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PROGRAMA DE PÓS-GRADUAÇÃO STRICTO SENSU EM CONSERVAÇÃO E
MANEJO DE RECURSOS NATURAIS – NÍVEL MESTRADO

MAILOR WELLINTON WEDIG AMARAL

DIATOMÁCEAS EPILÍTICAS DE RIACHOS SUBTROPICAIS COM ALTAS
CONCENTRAÇÕES DE ALUMÍNIO E FERRO, SUL DO BRASIL

CASCATEL-PR

Abril/2022

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Dissertação apresentado ao Programa de Pós-graduação *Stricto Sensu* em Conservação e Manejo de Recursos Naturais – Nível Mestrado, do Centro de Ciências Biológicas e da Saúde, da Universidade estadual do Oeste do Paraná, como requisito parcial para a obtenção do título de Mestre em Conservação e Manejo de Recursos Naturais

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Orientadora: Norma Catarina Bueno

Coorientadora: Thelma Alvim Veiga Ludwig

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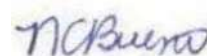
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ATA DA DEFESA PÚBLICA DA DISSERTAÇÃO DE MESTRADO DE MAILOR WELLINTON WEDIG AMARAL, ALUNO(A) DO PROGRAMA DE PÓS-GRADUAÇÃO EM CONSERVAÇÃO E MANEJO DE RECURSOS NATURAIS DA UNIVERSIDADE ESTADUAL DO OESTE DO PARANÁ - UNIOESTE, E DE ACORDO COM A RESOLUÇÃO DO PROGRAMA E O REGIMENTO GERAL DA UNIOESTE.

Aos 17 dias do mês de setembro de 2021 às 14h00min, por videoconferência, de forma remota, realizou-se a sessão pública da Defesa de Dissertação do candidato **MAILOR WELLINTON WEDIG AMARAL**, aluno do Programa de Pós-Graduação em Conservação e Manejo de Recursos Naturais - nível de Mestrado, na área de concentração em Ciências Ambientais. A comissão examinadora da Defesa Pública foi aprovada pelo Colegiado do Programa de Pós-Graduação em Conservação e Manejo de Recursos Naturais. Integraram a referida Comissão as Professoras Doutoras: Norma Catarina Bueno, Angela Maria da Silva-Lehmkuhl e Elaine Cristina Rodrigues Bartozek. Os trabalhos foram presididos pela Norma Catarina Bueno. Tendo satisfeito todos os requisitos exigidos pela legislação em vigor, o aluno foi admitido à Defesa de DISSERTAÇÃO DE MESTRADO, intitulada: "**Epilithic diatoms from subtropical streams with high concentrations of aluminum and iron, Southern Brazil**". A Senhora Presidente declarou abertos os trabalhos, e em seguida, convidou o candidato a discorrer, em linhas gerais, sobre o conteúdo da Dissertação. Feita a explanação, o candidato foi arguido sucessivamente, pelas professoras doutoras: Angela Maria da Silva-Lehmkuhl, Elaine Cristina Rodrigues Bartozek. Findas as arguições, o(a) Senhor(a) Presidente suspendeu os trabalhos da sessão pública, a fim de que, em sessão secreta, a Comissão expressasse o seu julgamento sobre a Dissertação. Efetuado o julgamento, o(a) candidato(a) foi aprovado. A seguir, a Senhora Presidente reabriu os trabalhos da sessão pública e deu conhecimento do resultado. E, para constar, o Coordenador do Programa de Pós-Graduação em Conservação e Manejo de Recursos Naturais, da Universidade Estadual do Oeste do Paraná – UNIOESTE - Campus de Cascavel, lavra a presente ata, e assina juntamente com os membros da Comissão Examinadora e o candidato.

Em tempos: _____



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Aos meus pais,
com amor

*“As coisas mais importantes não estão escritas num livro,
é preciso aprendê-las vivenciando-as sozinho.”*

Sakura Haruno em Naruto Shippuden

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RESUMO

As diatomáceas epilíticas são microrganismos fotossintéticos crescendo aderidos ao substrato rochoso. Estudos taxonômicos abordando floras de diatomáceas são cruciais para descrever novas espécies, assim como reconhecer estresses ambientais e avaliar o status ecológico dos sistemas aquáticos. Este trabalho teve o objetivo de conduzir um levantamento taxonômico das diatomáceas epilíticas de uma microbacia hidrográfica no sul do Brasil com concentrações naturalmente altas de ferro e alumínio. A amostragem das rochas, água e sedimentos foi realizada entre 2016 e 2019. As amostras de diatomáceas foram oxidadas e analisadas em microscopia eletrônica de luz e varredura. O primeiro artigo trata da descrição de uma nova espécie do gênero *Nupela*, encontrada em córregos com pH neutro-ácido, baixa condutividade e baixas a altas concentrações de nutrientes. No segundo artigo identificamos 221 espécies de diatomáceas em nível infragenérico, enfatizando suas formas teratológicas e caracterizando as condições limnológicas e concentrações de metais onde foram encontradas. *Fragilaria spectra*, *Fragilaria tenera* var. *nanana* e *Humidophila arcuatoides* foram registradas primeiramente no estado do Paraná e *Pinnularia laucensis* representa um novo registro para o Brasil. Valvas teratológicas foram encontradas em 34 táxons, contendo contorno modificado, alterações no padrão de estrias e área central duplicada. Os metais e variáveis ambientais na microbacia do Rio Cascavel provavelmente contribuíram para as teratologias nas diatomáceas. Nosso trabalho contribui para o conhecimento taxonômico das diatomáceas epilíticas na região, bem como para ampliar o registro de táxons teratológicos na literatura.

Palavras-chave: Bacillariophyta, ambientes lóticos, metais, morfologia, espécie nova, perifíton, taxonomia, teratologia

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ABSTRACT

Epilithic diatoms are photosynthetic microorganisms growing attached to rocky substrate. Taxonomic studies approaching diatom floras are crucial for describing new species, as well as recognizing environmental stresses and assessing the ecological status of aquatic systems. This work aimed to conduct a taxonomic survey of epilithic diatoms from a hydrographic microbasin in southern Brazil with naturally high iron and aluminum concentrations. Sampling of rocks, water and sediments were carried out from 2016 to 2019. Diatom samples were oxidized and analyzed using light and scanning electron microscopy. The first article deals with the description of a new species of the genus *Nupela*, which was found in streams with acidic–neutral pH, low conductivity and low to high nutrient concentrations. In the second article we identified 221 diatom species at infrageneric level, focusing on their teratological forms and characterizing the limnological conditions and metal concentrations where they were found. *Fragilaria spectra*, *Fragilaria tenera* var. *nanana* and *Humidophila arcuatoides* were firstly recorded in the state of Paraná, and *Pinnularia laucensis* represent a new record for Brazil. We found teratological valves in 34 taxa, containing modified outline, changes in the striae pattern, and doubled central area. The metals and environmental variables in the Cascavel River microbasin probably contributed to the diatom teratologies. Our work contributes to the taxonomic knowledge of epilithic diatoms in the region, as well as extending the record of teratological taxa in the literature.

Keywords: Bacillariophyta, lotic environments, metals, morphology, new taxa, periphyton, taxonomy, teratology

Nupela semifasciata (Bacillariophyceae), a new species from subtropical lotic environments in Western Paraná State, Brazil

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Abstract: During a survey on freshwater epilithic diatoms from subtropical lotic environments belonging to the Cascavel River microbasin, Paraná State, Brazil, we observed populations of a new *Nupela* Vyverman et Compère species. Morphological and meristic analyses were performed using light and scanning electron microscopy, resulting in the description of *Nupela semifasciata* sp. nov. This species is characterized by elliptic–lanceolate valves containing subcapitate apices in larger individuals and broadly rostrate–rounded apices in smaller ones, while the length/width ratio gradually decreases as the individuals become smaller. The central area unilaterally reaches the margin and always interrupts the row of areolae on the valve mantle. The valve mantle is externally composed of a second row of areolae near the apices. We cross-checked information with similar *Nupela* taxa, highlighting the main features that separate them. *Nupela semifasciata* was found in streams with acidic–neutral pH, low conductivity and low to high nutrient concentrations.

Key words: diatoms, morphology, new taxa, *Nupela*, periphyton, taxonomy

INTRODUCTION

One of the major obstacles in diatom taxonomy is that light microscopy is often insufficient for species level identification (POTAPOVA et al. 2003). This process is particularly accentuated in small-sized diatoms, such as *Nupela*, in which the individuals generally measure less than 20 µm (SPAULDING & EDLUND 2008). Ultrastructural analysis of frustules obtained by electron microscopy provides observation of additional features that improves accurate species diagnosis (ZIMMERMANN et al. 2014) and, thus, supports taxonomic robustness for biodiversity conservation purposes (THOMSON et al. 2018).

Nupela Vyverman et Compère was proposed as a distinct genus based on samples collected in high elevation ponds with a peaty bottom in Papua New Guinea, with *N. giluwensis* as the generitype. The authors pointed out morphological similarities with *Brachysira* Kützing and *Diadesmis* Kützing, such as striae composed of a few areolae transversally

elongated, a ridge or a hyaline area at the junction of the valve face and mantle, and simple or inconspicuous proximal raphe fissures (VYVERMAN & COMPÈRE 1991). However, recent phylogenetic investigations suggested that *Nupela* and *Brachysira* form a non-monophyletic group, with *Nupela* not being assigned to the family Brachysiraceae (KULIKOVSKIY et al. 2020). In addition, SIVER et al. (2007) expanded the circumscription of this genus to comprise cells with small size, terminal raphe ends externally curved to the secondary side, straight proximal raphe ends, Voigt faults indicating the secondary side of the valve, and a single row of elongated areolae throughout the valve mantle. The last feature appears to be well-established within the genus (VYVERMAN & COMPÈRE 1991; SIVER et al. 2007; FALASCO et al. 2015), along with the typical external openings of areolae covered by hymen that are larger than internal ones (SIVER et al. 2007; SPAULDING & EDLUND 2008). Regarding the raphe, *Nupela* possesses isovalvar forms with two fully developed raphe slits, such as in *N. giluwensis* (VYVERMAN & COMPÈRE

1991) and *N. amabilis* (TREMARIN et al. 2015); however, the vast majority of species are heterovalvar with one valve containing shortened raphe slits, as in *N. pardinhoensis* (BES et al. 2012), rudimentary slits, as in *N. scissura* (SIVER et al. 2007) and *N. rumrichorum* (LANGE–BERTALOT & MOSER 1994), or absent raphe slits as in *N. praecipuoides* (TREMARIN et al. 2015) and *N. major* (YU et al. 2017).

Since it was established, several species have been transferred from other genera to *Nupela*, mainly *Achnanthes* Bory, due to its monoraphid condition, and *Navicula* Bory, due to the areolae openings shape, which are externally elongated and internally rounded (see LANGE–BERTALOT & MOSER 1994; POTAPOVA 2013; TREMARIN et al. 2015). The number of new *Nupela* taxa is still increasing, consisting of 85 species taxonomically accepted for science, according to Algaebase (GUIRY & GUIRY 2020).

The genus generally occurs in oligotrophic waters with acidic to circumneutral pH (e.g., MONNIER et al. 2003; POTAPOVA et al. 2003; WOJTAL 2009; KULIKOVSKIY et al. 2010), being particularly well represented in the Neotropics (METZELTIN & LANGE–BERTALOT 1998; WOJTAL 2009). At present, seven taxa were described as new species for science based on Brazilian samples. One of them, *N. pardinhoensis* Bes, Torgan et Ector was found in slow moving waters with high dissolved oxygen, low biochemical oxygen demand, and high total phosphate concentration in the State of Rio Grande do Sul (BES et al. 2012) and the other six, *N. amabilis* Tremarin et Ludwig, *N. difficilis* Straube, Tremarin et Ludwig, *N. kocioleckii* Straube, Tremarin et Ludwig, *N. metzeltinii* Tremarin et Ludwig, *N. praecipuoides* Tremarin et Ludwig and *N. torganiae* Tremarin et Ludwig, were generally found in rivers with high water speed, low conductivity, and neutral

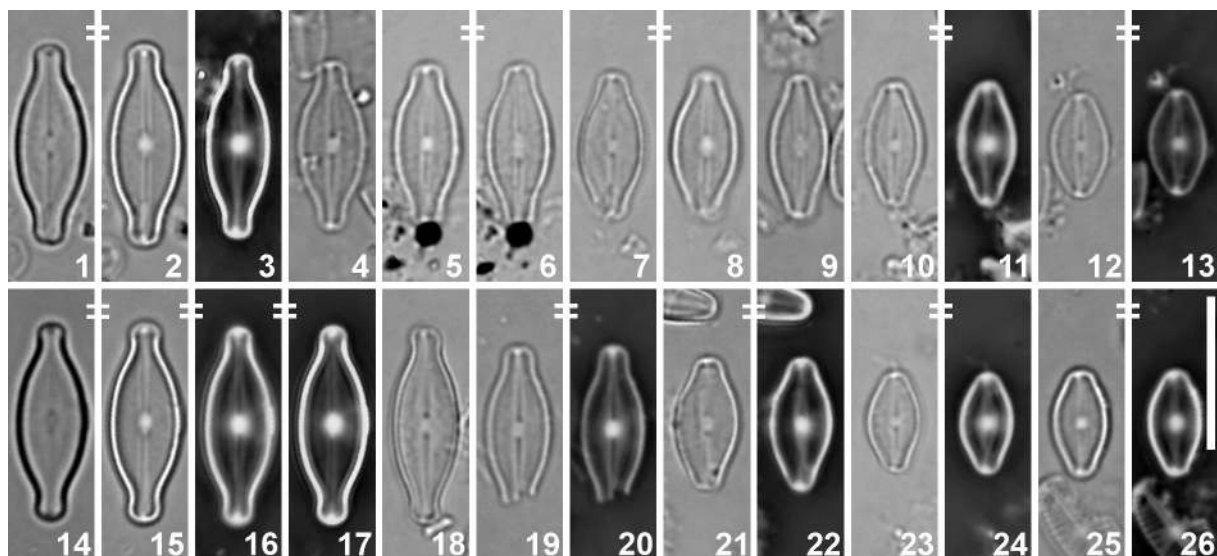
pH in Paraná State (TREMARIN et al. 2015).

The aim of this study was to describe and illustrate a new species of *Nupela* using light and scanning electron microscopy observations, and compare it with similar taxa, thus, expanding the knowledge on the genus.

MATERIAL AND METHODS

Study area. The Cascavel River (24°32' and 25°17'S; 53°05' and 53°50'W) is 17.5 km long and represents the principal source of water supply for Cascavel City, with the main tributaries within the urban perimeter (FUNDETEC 1995). The river is located in Western Paraná, a subtropical region characterized by hot and humid summers, with an average annual temperature of 22 °C (ALVARES et al. 2013).

For the study, we selected eight sampling sites (S) along the Cascavel River microbasin, as follows: S1 – urban perimeter, inside the conservation area of Paulo Gorski Ecological Park, with presence of *Hydrochoerus hydrochaeris* Linnaeus populations. Stream with visible silting up process. S2 – urban area, close to a highway, containing ciliary vegetation. S3 – urban area, close to a highway, lacking ciliary vegetation and visible silting up process. S4 – urban area, close to a garbage collection property, containing sparse ciliary vegetation and receiving water from storm drains. Presence of a bridge over the stream for pedestrian traffic. S5 – rural area, close to temporary croplands and a highway, containing ciliary vegetation. Presence of a bridge over the stream for pedestrian traffic. S6 – conservation area within a rural property, with goat farming, *Pinus* sp. plantation and temporary croplands. S7 – urban area, close to a deactivated fridge, containing ciliary vegetation. Stream with visible silting up process. S8 – rural area with temporary croplands, close to a basalt mining company using mineral deposits. Presence of a bridge over the stream for vehicle traffic.



Figs 1–26. *Nupela semifasciata* sp. nov., light microscopy images showing size diminution series and the variability of the valve outline: (1, 2) are the holotype, (3, 11, 13, 16, 17, 20, 22, 24, 26) were taken in phase contrast. The = symbol represents different valves of the same frustule. Scale bar represents 10 µm.

Table 1. Range of physical, chemical, and biological variables of the water in which *Nupela semifasciata* was found. Legend: (Temp) water temperature, (Cond) electrical conductivity, (DO) dissolved oxygen, (Turb) turbidity, (COD) chemical oxygen demand, (BOD) biochemical oxygen demand, (TKN) Kjeldahl nitrogen, (NO₃) nitrate, (N–NH₃) ammoniacal nitrogen, (TP) total phosphorus, (PO₄⁻) orthophosphate, (TS) total solids, (DS) dissolved solids, (SS) suspended solids, (CLa) chlorophyll-a, (TC) total coliforms, (*E. coli*) *Escherichia coli*.

Variables	Range (min – max)
Temp (°C)	16.67–23.70
Cond (mS.cm ⁻¹)	0.001–0.091
DO (mg.l ⁻¹)	6.53–14.27
pH	5.40–7.42
Turb (NTU)	0.06–41.90
COD (mg.l ⁻¹)	5.10–24.32
BOD (mg.l ⁻¹)	1.19–9.90
TKN (mg.l ⁻¹)	0.00–0.92
NO ₃ (mg.l ⁻¹)	0.32–14.00
N–NH ₃ (mg.l ⁻¹)	0.003–0.448
TP (mg.l ⁻¹)	0.005–0.060
PO ₄ ⁻ (mg.l ⁻¹)	0.002–0.030
TS (mg.l ⁻¹)	4.00–81.00
DS (mg.l ⁻¹)	3.30–66.70
SS (mg.l ⁻¹)	0.20–40.00
CLa (µg.l ⁻¹)	0.000–16.382
TC (NMP 100 ml ⁻¹)	1–606000
<i>E. coli</i> (NMP 100 ml ⁻¹)	1–606000

Field and laboratory procedures. Samplings were performed seasonally from 2016 to 2018, comprising eight samplings. We collected periphytic substrates (stones) in triplicates at each sampling site, totaling 204 qualitative samples. Water samples for physical, chemical, and biological analysis were collected one per sampling site.

The physical and chemical variables, such as water temperature, conductivity, dissolved oxygen, pH, and turbidity were measured in situ with a HORIBA U–5000 multiparameter probe. Additionally, water samples were collected using polyethylene bottles immersed in the surface of the water column, being adequately cooled, and kept in the dark, from which the environmental variables function of chemical oxidation and organic material, concentrations of total Kjeldahl nitrogen, nitrate, ammoniacal nitrogen, total phosphorus, orthophosphate, and total, dissolved, and suspended solids were measured. The biological variables chlorophyll-a, total coliforms, and *Escherichia coli* were also analyzed. All analyses were realized following the Standard Methods (APHA 2012). The flow (m³.s⁻¹) and depth (m) were measured using a ruler, metric tape, and polystyrene floating object, in a transect previously delimited for each stream. The flow was

calculated by multiplying the length of the transect and the average speed resulting from the object's displacement.

Diatoms were scraped off the stones and fixed in Transeau solution 1:1 according to BICUDO & MENEZES (2017). Subsamples of 10 ml were oxidized following the SIMONSEN (1974) technique, modified by MOREIRA-FILHO & VALENTE-MOREIRA (1981). Permanent slides were mounted from the cleaned diatom material using Naphrax® for light microscopy (LM) observations, using an Olympus BX60 microscope with a DP 71 capture camera attached, at 1000×. Cleaned samples were also placed in aluminum stubs, sputter-coated with gold in Balzers Union SCD 030, and examined with JEOL JSM 6360LV scanning electron microscope, operated at 15 kV and 9–10 mm of working distance. Species description follows the terminology of specialized literature.

