

Type analysis of *Achnantheidium minutissimum* and *A. catenatum* and description of *A. tropicocatenatum* sp. nov. (Bacillariophyta), a common species in Brazilian reservoirs

Gisele C. Marquardt^{1,*}, Livia F. Costa¹, Denise C. Bicudo¹,
Carlos E. de M. Bicudo¹, Saúl Blanco², Carlos E. Wetzel³ & Luc Ector³

¹Instituto de Botânica, Ecology Department, Av. Miguel Estéfano 3687, 04301–012, São Paulo, SP, Brazil

²University of León, Biodiversity and Environmental Management Department, 24071 León, Spain. Current address: Institute of the Environment, La Serna, 58, 24007 León, Spain

³Luxembourg Institute of Science and Technology (LIST), Environmental Research and Innovation Department (ERIN), 41 rue du Brill, 4422 Belvaux, Luxembourg

*Author for correspondence: giselecmarquardt@gmail.com

Background and aims – A commonly occurring *Achnantheidium* species has been observed in Brazilian reservoirs. The morphological features were described based on light and scanning electron microscope observations and it is described here as *A. tropicocatenatum* sp. nov.

Methods – The new species was compared with the type material of morphologically similar taxa: *Achnantheidium catenatum* and *A. minutissimum*. Comparisons with the most related species based on literature were also provided. Traditional weighted-averaging approaches (WA) were applied to model ecological optima.

Key results – Morphometric analysis of the valve shape supported the taxa separation; they can be easily differentiated by the morphological and ultrastructural features, including subcapitate to capitate apices, strongly radiate striae and a prominent axial area. This new species has been found in alkaline waters with low electrolyte content.

Conclusions – Records of *A. catenatum* in Brazil are considered questionable because the species may have been confused with *A. tropicocatenatum* sp. nov.

Key words – Distribution, ecological optimum, ecology, geometric morphometry, morphology, new species, taxonomy.

INTRODUCTION

The genus *Achnantheidium* Kütz. is a taxonomically difficult group of monoraphid diatoms (Round & Bukhtiyarova 1996). Despite its representatives being commonly recorded in worldwide freshwaters, the identification and delimitation of many taxa are still a challenge, because of both the small size of their valves, often requiring detailed scanning electron microscopy (SEM), and the lack of comprehensive descriptions of nomenclatural types and the undescribed variability of diagnostic features that often overlap (Hlúbiková et al. 2011).

Because of the high diversity in this group and its importance as indicators of water quality (Van de Vijver et al. 2011a, 2011b), *Achnantheidium* has received considerable attention and, during the last decade, the genus has been the

subject of intense revisions (e.g. Hlúbiková et al. 2011, Van de Vijver et al. 2011a, Wojtal et al. 2011, Novais et al. 2015), especially taxa belonging to the *A. minutissimum* (Kütz.) Czarn. complex (Ector 2011). These revisions have also contributed with more detailed information of the geographical distributions and biogeography of the genus in general.

Nowadays, *Achnantheidium* comprises more than 200 taxa (Fourtanier & Kociolek 2011) including many species described from various parts of the world in the last decade (e.g. Potapova & Ponader 2004, Cantonati & Lange-Bertalot 2006, Potapova 2006, Zidarova et al. 2009) and new species have regularly been described (e.g. Wojtal et al. 2010, Jüttner & Cox 2011, Van de Vijver et al. 2011b, Witkowski et al. 2012, Peres et al. 2014, Novais et al. 2015, Pinseel et al. 2015, Liu et al. 2016). However, the genus has received relatively little attention in Brazil, where *A. minutissimum* and

Table 1 – Data from sampling sites of *Achnanthyidum tropicocatenatum*, state of São Paulo, Brazil, habitat and the material number at the Herbarium of the Institute of Botany (SP).

Reservoir	Herbarium number	Habitat	Municipality	Main river	Sampling date	GPS coordinates
Cachoeira do França (type material)	SP469444	periphyton (stones)	Juquitiba	Juquiá	5 Aug. 2014	23°55'58.8"S 47°11'31.4"W
Jurupará	SP469208	surface sediment	Piedade	Peixes	25 Jun. 2014	23°56'00"S 47°22'18.0"W

A. catenatum (J.Bílý & Marvan) Lange-Bert. are among the most commonly recorded species (e.g. Bertolli et al. 2010, Faria et al. 2010, Faustino et al. 2016). Although these are widely used names, some misidentifications were observed and they are discussed in the present work. All these findings contributed to the generally accepted idea that *A. minutissimum* and *A. catenatum* species are globally distributed. Moreover, it is important to note that a number of records to the species above are ecological contributions (e.g. Santos & Ferragut 2013, Burliga et al. 2014, Fonseca et al. 2014) or publications discussing diatoms used as indicators of environmental change (e.g. Lobo et al. 2004a, 2004b, Hermany et al. 2006, Molisani et al. 2010, Bere & Tundisi 2011, Elias et al. 2017). These records emphasize the importance of accurately defining the identity of these most common taxa in order to improve the accuracy of the diagnostic tools that rely on diatom taxonomy and ecology (Wetzel & Ector 2014). Other records of these species can be found in unpublished doctoral dissertations (e.g. Carneiro 2003, Nascimento 2012) or do not show illustrations and/or descriptions (Souza & Oliveira 2007). *Achnanthyidum catenatum* is considered to be invasive in Europe (Bílý & Marvan 1959, Druart & Straub 1992, Coste & Ector 2000, Straub 2002, Hlúbíková et al. 2011) and more recently in East China (Ma et al. 2013).

The freshwater diatom flora of Brazil has been shown to contain many unique species (e.g. Tremarin et al. 2011, Wengrat et al. 2015, Marquardt et al. 2016). However, at present, no new *Achnanthyidum* species have been published. During a diatom survey in reservoirs of São Paulo State, an unknown *Achnanthyidum* species morphologically similar to *A. catenatum* was found, and it is proposed as a new species for science: *A. tropicocatenatum* sp. nov. The morphology is presented based on light (LM) and scanning electron microscopy (SEM) observations. Furthermore, we compared the new species with the type materials of morphologically similar species, *A. minutissimum* and *A. catenatum*. Examining type material has become a valuable tool to solve taxonomic issues (e.g. Cejudo-Figueiras et al. 2011). We also presented information based on ecology optima. In this way, apart from solving the taxonomic issues about the species, it is as well characterized from an autecological standpoint (e.g. Trobajo et al. 2013, Morales et al. 2015, Bicudo et al. 2016).

MATERIALS AND METHODS

Study area

The Jurupará and Cachoeira do França reservoirs (CBH-RB 2013) are located in the catchment area of the Ribeira de

Iguape/Litoral Sul basin which presents high availability of water in relation to the demand, due to a rich and extensive drainage network. The area is considered as a Conservation Unit (PEJU, Portuguese for Jurupará State Park) and widely covered with native forests. Information about the study area is summarized in table 1.

The Jurupará reservoir is part of a hydropower facility located in Piedade township at an elevation of 781 m along the Peixes River, tributary to the Juquiá River (SMA 2012). Built in 1947, the reservoir accumulation capacity is up to 42 10⁶ m³. This environment is predominantly mesotrophic, according to the Trophic Status Index (TSI) (Lamparelli 2004) calculated presently. The reservoir is also used for fish farming (fig. 1). The TSI results are in accordance with those of the PEJU Management Plan report (SMA 2012) in which observations carried out through ichthyofauna studies indicated a mesotrophic site in the network tanks region, probably a result of the aquaculture activities.

The Cachoeira do França reservoir is located in the region of Juquitiba at an elevation of 685 m. Its construction began in 1954 and was completed in 1957 and its operation started in the following year. It was the first hydroelectric plant built by Companhia Brasileira de Alumínio (CBA) for power generation which corresponds to the first of the cascading projects along the Juquiá River (SMA 2012). This reservoir is currently classified as oligotrophic (TSI) and presents an accumulation capacity of up to 135.2 10⁶ m³ (fig. 1).

Environmental variables

Water samples for chemical analysis were collected during austral summer and winter campaigns in 2014 with a Van Dorn sampler along a vertical profile. Temperature (°C), pH and specific conductance (µS cm⁻¹) were measured in the field using standard electrodes (Horiba U-53). The analytical procedure for dissolved oxygen (mg L⁻¹), ammonium (µg L⁻¹), alkalinity (mEq L⁻¹), nitrate (µg L⁻¹), soluble reactive silica (mg L⁻¹), total nitrogen (TN) and total phosphorus (TP) (µg L⁻¹), free carbon dioxide (mg L⁻¹) and bicarbonate ions (mg L⁻¹) followed Standard Methods (APHA 2005). Chlorophyll-*a* (µg L⁻¹), corrected for phaeophytin, was measured using 90 % ethanol (Sartory & Grobbelaar 1984). Details of the environmental conditions at the sampling sites are summarized in table 2.

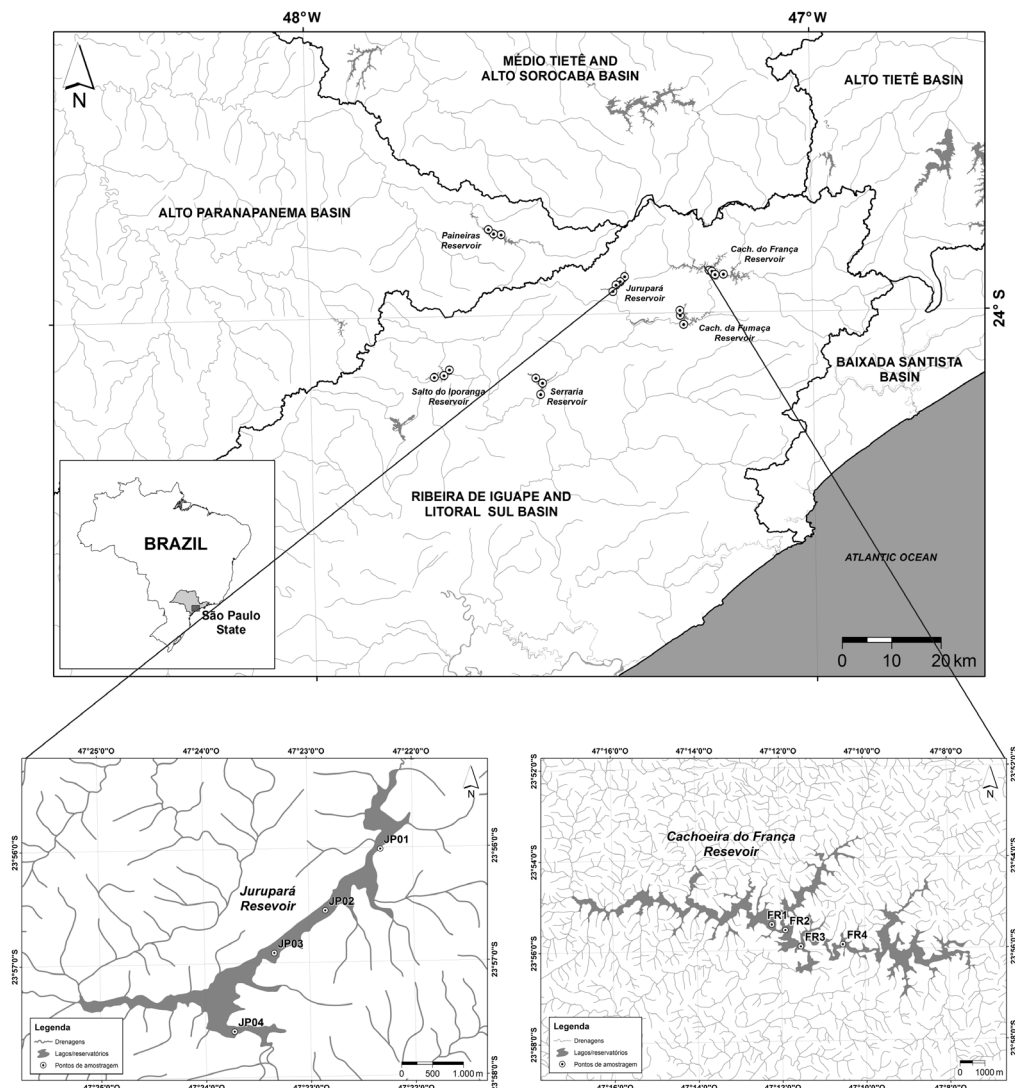
Sampling and diatom preparation

The following type materials were examined under LM and SEM microscopy, respectively:

Table 2 – Means (and standard deviation) of abiotic variables in the studied reservoirs in which *A. tropicocatenatum* occurred.

