sibling cells attached. 32. View of copula. Scale bar = 10 μ m.

As in *Stausosirella confusa* E.Morales the striae are very refractive under LM (Morales, 2005), yet the new species differs in the arrangement and density of the striae (Table 3). *Stausosirella acidophila* also shows similarity to other species currently in *Stausosirella* such as *S. oldenburgiana* (Hust.) E.Morales and *S. subcapitata* (Freng.) E.Morales. The new species differs from *S. oldenburgiana* in the width at the valve centre and the relatively small areolae present in *S. oldenburgiana* (Morales, 2005). In *S. subcapitata*, smaller specimens tend to lose their typical lanceolate outline, which does not occur in *S. acidophila*. The same shape variation trend can be observed in *S. dubia* (Grunow) E.Morales & Manoylov (Morales & Manoylov, 2006b), in which larger valves are lanceolate, but without expanded central area as in *S. acidophila*, and smaller valves are elliptical, not lanceolate or slightly rhombic as it occurs in *S. acidophila*.

Stausosirella acidophila also resembles smaller and medium-size representatives of *S. rhomboides* (Grunow) E.Morales & Manoylov that show rounded apices and similar sternum. However, *S. rhomboides* differs in the shape of valves and type of spines (Table 3). The maximum length observed in *S. acidophila* was 19.5 μ m, whereas in *S. rhomboides* the length range is 13-55 μ m with a more pronounced central expansion (Morales & Manoylov, 2006b).

The form factors calculated for the populations from Brazil were near 1, demonstrating that the form is strongly influenced by valve width. This metric corroborates the stability observed from larger to smaller valves. From scanned images, the form factor was calculated for *Stausosirella confusa*, *S. oldenburgiana*,



S. subcapitata, *S. rhomboides*, *Punctastriata lancettula* (Schum.) P.B.Hamilton & Siver, *P. mimetica* E.Morales, *Staurosira acutirostrata* (Metzeltin & Lange-Bert.) Metzeltin & Lange-Bert. and *Fragilaria pinnata* var. *acuminata* Ant.Mayer (Table 3). The mean of the values found for these species was similar to the average found for *S. acidophila* (average: 0.6), however for *Staurosira acutirostrata* and *F. pinnata* var. *acuminata* the values were smaller. Smaller values also were obtained by Rioual *et al.* (2014) for *Staurosira longwanensis* P.Rioual, E.Morales & Ector. Therefore, *Staurosira acutirostrata*, *F. pinnata* var. *acuminata* and *S. longwanensis* are species for which the form factor is controlled by valve length, with smaller specimens losing the narrowly lanceolate shape of larger specimens and becoming elliptic as size decreases.

Staurosirella acidophila has similar valve shape, length and width as *Punctastriata lancettula* and *P. mimetica*. However, the ultrastructure of the striae is different since it is composed of a net of transapical and apical silicified bars (Williams & Round, 1987; Morales, 2005; Hamilton & Siver, 2008). This net, gives a diffuse, blurry aspect to the striae under LM, instead of the refractive, brighter appearance in *S. acidophila*.

Another similar taxon is *Staurosira acutirostrata*, which has lanceolate valves, greater length (22-36 µm), central inflation less apparent, and the striae tend to be less radiate than in *S. acidophila* (Metzeltin & Lange-Bertalot, 1998). Nevertheless, under SEM, both of these taxa have incipient spines, and the striae and areolae also look similar. Although *S. acutirostrata* is currently ascribed to *Staurosirella*, it has characters of *Staurosirella* such as developed apical pore fields and lineolae. Despite the fact that too little microscopical information was given by Metzeltin & Lange-Bertalot (1998) at the time of description, and the reasons exposed by Metzeltin *et al.* (2005), the transfer of *S. acutirostrata* to *Staurosirella* is recommended (see below).

An additional taxon that is morphologically similar to *S. acidophila* is *Fragilaria pinnata* var. *acuminata*, at least as it appears depicted in some current references. The iconotype presented in Mayer (1937 see fig. 18) show this variety as having lanceolate valves, without a pronounced central inflation and apices not as protracted as in *S. acidophila*. Furthermore, the same author depicted his new variety as having slender and parallel striae, two features absent in *S. acidophila*. The current concept of *F. pinnata* var. *acuminata* is different from Mayer's, as adopted in studies by Reavie & Smol (1998), Camburn & Charles (2000) and Vermaire & Gregory-Eaves (2008), for example. A clarification of the taxonomy of this taxon through examination of the type material is required.