RESULTS

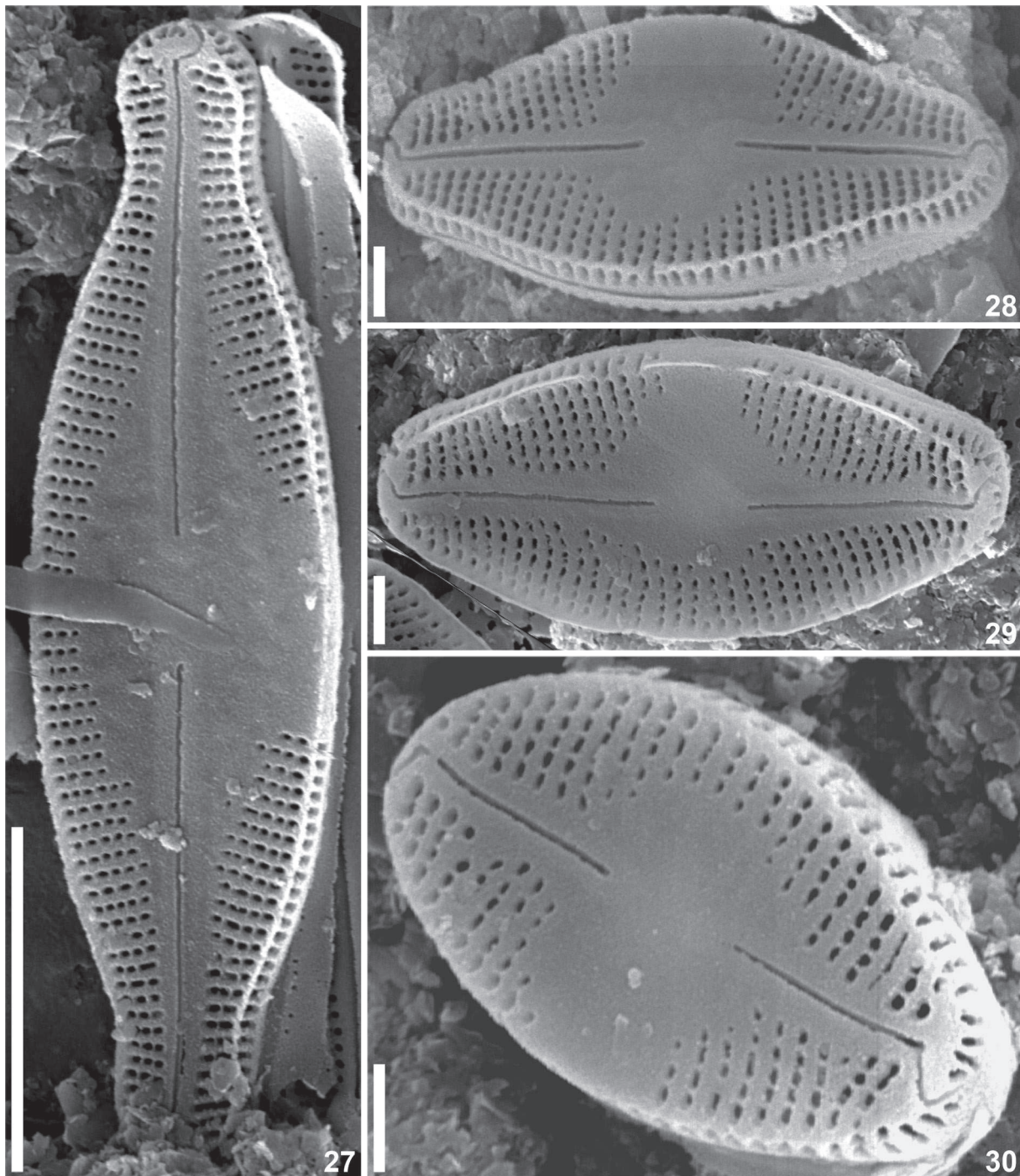
Nupela semifasciata Amaral, T.Ludwig et Bueno sp. nov. (Figs 1–50)

Description

Light microscopy (Figs 1–26): frustules heterovalvar regarding the raphe development, sometimes asymmetrical about apical and transapical planes (Figs 1–26). Length/width ratio gradually decreases as the valves become smaller, while the valve outline is modified. Valves elliptic-lanceolate containing subcapitate apices in larger individuals (Figs 1–3, 14–18) and rostrate in middle ones (Figs 4–11, 19–22), slightly drawn-out, becoming broadly rostrate-rounded in smaller individuals (Figs 12, 13, 23–26). Axial area narrowly lanceolate, slightly broadening toward the central area. Central area transversely expanded, asymmetric, unilaterally reaching the valve margin, discernible only in phase contrast images (Figs 3, 11, 13, 16, 17, 20, 22, 24, 26). One valve with long raphe slits and the other valve with slightly shorter raphe slits (compare Figs 1 to 2, 5 to 6, 7 to 8, 14 to 15).

Measurements (n= 89, occurring in 46 diatom samples): 6.7–16.4 µm long; 3.2–4.8 µm wide; 1.7–3.7 ratio length/width.

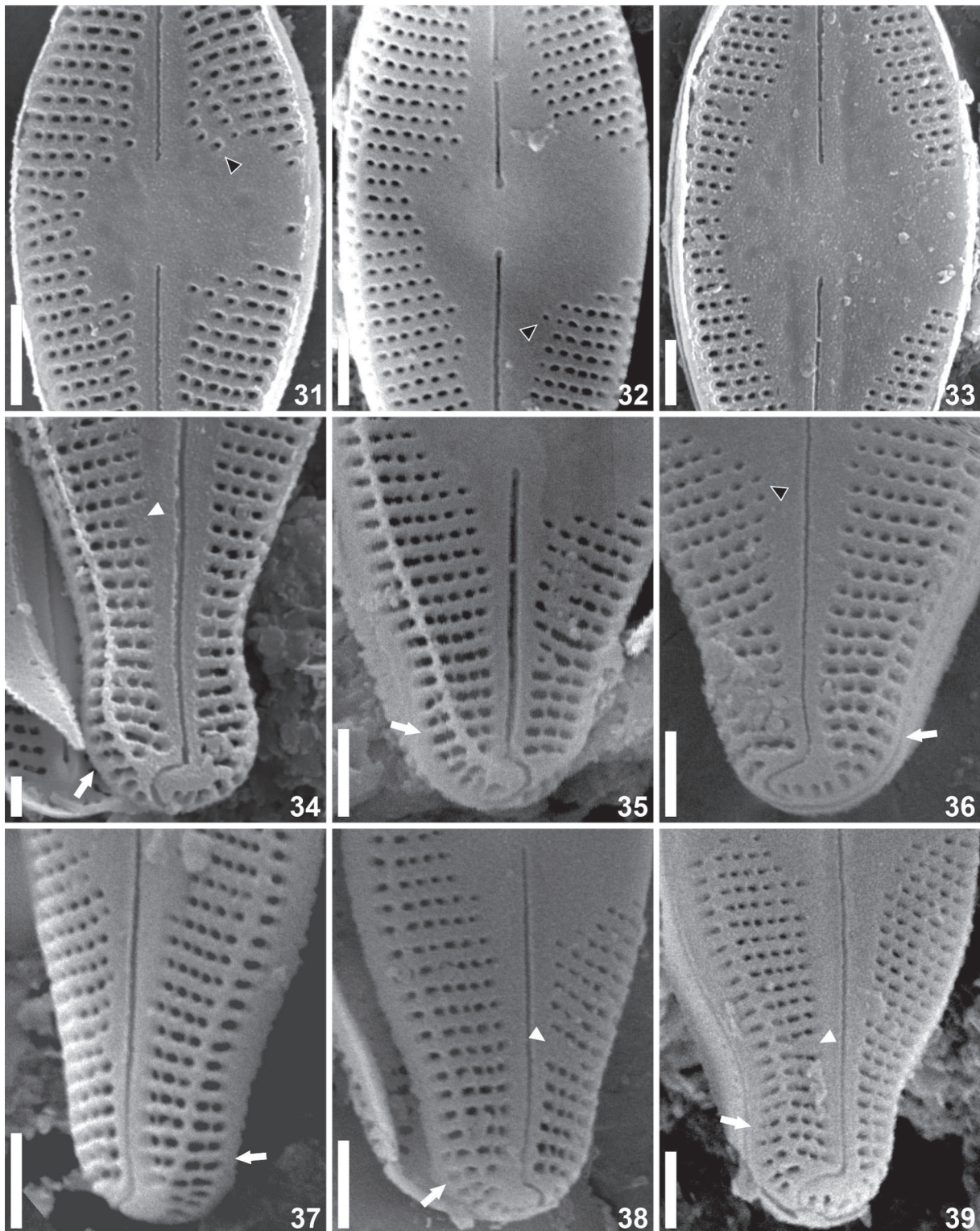
Scanning electron microscopy (Figs 27–39, 48–50 external views, Figs 41–47 internal views): axial area appears narrowly lanceolate (Figs 28–30, 41) or broadly lanceolate (Fig 27), sometimes with external siliceous thickenings surrounding the raphe and delicate depressions along the apical axis (Figs 27, 28, 31, 33, 49, 50). Central area broadly asymmetric, limited by irregular shortened marginal striae, ranging from rectangular (Fig 31) to rounded (Figs 28–30, 32) or rhombic (Figs 27, 33) shape, unilaterally reaching the secondary margin of the valve. The fascia is variable in shape, large or reduced, and always extends onto the valve mantle (Figs 27, 29, 31–33). Sometimes, a single areola appears in the fascia (Figs 31, 41). Valve mantle ornamented with one row of transversely elongated areolae along the valve (Fig 28) and at the end of



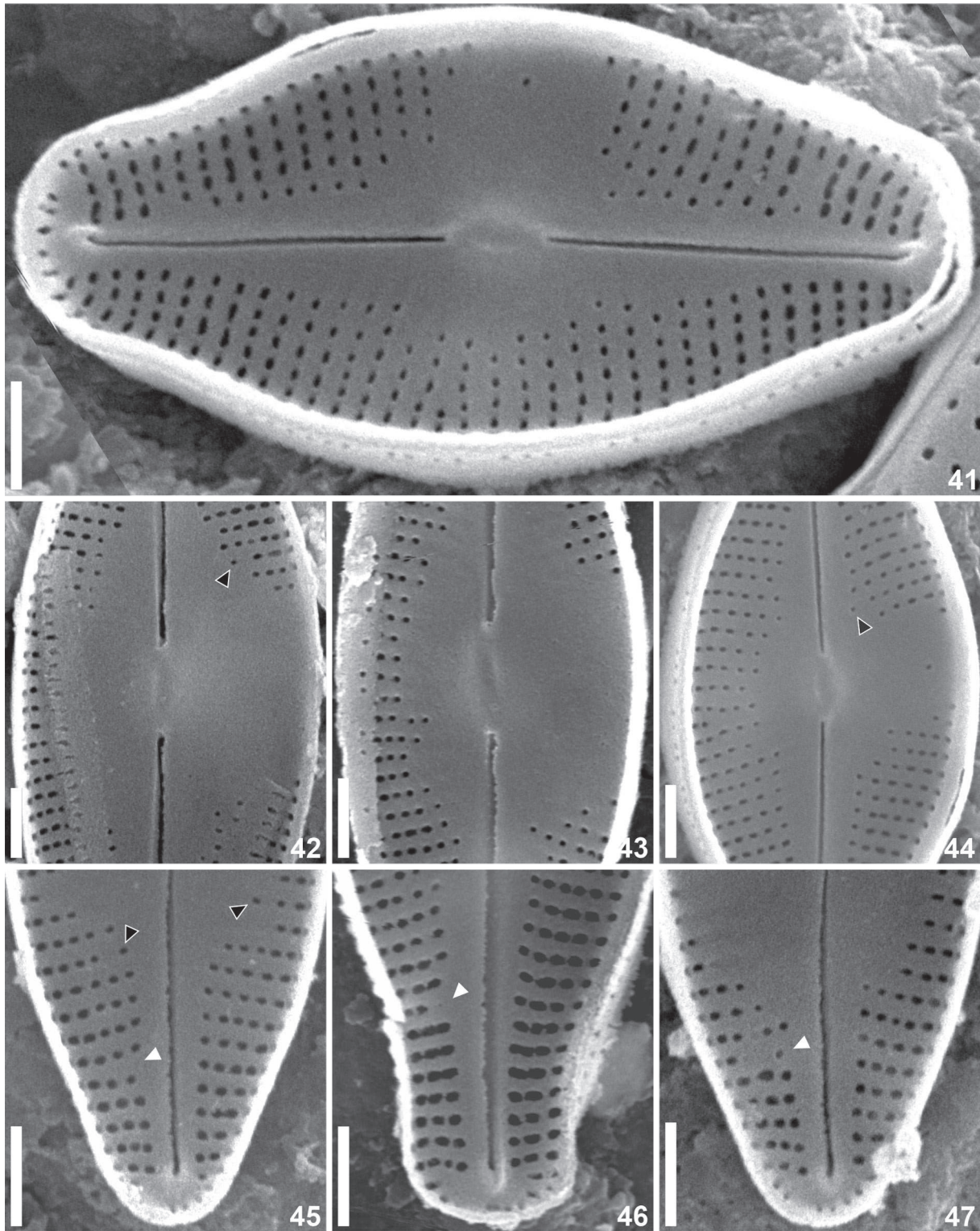
Figs 27–30. *Nupela semifasciata* sp. nov., SEM external whole valve views: (27) entire valve with shorter raphe slits showing the siliceous thickenings surrounding the raphe, delicate depressions along the apical axis, and the rhombic-shaped central area. Also note the ridge along the valve face–mantle junction and the interrupted mantle. (28, 29) entire valves with long raphe slits showing the mantle composed of one row of transversely elongated areolae along the whole valve, becoming two rows near the apices (28), and interrupted by the unilateral fascia (29). Note the asymmetrically rounded central area. (30) entire valve of a smaller individual with shorter raphe slits. Detail of the asymmetrically rounded central area and the hook-shaped terminal raphe fissure extending onto the valve mantle, interrupting the row of elongated areolae. Note the Voigt fault (27–30) on the secondary side of the valve, marked as a change in the striae pattern from radiate to convergent. Scale bars represent 5 μm (27), 1 μm (28–30).

the apex (Figs 30, 36), however, externally becoming two rows of elliptic areolae near the apices (white arrows in figs 34–39). Internally, the mantle appears to be ornamented with only one row of areolae (Figs 41, 47). The mantle is interrupted by the central area on

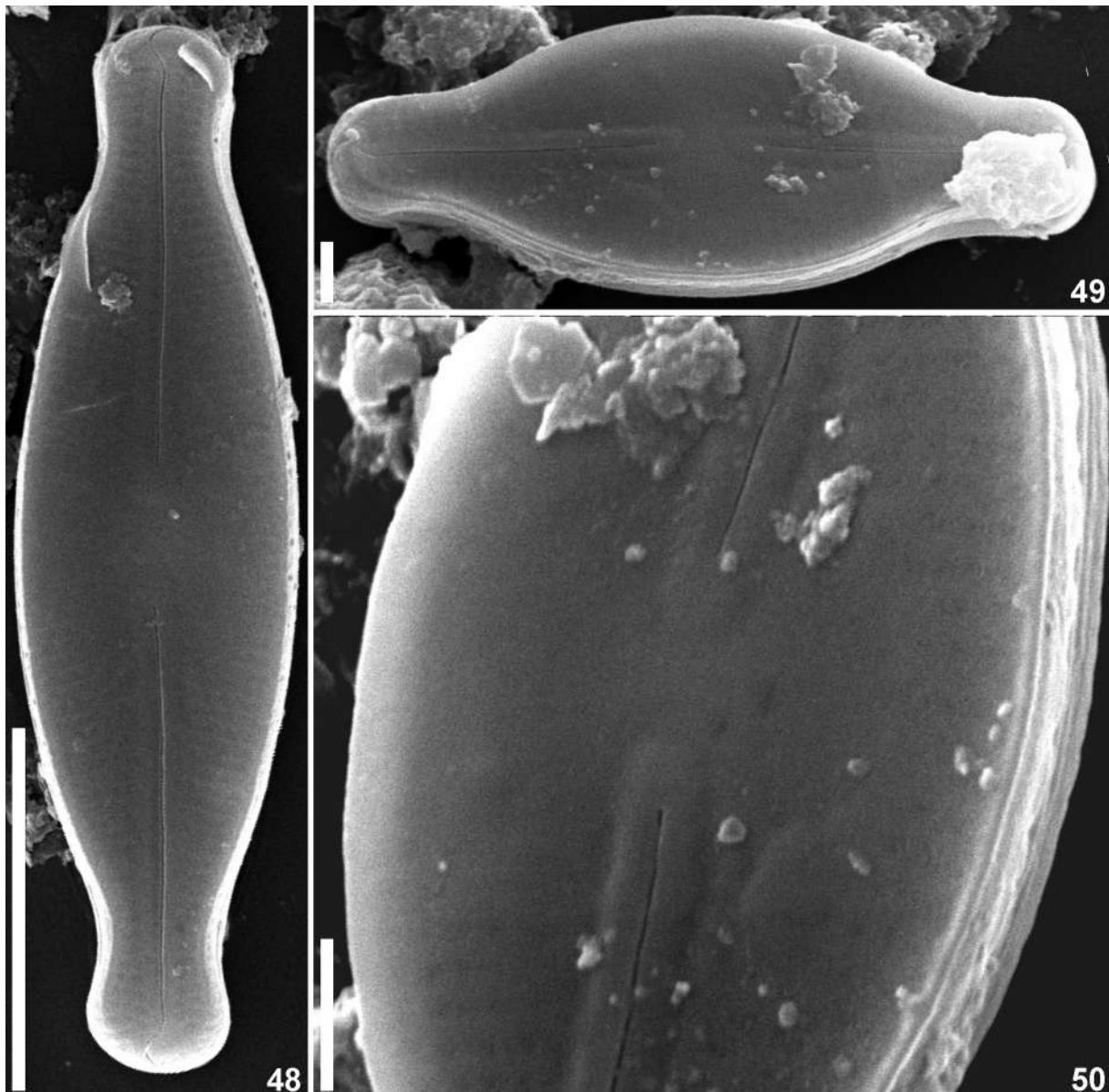
the secondary margin (Figs 27, 29) and by the terminal raphe fissures at the apices (Figs 30, 34–36, 38, 39). In some cases, a ridge is evident on the valve face–mantle junction (Figs 27–29). Raphe filiform, almost straight (Fig 28, 41, 49) or slightly sinuous (Figs 27,



Figs 31–39. *Nupela semifasciata* sp. nov., SEM external views: (31–33) asymmetrically shaped central area, rectangular (31), rounded (32), or rhombic (33). Note the single areola in the middle of the fascia (31). Raphe with long slits (32) or slightly shorter (31, 33). Proximal raphe endings simple (31, 33) or pore-like (32), straight (31) or slightly curved to the secondary side of the valve (32, 33). Note the siliceous thickenings surrounding the raphe and delicate depressions along the apical axis (31, 33). (34–39) valve apices. Second row of areolae on the valve mantle (white arrows in 34–39). White arrowheads indicate Voigt fault as a missing areola (34) or as a marked change in striae pattern (39). Black arrows indicate an isolated areola at the end of striae (31, 32, 36). Scale bars represent 1 μm .



Figs 41–47. *Nupela semifasciata* sp. nov., SEM internal views: (41) whole valve. Axial area narrowly lanceolate, raphe almost straight, and small inner areolae openings. Note the single areola in the middle of the fascia. (42–44) central area. Proximal raphe endings straight (42, 43) or bent (44), simple (44) or pore-like (42, 43). Note the mantle interrupted by the unilaterally expanded fascia. (45–47) valve apices. Terminal raphe ends finishing in small helictoglossa, straight (46) or slightly curved (45, 47). Note the Voigt fault as a marked change in striae pattern (white arrowhead in 45, 47) or like a missing areola (white arrowhead in 46). Also note isolated areola in (42, 44, 45) (black arrowheads). Scale bars represent 1 μ m.



Figs 48–50. *Nupela semifasciata* sp. nov., SEM external views showing areolae occluded by hymenes: (48–49) whole valve views. Raphe filiform, almost straight (49) or slightly sinuous (48), with simple proximal ends, slightly curved to one side, and terminal ends hook-shaped, reaching the mantle. (50) detail of central region of valve. Raphe with simple proximal raphe ends, curved to the same side, and surrounded by siliceous thickenings. Scale bars represent 5 μm (48), 1 μm (49, 50).

29, 30, 48). Proximal raphe ends are simple (Figs 31, 33, 44, 48, 49) or pore-like (Figs 32, 42, 43), straight (Figs 31, 42, 43) or somewhat bent to the primary side of the valve (Figs 32, 33, 44, 48, 49). Terminal raphe ends are externally hook-shaped, curved to the secondary side of the valve, reaching the valve mantle (Figs 34–39, 48, 49) and internally finishing in small helicoglossa, straight (Fig 46) or slightly curved (Figs 45, 47). Voigt fault occurs as a markedly changing striae pattern (white arrowheads in figs 38, 39, 45, 47) or seems like an areola is missing (white arrowhead in figs 34, 46). Striae radiate throughout the valve, becoming slightly (Figs 36–39, 45–47) to strongly (Figs 30, 34, 35) convergent near the apices, 40–48/10 μm . Striae are composed by a variable number of areolae

(commonly 4–6), arranged in continuous lines of areolae, although an isolate areola can be present at the end of some striae (black arrowheads in Figs 31, 32, 36, 42, 44, 45). Areolae small, rounded to transversally elongated in elliptic or rectangular shape (Figs 27–39), internally smaller in diameter relative to outer openings (Fig 41–47), 39–59/10 μm , commonly ca. 50. Outer openings of areolae are occluded by hymenes (Figs 48–50).

Holotype: slide no. 5364, sample site 4, designated here in figs 1 and 2, deposited in Norma C. Bueno collection at the Herbarium of Western Paraná State University (UNOPA), Cascavel municipality, Brazil.

Type locality: Brazil, State of Paraná, Cascavel municipality, Cascavel River, 24°32' and 25°17'S; 53°05' and

53°50'W, epilithic samples, collected by G. Medeiros et al. on 03/08/2018.

Etymology: the specific epithet refers to the wide central area unilaterally reaching the margin, always interrupting the single row of mantle areolae.