Abbreviations: Sec (Secchi depth; m), Temp (temperature; °C), Cond (conductivity; $\mu\text{S cm}^{-1}$), Alk (alkalinity; mEq L^{-1}), DO (dissolved oxygen; mg L^{-1}), N-NO_3 (nitrate; $\mu\text{g L}^{-1}$), P-PO_4 (phosphate; $\mu\text{g L}^{-1}$), TN (total nitrogen; $\mu\text{g L}^{-1}$), TP (total phosphorus; $\mu\text{g L}^{-1}$), SRS (soluble reactive silica; mg L^{-1}), Chlo (chlorophyll-*a*; $\mu\text{g L}^{-1}$), FR (Cachoeira do França reservoir), JP (Jurupará reservoir).

Reservoir	Sec	Temp	pH	Cond	Alk	DO	N-NO ₃	P-PO ₄	TN	TP	SRS	Chlo
FR1	1.7±0.1	23.1±6.8	7.3±0.9	24.5±0.7	0.2±0	7.5±0.2	8.7±1	4±0	151.8±162.9	5.5±2.2	2.4±1.3	5.4±4.8
FR2	1.9±0.2	24.2±8.1	7.3±1.3	24±0	0.2±0	7.4±0.9	8±0	4.2±0.2	191.1±45	10.0±7.5	2.39±1.2	4.12±2
FR3	1.7±0.1	24.1±8.3	7.9±0.6	23.5±2.1	0.2±0	7.3±1.6	8±0	4.6±0.9	231.2±21.2	6.6±3.7	2.3±1.4	20.2±26.5
FR4	1.7±0.1	24.0±7.9	8.6±0.6	24.5±3.5	0.2±0	8.2±1.6	9.1±1.6	4.1±1.9	280.0±153.7	6.8±2.5	2.2±1.3	22.8±29.7
JP1	1.5±0.8	24.1±6.7	6.4±1.3	24.0±2.8	0.2±0	7.8±0.8	35.4±38.8	4±0	464.3±89.4	19.5±4.2	3.9±0.3	16.8±1.8
JP2	1.9±0.4	24.6±6.5	6.6±0.1	23.5±3.5	0.2±0	6.7±1.3	40.6±46.1	4±0	351.4±201.4	16.6±0.3	3.8±0.3	10.1±0.7
JP3	2.2±0.2	25.4±6.9	6.6±0.1	24.0±2.8	0.2±0	7.5±0.3	31.8±33.6	4.5±0.8	453.3±8.2	13.5±0.7	3.7±0.3	7.1±1.2
JP4	2.1±0.4	25.0±7.1	6.8±0.2	23.5±3.5	0.2±0	7.1±1.1	37.6±41.8	4.2±0.2	434.1±103.8	13.3±1.9	4.0±0	6.6±3.7

**Figure 1 – Study area showing the *Achnanthydium tropicocatenatum* type locality (Cachoeira do França reservoir) and Jurupará reservoir.**

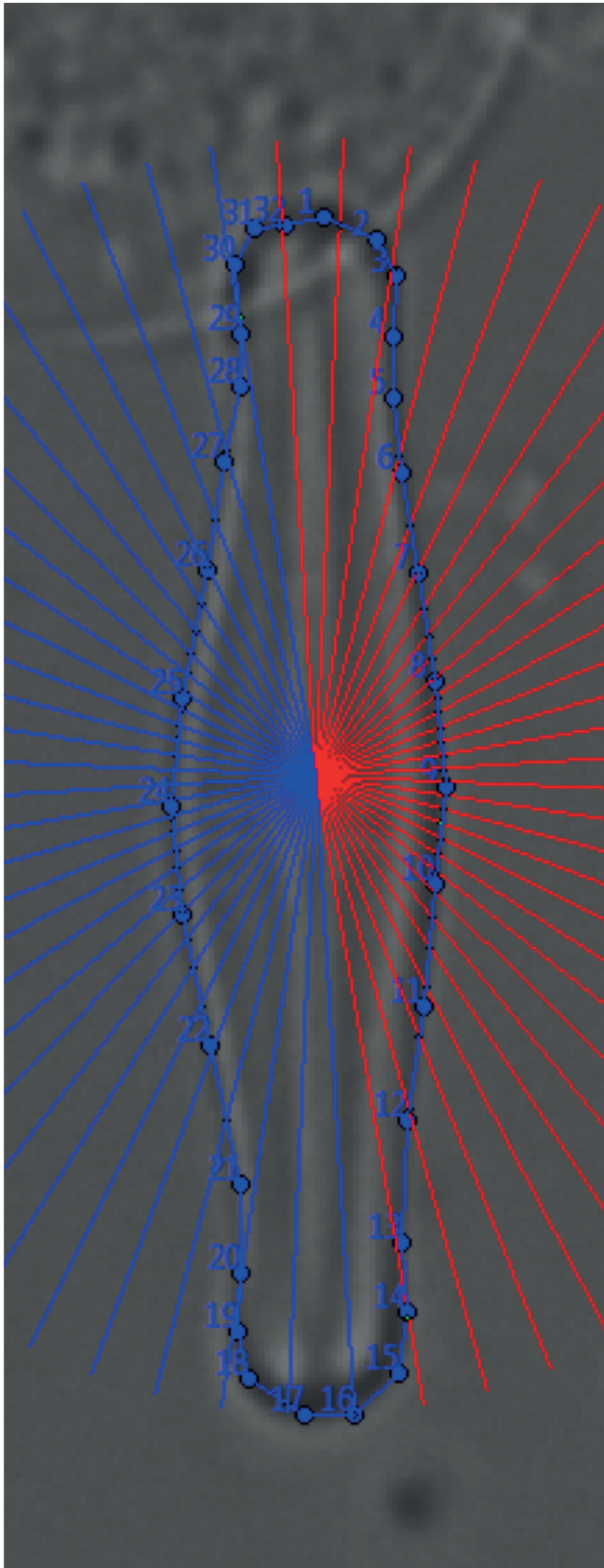


Figure 2 – Position of the pseudolandmarks on the valve outline used to perform the geometric morphometric analysis.

(1) *Achnantheidium minutissimum*: type material, Kützing's Algarum Aquae Dulcis Germanicarum, Decade VIII, no. 75, Kützing 301 (Van Heurck Collection, Botanic Garden Meise, Belgium). Type locality: Near Aschersleben, Germany; epiphytic on filamentous algae (Lange-Bertalot & Ruppel 1980, Potapova & Hamilton 2007, Novais et al. 2015).

(2) *Achnantheidium catenatum*: lectotype material, sample n° E9877 (Hustedt Collection, Alfred Wegener Institute in Bremerhaven, Germany). Type locality: Sedlice reservoir on Želivka River, Czech Republic (Bílý & Marvan 1959, Hlúbíková et al. 2011).

For the new species we selected two samples from two different habitats (see table 1). Periphytic material scraped off from stones during the summer and winter 2014 and surface sediment (2 cm deep) samples collected using a gravity core (UWITEC) only during the winter. Diatoms were cleaned from organic matter with hot hydrogen peroxide (35 %) and hydrochloric acid (37 %) (Battarbee et al. 2001). For LM observations, cleaned diatoms were mounted with Naphrax®. LM and morphometric measurements were performed with a Leica® DMRX brightfield microscope with 100x oil immersion objective, and light micrographs were taken with a Leica® DC500 camera. For scanning electron microscopy (SEM), parts of the oxidized suspensions were filtered with additional deionized water through a 3-µm Isopore™ polycarbonate membrane filter (Merck Millipore). Filters were mounted on aluminum stubs and coated with platinum using a Modular High Vacuum Coating System BAL-TEC MED 020 (BAL-TEC AG, Balzers, Liechtenstein). An ultra-high-resolution analytical field emission (FE) scanning electron microscope Hitachi SU-70 (Hitachi High-Technologies Corporation, 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 and up to 28° tilted. Plates were assembled by CorelDraw Graphics Suite X7®. Relative abundances were estimated following Battarbee et al. (2001) with a minimum of 400 valves counted per slide at 1000× magnification on up to six random transects and until reaching an efficiency of at least 90 % (Pappas & Stoermer 1996).

Holotype permanent slides, as well as the raw and cleaned samples were deposited at Herbário Científico do Estado “Maria Eneyda P. Kauffmann Fidalgo” (SP), São Paulo State Department of Environment, Brazil. Isotypes were deposited at BR, the Botanic Garden Meise, Belgium.

Morphological terminology followed Round et al. (1990) and Krammer & Lange-Bertalot (1991).

Diatom distribution

We estimated the new species ecological optima and tolerances with respect to pH, conductivity and total phosphorus based on weighted averaging (ter Braak & van Dam 1989), and tested its ecological profiles for differences in the ecological optima with respect to the similar species *A. catenatum*, by using a weighted comparison of means (Bland & Kerry 1998). The weighted average estimates of a species optimum is the mean of a measured environmental variable (such as total phosphorus concentration or pH) weighted by the abundance of the species in a sample data set, whereas

species tolerance is the weighted standard deviation (Porter et al. 2008).

Calculations were based on data from the Acquased project called “Base line diagnosis and reconstruction of anthropogenic impacts in the Guarapiranga Reservoir, focused on sustainable water supply and quality management in the reservoirs of the Alto Tietê and surrounding basins”, in which this study is included. This dataset involves 33 tropical reservoirs and 227 samples, covering a wide range of trophic states. In this sense, the chosen abiotic variables were considered relevant for water quality assessment.

Statistical analysis

Geometric morphometric techniques were applied to different *Achnanthyidium* groups to evaluate whether these morphotypes present differences in shape compared with *A. tropicocatenatum*. For this analysis, a total of 68 LM photographs were taken from *A. minutissimum* type material, 32

from *A. catenatum* type material, 100 from *A. tropicocatenatum* (population from JP reservoir) and 83 from *A. tropicocatenatum* (type material from FR reservoir) (fig. 2). The pseudolandmarks of the outlines to further identification and characterization were performed with CLIC (Collection of Landmarks for Identification and Characterization) software (Dujardin et al. 2010).

Matrices, consisting of pseudolandmarks defining outlines, were built based on the populations and shape variables were obtained through the Generalized Procrustes Analysis (GPA) superimposition algorithm (Rohlf 1990). After superimposition, shape differences can be described by the differences in coordinates of corresponding pseudolandmarks between objects (Adams et al. 2004). Principal component analysis (PCA) was carried out for the resulting normalized coordinates by means of the PAST software version 1.78 (Hammer et al. 2001). Finally, analysis of similarity (NPMANOVA) was performed between the Cartesian