Ecology and associated diatom flora: *Staurosirella acidophila* was found in shallow mesotrophic reservoirs (Gianesella-Galvão & Arcifa, 1988; Almeida & Bicudo, 2014) with higher relative abundance in the surface sediments than in phytoplankton. The highest abundance occurred in Cachoeira da Graça (Table 1), the shallower reservoir, with very low nutrient concentrations, and a well-illuminated water column, with occurrence of benthic species.

◀ Figs 33-41. SEM. *Staurosirella acidophila* from type material (SP427584). **33.** Valve in external view with spines. **34.** Valve in external view with a double line of spines (arrows). **35.** Narrow sternum and areolae. **36.** Frustule in girdle view showing copulae without ornamentation. **37.** Apical pore fields and initial semicircular areolae. **38.** Striae uniseriate with lineolae. **39-40.** Well-developed volae in internal view. **41.** Fimbriae on valvocopula. Scale bar represents 5 µm except in Fig. 38 where scale bar = 1 µm and Figs 35 and 37 where scale bar = 2 µm.

Table 2. Water chemistry data for Pedro Beicht (PB) and Cachoeira da Graça (CG) reservoirs (mean values for the water column) during winter (W) and summer (S). < below detection limit

Station	Temperature (°C)		Conductivity (µS cm ⁻¹)		pH		OD (mg L ⁻¹)		N-NH ₄ (µg L ⁻¹)		N-NO ₃ (µg L ⁻¹)		TN (µg L ⁻¹)		TP (µg L ⁻¹)		SRS (mg L ⁻¹)		free CO ₂ (µg L ⁻¹)		HCO ₃ (µg L ⁻¹)		Chlorophyll <i>a</i> (µg L ⁻¹)			
	W	S	W	S	W	S	W	S	W	S	W	S	W	S	W	S	W	S	W	S	W	S	W	S	W	S
PB1	16.9	22.9	13.3	16.2	6.2	6.0	8.6	4.8	12.5	26.6	14.7	22.5	380.2	283.8	15.4	16.0	2.3	2.9	7.0	11.8	5.7	5.9	8.0	20.2		
PB2	16.4	24.4	13.1	15.0	6.2	6.2	5.2	5.9	< 10	13.5	9.7	< 8	317.8	233.8	14.2	10.3	2.2	2.2	6.5	7.6	5.5	5.5	8.7	< 0.5		
PB3	16.1	23.9	13.2	16.4	6.0	6.1	6.5	5.1	< 10	21.5	15.2	< 8	266.6	192.6	14.0	9.7	2.0	2.2	10.2	17.0	5.8	5.9	11.4	10.1		
PB4	18.0	27.0	13.2	14.9	6.2	6.3	7.5	2.5	< 10	14.7	18.9	< 8	272.6	197.4	15.4	14.5	2.2	2.4	6.0	5.3	5.2	5.8	15.8	6.1		
PB5	16.5	25.0	13.2	15.2	6.4	6.2	7.2	4.6	< 10	39.8	11.4	< 8	234.6	168.1	14.6	13.6	2.3	2.8	4.8	6.4	5.7	5.0	6.4	10.5		
CG1	19.6	24.8	14.6	17.7	5.8	6.9	7.5	6.6	14.2	48.9	24.4	< 8	450.9	340.6	16.3	14.9	3.3	3.0	16.1	1.2	6.2	6.0	1.0	2.6		
CG2	15.8	24.3	14.6	17.2	5.9	6.5	9.7	6.7	< 10	18.8	29.4	< 8	335.1	313.3	15.1	18.5	3.4	3.1	14.5	3.5	6.9	6.3	2.5	11.4		

Table 3. Morphological features of *Stausosirella acidophila* sp. nov. and comparable species