Ecology: *Nupela semifasciata* was found in 46 of 204 epilithic samples analyzed, from which the average values of ecological parameters were calculated, considering the eight samplings performed. The new species occurred in streams with low flow (average of 0.24 m³.s⁻¹), low depth (average of 0.06 m), low conductivity (average of 0.05 mS.cm⁻¹), low to high nutrient concentrations (e.g., NO₃: 2.00–14.00 mg.l⁻¹), and low pH (average of 6.28) (Table 1). Other diatom taxa co-occurred in the samples, such as *Achnantheidium minutissimum* (Kützing) Czarnecki, *Humidophila contenta* (Grunow) Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot et Kopalová, *Sellaphora saugerresii* (Desmazières) Wetzel et Mann, *Sellaphora nigri* (De Notaris) Wetzel et Ector and some other unidentified species of *Eunotia* Ehrenberg and *Nupela* Vyverman et Compère.

DISCUSSION

Nupela semifasciata is characterized by elliptic-lanceolate valves containing subcapitate apices in larger individuals and broadly rostrate-rounded apices in smaller ones, while the length/width ratio gradually decreases as the individuals become smaller. The central area unilaterally reaches the margin and always interrupts the row of areolae on the valve mantle. The valve mantle is externally composed of a second row of areolae near the apices.

The new species shares morphological and morphometric similarities with some currently known species of the genus, but some features can be used to differentiate them (see Table 2). *N. deformis* Lange-Bertalot has deeper depressions along the apical axis, clearly visible in LM images, one valve with rudimentary raphe, and higher striae density (ca. 60 vs 40–48/10 µm) (LANGE-BERTALOT & MOSER 1994). *N. lesothensis* (Schoeman) Lange-Bertalot contains a considerably higher number of areolae in 10 µm (50–68 in SCHOEMAN 1973; 50–74 in SALA et al. 2014; 50–70 in KULIKOVSKIY et al. 2020 vs 39–59 in our study) and one valve with rudimentary raphe slits (SCHOEMAN 1973; SALA et al. 2014). Additionally, the central area does not always reach the valve margin (see SALA et al. 2014). The main features that differentiate *N. paludigena* (Scherer) Lange-Bertalot are the wider and often capitate apices, smaller central area, and proximal raphe ends, externally teardrop-shaped and internally t-shaped (LANGE-BERTALOT & MOSER 1994; SIVER et al. 2007). *N. tenuicephala* (Hustedt) Lange-Bertalot slightly resembles *N. semifasciata*, however,

it has dorsiventral valves with strongly capitate apices, the internal proximal raphe ends are hook-shaped, and the striae are generally higher in number (50–60 vs 40–48/10 µm), composed of wider areolae (LANGE-BERTALOT 1993). Lastly, *N. neotropica* Lange-Bertalot and *N. subpallavicinii* Metzeltin et Lange-Bertalot share the fascia that unilaterally reaches the valve margin, but in both taxa, the internal proximal raphe endings are hook-shaped (LANGE-BERTALOT & MOSER 1994; SALA et al. 2014, respectively). In addition, *N. subpallavicinii* has a radiate striae pattern along the whole valve (METZELTIN & LANGE-BERTALOT 1998), and *N. neotropica* has wider areolae (LANGE-BERTALOT & MOSER 1994).

Nupela wellneri (Lange-Bertalot) Lange-Bertalot, the most similar taxon, was first described in Germany as *Navicula wellneri* by LANGE-BERTALOT & KRAMMER (1987) and was later transferred to *Nupela* based on samples collected in the Andean region by RUMRICH et al. (2000). *Nupela semifasciata* and *Nupela wellneri* possess lanceolate valve outlines, one valve containing long raphe slits and the other valve containing shorter raphe slits, as well as a broad central area, expanded more pronouncedly to one side of the valve. However, in comparison to the protologue, the individuals found in our samples have broader variation in size (length: 6.7–16.4 vs 12–14 µm; width: 3.2–4.8 vs 4–4.8 µm), striae (40–48/10 µm vs ca. 45/10 µm) and areolae densities (39–59/10 µm vs ca. 50/10 µm). Previous studies registered *N. wellneri* with a larger range in size (8.5–15 µm long and 3–4.5 µm wide in POTAPOVA et al. 2003; 11.8–16.6 µm long and 3.7–4.4 µm wide in TREMARIN et al. 2015), striae (38–42/10 µm in POTAPOVA et al. 2003; ca. 42/10 µm in TREMARIN et al. 2015), and areolae (40–50/10 µm in TREMARIN et al. 2015). However, this species was not described and illustrated showing gradual length/width diminution associated with valve outline modification, as we observed in *N. semifasciata*. While in *N. semifasciata* the axial area varies from narrow to wide and the central area always extends onto the valve margin, LANGE-BERTALOT & KRAMMER (1987) originally described *N. wellneri* with broadly expanded axial and central areas, limited by very short striae. POTAPOVA et al. (2003), POTAPOVA (2010), and TREMARIN et al. (2015) illustrated individuals of *N. wellneri* similar to the protologue, but exemplars with a narrower axial area (Figs 76, 77; internal view; 137, 139, respectively) and the central area reaching the margin (Figs 78, 79 in POTAPOVA et al. 2003 and internal view in POTAPOVA 2010) are included, suggesting the close relationship of these specimens with *N. semifasciata*.

Considering the central area, its symmetry, shape, size, and extent are important morphological features within *Nupela* (see SIVER et al. 2007; WOJTAL 2009; BAHLS 2011; YU et al. 2017; GENKAL & YARUSHINA 2018; RYBAK et al. 2020). The central area unilaterally reaching the margin, consistently observed in *N.*

Table 2. Morphological information of *Nipela semifasciata* sp. nov. compared to similar taxa.

Species	<i>N. semifasciata</i>	<i>N. deformis</i>	<i>N. lesothensis</i>	<i>N. neotropica</i>	<i>N. paludigena</i>	<i>N. subpallavicinii</i>	<i>N. tenuicephala</i>	<i>N. wellheri</i>
Reference	Our study	LANGÉ-BERTALOT & MOSER (1994)	SCHOEMAN (1973) ¹ SALA et al. (2014) ²	LANGÉ-BERTALOT & MOSER (1994)	SVERER et al. (2007)	METZELTIN & LANGÉ-BERTALOT (1998) ¹ SALA et al. (2014) ²	HUSTEDT (1942) ¹ LANGÉ-BERTALOT (1993) ²	LANGÉ-BERTALOT & KRÄMMER (1987)
Length (µm)	6.7–16.4	11–18	5.3–13.5 ¹	12–18	10–20	11–16 ¹	11–15 ¹	12–14
Width (µm)	3.2–4.8	4–5.5	2.6–3.2 ¹	3–4	3–5	3.3–4.7 ¹	2.5–3 ¹	4–4.8
Length/width	1.7–3.7	n.d.	3–3.6 ²	n.d.	n.d.	n.d.	n.d.	n.d.
Striae in 10 µm	40–48	ca. 60	42–46 ¹	50–55	38–45	40–45 ¹	n.d.	45
Areolae in 10 µm	39–59	n.d.	50–68 ¹	n.d.	n.d.	28–37 ²	n.d.	50
Valve outline	Elliptical–lanceolate, with subcapitate to broadly rostrate–rounded apices	Elliptical–lanceolate, with rostrate apices	Elliptical–lanceolate, with capitate to broadly rounded apices ¹	Elliptical–lanceolate, with abruptly rostrate–protracted apices	Elliptical–lanceolate to linear–lanceolate, with rostrate to capitate apices	Elliptical, with capitate apices ¹	Linear, with small and capitate apices ¹	Lanceolate, with subcapitate–rounded protracted apices
Central area	Asymmetric, broadly rhombic to rounded, unilaterally reaching the mantle	Asymmetric, lanceolate, not reaching the mantle	Asymmetric, indistinct, unilaterally reaching the mantle ¹	Asymmetric ² , unilaterally reaching the mantle	Asymmetric ² , elliptical to rectangular, not reaching the mantle	Asymmetric ² , broadly triangular, unilaterally reaching the mantle ¹	Symmetric ² , indistinctly linear, unilaterally reaching the mantle ²	Asymmetric, broadly lanceolate, not reaching the mantle ²
Valve mantle	Single row of areolae, interrupted at center, becoming two rows near the apices	n.d.	Single row of areolae, interrupted near the apices ²	Single row of areolae, interrupted by the central area ²	Single row of areolae, not interrupted	Single row of areolae, interrupted at the apices and at the center ²	Single row of areolae, interrupted by the central area ²	n.d.
Raphe development	One valve with long raphe slits and the other valve with slightly shorter raphe slits	One valve with long raphe slits and the other valve with rudimentary raphe slits	One valve with long raphe slits and the other valve with rudimentary raphe slits ¹	Both valves with long raphe slits ²	Both valves with long raphe slits	At least one valve with long raphe slits ²	One valve with long raphe slits and the other valve with short raphe slits ² with “One valve with long raphe slits and the other valve with short raphe slits” ^{1,2}	Both valves with long raphe slits ²
External/internal proximal raphe ends	Simple or pore-like	Inconspicuous / n.d.	Slightly expanded ^{1,2}	Pore-like / n.d.	Tear-drop-shaped / T-shaped	Pore-like ¹ / Hook-shaped ²	Pore-like ² / Hook-shaped ²	Pore-like / Simple ²

*Personal observations based on cited literature. n.d.: no data.

semifasciata, is shared by some other *Nupela* taxa (see LANGE–BERTALOT 1993, 1999; LANGE–BERTALOT & MOSER 1994; METZELTIN & LANGE–BERTALOT 1998; SIVER & HAMILTON 2005; SIVER et al. 2007; POTAPOVA 2011; KULIKOVSKIY et al. 2015; TREMARIN et al. 2015; BAHLS 2017), indicating a well-established feature that should be considered for species level identification within this genus.

The mantle ornamentation of *N. semifasciata* highlights interesting morphological details. Firstly, the mantle partially lacks the row of transversely elongated areolae due to the central area that reaches the valve margin and extends toward the mantle. In fact, *Nupela* species with a central area expanded onto the margin are observed in *N. amabilis*, *N. torganiae* (see TREMARIN et al. 2015, fig 9 on page 81, and figs 124, 127 on page 89), *N. potapovae* (see BAHLS 2011, fig 14 on page 170 and figs 19, 21 on page 171), *N. elongata* and *N. vasta* (see KULIKOVSKIY et al. 2015, fig 10 on page 267, and fig 23 on page 271), but the row of areolae on the mantle is continuous, not interrupted as in *N. semifasciata*.

Further, the mantle is composed of a single row of elongated areolae throughout the whole valve, and a second row can be visualized near the apices. Taxa sharing similar morphological aspects were recently described by KULIKOVSKIY et al. (2015) based on samples from Lake Baikal, in south-eastern Siberia, Russia. *Nupela gomphosphenioides* Kulikovskiy et Lange–Bertalot possesses one or two areolae at the junction with the valve mantle and the second row appearing near the apices (Fig 24 on page 257). *N. neogracillima* subsp. *baicalensis* Kulikovskiy et Lange–Bertalot contains one or two areolae at the edge of the valve face and mantle, with the second row more evident in the median region of the valve (Figs 1, 2 on page 279). Despite that, those taxa appear to have areolae only at the junction between face and valve mantles, differing from *N. semifasciata*, in which the two areolae are always positioned in the mantle. Moreover, *N. potapovae* Bahls seems to have a second row of areolae near the apices on the valve mantle (BAHLS 2011, fig 16 on page 170), although the author has not pointed this out. Both of these aspects were not highlighted in the descriptions of *N. wellneri* provided by LANGE–BERTALOT & KRAMMER (1987), POTAPOVA et al. (2003), and TREMARIN et al. (2015), however, they represent important features to distinguish *Nupela* species.

Considering the striae, *N. wellneri* in LANGE–BERTALOT & KRAMMER (1987) has a radiate pattern throughout the valve, almost parallel in the apices, while POTAPOVA et al. (2003, figs 77–79 on page 304), POTAPOVA (2010, internal view), and TREMARIN et al. (2015, fig 139 on page 90) depicted some specimens with slightly convergent striae patterns near the apices. In *N. semifasciata*, the convergent pattern near the apices was quite common due to the occurrence of well-marked Voigt fault.

The proposition of the new species is supported by observations in our populations compared to descriptions of other *Nupela* taxa provided in the literature. Particularly, *Navicula wellneri* (= *Nupela wellneri*), which shares the closest morphological features, differs by the broadly expanded axial and central area limited by shortened striae, not reaching the valve margin, and by the radiate striae pattern along the valve. This contributes to support the idea that individuals sharing these same aspects, described as *Nupela wellneri* by POTAPOVA et al. (2003), POTAPOVA (2011), and TREMARIN et al. (2015), might be considered *Nupela semifasciata*. Furthermore, it is possible that the second row of areolae at the junction of the valve face and mantle, or even the second areolae positioned in the mantle, instead of a single row, appeared several times in *Nupela*, but further studies, especially those using molecular and phylogenetic techniques, are needed in order to clarify whether this feature represents a homoplasy. Considering that the number of *Nupela* taxa continues to increase, we suggest the central area unilaterally reaching the valve mantle, interrupting the row of areolae, and the second row of areolae on the valve mantle as morphological criteria that must be considered for species level identification within the genus *Nupela*.

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Taxonomy of epilithic diatoms and their teratological forms under the presence of metals in the surface sediment

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Abstract

Periphytic diatom species in eight sampling sites of the Cascavel River microbasin were taxonomically analyzed. The studied streams are located in a predominantly urban microbasin, with distroferic red latosol (rich in Fe and Al), being characterized by distinct metals, predominantly acidic pH, and high conductivity. Overall, 221 diatom species were identified at infrageneric level. *Pinnularia* was the most representative genus in number of species (28 spp.), followed by *Eunotia* (25 spp.), *Gomphonema* Ehrenberg (17 spp.), *Nitzschia* Hassall (14 spp.), and *Navicula* (11 spp.). This is the first record of *Fragilaria spectra*, *Fragilaria tenera* var. *nanana* and *Humidophila arcuatoides* for the state of Paraná, and the first record of *Pinnularia laucensis* in Brazil. We found teratological valves in 34 taxa, containing modified outline, changes in the striae pattern, and doubled central area. The genus *Eunotia* showed the highest number of altered taxa (8 spp.). Mixed teratologies (deformed valve outline + unusual striae pattern) were found only in *Ulnaria ulna*,

Encyonema neomesianum, and *Gomphonema graciledictum*. The metals and environmental variables in the Cascavel River microbasin probably contributed to the diatom teratologies. Our work contributes to the taxonomic knowledge of epilithic diatoms in the region, as well as extending the record of teratological taxa in the literature.

Keywords: Bacillariophyta, lotic environments, metals, morphology, periphyton, taxonomy, teratology

Introduction

Epilithic diatoms are photosynthetic microorganisms growing attached to rocky substrate (Townsend & Gell 2005, McGowan *et al.* 2018). The siliceous cell wall ornamentations are the basis for the group's taxonomy, as they allow the description and classification of individuals (Cavalier-Smith 2015, Schoefs *et al.* 2020). Taxonomic studies approaching diatom floras are crucial for recognizing environmental stresses and assessing the ecological status of aquatic systems (Morin *et al.* 2012, Olodo *et al.* 2019, Benito & Fritz 2020). This type of knowledge assists in decision-making for species conservation, especially in a scenario of constantly threatened biodiversity (Garnett & Christidis 2018, Azevedo-Santos *et al.* 2021).

The organic layer surrounding the diatom frustule contains metals that are essential for diatom metabolism (Sunda *et al.* 2005), preventing, for example, the loss of silica to the aquatic environment (Round *et al.* 1990). However, metals are potential stressors depending on their concentrations and under specific conditions (Falasco *et al.* 2009b, 2021). Diatom species from metal-polluted streams may lose diversity (da Silva *et al.* 2009, Barral-Fraga *et al.* 2016, Leguay *et al.* 2016, Pandey *et al.* 2018a), suffer physiological changes, such as in cytoplasmic content, lipid bodies number, and nuclear abnormalities (Morin *et al.* 2012, Gautam *et al.* 2017, Pandey *et al.* 2017), as well as undergo morphological changes involving community structure or individual scale (Cantonati *et al.* 2014, Lavoie *et al.* 2017, Olenici *et al.* 2017, Su *et al.* 2018, Falasco *et al.* 2021).

Establishing relationships between metal contamination and deformities in diatoms is difficult due to the various environmental and anthropogenic factors that are potential drivers of teratologies (Falasco *et al.* 2009a, Lavoie *et al.* 2017). Further, low frequency of deformed valves is considered natural in unpolluted environments (see Cattaneo *et al.* 2004, Lavoie *et al.* 2012, Morin *et al.* 2012, Pandey *et al.* 2014). Based on this, floristic studies provide an initial insight into aquatic systems, extending the recognition of deformities in diatoms, thus indicating possible metal contamination (Morin *et al.* 2012, Park *et al.* 2020, Falasco *et al.* 2021). Conducting prior taxonomic investigations of diatom assemblages is crucial in ecological studies of aquatic

environments, helping to identify the influence of metals on diatom abnormalities (see Yang & Duthie 1993, Szabó *et al.* 2005, Sgro *et al.* 2007, Tapia 2008, Falasco *et al.* 2009b, Ferreira da Silva *et al.* 2009, Pandey *et al.* 2018a). Although this type of approach is apparently becoming obsolete (Rimet 2012), the relationship between metals and teratologies in diatoms is underexplored in tropical environments as in Brazil. Detailed floristic studies would provide valuable information on aquatic systems for biomonitoring purposes using abnormalities in diatoms.

We conducted a taxonomic survey of epilithic diatoms from a hydrographic microbasin in southern Brazil, focusing on their teratological forms and characterizing the limnological conditions and metal concentrations where they were found.

Material & Methods

The Cascavel River is the principal source of water supply for the city of Cascavel, located in the Western Paraná State, and its tributaries are influenced by urbanized areas (FUNDETEC 1995). This subtropical region is characterized by the Cfa climate, with temperatures above 22 °C in the summer and around 30 mm of rain in the driest month (Alvares *et al.* 2013). The soil of the hydrographic microbasin is classified as Distrophic Red Latosol, with low fertility, high iron and aluminum concentrations, and usually clayey texture (dos Santos *et al.* 2018). Regarding trace metals, high bioavailability of Pb, Cu, and Mn have already been registered for the initial portion of the microbasin, at Paulo Gorski Lake (Remor *et al.* 2018).

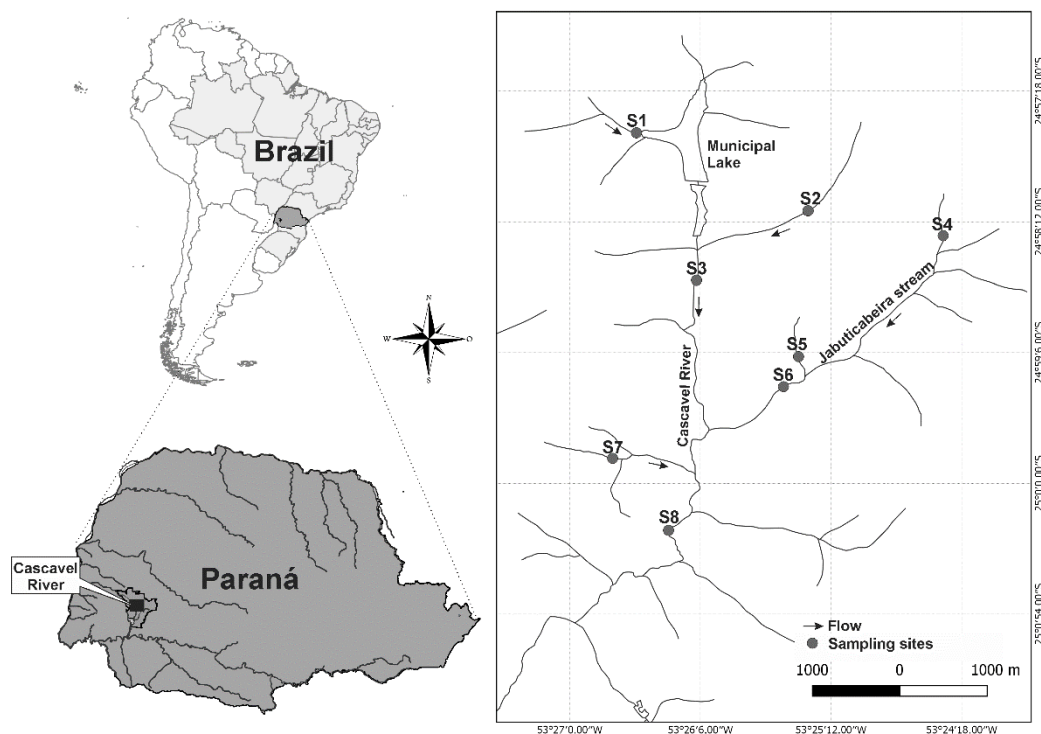


FIGURE 1. Location of the eight sampling sites (S1–S8) in the Cascavel River microbasin, Paraná State, Southern Brazil.

We analyzed the epilithic diatom flora collected from eight streams (Fig. 1), three of them located in the main channel of the Cascavel River, and five tributaries (see Table 1 in Appendix A for description of sampling sites), following the direction upstream to downstream of the river.