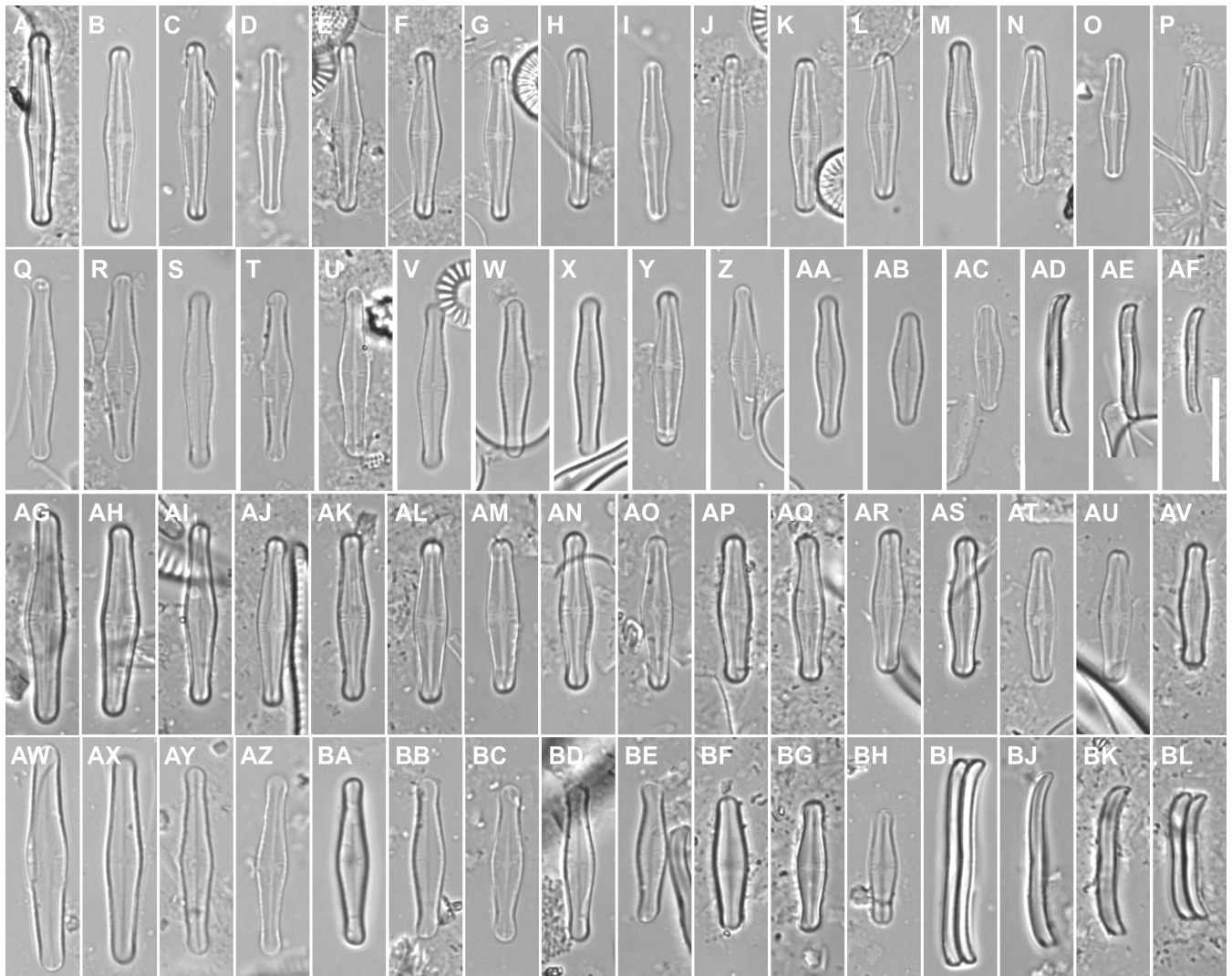


Figure 3 – *Achnanthyidium tropicocatenatum* sp. nov. LM: A–AF, specimens from type locality (Cachoeira do França reservoir, São Paulo, Brazil); A–P, raphe valves; Q–AC, rapheless valves; AD–AF, girdle view. AG–BL, specimens from Jurupará reservoir, São Paulo, Brazil; AG–AV, raphe valves; AW–BH, rapheless valves; BI–BL, girdle view. Scale bar = 10 µm.

coordinates of resulting groups in the PCA with the Chord distance measure.

RESULTS

Achnantheidium tropicocatenatum Marquardt, C.E. Wetzel & Ector, **sp. nov.**
Figs 3–5

Type: Brazil, São Paulo, Cachoeira do França Reservoir, 23°55'58.8"S 47°11'31.4"W, 5 Aug. 2014, *G. Marquardt & D. Bicudo* s.n. (holo-: SP!, slide 469444, Herbário Científico do Estado Maria Eneyda P. Kauffmann Fidalgo, Juquitiba, Brazil, depicted in fig. 3A; iso-: BR, slide 4503, Botanic Garden Meise, Belgium).

Frustules usually solitary (fig. 3A–BH); girdle view rectangular, slightly arched, with apices recurved to the rapheless valve (fig. 3BI–BL). **Valves** linear-lanceolate, slightly inflated in the central portion of the valve, with protracted, subcapitate to capitate apices (fig. 3A–BH); length 10.5–30.0 µm, width 2.5–3.5 µm in the middle; axial area narrowly linear and silicified, widening towards the central area (figs 3A–BH, 4A & B). **Raphe valve**: concave with small rounded central area, bordered by one more widely spaced stria on one or both sides (fig. 4A & B). Externally **raphe** straight, filiform, with slightly expanded proximal and distal raphe endings (fig. 4A & B); internally proximal raphe endings slightly deflected in opposite directions and distal endings terminating in small **helictoglossae** (fig. 5D). Transapical **striae** radiate throughout the valve (36–40 in 10 µm), becoming denser towards the apices (up to 45 in 10 µm), cannot be resolved in LM; **striae** mainly composed of four, sometimes five or six, rounded transapical **areolae** (fig. 4A & B), often slit-like near the valve margin and covered by a delicate silica membrane (hymen). **Rapheless valve**: convex with central area indistinct or narrow lanceolate, bordered by 1–2 more widely spaced striae on one or both sides (fig. 4C). Transapical **striae** radiate throughout the valve (38–40 in 10 µm), becoming denser towards the apices (up to 55 in 10 µm), cannot be resolved in LM; striae mainly composed of 5–6, sometimes 4, elliptical transapical **areolae** (fig. 5A–C), often slit-like near the valve margin and covered by a delicate silica membrane (hymen) (fig. 5A). On both valves, a single row of elongated areolae on the **mantle**, separated from the striae on the valve face by a hyaline area (fig. 4A & C).

Etymology – The epithet refers to the similarity of the new species, observed in a tropical region, to *A. catenatum*.

Morphological examination – Although the morphology of *A. tropicocatenatum* under LM could resemble *A. catenatum* at first sight, important features as the ends, protracted, subcapitate to capitate in the new species and not protracted, broadly rounded in *A. catenatum* as well as the shape of the frustule in girdle view can easily separate them. Also, valves are slender in *A. catenatum*, with widened central portion resulting in a more undulated valve margin whereas valves are only slightly inflated in *A. tropicocatenatum*. Thus, the valve appears more elongated in the latter. Besides, the axial area in *A. tropicocatenatum* is silicified and prominent (SEM) (fig. 5C & D).

The girdle view in *A. catenatum* resembles a “C” with the apices strongly curved and sharply pointed to the araphid valve, corresponding to one fifth of the frustule length (Hlúbíková et al. 2011). Also, it is commonly observed in small colonies, forming short chains of 2 (Straub 2002), 3 (Hlúbíková et al. 2011) and up to 8 cells in the planktonic type material of Bohemia (Bílý & Marvan 1959). Druart & Straub (1992) also observed in the Lac de Chaumeçon (France) that the *A. catenatum* cells are joined together in long, recognizable chains, a very rare character in *Achnantheidium* species. On the other hand, in *A. tropicocatenatum*, valves in girdle view are not so strongly curved, apices are not so sharply pointed and frustules are commonly observed as solitary (fig. 4BI–BL). Moreover, striae are more strongly radiate in the new species (fig. 5C).

Apices are also clearly different in *A. minutissimum*, ranging from subrostrate to protracted (fig. 7A–AD). Moreover, *A. minutissimum* differs in its valve dimensions (table 3) and in its not inflated central region.

The species is also similar to *A. lusitanicum* Novais & M. Morais (Novais et al. 2015). Nevertheless, the new species is longer (10.5–30.0 µm) compared to *A. lusitanicum* (5.3–13.0 µm). Additionally, the apices in the latter are rostrate and broadly rounded contrasting to the subcapitate to capitate in *A. tropicocatenatum* (table 3). *Achnantheidium lusitanicum* is described from two different sites: (1) Janeiro de Baixo, Zêzere River (Tejo basin) as the type material and (2) Boeiro, Sertão Stream (Tejo basin), which can have much larger valve dimensions (length: 8.3–19.3 and width: 2.3–4.3 µm). *Achnantheidium tropicocatenatum* is more similar to the second illustrated population, especially in girdle view. However, *A. lusitanicum* population 2 is wider in the middle of the valve than *A. tropicocatenatum* (2.8–3.2 µm). The species is also more rhomboidal compared to *A. tropicocatenatum*. Thereby, it is important to note the confusion regarding the circumscription of *A. lusitanicum*, as the second population seems to represent a distinct species.

Ecology, distribution and associated diatom flora – The type population of *A. tropicocatenatum* was observed in the Cachoeira do França reservoir in alkaline waters with low conductivity (24.5 µS cm⁻¹), low levels of nitrate (9.1 µg L⁻¹), total nitrogen (280.0 µg L⁻¹), total phosphorus (6.8 µg L⁻¹) and phosphate (4.1 µg L⁻¹) concentrations and pH of 8.6 (annual mean). Regarding its autecology, the species reached its optimum when the temperature was around 24.2 °C, values of pH were around 7.5, conductivity around 29 µS cm⁻¹, TN around 292.3 µg L⁻¹ and TP around 12.6 µg L⁻¹. Based on phosphate measurements, this species presented an optimum at oligotrophic conditions (table 2). The new species has a different autecology when compared with *A. catenatum*, although both taxa shared similar preferences with respect to pH (fig. 9A, *t* = 0.22, *p* = 0.83). *Achnantheidium tropicocatenatum* is an oligotrophic species (fig. 9B, *t* = 10.5, *p* < 0.01) that prefers waters with lower electrolyte content opposite to *A. catenatum*, which prefer eutrophic waters (fig. 9C, *t* = 9.2, *p* < 0.01).

Achnantheidium tropicocatenatum, dominant in the sample of Cachoeira do França (86.6 % relative abundance), was associated with *Brachysira neoexilis* Lange-Bert. (3.8 %)

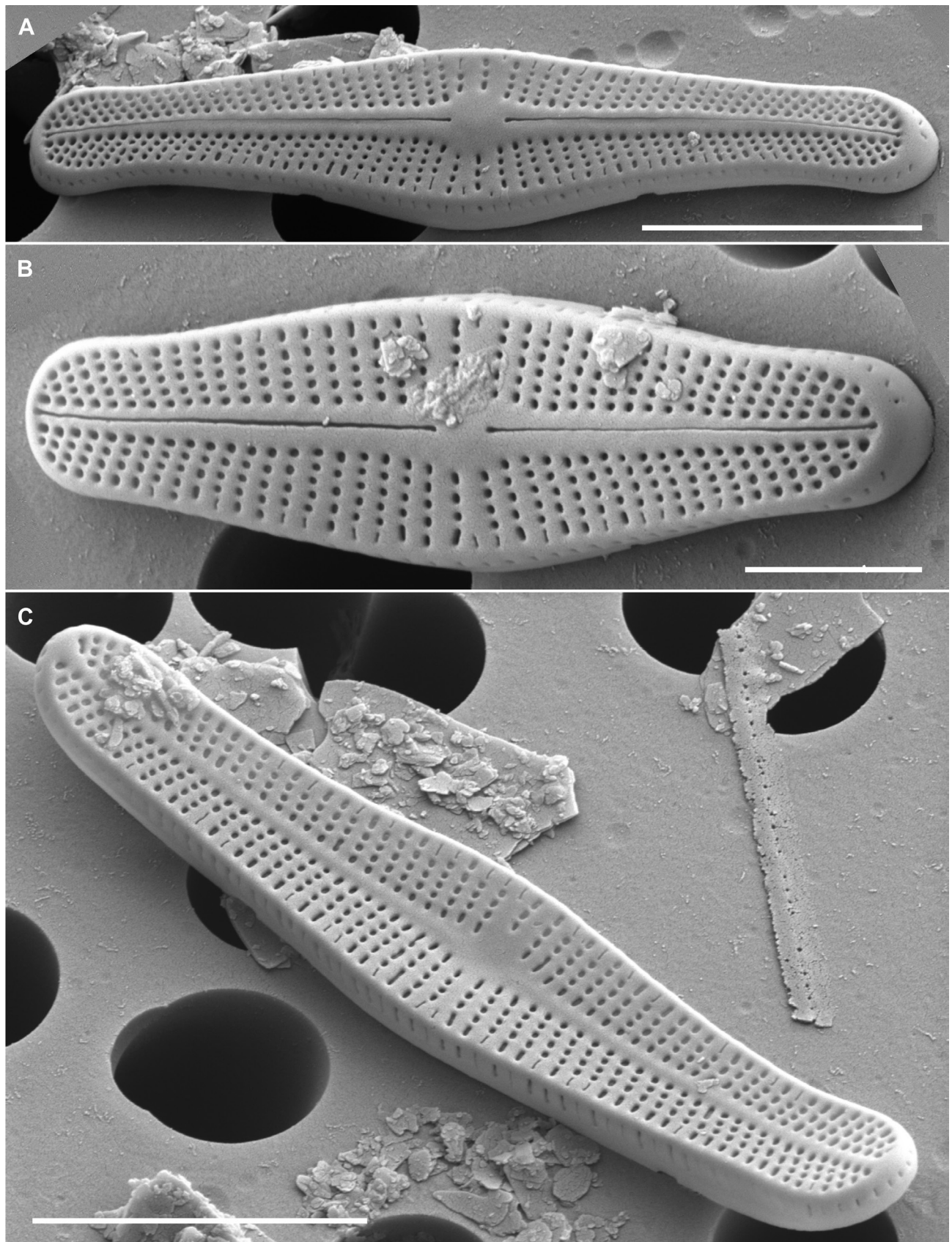


Figure 4 – *Achnantheidium tropicocatenatum* sp. nov. SEM: A–C, external views; A & B, raphe valves; C, rapheless valve. Population from type locality (Cachoeira do França reservoir, São Paulo, Brazil). Scale bars: A & C = 5 μ m, B = 2 μ m.