	<i>Stausosirella acidophila</i>	<i>Stausosirella confusa</i>	<i>Stausosirella oldenburgiana</i>	<i>Stausosirella subcapitata</i>	<i>Stausosirella rhomboidea</i>	<i>Punctosirella lanceollata</i>	<i>Punctosirella mimetica</i>	<i>Stausosira acutirostrata</i>	<i>Fragilaria pinata</i> var. <i>acuminata</i>
Reference	This study	Morales (2005)	Morales (2005)	Morales & Manoylov (2006b)	Morales & Manoylov (2006b)	Hamilton & Siver (2008)	Morales (2005)	Metzelin & Lange-Bertalot (1998), Metzelin <i>et al.</i> (2005)	Mayer (1937)
Valve shape	Isopolar to slightly heteropolar, rhombic-lanceolate	Slightly heteropolar, lanceolate	Isopolar, linear to lanceolate	Lanceolate	Rhomboid	Slightly heteropolar, elliptical-lanceolate	Slightly heteropolar, cruciform to rhomboid	Fusifforme, lanceolate	Lanceolate
Apices	Acuminate to rostrate-rounded	Acuminate	Rostrate to subcapitate, clearly protracted	Acute rostrate to subrostrate	Broadly rounded	Rostrate to lanceolate	Acuminate to subrostrate	Rostrate-protracted	Rounded to acuminate
Sternum	Variable, linear to lanceolate	Variable, linear to lanceolate	Variable, narrowly linear-lanceolate to linear	Lanceolate	Lanceolate	Variable, linear to lanceolate	Variable, linear to lanceolate	Variable, narrowly linear-lanceolate to linear	Linear
Length (µm)	10.8-19.5	11-20	13-24	8-27	13-55	5.7-14	7-22.5	22-36 µm	15-30
Width (µm)	4.5-6.8	3.5-4.5	2.5	4-5.5	5.5-9	4.4-5.2	5-7	4-6 µm	4-6
Striae in 10 µm	8-9	11	13-14	7-9	6.5-9	10-12	9-11	7-9	-
Striae	Alternate to slightly radiate	Parallel to slightly radial toward the apices	Parallel or alternate	Parallel to radial toward the apices	Parallel to slightly radial toward the apices	Linear, multiseriate, oval to elliptical	Parallel to slightly multiseriate	Alternate, transapical, linear	Parallel or alternate
Areolae	Slit-like, the first semicircular, evident	Slit-like	Small elliptical	Slit-like	Slit-like	Small and round	Small and round	Slit-like, the first semicircular, evident	-
Volae	Branched and originated from the sides of the areolae	Well-developed	Branched and originate from the sides of the areolae	Delicate and branched	Branched and originated from the sides of the areolae	Recessed	Not observed	Well-developed	-
Apical pore fields	Present at both apices, composed by round poroids, surrounded by siliceous rim	A few or numerous row of round poroids	Well developed at both apices, composed by round poroids, sometimes surrounded by siliceous rim	Well developed at both apices, composed by rows of large round poroids	Developed at both apices, probably heteropolar, composed by several round poroids	At one apex well-developed, the opposite apex smaller or not observed	Present, at both apices	Present, at both apices	-
Spines	Small, 2-3 located in costae valve, sometimes in double line	Solid, spatulate, 2-3 located in costae valve, sometimes absent	Externally, the costae bear rudimentary spines	Two kinds on the costae, sometimes absent	Flattened and located on the costae	Spatulate	Bifurcate, solid	Small, 2-3 located in costae valve	-
Girdle bands	Open, wide, without perforations	Open, numerous and without perforation	Open, without perforation	Numerous, unknown details	Several, valvocopulae larger than the other elements	Two to four copulae, unknown details	Open, without perforation	Unknown	-
Form factor ranges	0.2-0.9 (n = 68)	0.8-0.9 (n = 5)	0.5-1.0 (n = 12)	0.4-0.9 (n = 23)	0.5-1.0 (n = 8)	0.6-1.0 (n = 7)	0.6-1.0 (n = 14)	0.2 (n = 2)	0.3-0.6 (n = 3)

The low nutrient concentrations in the reservoirs (Table 2) are typical of tropical oligo- to mesotrophic environments (Arcifa *et al.*, 1981; Lopes *et al.*, 2005; Vercellino & Bicudo, 2006; Santos & Ferragut, 2013). The granulometric texture (0.2% sand, 19.1% clay and 80.7% silt) indicates environments with low hydrodynamic changes and not subjected to erosion (Fontana *et al.*, 2014). High C/N ratios (mean values of 13 and 12, respectively for Pedro Beicht and Cachoeira da Graça reservoir) indicate the presence of humic compounds derived from decomposition of vascular plants (Håkanson, 1984; Meyers, 2003). Given that the reservoirs are located in a well-protected area, their mesotrophic status probably derived from the early phase when vegetation was flooded by the dam construction, and later on by the organic matter from surrounding vegetation. The sediment TP concentrations (mean values of 0.280 mg g⁻¹ and 0.104 mg g⁻¹, respectively for Pedro Beicht and Cachoeira da Graça reservoir) are compatible with values found below 0.750 mg g⁻¹ in the continental crust (Wedepöhl, 1995; CETESB 2011).