We performed 12 seasonal samplings from 2016 to 2019. Initially, we collected 60 rocky substrates at S1, S3 and S8, 10 per sampling site, during autumn and spring 2016 (see details in Medeiros *et al.* 2020). Starting in 2017, we have additionally collected 240 stones in triplicates at the eight sampling sites. A total of 300 qualitative samples were collected (Appendix B). The biofilm containing the diatoms was scraped from all the stones using a toothbrush and distilled water, and stored in *Transeau* 1:1 solution (Bicudo & Menezes 2017) at the Universidade Estadual do Oeste do Paraná Herbarium (UNOPA).

A multiparameter probe (HORIBA U-5000) was used for *in situ* measuring of water temperature (Temp), electrical conductivity (Cond), dissolved oxygen (DO), pH, and turbidity (Turb). We also collected one water sample per sampling site, using polyethylene bottles immersed in the subsurface of the water column. These samples were kept in the dark and cooled, and subsequently analyzed to verify the physical, chemical, and biological properties of the water (Chlorophyll *a*: CLA; Total coliforms: TC; Biochemical Oxygen Demand: BOD; Chemical Oxygen Demand: COD; *Escherichia coli*: *E. coli*; Ammoniacal nitrogen: N-NH₃; Nitrate: NO₃; pH; Orthophosphate: PO₄⁻; Total phosphorus: TP; Dissolved solids: DS; Total solids: TS) (APHA 2012). The depth was manually measured by sinking a ruler to the bottom of the river. The flow was obtained by multiplying the length and the average speed of the displacement of a polystyrene object along a virtual transect in the river. The precipitation data was provided by the Paraná Meteorological System (SIMEPAR) (See Table 1 in Appendix C).

Three surface sediment samples were collected at each sampling site to form a composite sample, from which metals were extracted following CETESB & ANA (2011). Aluminum (Al) barium (Ba) cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), Iron (Fe) lead (Pb), manganese (Mn), nickel (Ni), and zinc (Zn) were read (See table 1 in Appendix D), according to U.S. Environmental Protection Agency 3050B method (USEPA 1996).

Ecological variables and metals were submitted to principal component analysis (PCA) aiming to characterize the sampling stations, as well as identify the variables that best differentiate them (Wiegand, 1980).

Permanent slides were prepared with subsamples oxidized according to Simonsen (1974), modified by Moreira-Filho & Valente-Moreira (1981). Light microscopy (LM) analyzes were performed at 1000× magnification, using an Olympus BX34 microscope equipped with a DP 71 capture camera and phase-contrast. Cleaned samples were also placed in aluminum stubs, sputter-

coated with gold in Balzers Union SCD 030, and examined in Scanning Electron Microscopy (SEM) with JEOL JSM 6360LV, operated at 15–20 kV and a working distance of 5–8 mm.

We provide morphometric and meristic data, occurrences at sampling sites, and literature used for taxa identification (Table 1). Photomicrographs of identified taxa are provided in optical (Figs 3–11) and scanning electron microscopy (Figs 12–16). In taxa showing teratologies, we described the morphological changes, as well as the ecological characteristics of the environment where abnormal individuals were found. New records were commented, and are accompanied by asterisk symbols, one for the Paraná State (*) and two for Brazil (**). First records were considered when the species was not previously recorded in published taxonomic articles, with measurements and photomicrographs.

Results

We identified 221 infrageneric taxa (Table 1), belonging to 52 genera. Regarding species richness, *Pinnularia* Ehrenberg was the most representative genus (28 spp.), followed by *Eunotia* Ehrenberg (25 spp.), *Gomphonema* Ehrenberg (17 spp.), *Nitzschia* Hassall (14 spp.), and *Navicula* (11 spp.). *Fragilaria spectra*, *Fragilaria tenera* var. *nanana* and *Humidophila arcuatooides* represent new records for the state of Paraná, and *Pinnularia laucensis* represent a first record for Brazil.

TABLE 1. Epilithic diatom species identified from Cascavel River microbasin. Morphological measurements, occurrences (UNOPA), and consulted literature. (L: length; W: width; L/W: length/width ratio; D: diameter; MH: mantle height; S: striae; DS: dorsal striae; VS: ventral striae; TS: transapical striae; LS: longitudinal striae; AC: alar channels; F: fibulae; A: areolae).

Taxa	Figures	Morphometric (μm) and meristic (in 10 μm) data	Occurrence	Followed literature
STEPHANODISCACEAE				
<i>Cyclotella distinguenda</i> Hustedt	2A	D: 15–18.1; S: 12–17 (n= 8)	S3, S8	Houk & Klee (2010)
<i>C. kingstonii</i> Julius, Downey, Theriot, et al.	2E; 11A	D: 8.3–22.4; S: 17–21; A: 18–21 (n= 83)	S1, S3, S4, S5, S8	Johansen <i>et al.</i> (2008) Downey <i>et al.</i> (2021)
<i>Discostella stelligera</i> (Cleve & Grunow) Houk & Klee	2B–2D; 11B–11C	D: 6.3–16.7; S: 12–17 (n= 268)	S1, S2, S3, S4, S5, S6, S7, S8	Houk & Klee (2004)
MELOSIRACEAE				
<i>Melosira varians</i> Agardh	2F	D: 10.1–20.7 (n= 25)	S2, S3, S4, S5, S8	Krammer & Lange-Bertalot (1991)
ORTHOSEIRACEAE				
<i>Orthoseira rooseana</i> (Rabenhorst) Pfitzer	2G	D: 11.6–16.4; S: 12–14; A: 11–14 (n= 5)	S1, S2, S5	Krammer & Lange-Bertalot (1991)
AULACOSEIRACEAE				
<i>Aulacoseira ambigua</i> (Grunow) Simonsen	2H; 11D	D: 4.3–13.9; MH: 4.5–13.2; S: 14–20; A: 12–20 (n= 157)	S1, S3, S4, S8	Tremarin <i>et al.</i> (2013)
<i>A. brasiliensis</i> Tremarin, Torgan & Ludwig	2I	D: 11–11.1; MH: 5.9–6.4; S: 13–14; A: 11–12 (n= 2)	S3	Tremarin <i>et al.</i> (2012)
<i>A. granulata</i> var. <i>angustissima</i> (Müller) Simonsen	2J	D: 2.5–3.3; MH: 9.2–17.5; S: 12–19; A: 14–20 (n= 40)	S2, S3, S4, S8	Krammer & Lange-Bertalot (1991)
<i>A. granulata</i> (Ehrenberg) Simonsen	2K	D: 4.4–4.6; MH: 9.9–14.5; S: 12–14; A: 10–14 (n= 5)	S3, S6, S8	Krammer & Lange-Bertalot (1991)
<i>A. pusilla</i> (Meister) Tuji & Houki	2L–2N; 11G	D: 4.5–6.6; MH: 2.3–3.8; A: 17–20 (n= 95)	S3, S4, S8	Tuji & Houki (2004)
<i>A. tenella</i> (Nygaard) Simonsen	2O–2Q; 11E–11F	D: 5.7–6.9; MH: 3.6–4.3; S: 19–26 (n= 95)	S1, S3, S4, S5, S8	Siver & Kling (1997) Camburn & Charles (2000)
FRAGILARIACEAE				
<i>Fragilaria fragilarioides</i> (Grunow) Cholnoky	2R–2T	L: 16.9–47.2; W: 4.3–6; S: 10–12 (n= 22)	S6, S8	Cholnoky (1963) Ludwig & Flóres (1997)
<i>F. gracilis</i> Østrup	2U–2AA; 11H–11I	L: 22.6–45.9; W: 2–3.2; S: 18–22 (n= 82)	S1, S3, S4, S8	Tuji (2007) Lange-Bertalot & Ulrich (2014)
<i>F. pectinalis</i> (Müller) Lyngbye	2AB–2AH; 11J–11K	L: 25.9–31.1; W: 2.7–3.6; S: 15–16; A: 57–61 (n= 97)	S1, S3, S4, S6, S8	Tuji & Williams (2008) Wetzel & Ector (2015)
<i>F. spectra</i> Almeida, Morales, Wetzel, Ector & Bicudo	2AP–2AR	L: 42.7–70.1; W: 1.8–3.1; S: 19–24 (n= 12)	S3, S8	Almeida <i>et al.</i> (2016)

Taxa	Figures	Morphometric (μm) and meristic (in 10 μm) data	Occurrence	Followed literature
<i>F. tenera</i> var. <i>tenera</i> (Smith) Lange-Bertalot	2AI–2AK	L: 39.9–66.7; 2.5–2.9; S: 15–17 (n= 44)	S1, S3, S6, S8	Lange-Bertalot & Ulrich (2014)
<i>F. tenera</i> var. <i>nanana</i> (Lange-Bertalot) Lange-Bertalot & Ulrich	2L–2AO	L: 45.1–67.1; W: 1.9–3.1; S: 20–22 (n= 30)	S3, S4, S6, S7, S8	Lange-Bertalot & Ulrich (2014)
<i>Fragilariforma javanica</i> (Hustedt) Wetzel, Morales & Ector	2AS–2AT	L: 20.5–95.9; W: 5.2–6.4; S: 19–20 (n= 39)	S1, S4, S5	Metzeltin & Lange-Bertalot (1998) Wetzel <i>et al.</i> (2013)
<i>Ulnaria contracta</i> (Østrup) Morales & Vis	2AU	L: 38.8–122.2; W: 6.7–8.9; S: 9–11 (n= 30)	S3, S8	Patrick & Reimer (1966) Morales & Vis (2007)
<i>U. delicatissima</i> (W.Smith) Aboal & Silva	2AV	L: 159.5–233.2; W: 4–5.5; S: 10–12 (n= 4)	S3, S6, S8	Tuji & Houki (2004)
<i>U. ulna</i> (Nitzsch) Compère	2AW–2AZ	L: 56.9–301.5; W: 5–8.5; S: 9–11 (n= 106)	S1, S2, S3, S4, S6, S7, S8	Patrick & Reimer (1966) Lange-Bertalot & Ulrich (2014)
EUNOTIACEAE				
<i>Actinella hermes-moreirae</i> Ruwer, Ludwig & Rodrigues	3A–3C	L: 15.8–49.6; W: 2.9–3.8; S: 15–18 (n= 43)	S2, S5, S7, S8	Ruwer <i>et al.</i> (2019)
<i>Eunotia bilunaris</i> (Ehrenberg) Schaarschmidt	3D–3J; 12A	L: 15.4–123; W: 2.9–4.3; S: 14–18; A: 42–44 (n= 353)	S1, S2, S3, S4, S5, S6, S7, S8	Lange-Bertalot <i>et al.</i> (2011) Costa <i>et al.</i> (2017a)
<i>E. botulitropica</i> Wetzel & Costa	3K–3P; 12B–12C	L: 9.8–32.9; W: 2.4–3.5; S: 13–18; A: 38–44 (n= 481)	S1, S2, S3, S4, S5, S6, S7, S8	Costa <i>et al.</i> (2017a)
<i>E. canicula</i> Furey, Lowe & Johansen	3Q–3R	L: 32.2–47.3; W: 3.9–4.8; S: 12–16 (n= 7)	S3, S5, S8	Furey <i>et al.</i> (2011)
<i>E. desmogonioides</i> Metzeltin & Lange-Bertalot	3S–3T; 12F–12G	L: 58.5–144.7; W: 3.4–6.6; S: 15–17; A: 36–37 (n= 49)	S1, S3, S8	Metzeltin & Lange-Bertalot (2002) Costa <i>et al.</i> (2017a)
<i>E. fallax</i> Cleve	3Y–3Z	L: 14.5–31.8; W: 2.4–3.1; S: 15–19 (n= 14)	S1, S3, S4, S5, S6, S7	Lange-Bertalot <i>et al.</i> (2011)
<i>E. formica</i> Ehrenberg	3AA	L: 42.7; W: 9.6; S: 9 (n= 1)	S1	Costa <i>et al.</i> (2017a)
<i>E. formicina</i> Lange-Bertalot	3AB	L: 86.3–116.2; W: 7.6–9.3; S: 8–10 (n= 5)	S1, S4, S7	Costa <i>et al.</i> (2017a)
<i>E. georgii</i> Metzeltin & Lange-Bertalot	3AY; 12H	L: 28.7–92.4; W: 6.8–9.9; S: 10–14; A: 28–29; O: 15–20 (n= 58)	S1, S2, S4, S5, S6, S7, S8	Costa <i>et al.</i> (2017a)
<i>E. intricans</i> Lange-Bertalot & Metzeltin	3AD	L: 23.3–49; W: 4.1–5.4; S: 11–15 (n= 52)	S1, S2, S3, S4, S5, S6, S8	Costa <i>et al.</i> (2017a)
<i>E. juettnerae</i> Lange-Bertalot	3AH–3AJ	L: 25.6–41.9; W: 2.2–3.2; S: 15–21 (n= 17)	S3, S8	Lange-Bertalot <i>et al.</i> (2011) Costa <i>et al.</i> (2017a)
<i>E. karenae</i> Metzeltin & Lange-Bertalot	3AC	L: 97.4–128.2; W: 8.4; S: 8–10 (n= 5)	S1, S6	Costa <i>et al.</i> (2017a)
<i>E. kruegeri</i> Lange-Bertalot	3AK–3AM	L: 12.1–19.2; W: 2.4–3.2; S: 18–24 (n= 57)	S1, S2, S3, S4, S5, S6, S8	Costa <i>et al.</i> (2017a)
<i>E. longicamelus</i> Costa, Bicudo & Wetzel	3AE	L: 25.5–49; W: 6.2–8.2; S: 10–12 (n= 10)	S1, S3, S8	Costa <i>et al.</i> (2017a)

Taxa	Figures	Morphometric (μm) and meristic (in 10 μm) data	Occurrence	Followed literature
<i>E. meridiana</i> Metzeltin & Lange-Bertalot	3AR–3AX	L: 14.5–34.9; W: 4.2–6.6; S: 11–16; A: 39 (n= 179)	S1, S2, S3, S4, S5, S6, S7, S8	Metzeltin & Lange-Bertalot (1998) Costa <i>et al.</i> (2017a)
<i>E. monodon</i> Ehrenberg	3AP–3AQ	L: 37.2–120.9; W: 8.6–10; S: 8–11 (n= 36)	S1, S2, S3, S6, S7, S8	Lange-Bertalot <i>et al.</i> (2011) Costa <i>et al.</i> (2017a)
<i>E. muscicola</i> Krasske	3AN–3AO	L: 11.7–17.9; W: 3.1–3.8; S: 20–25 (n= 21)	S3, S6, S8	Lange-Bertalot <i>et al.</i> (2011) Costa <i>et al.</i> (2017a)
<i>E. ossicula</i> (Metzeltin & Lange-Bertalot) Costa	4A	L: 111.8; W: 8.6–12.3; S: 14–17 (n= 7)	S1, S3, S8	Costa <i>et al.</i> (2017a)
<i>E. rabenhorstii</i> var. <i>monodon</i> Cleve & Grunow	3AF	L: 16.5–24.5; W: 5.9–7.3; S: 12–16 (n= 11)	S3, S6, S8	Costa <i>et al.</i> (2017a)
<i>E. rabenhorstii</i> var. <i>triodon</i> Cleve & Grunow	3AG	L: 22.8–26; W: 6.2–7.2; S: 12–15 (n= 8)	S1, S3	Costa <i>et al.</i> (2017a)
<i>E. rhomboidea</i> Hustedt	4B–4G; 12D–12E	L: 12.4–45.3; W: 4.1–5.6; S: 11–16 (n= 287)	S1, S2, S3, S4, S5, S6, S7, S8	Costa <i>et al.</i> (2017a)
<i>E. subarcuatooides</i> Alles, Nörpel & Lange-Bertalot	3U–3X	L: 8.3–19.3; W: 2.5–4.3; S: 21–23 (n= 24)	S1, S3, S4, S5, S6, S8	Costa <i>et al.</i> (2017a)
<i>E. tropico-arcus</i> Metzeltin & Lange-Bertalot	4H	L: 28.8–53.5; W: 6.3–9; S: 10–14 (n= 8)	S5, S7	Metzeltin & Lange-Bertalot (2007) Costa <i>et al.</i> (2017a)
<i>E. veneris</i> (Kützing) De Toni	4I–4K	L: 18.2–53.1; W: 4.4–6.2; S: 15–19 (n= 30)	S1, S2, S3, S5, S8	Lange-Bertalot <i>et al.</i> (2011) Costa <i>et al.</i> (2017a)
<i>E. yanomami</i> Metzeltin & Lange-Bertalot	4L	L: 20.1–113.9; W: 12.3–22.6; S: 11–13; A: 22–24 (n= 35)	S1, S3, S5, S6, S7	Metzeltin & Lange-Bertalot (1998) Costa <i>et al.</i> (2017a)
<i>E. yberai</i> Frenguelli	4M–4P	L: 20.7–89.2; W: 6.2–10.7; E: 6–10 (n= 52)	S1, S3, S4, S8	Costa <i>et al.</i> (2017a)
CYMBELLACEAE				
<i>Cymbopleura naviculiformis</i> (Auerswald) Krammer	4Q	L: 25.4–34.4; W: 7.1–9; L/W: 3.6–4.3; S: 13–17 (n= 83)	S1, S3, S4, S6, S8	Krammer (2003)
<i>Encyonema angustecapitatum</i> Krammer	4R–4S	L: 11.4–25; W: 4.1–5.2; S: 13–15 (n= 9)	S1, S4, S8	Rumrich <i>et al.</i> (2000) Vouilloud <i>et al.</i> (2010)
<i>E. exuberans</i> Tremarin, Wetzel & Ludwig	4AC–4AE	L: 33.5–39.8; W: 7.3–10; L/W: 3.7–5.2; DS: 7–9; VS: 11–13 (n= 34)	S8	Tremarin <i>et al.</i> (2011)
<i>E. minutiforme</i> Krammer	4AB	L: 16.1–23; W: 4–5.7; S: 10–12 (n= 23)	S3, S8	Krammer (1997a)
<i>E. neogracile</i> Krammer	4U	L: 17.2–40.6; W: 4.5–7.1; L/W: 3.6–6.1 S: 12–16 (n= 64)	S1, S3, S4, S8	Krammer (1997a)
<i>E. neomesianum</i> Krammer	4V–4X	L: 21.1–41.8; W: 5.6–8.5; S: 10–12 (14) (n= 54)	S3, S8	Krammer (1997a)
<i>E. perpusillum</i> (Cleve-Euler) Mann	4T	L: 14.4–21.7; W: 4–5.4; S: 9–13 (n= 102)	S3, S6, S8	Krammer (1997a)
<i>E. silesiacum</i> (Bleisch) Mann	4AY–4AA	L: 18.3–35.5; W: 6.8–8.3; S: 9–12 (n= 146)	S1, S2, S3, S4, S5, S6, S7, S8	Krammer (1997a)