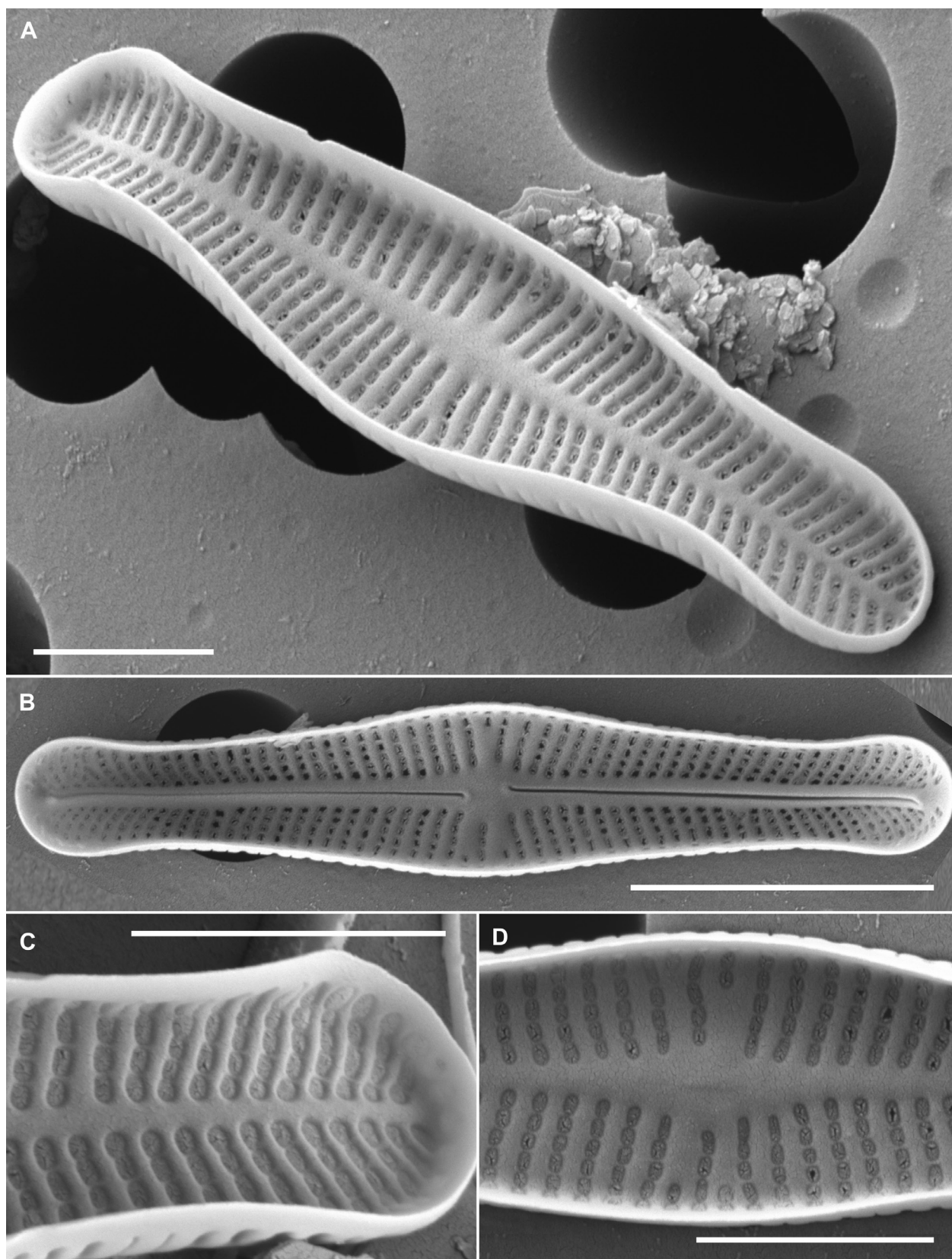


Figure 5 – *Achnanthisdium tropicocatenatum* sp. nov. SEM: A–D, internal views; A, C & D, rapheless valve; B, raphe valve; C, detail of apical striae and areolae; D, detail of central area and transapical striae. Population from type locality (Cachoeira do França reservoir, São Paulo, Brazil). Scale bars: A, C & D = 2 μ m, B = 5 μ m.

Table 3 – Comparison between *Achnanthyidum tropicocatenatum* and morphologically similar species.

	<i>Achnanthyidum tropicocatenatum</i> sp. nov.	<i>A. lusitanicum</i> Novais & M.Morais	<i>A. catenatum</i> (J.Bilý & Marvan) Lange-Bert.	<i>A. minutissimum</i> (Kütz.) Czarn.
Reference	This study	Novais et al. (2015)	Hlúbíková et al. (2011)	This study
Valve length (µm)	10.3–23.9	5.3–13	10–17.5	11.7–16.9
Valve width (µm)	2.7–3.5	2.3–3.0	2.8–3.6	2.6–3.3
Valve outline	Linear-lanceolate, slightly inflated in the central portion	Elliptic, linear-elliptic to linear-lanceolate, slightly inflated in the central portion	Slender with widened central portion resulting in an undulated valve margin	Linear-elliptic to linear-lanceolate
Valve apices	Protracted, subcapitate to capitate	Protracted, rostrate and broadly rounded	Broadly capitate to subcapitate	Protracted, rostrate to subcapitate
Striation pattern	Radiate throughout the valve, denser towards the apices	Slightly radiate in the central portion, more radiate and denser towards the apices	Radiate in the central portion and weakly radiate or almost parallel, denser towards the apices	Radiate, denser towards the apices
Raphe valve				
Central area	Small rounded, bordered by one more widely spaced stria on one or both sides	Small rounded	Rounded, almost absent	Almost absent, slightly rounded or as a rectangular fascia
Striae (in 10 µm)	36–40 (up to 45 near the apices)	35 (up to 40 near the apices)	30–32	30 (in the middle)
Number of areolae (per striae)	3–6	3–5	4–6	–
Rapheless valve				
Central area	Indistinct or narrow lanceolate	Absent or small, elliptical	Small, lanceolate to rhombic	Indistinct or narrow lanceolate
Striae (in 10 µm)	38–40 (up to 55 near the apices)	30–35	30–34	30–34 (in the middle)
Number of areolae (per striae)	3–6	3–4 (5–6)	4–6	–

and *Discostella stelligera* (Cleve & Grunow) Houk & Klee (2 %). *Navicula notha* J.H.Wallace, *Achnanthyidum hoffmannii* Van de Vijver, Ector, A.Mertens & Jarlman, *Eunotia* sp. and *Aulacoseira tenella* (Nygaard) Simonsen also occurred but with low relative abundance (< 2 %). The diatom flora is mainly composed of taxa indicating waters of low nutrient content.

Achnanthyidum tropicocatenatum probably corresponds to *A. cf. catenatum* in Morales et al. (2011, figs 128–134) from Maylanco (Bolivia), a small stream about 2 m wide and 30 cm deep (17°23.73'S 66°3.16'W, 2685 m a.s.l.) with alkaline waters, with relatively high conductivity (400 µS cm⁻¹), pH of 8.5 and temperature of 17.3 °C (Mesophytic, Province/Southern Puna, Mesophytic Sector).

Geometric morphometry – Morphological differences among the four studied populations were evidenced by their valve shape analysis (fig. 10). Similarity tests (NPMANOVA), performed on the Cartesian coordinates of resulting groups in the PCA, revealed statistically significant differences (*p* values: < 0.001, < 0.01, < 0.05) between *A. minutissimum* and *A. catenatum* compared with the *A. tropicoca-*

tenatum type and Jurupará populations, both of which belong to the same species (table 4).

DISCUSSION

Achnanthyidum tropicocatenatum clearly belongs to the complex of species around *A. minutissimum* based on the simple and straight distal raphe endings, contrary to the species in the *A. pyrenaicum* group that have clearly deflected to even hooked terminal raphe fissures (Kobayasi 1997, Potapova & Hamilton 2007, Van de Vijver & Kopalová 2014). This, widely distributed species in Brazilian reservoirs is characterized by a set of distinct morphological and ecological features that clearly separate it from all other similar *Achnanthyidum* species.

Landmark-based geometric morphometrics was a powerful tool in quantifying the shape variation in the studied species. Comparison and examination of *A. tropicocatenatum* and related species (type materials) showed significant overlapping of valve outlines (fig. 10) making the identification based only on LM difficult. However, NPMANOVA

Table 4 – NPMANOVA test performed on morphological differences resulting groups in the PCA using the Chord distance measure.
P values shown (< 0.001, < 0.01, < 0.05).

	<i>A. tropicocatenatum</i> (Jurupará)	<i>A. tropicocatenatum</i> (type material)	<i>A. catenatum</i> (type material)
<i>A. minutissimum</i> (type material)	< 0.05	< 0.01	< 0.05
<i>A. tropicocatenatum</i> (Jurupará)	–	0.4218	< 0.01
<i>A. tropicocatenatum</i> (type material)	–	–	< 0.01

statistically demonstrated differences in valve outline among them, supporting the placement of *A. tropicocatenatum* as a new taxon. The geometric morphometric approach has been increasingly applied in diatoms as a complementary tool for taxonomy, being very useful in the separation of morphologically similar taxa, either with *Achnantheidium* (e.g. Potapova & Hamilton 2007) or other genera (e.g. Falasco et al. 2009, Cejudo-Figueiras et al. 2011, Peng et al. 2014, Wengrat et al. 2015, Urbánková et al. 2016).

Currently, *A. minutissimum* s. lat. is regarded as a very generalized indicator species with a seemingly broad ecological tolerance and different North American *Achnantheidium* morphotypes exhibit different ecological preferences that could be useful in biomonitoring (Potapova & Hamilton 2007, Pinseel et al. 2017). The correct identity and biogeog-

graphical distribution of this taxon have long been obscured by force-fitting and taxonomic drift (Van de Vijver & Kopalová 2014). A recent study of Pinseel et al. (2017) has argued that the implementation of molecular data in the taxonomy of *Achnantheidium* will be essential to solve the taxonomic problems associated with this group resulting in better understanding of the biogeography and niche differentiation of different species belonging to the *A. minutissimum* complex. In Brazil, the species have been reported especially for the South (e.g. Bertolli et al. 2010, Faria et al. 2010, Marra et al. 2016, Nardelli et al. 2016; all of them in Paraná State) and the Southeast region (e.g. Faustino et al. 2016; São Paulo State). However, the specimens represented as *A. minutissimum* by Ferrari & Ludwig (2007) for the Ivaí basin (Paraná State) seem to represent two distinct taxa and probably the