In the studied samples, the diatom assemblage was composed of 98 taxa. Twenty three of these taxa (arranged in 11 genera) presented relative abundance $\geq 2\%$. The two most common genera were *Eunotia* Ehrenb. [*E. bilunaris* (Ehrenb.) M.G.M.Souza, *E. botuliformis* F.Wild, Nörpel & Lange-Bert., *E. incisa* W.Greg., *E. mucophila* (Lange-Bert. & Nörpel-Schempp) Lange-Bert., *E. muscicola* var. *tridentula* Nörpel-Schempp & Lange-Bert. and *E. veneris* (Kütz.) De Toni] and *Aulacoseira* Thwaites [*A. ambigua* (Grunow) Simonsen, *A. brasiliensis* Tremarin, Ludwig & Torgan, *A. granulata* (Ehrenb.) Simonsen, *A. herzogii* (Lemmermann) Simonsen and *A. tenella* (Nygaard) Simonsen]. The genus *Eunotia* has mainly been recorded from oligotrophic, slightly acidic and well-illuminated environments (Round *et al.*, 1990; Furey *et al.*, 2011, Fontana *et al.*, 2014). While *Aulacoseira* has a worldwide distribution and generally represents an important component of the phytoplankton of eutrophic lakes, lagoons and rivers, however it is also distributed in oligotrophic waters (Denys *et al.*, 2003; Tremarin *et al.*, 2013).

DISCUSSION

Staurosirella acidophila is a new species with several characters that allow its allocation into *Staurosirella* as far as we know the double line of incipient spines is for the first time reported for the genus *Staurosirella*. The new diatom occurs in naturally mesotrophic reservoirs that may be considered reference sites for pre-enriched conditions for the Metropolitan Region of São Paulo.

Despite the recent publications on South American diatoms, small araphids are not frequently reported and are usually identified only at the genus level (Metzeltin & Lange-Bertalot, 1998; Rumrich *et al.*, 2000; Metzeltin *et al.*, 2005), deriving in a poor representation of the araphid flora of the region. More recently, five new araphid species were described from the Bolivian Altiplano (Morales *et al.*, 2012, 2014b), indicating that taxonomic studies in this group can potentially contribute to the increase of species richness in tropical regions. Few species, belonging to the “small araphids” group, have been reported for Brazil, such as *Staurosirella leptostauron* (Ehrenb.) D.M.Williams & Round, *S. leptostauron* var. *dubia* (Grunow) Edlund, *S. martyi* (Héribaud) E.Morales & Manoylov, *S. pinnata* and *S. subcapitata* (Tremarin *et al.*, 2009; Silva *et al.*, 2011), *S. crassa* (Metzeltin & Lange-Bert.) F.C.P.Ribeiro & Torgan and *Staurosira acutirostrata* (Fontana &

Bicudo, 2009; Ribeiro *et al.*, 2010; Dunck *et al.*, 2012). However, few of these studies use a combination of LM and SEM, thus impeding taxonomical confirmations.

Our findings showed that *Staurosirella acidophila* has a benthic habitat, occurring in slightly acidic waters, with low nutrient concentrations and well-illuminated conditions.

Concerning biodiversity conservation, freshwater issues have globally acquired increasing importance (Dudgeon *et al.*, 2005; Kociolek & Stoermer, 2009). In this context, we describe herein a new species of fragilarioid diatom from reservoirs that hold good ecological conditions and are located in a biological reserve of Atlantic Forest, increasing the knowledge on biodiversity and ecology of this diatom group in Neotropical protected areas.

In addition, based on LM and SEM illustrations presented by Metzeltin & Lange-Bertalot (1998) and the reasons given above, *Staurosira acutirostrata* a species originally described for the Amazonian region (Demerara River, Guyana) is here transferred to the genus *Staurosirella*:

Staurosirella acutirostrata (Metzeltin & Lange-Bert.) P.D.Almeida & C.E.Wetzel **comb. nov.**

Basionym: *Fragilaria acutirostrata* Metzeltin & Lange-Bert. 1998, *Iconographia Diatomologica* vol. 5, p. 89, pl. 1, figs 18, 19; pl. 2, fig. 4.

≡ *Staurosira acutirostrata* (Metzeltin & Lange-Bert.) Metzeltin & Lange-Bert. in Metzeltin *et al.* 2005, *Iconographia Diatomologica*, vol. 15, p. 229.

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