Taxa	Figures	Morphometric (μm) and meristic (in 10 μm) data	Occurrence	Followed literature
<i>Geissleria punctifera</i> (Hustedt) Metzeltin, Lange-Bertalot & García-Rodríguez	4AT–4AU; 12I	L: 12.5–26.4; W: 5.6–7.1; S: 16–18; A: 48 (n= 83)	S1, S3, S4, S8	Metzeltin <i>et al.</i> (2005)
<i>Navigeia ignota</i> (Krasske) Bukhtiyarova	4AF–4AH	L: 11.1–19.4; W: 4.7–5.2; S: 15–16 (n= 9)	S1, S2, S3, S7	Bukhtiyarova & Pomazkina (2013)
<i>Placogeia kriegeri</i> (Krasske) Bukhtiyarova	4AI–4AK	L: 15–20.4; W: 6.8–8; S: 16–19 (n= 4)	S3, S8	Lange-Bertalot & Metzeltin (1996)
GOMPHONEMATACEAE				
<i>Encyonopsis difficilis</i> (Krasske) Krammer	4AN–4AO	L: 20–27.4; W: 4.7–6.2; S: 9–10 (n= 7)	S1, S3, S6, S8	Krammer (1997b) Marquardt <i>et al.</i> (2014)
<i>E. schubartii</i> (Hustedt) Krammer	4AP	L: 29.2–31.9; W: 5.9–7.5; R L/W: 4.2–5; S: 9–12 (n= 17)	S3, S4	Krammer (1997b)
<i>E. subminuta</i> Krammer & Reichardt	4AL–4AM	L: 18.1–19.5; W: 4.1–4.2; L/W: 4.3–4.8; S: 23–26 (n= 8)	S3	Krammer (1997b)
<i>E. thienemannii</i> (Hustedt) Krammer	4AQ–4AS	L: 16.9–18.1; W: 4.1–4.2; L/W: 4.1–4.3; E: 23–25 (n= 3)	S3	Krammer (1997b)
<i>Kurtkrammeria spicula</i> (Hustedt) Ohtsuka	4AV	L: 33.1–56.5; W: 5.9–7.2; L/W: 5.5–8.2; S: 15–17 (n= 31)	S3, S4	Ohtsuka (2018)
<i>Gomphonema affine</i> Kützing	5A–5B	L: 39.9–54.9; W: 7.4–7.7; S: 11–12 (n= 4)	S3, S8	Reichardt (1999)
<i>G. angustatum</i> (Kützing) Rabenhorst	5C–5H	L: 17.9–40.9; W: 4.6–6.6; S: 13–17 (n= 172)	S1, S2, S3, S4, S5, S6, S7, S8	Rumrich <i>et al.</i> (2000) Metzeltin <i>et al.</i> (2005)
<i>G. brasiliense</i> Grunow	5I	L: 17.3–34.5; W: 4.4–6.8; S: 11–15 (n= 28)	S3, S6, S8	Medeiros <i>et al.</i> (2018)
<i>G. brasiliense</i> subsp. <i>pacificum</i> Moser, Lange-Bertalot & Metzeltin	5J–5K	L: 19.8–34.3; W: 4.1–5.8; S: 12–14 (n= 5)	S3, S8	Medeiros <i>et al.</i> (2018)
<i>G. brasiliense</i> Metzeltin, Lange-Bertalot & García-Rodríguez	5L	L: 29.6–36.1; W: 6.6–6.9; S: 11–12 (n= 8)	S6, S8	Metzeltin <i>et al.</i> (2005) Medeiros <i>et al.</i> (2018)
<i>G. exilissimum</i> (Grunow) Lange-Bertalot & Reichardt	5M–4O	L: 18.4–31.4; W: 4.5–6.1; S: 12–16 (n= 53)	S1, S2, S3, S4, S5, S6, S7, S8	Jüttner <i>et al.</i> (2013)
<i>G. frequentiformis</i> (Metzeltin & Krammer) Wetzel & Almeida	5S	L: 41.9–48.7; W: 8.9–10.1; S: 11–14 (n= 2)	S3, S8	Metzeltin & Lange-Bertalot (1998)
<i>G. graciledictum</i> Reichardt	5T–5Y	L: 24.3–67.7; W: 5.6–8.4; S: 11–15 (n= 222)	S1, S3, S4, S6, S8	Reichardt (2015)
<i>G. guaraniarum</i> Metzeltin & Lange-Bertalot	5AC	L: 44.5–56.9; W: 7.2–9.7; S: 11–14; A: 23–27 (n= 48)	S1, S2, S3, S6, S8	Metzeltin & Lange-Bertalot (2007)
<i>G. hawaiiense</i> Reichardt	5AA–5AB	L: 32.7–50.8; W: 5.8–8.5; S: 12–16 (n= 15)	S2, S3, S6, S8	Reichardt (2005)
<i>G. lagenula</i> Kützing	5AN–5AS; 12J	L: 9–26.8; W: 4.8–7.2; S: 13–18; A: 32–34 (n= 400)	S1, S2, S3, S4, S5, S6, S7, S8	Metzeltin & Lange-Bertalot (1998)

Taxa	Figures	Morphometric (μm) and meristic (in 10 μm) data	Occurrence	Followed literature
<i>G. naviculoides</i> Smith	5AT–5AU	L: 32.2–59.8; W: 7.7–10.6; S: 12–14; A: 23–26 (n=128)	S1, S2, S3, S4, S6, S8	Reichardt (2015) Levkov <i>et al.</i> (2016)
<i>G. parvulum</i> (Kützing) Kützing	5P–5R	L: 11.5–23.2; W: 4.7–6.2; S: 13–17 (n=430)	S1, S2, S3, S4, S5, S6, S7, S8	Abarca <i>et al.</i> (2014) Levkov <i>et al.</i> (2016)
<i>G. perapicatum</i> Metzeltin & Lange-Bertalot	5Z	L: 50.9; W: 11.7; S: 9 (n=1)	S6	Metzeltin & Lange-Bertalot (2007)
<i>G. pseudoaugur</i> Lange-Bertalot	5AG–5AI	L: 22.4–38.1; W: 7.4–8.7; S: 12–15 (n=58)	S1, S2, S3, S4, S6, S8	Krammer & Lange-Bertalot (1986)
<i>G. pumilum</i> (Grunow) Reichardt & Lange-Bertalot	5AJ–5AM; 12K–12L	L: 9.6–26.3; W: 3.4–4.5; S: 11–16; A: 47–49 (n=104)	S1, S2, S3, S4, S6, S8	Reichardt (1997)
<i>G. saphrophilum</i> (Lange-Bertalot & Reichardt) Abarca, Jahn, Zimmermann & Enke	5AD–5AF	L: 15.2–31; W: 4.8–7.5; S: 12–17 (n=58)	S1, S3, S4, S6, S8	Abarca <i>et al.</i> (2014)
<i>Placoneis elginensis</i> (Gregory) Cox	6A	L: 21.2; W: 7.7; S: 14 (n=1)	S3	Cox (2003)
<i>P. hambergii</i> (Hustedt) Bruder	6B	L: 12.2–23.2; W: 5.9–7.9; S: 14–17 (n=45)	S1, S2, S3, S4, S5, S6, S7, S8	Bruder & Medlin (2007)
<i>P. undulata</i> (Østrup) Lange-Bertalot	6C	L: 19.5–24.7; W: 6.1–8.2; S: 14 (n=3)	S3, S8	Rumrich <i>et al.</i> (2000)
ACHNANTHIDIACEAE				
<i>Achnanthes inflata</i> (Kützing) Grunow	6D	L: 58.8; W: 16.5; S: 11–12; A: 12–13 (n=1)	S4	Patrick & Reimer (1966)
<i>Achnantheidium caledonicum</i> Lange-Bertalot	6E–6G; 13A	L: 16–23.9; W: 2.9–3.8; S: 26–30; A: 47–49 (n=21)	S3, S6, S8	Wojtal <i>et al.</i> (2011)
<i>A. macrocephalum</i> (Hustedt) Round & Bukhtiyarova	6H–6P; 13B	L: 8.7–14.6; W: 2.5–3.7; S: 28; A: 46 (n=59)	S1, S3, S4, S6, S7, S8	Potapova & Hamilton (2007) Morales <i>et al.</i> (2011)
<i>A. minutissimum</i> (Kützing) Czarnecki	6S–6Z; 13C–13E	L: 5.9–17.7; W: 2–3.8; S: 24–34; A: 40–56 (n=471)	S1, S2, S3, S4, S5, S6, S7, S8	Krammer & Lange-Bertalot (1991) Potapova & Hamilton (2007)
<i>A. modestiforme</i> (Lange-Bertalot) Van de Vijver	6AD–6AH	L: 6–14.1; W: 3.5–5; S: 19–24 (n=91)	S1, S2, S3, S4, S5, S6, S7, S8	Morales <i>et al.</i> (2011)
<i>A. tropicocatenatum</i> Marquardt, Wetzel & Ector	6Q–6T; 13F–13G	L: 12–18.1; W: 2.8–4; S: 35–38; A: 44–50 (n=88)	S2, S3, S4, S6, S8	Marquardt <i>et al.</i> (2017)
<i>Gogorevia constricta</i> (Torka) Kulikovskiy & Kociolek	6I–6J	L: 10.5–16.3; W: 4.4–6.8; S: 19–22 (n=32)	S1, S8	Kulikovskiy <i>et al.</i> (2020)
<i>Planothidium bagualensis</i> Wetzel & Ector	6AK	L: 13.9–21.2; W: 6.4–8.1; S: 11–15 (n=4)	S1, S8	Wetzel <i>et al.</i> (2014)
<i>P. frequentissimum</i> (Lange-Bertalot) Lange-Bertalot	6AQ–6AR	L: 7.2–16.1–16.6; W: 3.7–5.6; S: 14–17 (n=17)	S1, S2, S3, S4, S7, S8	Lange-Bertalot (1999) Jahn <i>et al.</i> (2017)
<i>P. lagerheimii</i> (Cleve) Wetzel & Ector	6AL	L: 24.7; W: 12.6; S: 8 (n=1)	S8	Wetzel <i>et al.</i> (2014)
<i>P. rostratum</i> (Østrup) Round & Bukhtiyarova	6AM–6AP	L: 13.2–14.4; W: 5.2–5.8; S: 14–15 (n=8)	S3, S8	Round & Bukhtiyarova (1996) Krammer & Lange-Bertalot (1991)

Taxa	Figures	Morphometric (μm) and meristic (in 10 μm) data	Occurrence	Followed literature
<i>Psammothidium hustedtii</i> (Krasske) Mayama	6AS–6AT	L: 10.1–14.9; W: 4.5–6.7; S: 16–22 (n= 80)	S1, S2, S5, S6, S7, S8	Hofmann <i>et al.</i> (2013) Mayama <i>et al.</i> (2002)
COCCONEIDACEAE				
<i>Cocconeis fluviatilis</i> Wallace	6AAD	L: 19.4–19.7; W: 12.3–12.4; S: 12–16; A: 9–10 (n= 2)	S3, S8	Patrick & Reimer (1966)
<i>C. lineata</i> Ehrenberg	6AAE	L: 16.9–24.7; W: 11.4–12.5; S: 24–25; A: 11–16 (n= 3)	S2, S3, S8	Patrick & Reimer (1966) Krammer & Lange-Bertalot (1991b)
DIADESMIDACEAE				
<i>Humidophila arcuatoides</i> Lange-Bertalot	6AU–6AV	L: 12.1–14.6; W: 2.9–3.2 (n= 2)	S2, S8	Lange-Bertalot (2004) Lowe <i>et al.</i> (2014)
<i>H. contenta</i> (Grunow) Lowe et al.	6AX–6AZ	L: 7.2–15.7; W: 2.1–3.3 (n= 115)	S1, S2, S3, S4, S5, S6, S7, S8	Metzeltin & Lange-Bertalot (2007) Lowe <i>et al.</i> (2014)
<i>H. implicata</i> (Moser, Lange-Bertalot & Metzeltin) Lowe et al.	6AW	L: 9.4–13.1; W: 2.8–3.5 (n= 4)	S1, S5, S6	Lowe <i>et al.</i> (2014)
<i>H. subtropica</i> (Metzeltin, Lange-Bertalot & García-Rodríguez) Lowe et al.	6AAA–6AAC	L: 9.6–15.8; W: 2.3–3.2 (n= 42)	S1, S2, S3, S4, S6, S7, S8	Lowe <i>et al.</i> (2014)
<i>Luticola charlatii</i> (Peragallo) Metzeltin & Lange-Bertalot	6AAH	L: 37.6–39; W: 10.6–12.1; S: 12–15; A: 12 (n= 2)	S1, S7	Metzeltin <i>et al.</i> (2005)
<i>L. ectorii</i> Levkov, Metzeltin & Pavlov	6AAF; 13I	L: 11.3–17.7; W: 5.2–7.1; S: 20–27; A: 26–28 (n= 20)	S1, S3, S8	Levkov <i>et al.</i> (2013)
<i>L. goeppertiana</i> (Bleisch) Mann	6AAL–6AAN	L: 10.3–34.8; W: 4.8–8.8; S: 18–22 (n= 68)	S2, S3, S4, S5, S6, S8	Levkov <i>et al.</i> (2013)
<i>L. hustedtii</i> Levkov, Metzeltin & Pavlov	6AAG	L: 15.6–22.4; W: 7–7.8; S: 22–24 (n= 10)	S1, S3, S4, S5, S6, S8	Levkov <i>et al.</i> (2013)
<i>L. permuticoides</i> Metzeltin & Lange-Bertalot	6AAI–AAJ	L: 11.2–18.2; W: 6.3–8.7; S: 21–25 (n= 26)	S1, S2, S3, S4, S5, S6, S7, S8	Levkov <i>et al.</i> (2013)
<i>L. ventricosa</i> (Kützing) Mann	6AAK	L: 17.5; W: 6; S: 18 (n= 1)	S1	Levkov <i>et al.</i> (2013)
AMPHIPLEURACEAE				
<i>Amphipleura lindheimeri</i> Grunow	7A	L: 175–194; W: 28–29 (n= 4)	S8	Metzeltin <i>et al.</i> (2005)
<i>Frustulia acidophilissima</i> Wydrzycka & Lange-Bertalot	7B	L: 39.2–57.4; W: 11.2–14.6; TS: 27–30; LS: 28–30 (n= 27)	S2, S3, S4, S5, S6, S7, S8	Metzeltin & Lange-Bertalot (2007)
<i>F. australocrassinervia</i> Casa, Mataloni & Van de Vijver	7C; 13K–13L	L: 25–61.2; W: 7.7–11.8; TS: 28–35; LS: 28–37 (n= 223)	S1, S2, S3, S4, S5, S6, S7, S8	Casa <i>et al.</i> (2018)
<i>F. guayanensis</i> Metzeltin & Lange Bertalot	7D	L: 36.9–56.1; W: 8.8–9.2 (n= 4)	S3, S5, S8	Metzeltin & Lange-Bertalot (1998)
<i>F. neomundana</i> Lange-Bertalot & Rumrich	7E; 13M	L: 29.2–39.7; W: 6.4–9.1; S: 36; A: 31 (n= 64)	S1, S2, S3, S4, S5, S6, S7, S8	Rumrich <i>et al.</i> (2000)
<i>F. pumilio</i> Lange-Bertalot & Rumrich	7K–7L	L: 14–27.5; W: 4.5–6.1 (n= 35)	S1, S2, S3, S4, S6, S7, S8	Rumrich <i>et al.</i> (2000)

Taxa	Figures	Morphometric (μm) and meristic (in 10 μm) data	Occurrence	Followed literature
<i>F. saxonica</i> Rabenhorst	7I–7J	L: 46.1–71.6; W: 10.9–14.9; TS: 24–30; LS: 23–30 (n= 105)	S1, S2, S3, S4, S5, S6, S7, S8	Lange-Bertalot (2001)
<i>F. undosa</i> Metzeltin & Lange-Bertalot	7F	L: 33.4–43.8; W: 7.6–10 (n= 32)	S1, S2, S3, S4, S5, S6, S7, S8	Lange-Bertalot (2001)
<i>F. vulgaris</i> (Thwaites) De Toni	7H	L: 41.2–46.9; W: 7.5–9.1 (n= 30)	S1, S2, S3, S4, S6, S7, S8	Lange-Bertalot (2001)
<i>F. weinholdii</i> Hustedt	7G; 13J	L: 24.6–37.9; W: 6.8–8.3; S: 32; A: 29 (n= 65)	S1, S2, S3, S4, S6, S7, S8	Lange-Bertalot (2001)
BRACHYSIRACEAE				
<i>Brachysira brebissonii</i> Ross	7N	L: 18.3–25; W: 5.1–6.9; S: 27–28 (n= 14)	S3, S4, S5, S6, S8	Lange-Bertalot & Moser (1994) Kennedy & Allott (2017)
<i>B. microcephala</i> (Grunow) Compère	7O; 13H	L: 11.3–26.4; W: 3.8–5.2; S: 34–36 (n= 116)	S2, S3, S4, S6, S7, S8	Lange-Bertalot & Moser (1994) Kennedy & Allott (2017)
<i>B. serians</i> var. <i>acuta</i> (Hustedt) Vyverman	7M	L: 37.4–62.5; W: 8.7–11.4; S: 21–26; A: 13–14 (n= 6)	S1, S3, S8	Vyverman (1991)
<i>N. praecipuoides</i> Tremarin & Ludwig	7P	L: 15.4–18.7; W: 4.7–6 (n= 38)	S1, S3, S8	Tremarin <i>et al.</i> (2015)
<i>N. semifasciata</i> Amaral, Ludwig & Bueno	7Q–7R	L: 6.7–16.4; W: 3.2–4.8; L/W: 1.7–3.7 (n= 222)	S1, S2, S3, S4, S5, S6, S7, S8	Amaral <i>et al.</i> (2021)
NEIDIACEAE				
<i>Neidium affine</i> var. <i>amphirhynchus</i> (Ehrenberg) Cleve	7U	L: 36.3–51.7; W: 9.9–11.9; S: 22–25; A: 18–24 (n= 10)	S3, S8	Patrick & Reimer (1966)
<i>N. catarinense</i> (Krasske) Lange-Bertalot	7Y	L: 18.8–23.5; W: 4.2–4.7; S: 21–23 (n= 5)	S1, S6, S8	Metzeltin & Lange-Bertalot (1998)
<i>N. essequiboanum</i> Metzeltin & Krammer	7W–7X	L: 31.6–40.2; W: 5.4–6.5 (n= 5)	S5	Metzeltin & Lange-Bertalot (1998, 2007)
<i>N. gracile</i> f. <i>aequale</i> Hustedt	7S	L: 48.2–49.8; W: 10–10.6; S: 21–23 (n= 4)	S3	Patrick & Reimer (1966)
<i>N. iridis</i> (Ehrenberg) Cleve	7T	L: 44–57.6; W: 12.1–13.6; S: 22–23; A: 19–20 (n= 2)	S3	Patrick & Reimer (1966) Krammer & Lange-Bertalot (1986)
<i>N. tenuissimum</i> Hustedt	7V	L: 15–19.8; W: 3.8–4.9 (n= 8)	S1, S2, S3, S4, S5, S8	Hustedt (1943) Patrick & Reimer (1966)
PINNULARIACEAE				
<i>Pinnularia acoricola</i> Hustedt	7Z–7AA; 14A	L: 15.3–25.5; W: 3.7–5; S: 16–19 (n= 53)	S1, S2, S3, S4, S5, S7, S8	Hustedt (1935) Tremarin <i>et al.</i> (2010)
<i>P. acrosphaeria</i> (Brébisson) Smith	7AB	L: 49–54.5; W: 10.2–11.9; S: 12–14 (n= 4)	S2, S3	Krammer (2000)
<i>P. borealis</i> var. <i>rectangularis</i> Carlson	7AD	L: 23–42; W: 6.4–8.2; S: 5–6 (n= 7)	S1, S3, S6, S7, S8	Krammer (2000)
<i>P. borealis</i> var. <i>sublinearis</i> Krammer	7AE	L: 19.6–44.5; W: 6–9.7; S: 5–7 (n= 2)	S1, S3	Krammer (2000)

Taxa	Figures	Morphometric (μm) and meristic (in 10 μm) data	Occurrence	Followed literature
<i>P. brauniana</i> (Grunow) Studnicka	7AF	L: 34.6–55.8; W: 6.3–8.9; S: 10–13 (n= 19)	S1, S2, S3, S5	Krammer (1992)
<i>P. butantanum</i> (Krasske) Metzeltin	7AH	L: 63.6–102; W: 12–13.5; S: 15–17 (n= 13)	S3, S6, S8	Metzeltin & Lange-Bertalot (1998)
<i>P. certa</i> Krammer & Metzeltin	8L–8M	L: 38.2–41.9; W: 7.7–8.5; S: 11–12 (n= 3)	S3	Metzeltin & Lange-Bertalot (1998)
<i>P. decrescens</i> var. <i>ignorata</i> (Krammer) Krammer	7AK	L: 41.7–52.1; W: 11.4–12.8; S: 11–12 (n= 4)	S3	Krammer (2000)
<i>P. divergens</i> Smith	7I–7J	L: 41.8–73.9; W: 9.2–12.2; S: 9–12 (n= 20)	S1, S2, S3, S4, S5, S6	Krammer (1992)
<i>P. divergens</i> var. <i>media</i> Krammer	7AG	L: 39.6–72.8; W: 8.9–13.3; S: 10–13 (n= 85)	S1, S2, S3, S4, S6	Krammer (2000)
<i>P. divergens</i> var. <i>mesoleptiformis</i> Krammer & Metzeltin	7AL	L: 63.2–89.2; W: 12.1–13.9; S: 11–12 (n= 9)	S1, S3, S4, S8	Metzeltin & Lange-Bertalot (1998)
<i>P. gaiserae</i> Metzeltin & Lange-Bertalot	7AC	L: 46.6–56.2; W: 7.3–8.9; S: 11 (n= 7)	S1, S5, S8	Metzeltin & Lange-Bertalot (2007)
<i>P. graciloides</i> var. <i>latecapitata</i> Metzeltin & Krammer	8A–8B	L: 38.6–65.4; W: 6.7–9.4; S: 11–13 (n= 45)	S1, S2, S3, S4, S5, S6, S8	Metzeltin & Lange-Bertalot (1998)
<i>P. graciloides</i> var. <i>triundulata</i> (Fontell) Krammer	8C	L: 65.3; W: 9.1; S: 12 (n= 1)	S8	Krammer (2000)
<i>P. joculata</i> (Manguin) Krammer	8V	L: 18.6–19.4; W: 3.8; S: 18–20 (n= 2)	S1, S2	Krammer (2000)
<i>P. laucensis</i> Lange-Bertalot	8J–8K	L: 15.5–26.3; W: 3.6–4.5; S: 12–15 (n= 23)	S1, S3, S5, S7, S8	Rumrich <i>et al.</i> (2000)
<i>P. meridiana</i> Metzeltin & Krammer	8Q	L: 32.2–45.; W: 8.8–10; S: 11–13 (n= 12)	S1, S2, S3, S4	Metzeltin & Lange-Bertalot (1998)
<i>P. microstauron</i> var. <i>rostrata</i> Krammer	8N	L: 24.6–34.7; W: 5.2–8; S: 12–14 (n= 21)	S1, S2, S3, S4, S5, S6, S8	Krammer (2000)
<i>P. obscura</i> Krasske	8T–8U	L: 16.8–30.1; W: 4.6–6.2; S: 13–16 (n= 37)	S1, S2, S3, S4, S5, S7, S8	Krasske (1932) Krammer (2000)