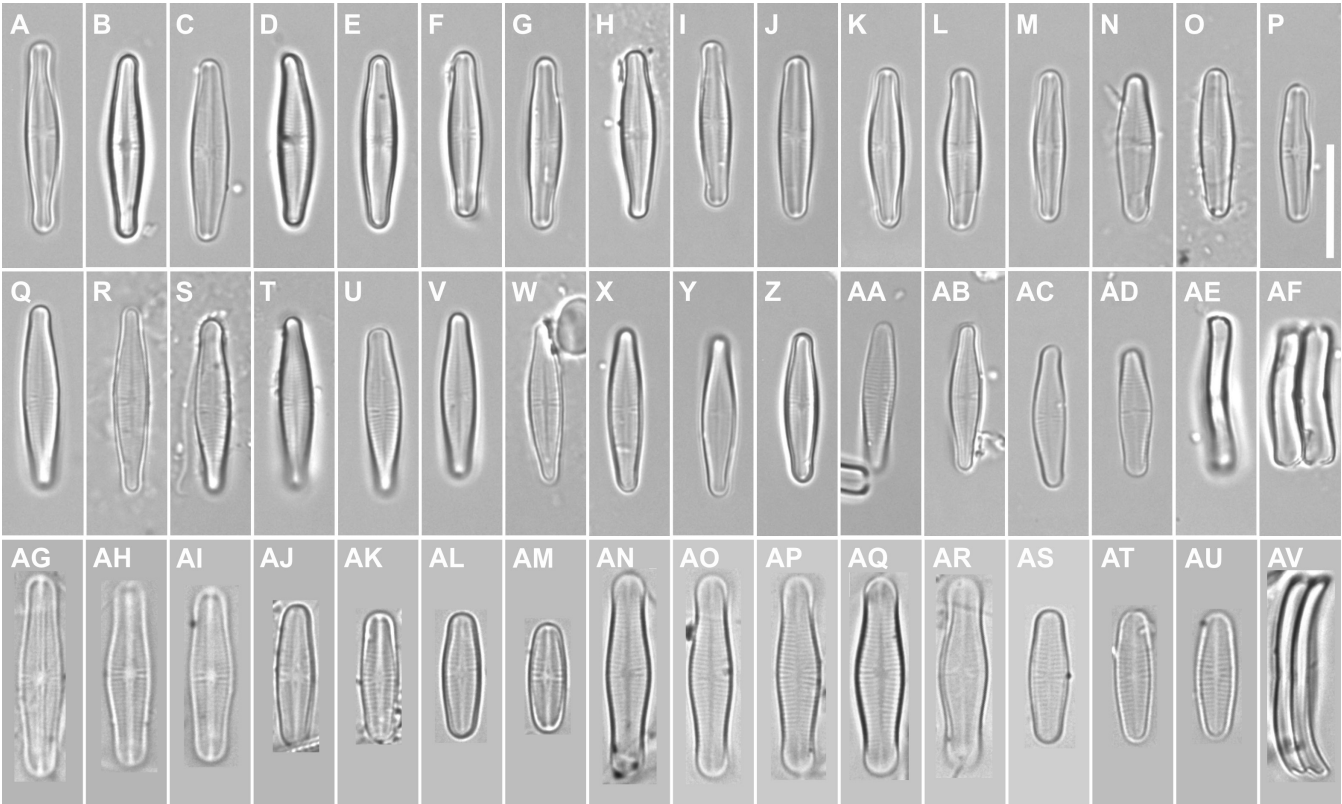


Figure 6 – *Achnantheidium minutissimum* LM: A–AF, specimens from type material (type locality: near Aschersleben, Germany); A–P, raphe valves; Q–AD, rapheless valves; AE & AF, girdle view. *Achnantheidium catenatum* LM: AG–AV, specimens from lectotype material (type locality: Sedlice reservoir on Želivka River, Czech Republic); AG–AM, raphe valves; AN–AU, rapheless valves; AV, girdle view. Scale bar = 10 µm.

diatom illustrated in the fig. 30 is closer to *A. catenatum*. Likewise, the images 120–122 in Silva et al. (2010) from the Iraí reservoir, Paraná state (eutrophic system) are not related to *A. minutissimum*.

Similarly, *Achnantheidium catenatum* is also an indicator of organic pollution (Berthon et al. 2011). The species is widely reported from around the world and it is the only planktonic species of the genus, so records of blooms

in which *A. catenatum* is dominant are not uncommon (e.g. Straub 2002, Ma et al. 2013).

Nevertheless, its distribution in Brazil is uncertain and *A. catenatum* have been recorded by diatomists especially in samples from the South and the Southwest regions of the country. However, some of the cells illustrated bear little resemblance to *A. catenatum*. Mostly cited to São Paulo localities (e.g. Faustino et al. 2016), this species has been re-

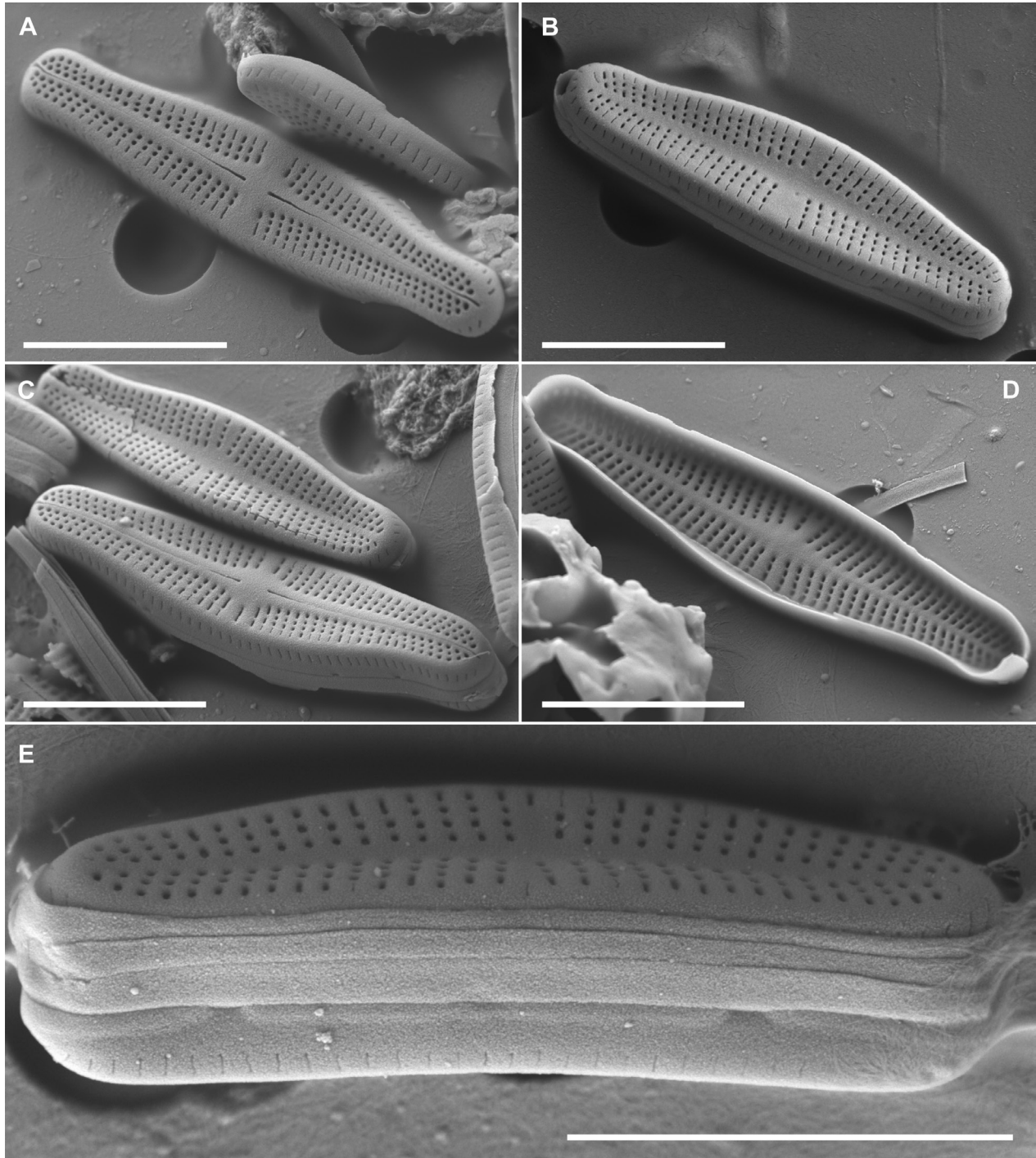


Figure 7 – *Achnantheidium minutissimum* SEM: A–C, external views; D, internal view; E, girdle view; A, raphe valve; B & D, rapheless valve; C, raphe and rapheless valves. Specimens from type material (type locality: near Aschersleben, Germany). Scale bars = 5 μm.

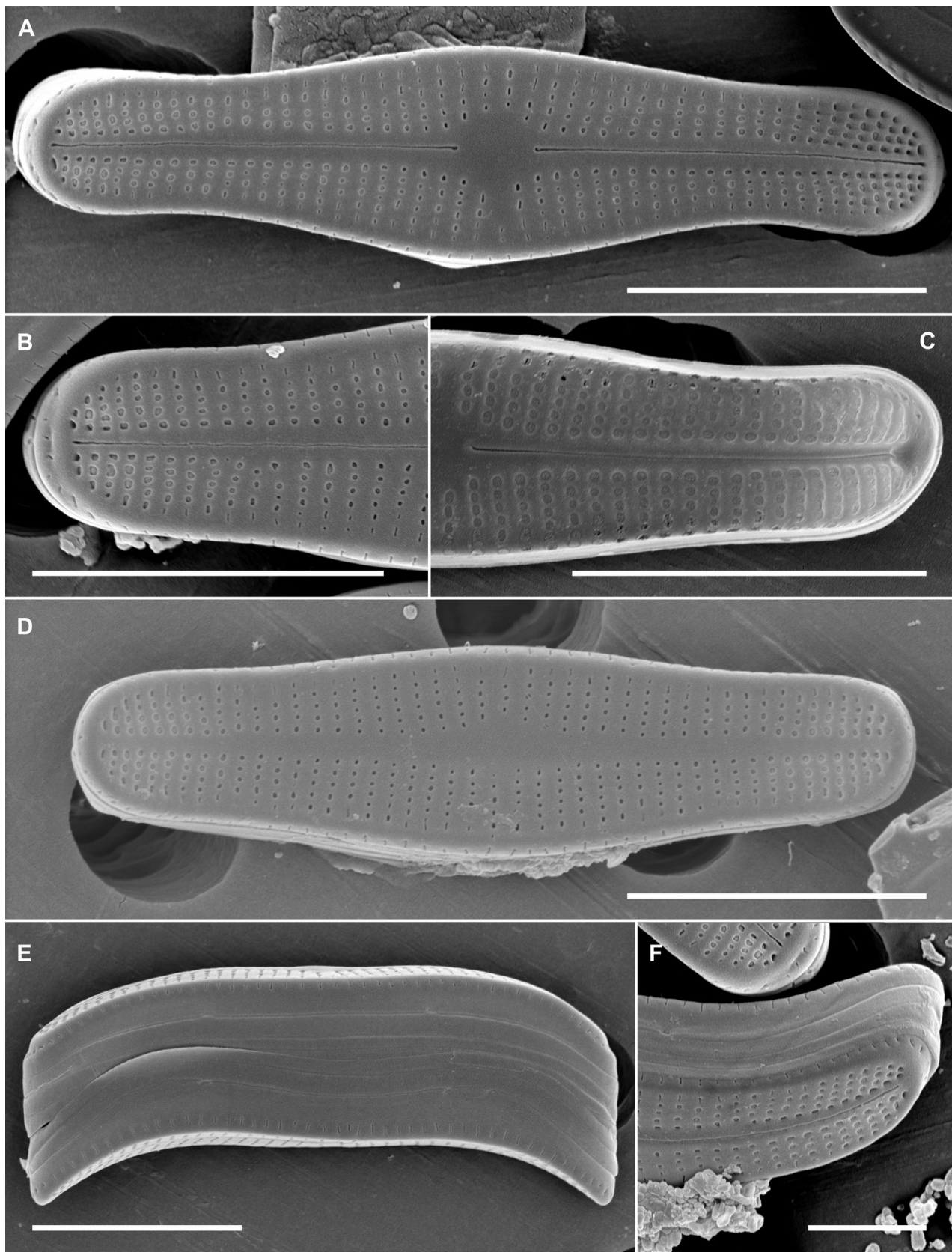


Figure 8 – *Achnantheidium catenatum* SEM: A & B, D, F, external views; C, internal view; E & F, girdle view, B & C, detail of apices with its distal raphe endings; F, detail of apex. Specimens from lectotype material (type locality: Sedlice reservoir on Želivka River, Czech Republic). Scale bars: A–E = 5 µm, F = 2 µm.

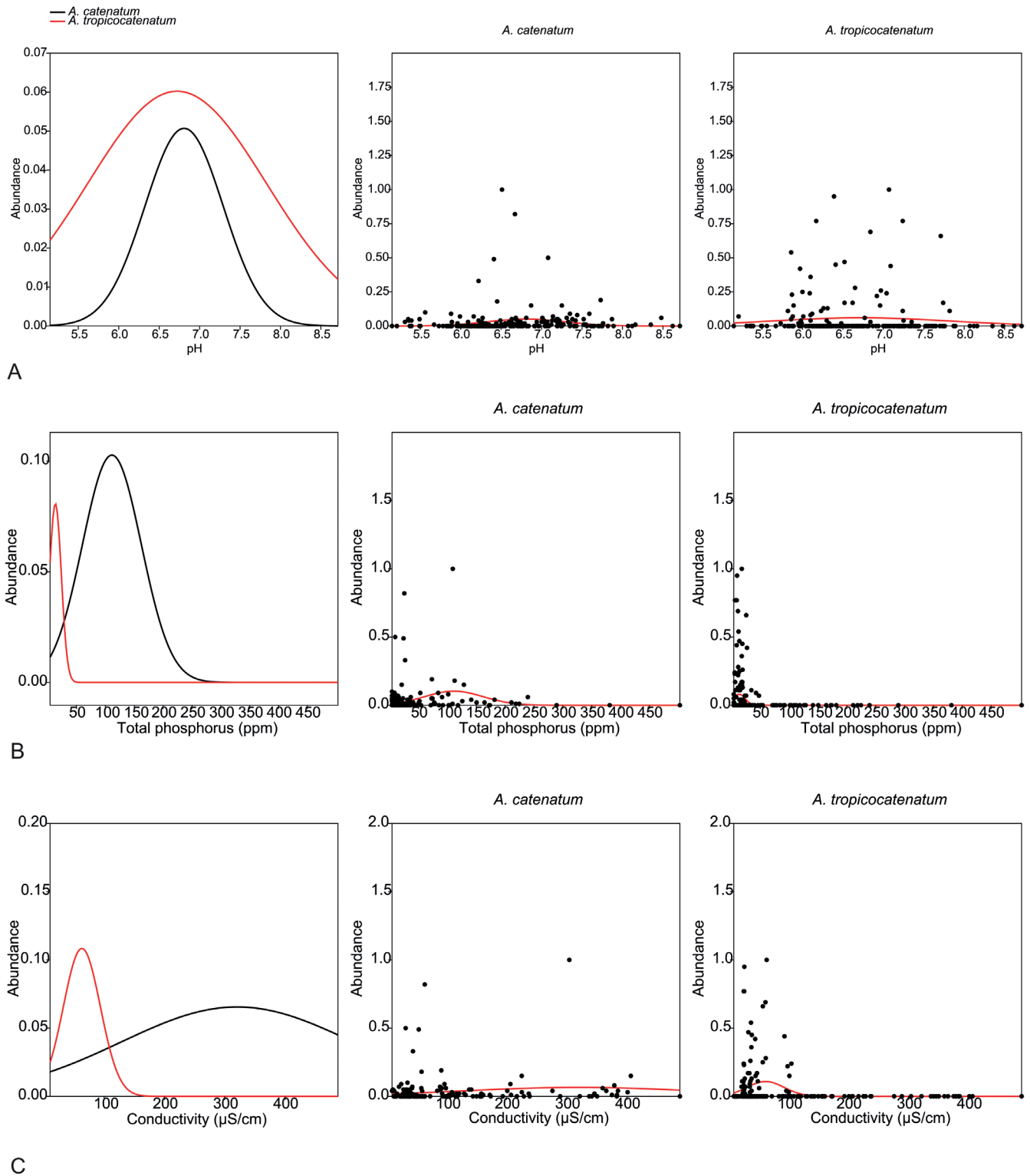


Figure 9 – Relative abundance (%) of *Achnanthyidium catenatum* (black lines) and *Achnanthyidium tropicocatenatum* (red lines) in São Paulo reservoirs, Brazil and relationships between pH (A), total phosphorus (B; $\mu\text{g L}^{-1}$) and conductivity (C; $\mu\text{S cm}^{-1}$). Percentages based in counting of at least 400 valves per sample. Lines indicates weighted average optima.

ported as an indicator of an environmental shift particularly associated with the eutrophication process in paleolimnological reconstruction studies (Costa-Böddeker et al. 2012, Fontana et al. 2014). However, no illustration of the taxon was available. Also, *A. catenatum* was reported by Fontana & Bicudo (2012) from surface sediments in the cascading reservoirs of Paranapanema River (São Paulo and Paraná States, Brazil). Nevertheless, the species is described to possess smaller dimensions for length (9.6–9.8 µm) and valve width (1.4–1.7 µm), not compatible with the *A. catenatum* lectotype material measurements (table 3). Finally, the species reported by Marra et al. (2016: figs 110–112) in a mesotrophic reservoir in Paraná State is probably conspecific with *A. tropicocatenatum*.

Originally applied to paleolimnological research, weighted-averaging regression and calibration methods (Birks et al. 1990) have been increasingly used to quantify relations between species and various environmental variables (Kelly & Whitton 1995, Pan & Stevenson 1996, Leland & Porter 2000, Winter & Duthie 2000, Leland et al. 2001, Potapova et al. 2004). *Achnantheidium tropicocatenatum* not only differs morphologically but it is also ecologically distinct compared with *A. catenatum* and *A. minutissimum*. Thereafter, the presence of a taxon can be used to indicate the probable availability of its preferred conditions at the time of collection. However, if subsequently this taxon is confused with, or

wrongly assumed to include another taxon with different and unknown ecological preferences (perhaps because fine morphological differences have not been noted), records of these algae will not indicate the prevalence of any particular conditions, and the bioindicator system breaks down (Cox 1987).

Achnantheidium minutissimum and *A. catenatum* are considered two of the most commonly reported taxa in floristic and ecological works worldwide. 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 (Morales et al. 2013). The description of *A. tropicocatenatum* improves our knowledge about the biogeography of species in this complex and allows their use in more precise ecological and biogeographical studies.

ACKNOWLEDGEMENTS

We gratefully acknowledge Angela Silva for providing the *A. catenatum* AcquaSed data improving the manuscript. This study was carried out within the framework of the AcquaSed project supported by funds from FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo, grant nº 2009/53898-9), and was undertaken as part of GCM thesis

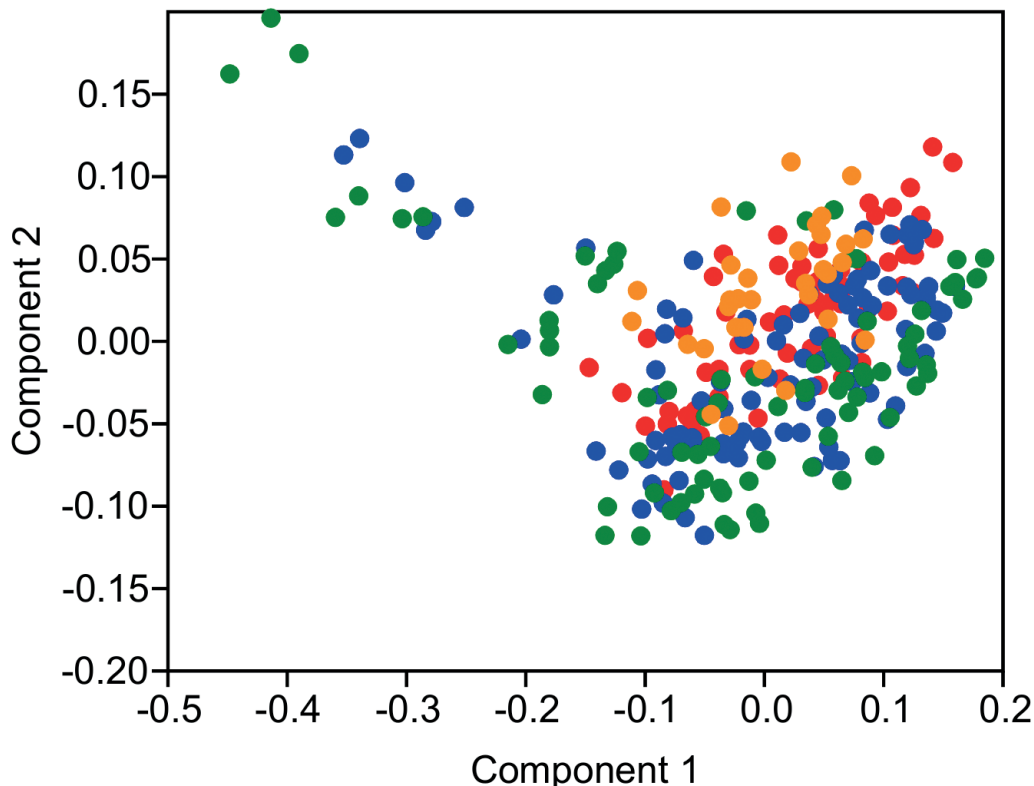


Figure 10 – PCA plot of Procrustes-transformed valve outline pseudolandmark coordinates among *A. catenatum* (32 valve measurements) and *A. minutissimum* (68 valve measurements) lectotype materials, *A. tropicocatenatum* (population from Jurupará reservoir, 100 valve measurements), and from *A. tropicocatenatum* (type material from Cachoeira do França reservoir, 83 valve measurements). Legend symbols: orange: *A. catenatum*, red: *A. minutissimum*; blue: *A. tropicocatenatum* (Jurupará reservoir) and green: *A. tropicocatenatum* (type material).

at the Instituto de Botânica, São Paulo, Brazil (FAPESP fellowship n° 2013/10314-2). Funding for this research was also provided in the framework of the project DIATOMS (LIST–Luxembourg Institute of Science and Technology). CEMB and DCB thanks CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for Research Fellowship (n° 309474/2010-8 and 310940/2013-3). We deeply appreciate the valuable assistance of personnel from Votorantim Energia for their valuable logistical support during the fieldwork. We are also grateful to Prof. William de Queiróz (Universidade de Guarulhos, Laboratório de Geoprocessamento) for the illustration of the study area.