Taxa	Figures	Morphometric (μm) and meristic (in 10 μm) data	Occurrence	Followed literature
<i>P. rumrichae</i> Krammer	8R	L: 27.6–40.7; W: 5.1–6.8; S: 11–13 (n= 17)	S1, S2, S3	Krammer (2000)
<i>P. schoenfelderi</i> Krammer	8S	L: 24.8–40.9; W: 5.6–7.9; S: 13–15 (n= 10)	S1, S2, S3, S5, S6	Krammer (1992)
<i>P. stidolphii</i> Krammer	8I	L: 150.2; W: 24; S: 12 (n= 1)	S4	Krammer (2000)
<i>P. subanglica</i> Krammer	8P	L: 30.4–47.8; W: 7.5–9.9; S: 11–13 (14) (n= 98)	S1, S2, S3, S4, S5, S6, S8	Krammer (2000)
<i>P. subcapitata</i> W.Gregory	8O	L: 33.3–44; W: 5.5–7.2; S: 13–15 (n= 29)	S1, S2, S3, S4, S5, S8	Krammer (2000)
<i>P. subgibba</i> Krammer	8H	L: 61.9–78.7; W: 9.4–11.6; S: 9 (n= 2)	S1, S3	Krammer (1992)
<i>P. subgibba</i> var. <i>undulata</i> Krammer	8F–8G	L: 35.7–83.3; W: 8.1–11; S: 10–12 (n= 80)	S1, S2, S3, S4, S5, S6, S7, S8	Krammer (2000)
<i>P. tabellaria</i> Ehrenberg	8E	L: 70.6–75.3; W: 10.3–12.1; S: 13–15 (n= 3)	S3	Krammer (2000)
<i>P. viridiformis</i> Krammer	8D	L: 72–79.5; W: 12.5–13.2; S: 10–11 (n= 2)	S3	Krammer (2000)
SELLAPHORACEAE				
<i>Fallacia insociabilis</i> (Krasske) Mann	8W	L: 8.8–13; W: 5.4–6.6; S: 22–24 (n= 5)	S1, S3	Krammer & Lange-Bertalot (1986)
<i>Sellaphora laevis</i> (Kützing) Mann	8Z	L: 33.4–39.8; W: 6.8–10.2; S: 22–25 (n= 7)	S2, S3, S6	Mann <i>et al.</i> (2008)
<i>S. nigri</i> (De Notaris) Wetzel & Ector	8X–8Y; 14C–14D	L: 6.5–11.3; W: 3.4–4.5; S: 23–30; A: 42–54 (n= 192)	S1, S2, S3, S4, S5, S6, S7, S8	Wetzel <i>et al.</i> (2015)
<i>S. pupula</i> (Kützing) Mereschkowsky	8AB	L: 14.7–39.4; W: 5.6–8.7; S: 20–26 (n= 55)	S1, S2, S3, S5, S6, S8	Mann <i>et al.</i> (2004)
<i>S. rhombicarea</i> Metzeltin, Lange-Bertalot & García-Rodríguez	8AA	L: 36.3; W: 9.8; S: 16 (n= 1)	S8	Metzeltin <i>et al.</i> (2005)
<i>S. sassiana</i> (Menzies) Metzeltin & Lange-Bertalot Wetzel	8AG	L: 12.1–15.4; W: 4.3–5.2; S: 23–26 (n= 71)	S1, S3, S4, S8	Metzeltin & Lange-Bertalot (1998)
<i>S. saugerresii</i> (Desmazières) Wetzel & Mann	8AC–8AF; 14E	L: 5.2–16.6; W: 3–5.2; 19–23 (n= 291)	S1, S2, S3, S4, S5, S6, S7, S8	Wetzel <i>et al.</i> (2015)
<i>S. tridentula</i> (Krasske) Wetzel	8AH	L: 13.8–20.2; W: 3.8–4.9 (n= 8)	S1, S3	Wetzel <i>et al.</i> (2015)
DIPLONEIDACEAE				
<i>Diploneis subovalis</i> Cleve	8AI	L: 17–34.4; W: 10.1–19.1; S: 10–13; A: 18–22 (n= 27)	S1, S2, S3, S4, S6, S7, S8	Krammer & Lange-Bertalot (1986)
NAVICULACEAE				
<i>Caloneis bacillum</i> (Grunow) Cleve	8AL–8AM	L: 25.7–37.3; W: 4.7–7.3; S: 19–23 (n= 57)	S1, S2, S3, S4, S7, S8	Krammer & Lange-Bertalot (1986)
<i>C. hyalina</i> Hustedt	8AJ	L: 14.8–21.4; W: 4.5–5.7 (n= 61)	S1, S2, S3, S4, S5, S6, S7, S8	Patrick & Reimer (1966)
<i>C. westii</i> (Smith) Hendey	8AK	L: 49.6–59.8; W: 12.1; S: 14–15 (n= 3)	S1, S2, S3, S5	Hendey (1964)

Taxa	Figures	Morphometric (μm) and meristic (in 10 μm) data	Occurrence	Followed literature
<i>Capartogramma crucicula</i> (Grunow) Ross	9A	L: 30.5–30.7; W: 8.4–8.5; S: 23–25 (n= 2)	S3	Patrick & Reimer (1966)
<i>Gyrosigma acuminatum</i> (Kützing) Rabenhorst	9B	L: 100.4; W: 12.8; S: 21 (n= 1)	S1	Metzeltin <i>et al.</i> (2005)
<i>G. obtusatum</i> (Sullivant & Wormley) Boyer	9C	L: 56.4–65.8; W: 9.5–10.1; S: 24–25 (n= 28)	S1, S2, S3, S4, S6, S7, S8	Patrick & Reimer (1966) Metzeltin <i>et al.</i> (2005)
<i>Navicula angusta</i> Grunow	9J	L: 36.9–49.8; W: 5.7–6.6; S: 12–14 (n= 101)	S1, S2, S3, S4, S5, S6, S7, S8	Krammer & Lange-Bertalot (1997)
<i>N. cryptocephala</i> Kützing	9F–9I; 14F	L: 21.2–43.9; W: 4.7–7.1; S: 15–18; A: 40 (n= 280)	S1, S2, S3, S4, S5, S6, S7, S8	Lange-Bertalot (2001) Jüttner <i>et al.</i> (2020)
<i>N. cryptotenella</i> Lange-Bertalot	9L–9M	L: 15.4–36; W: 5–6.7; S: 14–17; A: 37 (n= 108)	S1, S2, S3, S4, S5, S6, S7, S8	Krammer & Lange-Bertalot (1997) Lange-Bertalot (2001)
<i>N. eichhorniaephila</i> Manguin ex Kociolek & Reiers	9D–9E	L: 29.8–34.7; W: 4.9–6.1; S: 12–14 (n= 36)	S1, S2, S3, S4, S6, S7, S8	Rumrich <i>et al.</i> (2000) Metzeltin <i>et al.</i> (2007)
<i>N. leptostriata</i> Jørgensen	9K	L: 47.2–55.8; W: 6.8–9.3; S: 13–14 (n= 27)	S2, S6, S7, S8	Lange-Bertalot (2001) Metzeltin & Lange-Bertalot (2007)
<i>N. radiosa</i> Kützing	9N	L: 49.6–85.4; W: 8.3–10; S: 9–11 (n= 42)	S1, S2, S3, S4, S6, S8	Lange-Bertalot (2001)
<i>N. rostellata</i> Kützing	9S	L: 35.3–42.8; W: 8.6–10.7; S: 12–15 (n= 49)	S1, S2, S3, S6, S8	Rumrich <i>et al.</i> (2000) Lange-Bertalot (2001)
<i>N. salinicola</i> Hustedt	9T–9U; 14G	L: 10–18.9; W: 3.1–4.4; S: 17–20; A: 38–43 (n= 125)	S1, S2, S3, S4, S5, S6, S7, S8	Krammer & Lange-Bertalot (1997) Lange-Bertalot (2001)
<i>N. schwabei</i> Krasske	9R	L: 53.3; W: 9.9; S: 21–22; A: 22–23 (n= 1)	S3	Lange-Bertalot <i>et al.</i> (1996)
<i>N. symmetrica</i> Patrick	9Q; 14H–14I	L: 24.8–40.9; W: 5.9–7.9; S: 13–15; A: 23–29 (n= 148)	S1, S2, S3, S4, S6, S7, S8	Lange-Bertalot (2001)
<i>N. tenelloides</i> Hustedt	9O–9P	L: 11.5–18.3; W: 2.9–4; S: 18–21 (n= 8)	S1, S8	Krammer & Lange-Bertalot (1997) Lange-Bertalot (2001)
STAURONEIDACEAE				
<i>Craticula ambigua</i> (Ehrenberg) Mann	9X	L: 54.8–82.1; W: 14.6–21.5; S: 16–20; A: 27–29 (n= 3)	S1, S3	Lange-Bertalot (2001) Levkov <i>et al.</i> (2016)
<i>C. riparia</i> (Hustedt) Lange-Bertalot	9Y–9Z	L: 32.7–48.1; W: 7.6–10.6; S: 18–22 (n= 25)	S3, S4, S8	Lange-Bertalot (2001)
<i>C. submolesta</i> (Hustedt) Lange-Bertalot	9V–9W	L: 14.2–23.2; W: 4.1–5.8; S: 18–24 (n= 27)	S1, S2, S3, S4, S5	Lange-Bertalot (2001)
<i>Stauroneis gracilis</i> Ehrenberg	9AC	L: 54–78.3; W: 9.9–14.8; S: 19–25; A: 20–23 (n= 3)	S3, S6	Reichardt (1995) Bahls (2010)
<i>S. neohyalina</i> Lange-Bertalot & Krammer	9AD	L: 44.4–50.2; W: 8.8–9.1 (n= 2)	S3, S4	Lange-Bertalot & Genkal (1999)

Taxa	Figures	Morphometric (μm) and meristic (in 10 μm) data	Occurrence	Followed literature
<i>S. phoenicenteron</i> (Nitzsch) Ehrenberg	9AE	L: 112–160; W: 21.7–28.9; S: 16–18; A: 15–19 (n= 3)	S3, S8	Krammer & Lange-Bertalot (1986) Patrick & Reimer (1966)
<i>S. smithii</i> Grunow	9AA–9AB	L: 21.1–21.7; W: 3.7–4.1 (n= 7)	S2, S4, S5, S6	Bahls (2010) Levkov <i>et al.</i> (2016)
NAVICULALES INCERTAE SEDIS				
<i>Chamaepinnularia brasilianopsis</i> Metzeltin & Lange-Bertalot	9AF–9AG; 14B	L: 8.5–13.3; W: 3–4.1; S: 23–26 (n= 22)	S2, S3, S4, S5, S7	Metzeltin & Lange-Bertalot (1998)
<i>Kobayasiella parasubtilissima</i> (Kobayasi & Nagumo) Lange-Bertalot	9AI–AJ	L: 16.8–30.9; W: 3.7–5.5 (n= 40)	S1, S3, S8	Kobayasi & Nagumo (1988) Buczko <i>et al.</i> (2009)
<i>Pseudofallacia monoculata</i> (Hustedt) Liu, Kociolek & Wang	9AH	L: 12.8–16.2; W: 4.8–5; S: 22–24 (n= 2)	S3	Hustedt (1945) Krammer & Lange-Bertalot (1985)
CATENULACEAE				
<i>Amphora copulata</i> (Kützing) Schoeman & Archibald	9AM	L: 28.1–33.9; W: 6.2–7.8; S: 14 (n= 2)	S3	Schoeman & Archibald (1986) Levkov (2009)
<i>Halamphora montana</i> (Krasske) Levkov	9AK	L: 12.9–18.2; W: 2.9–4.2 (n= 61)	S1, S2, S3, S4, S6, S7, S8	Krammer & Lange-Bertalot (1997) Hofmann <i>et al.</i> (2013)
<i>H. normanii</i> (Rabenhorst) Levkov	9AL	L: 21.6–31.7; W: 4.5–6.2; S: 17–19 (n= 40)	S1, S3, S4, S6, S7, S8	Levkov (2009)
BACILLARIACEAE				
<i>Hantzschia amphioxys</i> (Ehrenberg) Grunow	10A	L: 19.4–38.7; W: 3.6–7.5; S: 8–12 (14); S: 22–27 (n= 48)	S1, S2, S3, S4, S5, S6, S7, S8	Krammer & Lange-Bertalot (1988) Siver & Hamilton (2011)
<i>Nitzschia amphibia</i> Grunow	10U–10V	L: 9–27.4; W: 3.2–4.1; F: 8–11; S: 16–19 (n= 23)	S1, S4, S8	Rumrich <i>et al.</i> (2000)
<i>N. brevisissima</i> Grunow	10Q	L: 27.7–31.6; W: 5.5–5.6; F: 8 (n= 2)	S4, S7	Krammer & Lange-Bertalot (1988)
<i>N. clausii</i> Grunow	9AJ; 15A–15B	L: 22.4–42.1; W: 3.6–5.1; F: 9–14; S: 37; A: 38 (n= 58)	S1, S2, S3, S4, S5, S6, S7, S8	Krammer & Lange-Bertalot (1988) Hofmann <i>et al.</i> (2013)
<i>N. dissipata</i> (Kützing) Rabenhorst	10B	L: 29.1–33.5; W: 4.3–4.9; F: 13 (n= 3)	S3	Krammer & Lange-Bertalot (1988)
<i>N. inconspicua</i> Grunow	10N–10P; 15C–15D	L: 8.5–18.3; W: 2.5–3.3; F: 9–13; S: 23–25; A: 28 (n= 17)	S1, S2, S3, S4, S7, S8	Trobajo <i>et al.</i> (2013)
<i>N. intermedia</i> Hantzsch ex Cleve & Grunow	10I	L: 23.5–71; W: 2.9–6; F: 10–16 (n= 73)	S1, S2, S3, S6, S8	Krammer & Lange-Bertalot (1988)
<i>N. linearis</i> (Agardh) Smith	10K	L: 49–119.2; W: 5.7–6; F: 7–13 (n= 29)	S2, S3, S4, S6, S8	Krammer & Lange-Bertalot (1988)
<i>N. lorenziana</i> Grunow	10J	L: 49.3–79.6; W: 3–3.8; F: 8–10; S: 17–18 (n= 9)	S2, S3, S4, S6, S8	Rumrich <i>et al.</i> (2000)

Taxa	Figures	Morphometric (μm) and meristic (in 10 μm) data	Occurrence	Followed literature
<i>N. palea</i> (Kützing) Smith	10C–10F	L: 15.7–44.4; W: 3–5.1; F: 10–16 (n= 155)	S1, S2, S3, S4, S5, S6, S7, S8	Rumrich <i>et al.</i> (2000) Levkov <i>et al.</i> (2007)
<i>N. palea</i> var. <i>debilis</i> (Kützing) Grunow	10L–10M	L: 13.1–53.6; W: 2.3–3.6; F: 11–14 (n= 29)	S1, S2, S3, S4, S6	Krammer & Lange-Bertalot (1988)
<i>N. perminuta</i> Grunow in Van Heurck	10R–10S; 15E–15F	L: 10.5–19.1; W: 2.6–3.4; F: 10–13; S: 22–33; A: 47 (n= 36)	S1, S2, S3, S4, S6, S7, S8	Krammer & Lange-Bertalot (1988) Levkov <i>et al.</i> (2007)
<i>N. recta</i> Hantzsch ex Rabenhorst	10H	L: 24–63.4; W: 3.3–5.5; F: 5–9 (n= 56)	S1, S2, S3, S4, S5, S6, S7, S8	Krammer & Lange-Bertalot (1988)
<i>N. semirobusta</i> Lange-Bertalot	10W	L: 16–24.7; W: 3.4–4.2; F: 7–8; S: 17 (n= 2)	S1	Lange-Bertalot (1993)
<i>N. terrestris</i> (Petersen) Hustedt	10T	L: 38.3–55.9; W: 4.3–4.4; F: 6–7 (n= 2)	S3	Krammer & Lange-Bertalot (1988)
<i>Tryblionella debilis</i> Arnott ex O'Meara	10X	L: 20.5–21.6; W: 8.7–9.1; S: 17–18 (n= 2)	S3, S4	Krammer & Lange-Bertalot (1988)
RHOPALODIACEAE				
<i>Epithemia gibberula</i> (Ehrenberg) Kützing	10Y	L: 29.4–34; W: 7.8–8.2; AC: 3–4; S: 18–20; A: 14–20 (n= 7)	S2, S3, S6	Cocquyt <i>et al.</i> (2018)
SURIRELLACEAE				
<i>Iconella curvula</i> (Smith) Ruck & Nakov	10Z–10AA; 15G–15H	L: 166.3–214; W: 5.6–6.8; AC: 5–6; S: 23–25 (n= 2)	S3	Metzeltin & Lange-Bertalot (1998)
<i>I. delicatissima</i> (Lewis) Ruck & Nakov	10AB; 15I	L: 37.4–65.6; W: 4.9–6.4; AC: 6–7 (n= 2)	S3, S5	Metzeltin & Lange-Bertalot (1998)
<i>I. splendida</i> (Ehrenberg) Kützing	10AC	L: 83.5–86.3; W: 34.4–38; AC: 2 (n= 5)	S1, S3, S6, S8	Krammer & Lange-Bertalot (1988)
<i>I. tenuissima</i> (Hustedt) Kapustin & Kulikovskiy	10AD	L: 12–31.2; W: 5.2–10; AC: 4–6 (n= 99)	S1, S2, S3, S4, S5, S6, S7, S8	Krammer & Lange-Bertalot (1988) Kapustin & Kulikovskiy (2018)
<i>Surirella angusta</i> Kützing	10AF	L: 16.9–35.4; W: 7.2–8.3; AC: 5–7; S: 27 (n= 23)	S1, S2, S3, S4, S7, S8	Krammer & Lange-Bertalot (1988)
<i>S. biseriata</i> var. <i>constricta</i> Grunow ex Hustedt	10AG	L: 74.3–97.9; W: 15.5–18.7; AC: 2–3 (n= 2)	S3	Hustedt (1930)
<i>S. grunowii</i> Kulikovskiy, Lange-Bertalot & Witkovski	10AH	L: 60.6; W: 13.1–16.8; AC: 3 (n= 2)	S3	Kulikovskiy <i>et al.</i> (2010)
<i>S. stalagma</i> Hohn & Hellerman	10AE	L: 12.8; 4.7; AC: 9 (n= 1)	S4	Hohn & Hellerman (1963)

¹Van Dam *et al.* (1994); ²Lobo *et al.* (2015); ³Faustino *et al.* (2016); ⁴Marra *et al.* (2016); ⁵Hofmann (1994); ⁶Yang & Dickman (1993); ⁷Levkov *et al.* (2016); ⁸Costa *et al.* (2017a); ⁹Jüttner *et al.* (2013); ¹⁰Lange-Bertalot (2001); ¹¹Wojtal *et al.* (2011); ¹²Krammer & Lange-Bertalot (1991a); ¹³Krammer & Lange-Bertalot (1997); ¹⁴Lange-Bertalot & Ulrich (2014); ¹⁵Tuji & Williams (2007); ¹⁶Wetzel *et al.* (2002); ¹⁷Lepskaya *et al.* (2010); ¹⁸Liu *et al.* (2011); ¹⁹Taylor *et al.* (2007); ²⁰Almeida *et al.* (2016); ²¹Silva *et al.* (2010); ²²Moro & Fürstenberger (1997); ²³Krammer (1997a); ²⁴Krammer (1997b); ²⁵Wetzel *et al.* (2014); ²⁶Potapova & Charles (2007); ²⁷Stenger-Kovács *et al.* (2007); ²⁸Krammer (2000); ²⁹Ferreira &

Bicudo (2017); ³⁰Levkov *et al.* (2013); ³¹Moresco *et al.* (2011); ³²Reichardt (2015); ³³Morales & Vis (2007); ³⁴Hofmann *et al.* (2011); ³⁵Patrick & Reimer (1966); ³⁶Silva-Lehmkuhl *et al.* (2019); ³⁷Bicudo *et al.* (2016); ³⁸Wengrat *et al.* (2015); ³⁹Faria *et al.* (2010); ⁴⁰Lange-Bertalot & Metzeltin (1996); ⁴¹Bertolli *et al.* 2010; ⁴²Luchini & Verona (1972); ⁴³Bartozek *et al.* (2018); ⁴⁴Nardelli *et al.* (2016); ⁴⁵Metzeltin *et al.* (2005); ⁴⁶Marquardt *et al.* (2014); ⁴⁷Weiler *et al.* (2016); ⁴⁸Kapetanovic *et al.* (2011); ⁴⁹Wunsam *et al.* (1995); ⁵⁰Udovic *et al.* (2017); ⁵¹Costa *et al.* (2017b); ⁵²Almeida *et al.* (2020); ⁵³Marquardt *et al.* (2017); ⁵⁴Medeiros *et al.* (2018); ⁵⁵Lobo *et al.* (2004); ⁵⁶Heinrich *et al.* (2014); ⁵⁷da Silva *et al.* (2016); ⁵⁸Lobo *et al.* (2016); ⁵⁹Levkov *et al.* (2016); ⁶⁰Morais *et al.* (2018); n.d.: no data.