REFERENCES

- Adams D.C., Rohlf F.J., Slice D.E. (2004) Geometric morphometrics: ten years of progress following the 'revolution'. *Italian Journal of Zoology* 71: 5–16. <https://doi.org/10.1080/11250000409356545>
- APHA – American Public Health Association (2005) Standard Methods for the Examination of Water and Wastewater. 21st Ed. Washington, DC, American Public Health Association.
- Battarbee R.W., Jones V.J., Flower R.J., Cameron N.G., Bennion H., Carvalho L., Juggins S. (2001) Diatoms. In: Smol J.P., Birks H.J.B., Last W.M. (eds) *Tracking environmental change using lake sediments. Volume 3. Terrestrial, algal, and siliceous indicators*: 155–202. Dordrecht, Kluwer Academic Publishers. https://doi.org/10.1007/0-306-47668-1_8
- Bere T., Tundisi J.G. (2011) Applicability of borrowed diatom-based water quality assessment indices in streams around São Carlos-SP, Brazil. *Hydrobiologia* 673: 179–192. <https://doi.org/10.1007/s10750-011-0772-7>
- Berthon V., Bouchez A., Rimet F. (2011) Using diatom life-forms and ecological guilds to assess organic pollution and trophic level in rivers: a case study of rivers in south-eastern France. *Hydrobiologia* 673: 259–271. <https://doi.org/10.1007/s10750-011-0786-1>
- Bertolli L.M., Tremarin P.I., Ludwig T.A.V. (2010) Diatomáceas perifíticas em Polygonum hydropiperoides Michaux, reservatório do Passaúna, Região Metropolitana de Curitiba, Paraná, Brasil. *Acta Botanica Brasílica* 24: 1065–1081. <https://doi.org/10.1590/S0102-33062010000400022>
- Bicudo D.C., Tremarin P.I., Almeida P.D., Zorzal-Almeida S., Wengrat S., Faustino S.B., Costa L.F., Bartozek E.C.R., Rocha A.C.R., Bicudo C.E.M., Morales E.A. (2016) Ecology and distribution of Aulacoseira species (Bacillariophyta) in tropical reservoirs from Brazil. *Diatom Research* 31: 199–215. <https://doi.org/10.1080/0269249X.2016.1227376>
- Bílý J., Marvan P. (1959) *Achnanthes catenata* sp. n. *Preslia* 31: 34–35, pl. 8.
- Birks H.J.B., ter Braak C.J.F., Line J.M., Juggins S., Stevenson A.C. (1990) Palaeolimnology and lake acidification - Diatoms and pH reconstruction. *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences*, 327: 263–278. <https://doi.org/10.1098/rstb.1990.0062>
- Bland J.M., Kerry S.M. (1998) Weighted comparison of means. *British Medical Journal* 316: 129. <https://doi.org/10.1136/bmj.316.7125.129>
- Burliga A.L., Torgan L.C., Andrade E.A.N., Sutil C., Beaumord A.C., Laux M., Kociólek J.P. (2014) Changes in diatom associations with altitudinal gradient and land use in Itajaí-Mirim River, Southern Brazil. *Iheringia, Sér. Bot.* 69: 451–464.
- Cantonati M., Lange-Bertalot H. (2006) *Achnanthyidium dolomitum* sp. nov. (Bacillariophyta) from oligotrophic mountain springs and lakes fed by dolomite aquifers. *Journal of Phycology* 42: 1184–1188. <https://doi.org/10.1111/j.1529-8817.2006.00281.x>
- Carneiro L.A. (2003) *Ordem Achnanthes (Bacillariophyceae) de águas doces do estado de São Paulo: levantamento florístico*. PhD thesis, Universidade Estadual Paulista, Instituto de Biociências de Rio Claro, Brazil.
- CBH-RB - Comitê da Bacia Hidrográfica do Ribeira de Iguape e Litoral Sul (2013) Relatório de Situação dos Recursos Hídricos da Unidade de Gerenciamento n° 11: Ano-Base 2011. UGRHI 11. Registro, SP. Available from http://www.sigrb.com.br/app/pdf/RELATORIO_SITUACAO_RB_2012.pdf [accessed 26 Jul. 2017].
- Cejudo-Figueiras C., Morales E.A., Wetzel C.E., Blanco S., Hoffmann L., Ector L. (2011) Analysis of the type of *Fragilaria construens* var. *subsalina* (Bacillariophyceae) and description of two morphologically related taxa from Europe and the United States. *Phycologia* 50: 67–77. <https://doi.org/10.2216/09-40.1>
- Costa-Bödeker S., Bennion H., de Jesus T.A., Albuquerque A.L.S., Figueira R.C.L., Bicudo D.C. (2012) Paleolimnologically inferred eutrophication of a shallow, tropical, urban reservoir in southeast Brazil. *Journal of Paleolimnology* 48: 751–766. <https://doi.org/10.1007/s10933-012-9642-1>
- Coste M., Ector L. (2000) Diatomées invasives exotiques ou rares en France: principales observations effectuées au cours des dernières décennies. *Systematics and Geography of Plants* 70: 373–400. <https://doi.org/10.2307/3668651>
- Cox E.J. (1987) *Placoneis Mereschkowsky*: the re-evaluation of a diatom genus originally characterized by its chloroplast type. *Diatom Research* 2: 145–157. <https://doi.org/10.1080/0269249X.1987.9704994>
- Druart J.-C., Straub F. (1992) *Achnanthes catenata* Bily & Marvan, (Diatomophyceae), diatomée planctonique nouvelle pour les eaux douces françaises. *Cryptogamie, Algologie* 14(2–3): 95–98.
- Dujardin J.P., Kaba D., Henry A.B. (2010) The exchangeability of shape. *BMC Research Notes* 3: 266.
- Ector L. (2011) 1st European Workshop on Diatom Taxonomy (1st EWDT). *Algological Studies* 136/137: 1–4. <https://doi.org/10.1127/1864-1318/2011/0136-0001>
- Elias C.L., Rocha R.J.M., Feio M.J., Figueira E., Almeida S.F.P. (2017) Influence of the colonizing substrate on diatom assemblages and implications for bioassessment: a mesocosm experiment. *Aquatic Ecology* 51: 145–158. <https://doi.org/10.1007/s10452-016-9605-0>
- Falasco E., Blanco S., Bona F., Gomà J., Hlúbíková D., Novais M.H., Hoffmann L., Ector L. (2009) Taxonomy, morphology and distribution of the *Sellaphora stroemii* complex (Bacillariophyceae). *Fottea* 9: 243–256. <https://doi.org/10.5507/fot.2009.025>
- Faria D.M., Tremarin P.I., Ludwig T.A.V. (2010) Diatomáceas perifíticas da represa Itaqui, São José dos Pinhais, Paraná: *Fragilariiales*, *Eunotiales*, *Achnanthes* e *Gomphonema* Ehrenberg. *Biota Neotropica* 10: 415–427. <https://doi.org/10.1590/S1676-06032010000300035>
- Faustino S.B., Fontana L., Bartozek E.C.R., Bicudo C.E.M., Bicudo D.C. (2016) Composition and distribution of diatom assemblages from core and surface sediments of a water supply reservoir in Southeastern Brazil. *Biota Neotropica* 16: e20150129. <https://doi.org/10.1590/1676-0611-BN-2015-0129>

- Ferrari F., Ludwig T.A.V. (2007) Coscinodiscophyceae, Fragilariophyceae e Bacillariophyceae (Achnanthales) dos rios Ivaí, São João e dos Patos, bacia hidrográfica do rio Ivaí, município de Prudentópolis, PR, Brasil. *Acta Botanica Brasilica* 21: 421–441. <https://doi.org/10.1590/S0102-33062007000200016>
- Fonseca B.M., Ferragut C., Tucci A., Crossetti L.O., Ferrari F., Bicudo D.C., Sant'Anna C.L., Bicudo C.E.M. (2014) Biovolume de cianobactérias e algas de reservatórios tropicais do Brasil com diferentes estados tróficos. *Hoehnea* 41: 9–30. <https://doi.org/10.1590/S2236-89062014000100002>
- Fontana L., Bicudo D.C. (2012) Biodiversidade e distribuição das diatomáceas (Bacillariophyceae) de sedimentos superficiais nos reservatórios em cascata do rio Paranapanema, SP/PR, Brasil. *Hoehnea* 39: 587–612. <https://doi.org/10.1590/S2236-89062012000400007>
- Fontana L., Albuquerque A.L.S., Brenner M., Bonotto D.M., Sbaris T.P.P., Pires M.A.F., Cotrim M.E.B., Bicudo D.C. (2014) The eutrophication history of a tropical water supply reservoir in Brazil. *Journal of Paleolimnology* 51: 29–43. <https://doi.org/10.1007/s10933-013-9753-3>
- Fourtanier E., Kociolek J.P. (2011) Catalogue of Diatom Names. Updated 19 September 2011. [online]. Available from <http://researcharchive.calacademy.org/research/diatoms/names/index.asp> [accessed 26 Sep. 2017].
- Hammer Ø., Harper D.A.T., Ryan P.D. (2001) PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4(1): art. 4: 1–9. Available from http://palaeo-electronica.org/2001_1/past/past.pdf [accessed 28 Sep. 2017].
- Hermany G., Schwarzbald A., Lobo E.A., Oliveira M.A. (2006) Ecology of the epilithic diatom community in a low-order stream system of the Guaíba hydrographical region: subsidies to the environmental monitoring of southern Brazilian aquatic systems. *Acta Limnologica Brasiliensia* 18(1): 9–27.
- Hlúbíková D., Ector L., Hoffmann L. (2011) Examination of the type material of some diatom species related to *Achnanthes minutissimum* (Kütz.) Czarn. (Bacillariophyceae). *Algological Studies* 136/137: 19–43. <https://doi.org/10.1127/1864-1318/2011/0136-0019>
- Jüttner I., Cox E.J. (2011) *Achnanthes pseudoconspicuum* comb. nov.: morphology and ecology of the species and a comparison with related taxa. *Diatom Research* 26: 21–28. <https://doi.org/10.1080/0269249X.2011.573707>
- Kelly M.G., Whitton B.A. (1995) The Trophic Diatom Index: a new index for monitoring eutrophication in rivers. *Journal of Applied Phycology* 7: 433–444. <https://doi.org/10.1007/BF00003802>
- Kobayasi H. (1997) Comparative studies among four linear-lanceolate *Achnanthes* species (Bacillariophyceae) with curved terminal raphe endings. *Nova Hedwigia* 65(1–4): 147–163.
- Krammer K., Lange-Bertalot H. (1991) Bacillariophyceae 4. Teil: Achnanthaceae, Kritische Ergänzungen zu Navicula (Lineolatae) und Gomphonema. *Gesamtliteraturverzeichnis Teil 1–4*. In: Ettl H., Gärtner G., Gerloff J., Heynig H., Mollenhauer D. (eds) *Süßwasserflora von Mitteleuropa*. Band 2/4. Stuttgart, Gustav Fischer Verlag.
- Lamparelli M.C. (2004) Graus de trofia em corpos d'água do Estado de São Paulo: Avaliação dos métodos de monitoramento. PhD thesis, Universidade de São Paulo, São Paulo, Brazil.
- Lange-Bertalot H., Ruppel M. (1980) Zur Revision taxonomisch problematischer, ökologisch jedoch wichtiger Sippen der Gattung *Achnanthes* Bory. *Archiv für Hydrobiologie/Suppl.* 60 *Algological Studies* 26: 1–31.
- Leland H.V., Porter S.D. (2000) Distribution of benthic algae in the upper Illinois River basin in relation to geology and land use. *Freshwater Biology* 44: 279–301. <https://doi.org/10.1046/j.1365-2427.2000.00536.x>
- Leland H.V., Brown L.R., Mueller D.K. (2001) Distribution of algae in the San Joaquin River, California, in relation to nutrient supply, salinity and other environmental factors. *Freshwater Biology* 46: 1139–1167. <https://doi.org/10.1046/j.1365-2427.2001.00740.x>
- Liu B., Blanco S., Long H., Xu J., Jiang X. (2016) *Achnanthes sinense* sp. nov. (Bacillariophyta) from the Wuling Mountains Area, China. *Phytotaxa* 284: 194–202. <https://doi.org/10.11646/phytotaxa.284.3.4>
- Lobo E.A., Callegaro V.L.M., Hermany G., Bes D., Wetzel C.E., Oliveira M.A. (2004a) Use of epilithic diatoms as bioindicators from lotic system in southern Brazil, with special emphasis on eutrophication. *Acta Limnologica Brasiliensia* 16(1): 25–40. Available from: http://www.ablimno.org.br/acta/pdf/acta_limnologica_contents1601E_files/art3_16%281%29.pdf [accessed 28 Sep. 2017].
- Lobo E.A., Callegaro V.L.M., Wetzel C.E., Hermany G., Bes D. (2004b) Water quality study of Condor and Capivara streams, Porto Alegre municipal district, RS, Brazil, using epilithic diatoms biocenoses as bioindicators. *Oceanological and Hydrobiological Studies* 33(2): 77–93.
- Ma P., Shi L., Zhao X., Zhang J., Chen W., Hu J. (2013) A bloom-forming freshwater diatom: *Achnanthes catenatum*. *Journal of Lake Sciences* 25: 156–162. [in Chinese] <https://doi.org/10.18307/2013.0120>
- Marquardt G.C., da Rocha A.C.R., Wetzel C.E., Ector L., Bicudo C.E.M. (2016) *Encyonema aquasensis* sp. nov. and *Kurtkrammeria salesopolensis* sp. nov.: two new freshwater diatom species (Cymbellales, Bacillariophyceae) from an oligotrophic reservoir in southeastern Brazil. *Phytotaxa* 247: 62–74. <https://doi.org/10.11646/phytotaxa.247.1.4>
- Marra R.C., Tremarin P.I., Algarte V.M., Ludwig T.V. (2016) Epiphytic diatoms (Diatomeae) from Piraquara II urban reservoir, Paraná state. *Biota Neotropica* 16: e20160200. <https://doi.org/10.1590/1676-0611-BN-2016-0200>
- Molisaní M.M., Barroso H.S., Becker H., Moreira M.O.P., Hijo C.A.G., Monte T.M., Vasconcellos G.H. (2010) Trophic state, phytoplankton assemblages and limnological diagnosis of the Castanhão Reservoir, CE, Brazil. *Acta Limnologica Brasiliensia* 22: 1–12. <https://doi.org/10.4322/actalb.02201001>
- Morales E.A., Ector L., Fernández E., Novais M.H., Hlúbíková D., Hamilton P.B., Blanco S., Vis M.L., Kociolek J.P. (2011) The genus *Achnanthes* Kütz. (Achnanthales, Bacillariophyceae) in Bolivian streams: a report of taxa found in recent investigations. *Algological Studies* 136/137: 89–130. <https://doi.org/10.1127/1864-1318/2011/0136-0089>
- Morales E.A., Guerrero J.M., Wetzel C.E., Sala S., Ector L. (2013) Unraveling the identity of *Fragilaria pinnata* Ehrenberg and *Staurosira pinnata* Ehrenberg: research in progress on a convoluted story. *Cryptogamie, Algologie* 34: 89–102. <https://doi.org/10.7872/crya.v34.iss2.2013.89>
- Morales E.A., Rivera S.F., Wetzel C.E., Hamilton P.B., Bicudo D.C., Bibernat R.A., Ector L. (2015) Hipótesis: la agrupación *Microcystis aeruginosa* Kütz.-Nitzschia palea (Kütz.) W. Sm.-bacterias en la laguna Alalay, Cochabamba, Bolivia es de tipo simbiótico. *Acta Nova* 7: 122–142. Available from http://www.scielo.org.bo/scielo.php?pid=S1683-07892015000200004&script=sci_arttext [accessed 28 Sep. 2017].