The Principal Component Analysis (PCA) for the ecological variables accounted for 52.52% of the total variability in the data in the first two axes (Fig 2a). The first axis of the PCA explains the variability primary related to total phosphorus positively (correlation value: 0.54), and pH negatively (-0.51). The distribution of these scores tends to cluster points S5, S6, S7 and S8 to the negative side of the axis, relating to higher values of pH. The second axis is positively related with orthophosphate (0.39) and negatively with nitrate (-0.63), and electrical conductivity (-0.54). The distribution of these scores tends to separate sampling sites S2 and S4 from S5, S6, S7, and S8.

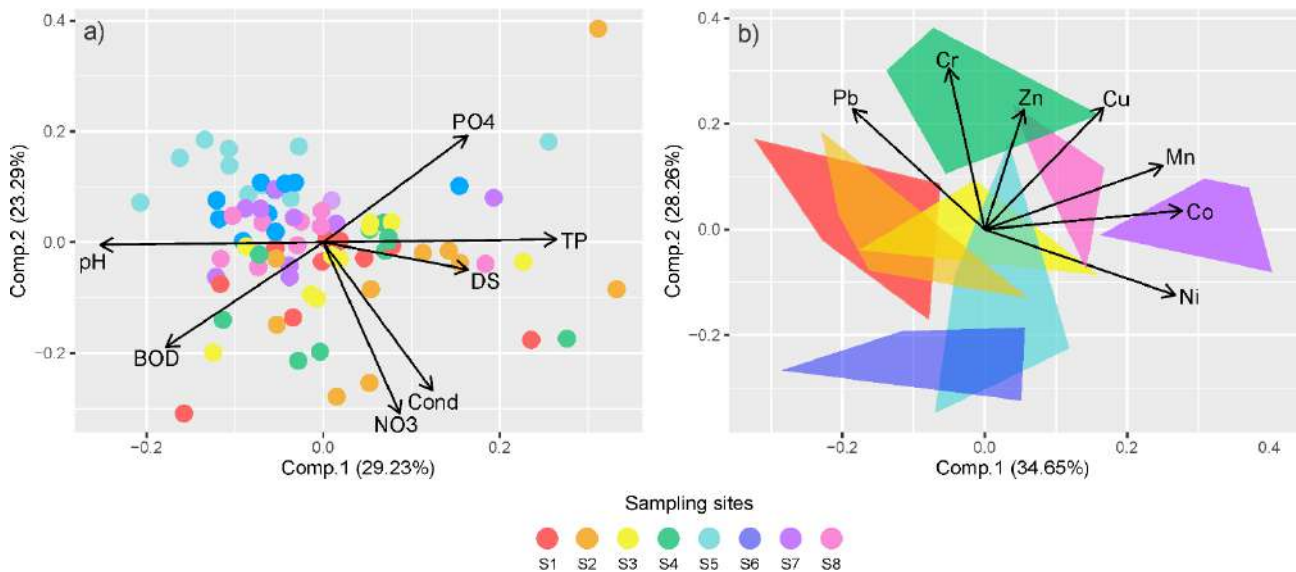


FIGURE 2. Variability of environmental data for the eight sampling sites in the Cascavel River microbasin, Paraná State, Brazil. a) Principal Component Analysis for abiotic data analyzed from water samples (BOD: Biochemical Oxygen Demand; NO₃: nitrate; Cond: electrical conductivity; DS: dissolved solids; TP: total phosphorus; PO₄⁻: orthophosphate). b) Principal Component Analysis for metals data analyzed in the surface sediment (Co: cobalt, Cr: chromium, Cu: copper, Mn: manganese, Ni: nickel, Pb: lead, Zn: zinc). The sampling sites for surface sediment were represented by polygons.

Principal Component Analysis for the metals accounted for 62.91% of the total variability in the data in the first two axes (Fig 2b). The first axis of the PCA explains the variability primary related to cobalt (correlation value: 0.53), nickel (0.51), and manganese (0.47) positively, and lead negatively (-0.35). The distribution of these scores demonstrates a separation of sampling sites S7 and S8 from S1 and S2. Moreover, the second axis is positively related with chromium (0.58), copper (0.44), lead (0.43), and zinc (0.42) positively, and nickel negatively (-0.24). The distribution of these scores demonstrates a separation of sampling sites S5 and S6 from the others.

During our study, we identified diatoms with deformed valve outlines, changes concerning the striae pattern, and with doubled central area in the eight sampling sites (Table 2). Teratological valves were found in 34 taxa, belonging to 15 genera, with *Eunotia* containing the greater number of altered taxa (8 spp.). Mixed teratologies (deformed valve outline + unusual striae pattern) were found only in *Ulnaria ulna*, *Encyonema neomesianum*, and *Gomphonema graciledictum*. In SEM, we found only *Eunotia bilunaris* (n = 1) and *Gomphonema lagenula* (n = 1) with teratologies,

showing a deformed valve outline. Teratological taxa are described below, showing morphological changes and ecological characteristics of the environment where abnormal individuals were found.

TABLE 2. Teratological diatoms found in Cascavel river microbasin, with occurrences in sampling sites, and related metals.

Taxa	Figures	Type of teratology*	Occurrence	Metals	
				Literature*	Our study
<i>Discostella stelligera</i>	2D–2E	Type 3	S3	x	Undefined
<i>Fragilaria gracilis</i>	2X–2AA	Type 1	S3, S8	As, Cd, Cu, Hg, Pb, Zn	Cu, Mn, Ni
<i>F. pectinalis</i>	2AE–2AH	Type 1	S3	x	Undefined
<i>F. tenera</i> var. <i>nanana</i>	2AL–2AO	Type 1	S3	x	Undefined
<i>Ulnaria ulna</i>	2AX–2AZ	Type 1 Type 7	S1, S2, S3, S6, S8	Cd, Cr, Cu, Fe, Sr, Zn	Undefined
<i>Eunotia bilunaris</i>	3G–3J; 12A	Type 1	S1, S2, S3, S4, S6, S8	x	Undefined
<i>E. botulitropica</i>	3P	Type 1	S5	x	Ni, Zn
<i>E. desmogonioides</i>	3T	Type 1	S3, S8	x	Cu, Mn, Ni
<i>E. meridiana</i>	3AV–3AX	Type 1	S3, S8	x	Cu, Mn, Ni
<i>E. monodon</i>	3AQ	Type 1	S2	x	Pb
<i>E. rhomboidea</i>	4E–4G	Type 1	S2	x	Pb
<i>E. subarcuatooides</i>	3W–3X	Type 1	S3, S8	Al, Ba, Mn	Cu, Mn, Ni
<i>E. yberai</i>	4N–4P	Type 1 Type 2	S1, S4	x	Cr, Pb
<i>Encyonema neomesianum</i>	4X	Type 7	S3	x	Undefined
<i>E. silesiacum</i>	4AA	Type 1	S2	Cd, Cu, Pb, Zn	Pb
<i>Gomphonema angustatum</i>	5F–5H	Type 1	S2, S3, S6	x	Pb
<i>G. graciledictum</i>	5W–5X	Type 2 Type 7	S3	x	Undefined
<i>G. lagenula</i>	5AQ–5AS; 12J	Type 1	S1, S2, S3, S4, S6, S7, S8	x	Undefined
<i>G. naviculoides</i>	5AU	Type 2	S3	x	Undefined
<i>G. pumilum</i>	5AM	Type 1	S8	x	Cu, Mn
<i>Achnanthydium macrocephalum</i>	6L–6P	Type 1	S3, S4, S8	Cu, Zn	Cr, Zn
<i>A. minutissimum</i>	6X–6AC	Type 1	S1, S2, S3, S4, S6, S7	Cd, Cr, Cu, Pb, Sr, Zn	Undefined
<i>A. modestiforme</i>	6AF–AH	Type 1	S3, S5, S7	x	Ni
<i>Planothidium rostratum</i>	6AO–AP	Type 1	S3	x	Undefined
<i>Frustulia saxonica</i>	7I	Type 1	S1, S2	x	Pb
<i>Pinnularia divergens</i>	7AJ	Type 1	S1	x	Pb
<i>P. graciloides</i> var. <i>latecapitata</i>	8B	Type 1	S2, S4, S7	x	Cr, Ni, Pb
<i>Sellaphora saugerresii</i>	8AD–8AF	Type 1	S1, S2, S7, S8	As, Cd, Pb, Zn	Ni, Pb
<i>Caloneis bacillum</i>	8AM	Type 1	S8	Cd, Cu, Pb, Zn	Cu, Mn
<i>Navicula cryptocephala</i>	9G–9I	Type 1	S2	x	Pb
<i>N. cryptotenella</i>	9M	Type 1	S2, S3	Cd, Cu, Pb, Zn	Pb
<i>Craticula riparia</i>	9Z	Type 1	S3	x	Undefined
<i>C. submolesta</i>	9W	Type 1	S3	x	Undefined
<i>Nitzschia palea</i>	10D–10F	Type 1	S3	As, Cd, Cu, Hg, Ni, Pb, Zn	Undefined

* See Falasco *et al.* (2021) and references therein.

Family STEPHANODISCACEAE

Discostella stelligera (Figs 3D–3E)

Duplication of the stellate pattern of the alveoli in the central area (n = 2).

Ecological characteristics: Cond: 47.00 $\mu\text{S cm}^{-1}$; BOD: 3.00 mg L^{-1} ; COD: 15.50 mg L^{-1} ; N-NH₃: 200 $\mu\text{g L}^{-1}$; NO₃: 1.40 mg L^{-1} ; DO: 16.30 mg L^{-1} ; pH: 6.05; PO₄⁻: 0.022 mg L^{-1} ; TP: 0.05 mg L^{-1} ; TS: 58.00 mg L^{-1} ; Temp: 18.31 °C; Turb: 7.26; Flow: 0.93 $\text{m}^3 \text{s}^{-1}$; Average depth: 0.15 m; Prec: 2.73 mm.

Metal Concentrations: Co: 33.57 mg L^{-1} ; Cr: 27.33 mg L^{-1} ; Cu: 197.55 mg L^{-1} ; Mn: 398.50 mg L^{-1} ; Ni: 31.80 mg L^{-1} ; Pb: 46.56 mg L^{-1} ; Zn: 93.81 mg L^{-1} .

Family FRAGILARIACEAE

Fragilaria gracilis (Figs 3X–3AA)

Deformed valve outline, undulated, containing protrusions, or bent apices (n = 6).

Ecological characteristics: Cond: 39.00–40.00 $\mu\text{S cm}^{-1}$; BOD: 1.19–2.38 mg L^{-1} ; COD: 5.95–13.60 mg L^{-1} ; N-NH₃: 58.00–37.80 $\mu\text{g L}^{-1}$; NO₃: 0.34–0.37 mg L^{-1} ; DO: 14.27–14.80 mg L^{-1} ; pH: 6.19–6.26; PO₄⁻: 0.004–0.006 mg L^{-1} ; TP: 0.007–0.016 mg L^{-1} ; TS: 10.00–48.00 mg L^{-1} ; Temp: 17.37–17.90 °C; Turb: 7.96–10.70; Flow: 0.47–1.16 $\text{m}^3 \text{s}^{-1}$; Average depth: 0.19–0.29 m; Prec: 9.96 mm.

Metal Concentrations: Co: 23.91–39.39 mg L^{-1} ; Cr: 27.33–85.31 mg L^{-1} ; Cu: 170.55–264.18 mg L^{-1} ; Mn: 398.50–606.73 mg L^{-1} ; Ni: 31.80–73.27 mg L^{-1} ; Pb: 31.05–46.56 mg L^{-1} ; Zn: 79.00–172.60 mg L^{-1} .

F. pectinalis (Figs 3AE–3AH)

Abnormal valve outline, undulated (n = 11).

Ecological characteristics: Cond: : 39.00–40.00 $\mu\text{S cm}^{-1}$; BOD: 0.89– 2.38 mg L^{-1} ; COD: 8.08–13.60 mg L^{-1} ; N-NH₃: 323–378 $\mu\text{g L}^{-1}$; NO₃: 0.34–0.37 mg L^{-1} ; DO: 14.62–14.80 mg L^{-1} ; pH: 6.04–6.26; PO₄⁻: 0.005–0.006 mg L^{-1} ; TP: 0.009–0.016 mg L^{-1} ; TS: 10.00–38.00 mg L^{-1} ; Temp: 17.37–17.68 °C; Turb: 9.25–10.70; Flow: 0.47–0.62 $\text{m}^3 \text{s}^{-1}$; Average depth: 0.23–0.31 m; Prec: 9.96 mm.

Metal Concentrations: Co: 23.91–33.57 mg L^{-1} ; Cr: 27.33–78.86 mg L^{-1} ; Cu: 197.55–235.49 mg L^{-1} ; Mn: 398.50–444.52 mg L^{-1} ; Ni: 31.80–69.89; Pb: 35.58–46.56 mg L^{-1} ; Zn: 93.81–167.31 mg L^{-1} .

**F. spectra* (Figs 3AP–3AR)

Length: 42.7–70.1; width: 1.8–3.1; striae: 19–24 in 10 μm

The individuals found in our samples presented greater width (1.8–3.1 \times 1.5–2.5 μm) and lower striae density (19–24 \times 24–25 in 10 μm) in comparison to the original description (Almeida *et al.* 2016), however, the delicate appearance of the valves are similar. In our samples, *Fragilaria tenera* var. *tenera* was distinguished by lower striae density (15–16 in 10 μm) and *F. tenera* var. *nanana* by the capitate apices (Lange-Bertalot & Ulrich 2014, Almeida *et al.* 2020).

**F. tenera* var. *nanana* (Figs 3AL–3AO)

Length: 45.1–67.1 μm ; width: 1.9–3.1 μm ; striae: 20–22 in 10 μm

The individuals from Cascavel river microbasin contain a higher striae number (18.5–20 in 10 μm) than the original description. However, the authors pointed out that their specimens had lower striae density compared to the protologue provided by Meister (Lange-Bertalot & Ulrich 2014).

Deformed valve outline, undulated (n = 3).

Ecological characteristics: Cond: 38.00–40.00 $\mu\text{S cm}^{-1}$; BOD: 1.49–3.27 mg L^{-1} ; COD: 7.23–8.08 mg L^{-1} ; N-NH₃: 316–378 $\mu\text{g L}^{-1}$; NO₃: 0.37 mg L^{-1} ; DO: 11.42–14.57 mg L^{-1} ; pH: 5.79–6.19; PO₄⁻: 0.006–0.008 mg L^{-1} ; TP: 0.010–0.017 mg L^{-1} ; TS: 38.00–66.00 mg L^{-1} ; Temp: 17.37–17.50 °C; Turb: 7.03–10.70; Flow: 0.47 $\text{m}^3 \text{s}$; Average depth: 0.19–0.23 m; Prec: 9.96 mm.

Metal Concentrations: Co: 23.91–33.57 mg L^{-1} ; Cr: 27.33–78.86 mg L^{-1} ; Cu: 197.55–235.49 mg L^{-1} ; Mn: 398.50–444.52 mg L^{-1} ; Ni: 31.80–69.89; Pb: 35.58–46.56 mg L^{-1} ; Zn: 93.81–167.31 mg L^{-1} .

Ulnaria ulna (Figs 3AX–3AZ)

Deformed valve outline, undulated or with strong constriction in the median portion of the valve. Slight alteration in the striae pattern (n = 18).

Ecological characteristics: Cond: 38.00–78.00 $\mu\text{S cm}^{-1}$; BOD: 0.1–9.9 mg L^{-1} ; COD: 1.22–24.32 mg L^{-1} ; N-NH₃: 8–316 $\mu\text{g L}^{-1}$; NO₃: 0.34–17.20 mg L^{-1} ; DO: 5.84–22.80 mg L^{-1} ; pH: 5.31–7.09; PO₄⁻: 0.005–0.030 mg L^{-1} ; TP: 0.005–0.82 mg L^{-1} ; TS: 10.00–72.00 mg L^{-1} ; Temp: 17.37–23.24 °C; Turb: 1.93–10.7; Flow: 0.027–4.17 $\text{m}^3 \text{s}$; Average depth: 0.02–0.29 m; Prec: 2.36–9.98 mm.

Metal Concentrations: Co: 18.10–55.52 mg L^{-1} ; Cr: 24.51–85.01 mg L^{-1} ; Cu: 170.45–245.83 mg L^{-1} ; Mn: 345.77–639.87 mg L^{-1} ; Ni: 32.41–78.27 mg L^{-1} ; Pb: 24.07–52.76 mg L^{-1} ; Zn: 123.32–412.52.

Family EUNOTIACEAE

Eunotia bilunaris (Figs 4G–4J; 13A)

Deformed valve outline, undulated, with protrusion, slight or strong constriction in the median portion of the valve, or strong bent ventrally or near the apices (n = 49).

Ecological characteristics: Cond: 41.00–70.00 $\mu\text{S cm}^{-1}$; BOD: 0.89–9.35 mg L^{-1} ; COD: 1.20–20.32 mg L^{-1} ; N-NH₃: 18–316 $\mu\text{g L}^{-1}$; NO₃: 0.32–9.31 mg L^{-1} ; DO: 7.70–19.50 mg L^{-1} ; pH: 5.79–6.99; PO₄⁻: 0.003–0.022 mg L^{-1} ; TP: 0.007–0.040 mg L^{-1} ; TS: 10.00–174.00 mg L^{-1} ; Temp: 17.37–23.56 °C; Turb: 2.47–37.50; Flow: 0.07–5.49 $\text{m}^3 \text{s}$; Average depth: 0.04–0.42 m; Prec: 2.37–11.17 mm.

Metal Concentrations: Co: 17.50–47.24 mg L⁻¹; Cr: 21.98–81.32 mg L⁻¹; Cu: 160.64–263.62 mg L⁻¹; Mn: 349.80–502.78 mg L⁻¹; Ni: 27.57–66.65 mg L⁻¹; Pb: 33.49–66.21 mg L⁻¹; Zn: 78.97–170.48 mg L⁻¹.

E. botulitropica (Fig 4P)

Deformed valve outline, with constriction in the median portion of the valve (n = 1).

Ecological characteristics: Cond: 10.00 μS cm⁻¹; BOD: 1.19 mg L⁻¹; COD: 4.28 mg L⁻¹; N-NH₃: 200 μg L⁻¹; NO₃: 0.10 mg L⁻¹; DO: 18.64 mg L⁻¹; pH: 7.18; PO₄⁻: 0.005 mg L⁻¹; TP: 0.005 mg L⁻¹; TS: 13.00 mg L⁻¹; Temp: 21.27 °C; Turb: 0.96; Flow: 0.03 m³ s; Average depth: 0.02 m; Prec: 3.81 mm.

Metal Concentrations: Co: 26.94 mg L⁻¹; Cr: 72.40 mg L⁻¹; Cu: 234.78 mg L⁻¹; Mn: 312.44 mg L⁻¹; Ni: 91.80 mg L⁻¹; Pb: 25.81 mg L⁻¹; Zn: 162.55 mg L⁻¹.

E. desmogonioides (Fig 4T)

Deformed valve outline, undulated (n = 34)

Ecological characteristics: Cond: 6.00–53.00 μS cm⁻¹; BOD: 0.89–5.83 mg L⁻¹; COD: 4.68–17.50 mg L⁻¹; N-NH₃: 200–680 μg L⁻¹; NO₃: 0.32–7.50 mg L⁻¹; DO: 7.66–14.62 mg L⁻¹; pH: 5.79–7.09; PO₄⁻: 0.004–0.030 mg L⁻¹; TP: 0.007–0.017 mg L⁻¹; TS: 11.00–66.00 mg L⁻¹; Temp: 17.40–23.53 °C; Turb: 7.03–13.70; Flow: 0.45–1.06 m³ s; Average depth: 0.19–0.34 m; Prec: 3.48–9.96 mm.

Metal Concentrations: Co: 23.73–60.76 mg L⁻¹; Cr: 27.33–88.85 mg L⁻¹; Cu: 160.64–264.18 mg L⁻¹; Mn: 398.50–606.73 mg L⁻¹; Ni: 27.57–73.27 mg L⁻¹; Pb: 31.05–66.21 mg L⁻¹; Zn: 78.97–173.12 mg L⁻¹.

E. meridiana (Figs 4AV–4AX)

Deformed valve outline, with protrusion or constriction near the apices (n = 4)

Ecological characteristics: Cond: 47.00–52.00 μS cm⁻¹; BOD: 0.89–5.05 mg L⁻¹; COD: 10.20–11.05 mg L⁻¹; N-NH₃: 363–448 μg L⁻¹; NO₃: 0.37 mg L⁻¹; DO: 11.15–14.62 mg L⁻¹; pH: 6.04–6.47; PO₄⁻: 0.005–0.006 mg L⁻¹; TP: 0.009 mg L⁻¹; TS: 21.00–49.00 mg L⁻¹; Temp: 17.35–17.40 °C; Turb: 7.79–9.25; Flow: 0.47–1.32 m³ s; Average depth: 0.19–0.33 m; Prec: 9.96 mm.