- Nardelli M.S., Bueno N.C., Ludwig T.A.V., Guimarães A.T.B. (2016) Structure and dynamics of the planktonic diatom community in the Iguassu River, Paraná State, Brazil. *Brazilian Journal of Biology* 76: 374–386. <https://doi.org/10.1590/1519-6984.16114>
- Nascimento M.N. (2012) Biodiversidade e distribuição das diatomáceas planctônicas e de sedimento superficial em represa profunda oligotrófica (Sistema Cantareira, Represa Jaguari-Jacaré). Dissertação (Mestrado), Instituto de Botânica da Secretaria de Estado do Meio Ambiente, São Paulo, Brazil.
- Novais M.H., Jüttner I., Van de Vijver B., Morais M.M., Hoffmann L., Ector L. (2015) Morphological variability within the *Achnanthyidium minutissimum* species complex (Bacillariophyta): comparison between the type material of *Achnanthes minutissima* and related taxa, and new freshwater *Achnanthyidium* species from Portugal. *Phytotaxa* 224: 101–139. <https://doi.org/10.11646/phytotaxa.224.2.1>
- Pan Y., Stevenson R.J. (1996) Gradient analysis of diatom assemblages in western Kentucky wetlands. *Journal of Phycology* 32: 222–232. <https://doi.org/10.1111/j.0022-3646.1996.00222.x>
- Pappas J.L., Stoermer E.F. (1996) Quantitative method for determining a representative algal sample count. *Journal of Phycology* 32: 693–696. <https://doi.org/10.1111/j.0022-3646.1996.00693.x>
- Peng Y., Rioual P., Levkov Z., Williams D.M., Jin Z. (2014) Morphology and ultrastructure of *Hippodonta qinghaiensis* sp. nov. (Bacillariophyceae), a new diatom from Lake Qinghai, China. *Phytotaxa* 186: 61–74. <https://doi.org/10.11646/phytotaxa.186.2.1>
- Peres F., Le Cohu R., Delmont D. (2014) *Achnanthyidium barbei* sp. nov. and *Achnanthyidium costei* sp. nov., two new diatom species from French rivers. *Diatom Research* 29: 387–397. <https://doi.org/10.1080/0269249X.2014.890956>
- Pinseel E., Van de Vijver B., Kopalová K. (2015) *Achnanthyidium petuniabuktianum* sp. nov. (Achnanthidiaceae, Bacillariophyta), a new representative of the *A. pyrenaicum* group from Spitsbergen (Svalbard Archipelago, High Arctic). *Phytotaxa* 226: 63–74. <https://doi.org/10.11646/phytotaxa.226.1.6>
- Pinseel E., Vanormelingen P., Hamilton P.B., Vyverman W., Van de Vijver B., Kopalová K. (2017) Molecular and morphological characterization of the *Achnanthyidium minutissimum* complex (Bacillariophyta) in *Petuniabukta* (Spitsbergen, High Arctic) including the description of *A. digitatum* sp. nov. *European Journal of Phycology* 52: 264–280. <https://doi.org/10.1080/0967026.2.2017.1283540>
- Porter S.D., Mueller D.K., Spahr N.E., Munn M.D., Dubrovsky N.M. (2008) Efficacy of algal metrics for assessing nutrient and organic enrichment in flowing waters. *Freshwater Biology* 53: 1036–1054. <https://doi.org/10.1111/j.1365-2427.2007.01951.x>
- Potapova M. (2006) *Achnanthyidium zhakovschikovii* sp. nov. (Bacillariophyta) and related species from rivers of North-western Russia. *Nova Hedwigia* 82: 399–408. <https://doi.org/10.1127/0029-5035/2006/0082-0399>
- Potapova M., Hamilton P.B. (2007) Morphological and ecological variation within the *Achnanthyidium minutissimum* (Bacillariophyceae) species complex. *Journal of Phycology* 43: 561–575. <https://doi.org/10.1111/j.1529-8817.2007.00332.x>
- Potapova M.G., Ponader K.C. (2004) Two common North American diatoms, *Achnanthyidium rivulare* sp. nov. and *A. deflexum* (Reimer) Kingston: morphology, ecology and comparison with related species. *Diatom Research* 19: 33–57. <https://doi.org/10.1080/0269249X.2004.9705606>
- Potapova M.G., Charles D.F., Ponader K.C., Winter D.M. (2004) Quantifying species indicator values for trophic diatom indices: a comparison of approaches. *Hydrobiologia* 517: 25–41. <https://doi.org/10.1023/B:HYDR.0000027335.73651.ea>
- Rohlf F.J. (1990) Morphometrics. *Annual Review of Ecology and Systematics* 21: 299–316. <https://doi.org/10.1146/annurev.es.21.110190.001503>
- Round F.E., Bukhtiyarova L. (1996) Four new genera based on *Achnanthes* (*Achnanthyidium*) together with a re-definition of *Achnanthyidium*. *Diatom Research* 11: 345–361. <https://doi.org/10.1080/0269249X.1996.9705389>
- Round F.E., Crawford R.M., Mann D.G. (1990) The diatoms: biology & morphology of the genera. Cambridge, Cambridge University Press.
- Santos T.R., Ferragut C. (2013) The successional phases of a periphytic algal community in a shallow tropical reservoir during the dry and rainy seasons. *Limnetica* 32: 337–352.
- Sartory D.P., Grobbelaar J.U. (1984) Extraction of chlorophyll a from freshwater phytoplankton for spectrophotometric analysis. *Hydrobiologia* 114: 177–187. <https://doi.org/10.1007/BF00031869>
- Silva A.M., Ludwig T.A.V., Tremarin P.I., Vercellino I.S. (2010) Diatomáceas perifíticas em um sistema eutrófico brasileiro (Reservatório do Iraí, estado do Paraná). *Acta Botanica Brasiliense* 24: 997–1016. <https://doi.org/10.1590/S0102-33062010000400015>
- SMA - Secretaria do Meio Ambiente. Fundação Florestal do Estado de São Paulo (2012) Parque Estadual do Jurupará. Meio Físico. Plano de Manejo. São Paulo, SP, Governo do Estado de São Paulo.
- Souza M.G.M., Oliveira R.I.R. (2007) Levantamento da diatomoflora epilítica da bacia do rio Paranã, Goiás, Brasil. In: Martins Silva M.J. (org.) Inventário da biota aquática com vistas a conservação e utilização sustentável do bioma Cerrado (Serra e Vale do rio Paranã), vol. 1: 72–94. Brasília, Ministério do Meio Ambiente.
- Straub F. (2002) Note algologique II. Apparition envahissante de la diatomée *Achnanthes catenata* Bily & Marvan (Heterokontophyta-Bacillariophyceae) dans le lac de Neuchâtel (Suisse). *Bulletin de la Société Neuchâteloise des Sciences Naturelles* 125: 59–65.
- ter Braak C.J.F., van Dam H. (1989) Inferring pH from diatoms: a comparison of old and new calibration methods. *Hydrobiologia* 178: 209–223. <https://doi.org/10.1007/BF00006028>
- Tremarin P.I., Wetzel C.E., Ludwig T.A.V., Ector L. (2011) *Encyonema exuberans* sp. nov. (Bacillariophyceae) from southern Brazilian lotic systems. *Nova Hedwigia* 92: 107–120. <https://doi.org/10.1127/0029-5035/2011/0092-0107>
- Trobajo R., Rovira L., Ector L., Wetzel C.E., Kelly M., Mann D.G. (2013) Morphology and identity of some ecologically important small *Nitzschia* species. *Diatom Research* 28: 37–59. <https://doi.org/10.1080/0269249X.2012.734531>
- Urbánková P., Scharfen V., Kulichová J. (2016) Molecular and automated identification of the diatom genus *Frustulia* in northern Europe. *Diatom Research* 31: 217–229. <https://doi.org/10.1080/0269249X.2016.1224780>
- Van de Vijver B., Kopalová K. (2014) Four *Achnanthyidium* species (Bacillariophyta) formerly identified as *Achnanthyidium minutissimum* from the Antarctic Region. *European Journal of Taxonomy* 79: 1–19. <https://doi.org/10.5852/ejt.2014.79>
- Van de Vijver B., Ector L., Beltrami M.E., de Haan M., Falasco E., Hlúbíková D., Jarlman A., Kelly M., Novais M.H., Wojtal A.Z. (2011a) A critical analysis of the type material of *Achnanthyidium lineare* W. Sm. (Bacillariophyceae). *Algological Studies* 136/137: 167–191. <https://doi.org/10.1127/1864-1318/2011/0136-0167>

- Van de Vijver B., Jarlman A., Lange-Bertalot H., Mertens A., de Haan M., Ector L. (2011b) Four new European Achnantheidium species (Bacillariophyceae). *Algological Studies* 136/137: 193–210. <https://doi.org/10.1127/1864-1318/2011/0136-0193>
- Wengrat S., Marquardt G.C., Bicudo D.C., Bicudo C.E.M., Wetzel C.E., Ector L. (2015) Type analysis of *Cymbella schubartii* and two new *Encyonopsis* species (Bacillariophyceae) from southeastern Brazil. *Phytotaxa* 221: 247–264. <https://doi.org/10.11646/phytotaxa.221.3.3>
- Wetzel C.E., Ector L. (2014) Taxonomy, distribution and autecology of *Planothidium bagualensis* sp. nov. (Bacillariophyta) a common monoraphid species from southern Brazilian rivers. *Phytotaxa* 156: 201–210. <https://doi.org/10.11646/phytotaxa.156.4.2>
- Winter J.G., Duthie H.C. (2000) Epilithic diatoms as indicators of stream total N and total P concentration. *Journal of the North American Benthological Society* 19: 32–49. <https://doi.org/10.2307/1468280>
- Witkowski A., Kulikovskiy M., Riaux-Gobin C. (2012) *Achnantheidium sieminskae*, a new diatom species from the Kerguelen Archipelago (Austral Islands). In: Wołowski K., Kaczmarska I., Ehrman J.M., Wojtal A.Z. (eds) *Current advances in algal taxonomy and its applications: phylogenetic, ecological and applied perspective*: 61–68. Kraków, W. Szafer Institute of Botany, Polish Academy of Sciences.
- Wojtal A.Z., Lange-Bertalot H., Nautiyal R., Verma J., Nautiyal P. (2010) *Achnantheidium chitrakootense* spec. nov. from rivers of northern and central India. *Polish Botanical Journal* 55(1): 55–64.
- Wojtal A.Z., Ector L., Van de Vijver B., Morales E.A., Blanco S., Piatek J., Smieja A. (2011) The *Achnantheidium minutissimum* complex (Bacillariophyceae) in southern Poland. *Algological Studies* 136/137: 211–238. <https://doi.org/10.1127/1864-1318/2011/0136-0211>
- Zidarova R., Van de Vijver B., Mataloni G., Kopalová K., Nedbalová L. (2009) Four new freshwater diatom species (Bacillariophyceae) from Antarctica. *Cryptogamie Algologie* 30(4): 295–310.

Manuscript received 10 Jan. 2017; accepted in revised version 2 Aug. 2017.

Communicating Editor: Bart Van de Vijver.