Metal Concentrations: Co: 23.73–60.76 mg L⁻¹; Cr: 27.33–88.85 mg L⁻¹; Cu: 160.64–264.18 mg L⁻¹; Mn: 398.50–606.73 mg L⁻¹; Ni: 27.57–73.27 mg L⁻¹; Pb: 31.05–66.21 mg L⁻¹; Zn: 78.97–173.12 mg L⁻¹.

E. monodon (Fig 4AQ)

Deformed valve outline, undulated (n = 1).

Ecological characteristics: Cond: 70.00 $\mu\text{S cm}^{-1}$; BOD: 1.00 mg L^{-1} ; COD: 2.62 mg L^{-1} ; N-NH₃: 200 $\mu\text{g L}^{-1}$; NO₃: 3.30 mg L^{-1} ; DO: 15.81 mg L^{-1} ; pH: 6.30; PO₄⁻: 0.05 mg L^{-1} ; TP: 0.005 mg L^{-1} ; TS: 174.00 mg L^{-1} ; Temp: 18.23 °C; Turb: 2.47; Flow: 0.88 $\text{m}^3 \text{s}^{-1}$; Average depth: 0.14 m; Prec: 2.37 mm.

Metal Concentrations: Co: 17.50 mg L^{-1} ; Cr: 79.78 mg L^{-1} ; Cu: 244.29 mg L^{-1} ; Mn: 370.99 mg L^{-1} ; Ni: 66.65 mg L^{-1} ; Pb: 33.49 mg L^{-1} ; Zn: 146.17 mg L^{-1} .

E. rhomboidea (Figs 5E–5G)

Deformed valve outline, with protrusion or bent of the valve (n = 9).

Ecological characteristics: Cond: 1.00–76.00 $\mu\text{S cm}^{-1}$; BOD: 2.03–3.50 mg L^{-1} ; COD: 4.79–27.60 mg L^{-1} ; N-NH₃: 200–310 $\mu\text{g L}^{-1}$; NO₃: 2.00–2.53 mg L^{-1} ; DO: 11.62–17.23 mg L^{-1} ; pH: 5.11–5.79; PO₄⁻: 0.05–0.323 mg L^{-1} ; TP: 0.17–0.35 mg L^{-1} ; TS: 61.00–83.00 mg L^{-1} ; Temp: 19.44–21.55 °C; Turb: 1.09–4.73; Flow: 0.33–0.82 $\text{m}^3 \text{s}^{-1}$; Average depth: 0.07–0.15 m; Prec: 2.73–3.81 mm.

Metal Concentrations: Co: 19.52–23.11 mg L^{-1} ; Cr: 25.92–78.24 mg L^{-1} ; Cu: 196.71–253.92 mg L^{-1} ; Mn: 329.01–434.49 mg L^{-1} ; Ni: 33.21–84.74 mg L^{-1} ; Pb: 24.42–38.28 mg L^{-1} ; Zn: 78.35–182.64 mg L^{-1} .

E. subarcuatoidea (Figs 4W–4X)

Deformed valve outline, undulated in the ventral side of the valve (n = 3).

Ecological characteristics: Cond: 44.00–53.00 $\mu\text{S cm}^{-1}$; BOD: 1.49–5.66 mg L^{-1} ; COD: 8.08–16.67 mg L^{-1} ; N-NH₃: 200–378 $\mu\text{g L}^{-1}$; NO₃: 0.37–5.46 mg L^{-1} ; DO: 10.60–14.57 mg L^{-1} ; pH: 6.19–6.58; PO₄⁻: 0.006–0.030 mg L^{-1} ; TP: 0.006–0.010 mg L^{-1} ; TS: 38.00–44.00 mg L^{-1} ; Temp: 17.35–21.57 °C; Turb: 10.70–24.40; Flow: 0.47–6.34 $\text{m}^3 \text{s}^{-1}$; Average depth: 0.19–0.37 m; Prec: 3.48–9.96 mm.

Metal Concentrations: Co: 23.91–60.76 mg L^{-1} ; Cr: 27.33–88.85 mg L^{-1} ; Cu: 163.45–241.67 mg L^{-1} ; Mn: 398.50–557.50 mg L^{-1} ; Ni: 31.80–69.89 mg L^{-1} ; Pb: 35.58–48.63 mg L^{-1} ; Zn: 83.42–173.12 mg L^{-1} .

E. yberai (Figs 5N–5P)

Deformed valve outline, with depression in the ventral region of the valve. Some individuals presented valves more curved ventrally. Unusual striae pattern (n = 5).

Ecological characteristics: Cond: 58.00–78.00 mS cm^{-1} ; BOD: 1.10–5.50 mg L^{-1} ; COD: 1.00–18.10 mg L^{-1} ; N-NH₃: 200 $\mu\text{g L}^{-1}$; NO₃: 2.10–14.30 mg L^{-1} ; DO: 6.17–19.50 mg L^{-1} ; pH: 6.09–6.75;

PO₄⁻: 0.004–0.046 mg L⁻¹; TP: 0.009–0.943 mg L⁻¹; TS: 32.00–104.00 mg L⁻¹; Temp: 18.01–20.93 °C; Turb: 2.81–6.38; Flow: 0.10–0.45 m³ s; Average depth: 0.04–0.12 m; Prec: 2.39–11.17 mm.
Metal Concentrations: Co: 23.67–44.91 mg L⁻¹; Cr: 23.67–83.01 mg L⁻¹; Cu: 210.07–273.01 mg L⁻¹; Mn: 349.80–523.81 mg L⁻¹; Ni: 28.58–58.71 mg L⁻¹; Pb: 35.17–61.04 mg L⁻¹; Zn: 81.88–239.18 mg L⁻¹.

Family CYMBELLACEAE

Encyonema neomesianum (Fig 5X)

Deformed valve outline, slight constriction in the median portion of the valve, on the ventral side. Slight change in striae pattern in the dorsal margin (n = 2).

Ecological characteristics: Cond: 41.00–52.00 μS cm⁻¹; BOD: 2.32–4.16 mg L⁻¹; COD: 5.10–17.43 mg L⁻¹; N-NH₃: 313–316 mg L⁻¹; NO₃: 0.32–1.70 mg L⁻¹; DO: 7.24–12.72 mg L⁻¹; pH: 6.03–6.90; PO₄⁻: 0.004–0.006 mg L⁻¹; TP: 0.010–0.012 mg L⁻¹; TS: 10.00–16.00 mg L⁻¹; Temp: 17.90–23.23 °C; Turb: 8.84–9.60; Flow: 0.45–0.75 m³ s; Average depth: 0.19–0.29 m; Prec: 9.96 mm.

Metal Concentrations: Co: 23.73–47.24 mg L⁻¹; Cr: 27.33–80.86 mg L⁻¹; Cu: 160.64–235.49 mg L⁻¹; Mn: 398.05–444.52 mg L⁻¹; Ni: 27.57–69.89 mg L⁻¹; Pb: 35.58–66.21 mg L⁻¹; Zn: 78.97–167.31 mg L⁻¹.

E. silesiacum (Fig 5AA)

Deformed valve outline, with slight constriction in the median portion of the valve, on the ventral side (n = 1).

Ecological characteristics: Cond: 70.00 μS cm⁻¹; BOD: 1.00 mg L⁻¹; COD: 2.62 mg L⁻¹; N-NH₃: 200 μg L⁻¹; NO₃: 3.30 mg L⁻¹; DO: 15.81 mg L⁻¹; pH: 6.30; PO₄⁻: 0.05 mg L⁻¹; TP: 0.005 mg L⁻¹; TS: 174.00 mg L⁻¹; Temp: 18.28 °C; Turb: 2.47; Flow: 0.88 m³ s; Average depth: 0.14 m; Prec: 2.37 mm.

Metal Concentrations: Co: 17.50 mg L⁻¹; Cr: 79.78 mg L⁻¹; Cu: 244.29 mg L⁻¹; Mn: 370.99 mg L⁻¹; Ni: 66.65 mg L⁻¹; Pb: 33.49 mg L⁻¹; Zn: 146.17 mg L⁻¹.

Family GOMPHONEMATACEAE

Gomphonema angustatum (Figs 6F–6H)

Deformed valve outline, with protrusion, or bent apices. Unusual striae pattern, usually becoming more radiate (n = 6).

Ecological characteristics: Cond: 21.00–53.00 μS cm⁻¹; BOD: 1.49–10.03 mg L⁻¹; COD: 8.08–24.32 mg L⁻¹; N-NH₃: 200–378 μg L⁻¹; NO₃: 0.37–9.69 mg L⁻¹; DO: 8.98–14.57 mg L⁻¹; pH: 6.02–6.80; PO₄⁻: 0.002–0.030 mg L⁻¹; TP: 0.01–0.04 mg L⁻¹; TS: 20.00–72.00 mg L⁻¹; Temp:

17.37–21.82 °C; Turb: 1.93–14.57; Flow: 0.31–2.78 m³ s; Average depth: 0.09–0.29 m; Prec: 3.48–9.96 mm.

Metal Concentrations: Co: 17.50–58.28 mg L⁻¹; Cr: 23.10–85.16 mg L⁻¹; Cu: 153.45–259.07 mg L⁻¹; Mn: 370.99–622.76 mg L⁻¹; Ni: 31.80–69.89 mg L⁻¹; Pb: 33.49–57.94 mg L⁻¹; Zn: 71.00–412.52 mg L⁻¹.

G. graciledictum (Figs 6W–6X)

Deformed valve outline, with bent apices. Unusual striae pattern, throughout the valve or strongly radiate in the median portion of the valve (n = 7).

Ecological characteristics: Cond: 50.00–53.00 μS cm⁻¹; BOD: 0.89–3.27 mg L⁻¹; COD: 7.23–13.60 mg L⁻¹; N-NH₃: 316–378 μg L⁻¹; NO₃: 0.34–0.37 mg L⁻¹; DO: 11.42–14.80 mg L⁻¹; pH: 5.79–6.26; PO₄⁻: 0.005–0.008 mg L⁻¹; TP: 0.009–0.017 mg L⁻¹; TS: 10.00–66.00 mg L⁻¹; Temp: 17.40–17.68 °C; Turb: 7.03–10.70; Flow: 0.47–0.62 m³ s; Average depth: 0.19–0.24 m; Prec: 9.96 mm.

Metal Concentrations: Co: 23.91–33.57 mg L⁻¹; Cr: 27.33–78.86 mg L⁻¹; Cu: 197.55–235.49 mg L⁻¹; Mn: 398.50–444.52 mg L⁻¹; Ni: 31.80–69.89; Pb: 35.58–46.56 mg L⁻¹; Zn: 93.81–167.31 mg L⁻¹.

G. lagenula (Figs 6AQ–6AS; 13J)

Deformed valve outline, undulated, or with bent apices. Unusual striae pattern, usually strongly radiate in the median portion of the valve (n = 23).

Ecological characteristics: Cond: 1.00–63.00 μS cm⁻¹; BOD: 1.19–13.96 mg L⁻¹; COD: 1.23–24.65 mg L⁻¹; N-NH₃: 3–448 μg L⁻¹; NO₃: 0.32–13.60 mg L⁻¹; DO: 7.18–26.24 mg L⁻¹; pH: 5.28–6.79; PO₄⁻: 0.002–0.050 mg L⁻¹; TP: 0.005–0.036 mg L⁻¹; TS: 4.00–84.0 mg L⁻¹; Temp: 16.60–21.82 °C; Turb: 1.93–10.70; Flow: 0.06–5.88 m³ s; Average depth: 0.02–1.32 m; Prec: 2.39–11.17 mm.

Metal Concentrations: Co: 17.50–60.76 mg L⁻¹; Cr: 23.10–79.78 mg L⁻¹; Cu: 163.45–301.34 mg L⁻¹; Mn: 370.99–809.37 mg L⁻¹; Ni: 29.18–87.39 mg L⁻¹; Pb: 33.49–57.94 mg L⁻¹; Zn: 71.00–296.79 mg L⁻¹.

G. naviculoides (Fig 6AU)

Unusual striae pattern, strongly radiate in the middle portion of the valve (n = 3).

Ecological characteristics: Cond: 52.00–53.00 μS cm⁻¹; BOD: 0.89–3.27 mg L⁻¹; COD: 7.23–11.05 mg L⁻¹; N-NH₃: 316–378 mg L⁻¹; NO₃: 0.37 mg L⁻¹; DO: 11.42–14.62 mg L⁻¹; pH: 5.79–6.19; PO₄⁻: 0.005–0.008 mg L⁻¹; TP: 0.009–0.017 mg L⁻¹; TS: 21.00–66.00 mg L⁻¹; Temp: 17.37–17.50 °C; Turb: 7.03–10.70; Flow: 0.66–0.78 m³ s; Average depth: 0.22–0.34 m; Prec: 9.96 mm.

Metal Concentrations: Co: 23.91–33.57 mg L⁻¹; Cr: 27.33–78.86 mg L⁻¹; Cu: 197.55–235.49 mg L⁻¹; Mn: 398.50–444.52 mg L⁻¹; Ni: 31.80–69.89; Pb: 35.58–46.56 mg L⁻¹; Zn: 93.81–167.31 mg L⁻¹.

G. pumilum (Fig 6AM)

Deformed valve outline, with bent of the valve (n = 1)

Ecological characteristics: Cond: 44.00 $\mu\text{S cm}^{-1}$; BOD: 3.27 mg L^{-1} ; COD: 11.05 mg L^{-1} ; N-NH₃: 18 $\mu\text{g L}^{-1}$; NO₃: 0.32 mg L^{-1} ; DO: 13.39 mg L^{-1} ; pH: 6.43; PO₄⁻: 0.004 mg L^{-1} ; TP: 0.007 mg L^{-1} ; TS: 42.00 mg L^{-1} ; Temp: 17.71 °C; Turb: 8.27; Flow: 1.12 $\text{m}^3 \text{s}$; Average depth: 0.33 m; Prec: 9.96 mm.

Metal Concentrations: Co: 34.35–39.39 mg L^{-1} ; Cr: 28.18–85.31 mg L^{-1} ; Cu: 170.55–264.18 mg L^{-1} ; Mn: 503.78–606.73 mg L^{-1} ; Ni: 32.81–73.27 mg L^{-1} ; Pb: 31.05–39.31 mg L^{-1} ; Zn: 79.00–172.60 mg L^{-1} .

Family ACHNANTHIDIACEAE

Achnanthydium macrocephalum (Figs 7L–7P)

Deformed valve outline, usually with constriction near the apices (n = 6).

Ecological characteristics: Cond: 22.00–64.00 $\mu\text{S cm}^{-1}$; BOD: 1.49–4.57 mg L^{-1} ; COD: 5.53–18.10 mg L^{-1} ; N-NH₃: 130–378 $\mu\text{g L}^{-1}$; NO₃: 0.37–2.20 mg L^{-1} ; DO: 7.71–19.35 mg L^{-1} ; pH: 5.51–6.19; PO₄⁻: 0.006–0.046 mg L^{-1} ; TP: 0.010–0.060 mg L^{-1} ; TS: 4.00–104.00 mg L^{-1} ; Temp: 17.37–23.00 °C; Turb: 6.38–10.70; Flow: 0.44–1.20 $\text{m}^3 \text{s}$; Average depth: 0.09–0.34 m; Prec: 2.73–9.96 mm.

Metal Concentrations: Co: 23.91–60.76 mg L^{-1} ; Cr: 23.67–88.85 mg L^{-1} ; Cu: 163.45–241.67 mg L^{-1} ; Mn: 393.75–557.50 mg L^{-1} ; Ni: 31.80–69.89; Pb: 35.58–48.63 mg L^{-1} ; Zn: 83.42–173.12 mg L^{-1} .

A. minutissimum (Figs 7X–7AC)

Deformed valve outline, usually with constriction or bent apices. Sometimes with strong constriction in the median portion of the valve. Sigmoid shape, or unidentified (n = 38).

Ecological characteristics: Cond: 25.00–91.00 $\mu\text{S cm}^{-1}$; BOD: 0.77–9.90 mg L^{-1} ; COD: 1.00–24.32 mg L^{-1} ; N-NH₃: 200–540 $\mu\text{g L}^{-1}$; NO₃: 0.37–13.60 mg L^{-1} ; DO: 6.54–19.35 mg L^{-1} ; pH: 5.01–6.92; PO₄⁻: 0.005–0.050 mg L^{-1} ; TP: 0.005–0.922 mg L^{-1} ; TS: 19.00–174.00 mg L^{-1} ; Temp: 17.40–23.56 °C; Turb: 0.06–37.50; Flow: 0.10–5.09 $\text{m}^3 \text{s}$; Average depth: 0.04–0.41 m; Prec: 2.37–11.17 mm.

Metal Concentrations: Co: 16.73–58.28 mg L^{-1} ; Cr: 21.98–85.31 mg L^{-1} ; Cu: 160.64–292.30 mg L^{-1} ; Mn: 347.86–745.82 mg L^{-1} ; Ni: 27.57–85.63 mg L^{-1} ; Pb: 33.49–66.21 mg L^{-1} ; Zn: 73.15–412.52 mg L^{-1} .

A. modestiforme (Figs 7AF–7AH)

Deformed valve outline, with constriction near the apices (n = 8).

Ecological characteristics: Cond: 11.00–50.00 $\mu\text{S cm}^{-1}$; BOD: 2.49–9.90 mg L^{-1} ; COD: 7.57–24.32 mg L^{-1} ; N-NH₃: 200 $\mu\text{g L}^{-1}$; NO₃: 0.40–9.69 mg L^{-1} ; DO: 8.98–19.93 mg L^{-1} ; pH: 6.70–6.92; PO₄⁻: 0.002–0.050 mg L^{-1} ; TP: 0.005–0.019 mg L^{-1} ; TS: 23.00–48.00 mg L^{-1} ; Temp: 20.12–21.23 °C; Turb: 0.06–8.40; Flow: 0.13–2.78 $\text{m}^3 \text{s}^{-1}$; Average depth: 0.05–0.29 m; Prec: 3.76–4.96 mm.

Metal Concentrations: Co: 26.05–57.56 mg L^{-1} ; Cr: 19.44–85.16 mg L^{-1} ; Cu: 149.34–268.89 mg L^{-1} ; Mn: 234.22–745.82 mg L^{-1} ; Ni: 33.21–85.63 mg L^{-1} ; Pb: 33.84–61.04 mg L^{-1} ; Zn: 68.52–258.21 mg L^{-1} .

Planothidium rostratum (Figs 7AO–7AP)

Deformed valve outline, with loss of transapical symmetry (n = 2).

Ecological characteristics: Cond: 42.00–50.00 $\mu\text{S cm}^{-1}$; BOD: 1.43–2.38 mg L^{-1} ; COD: 4.68–13.68 mg L^{-1} ; N-NH₃: 323–680 mg L^{-1} ; NO₃: 0.34–1.00 mg L^{-1} ; DO: 7.79–14.80 mg L^{-1} ; pH: 6.26–6.28; PO₄⁻: 0.005–0.006 mg L^{-1} ; TP: 0.007–0.016 mg L^{-1} ; TS: 10.00–11.00 mg L^{-1} ; Temp: 17.68–23.25 °C; Turb: 10.30–10.40; Flow: 0.62–0.66 $\text{m}^3 \text{s}^{-1}$; Average depth: 0.22–0.24 m; Prec: 9.96 mm.

Metal Concentrations: Co: 23.73–47.24 mg L^{-1} ; Cr: 27.33–80.86 mg L^{-1} ; Cu: 160.64–235.49 mg L^{-1} ; Mn: 398.05–444.52 mg L^{-1} ; Ni: 27.57–69.89 mg L^{-1} ; Pb: 35.58–66.21 mg L^{-1} ; Zn: 78.97–167.31 mg L^{-1} .

Family DIADESMIDACEAE

****Humidophila arcuatoides*** (Figs 7AU–7AV)

Length: 12.1–14.6 μm ; width: 2.9–3.2 μm

Humidophila arcuatoides (= *Diadsmis arcuatoides*), described originally by Werum & Lange-Bertalot (2004), has 16–26 μm in length and 5–6 μm in width, however, the taxon has been already registered with 8–14 μm in length and 2.6–4 μm in width by Wetzel (2011). *Humidophila arcuata* Lange-Bertalot is easily differentiated by the less inflated central portion of the valve (Werum & Lange-Bertalot 2004).

Family AMPHIPLEURACEAE

Frustulia saxonica (Fig 8I)

Deformed valve outline, slightly undulated in one side of the valve (n = 2).

Ecological characteristics: Cond: 60.00–94.00 $\mu\text{S cm}^{-1}$; BOD: 6.62–13.63 mg L^{-1} ; COD: 7.14–25.30 mg L^{-1} ; N-NH₃: 200 $\mu\text{g L}^{-1}$; NO₃: 11.80–12.70 mg L^{-1} ; DO: 7.52–9.50 mg L^{-1} ; pH: 6.18–6.78; PO₄⁻: 0.002–0.055 mg L^{-1} ; TP: 0.01–0.09 mg L^{-1} ; TS: 44.00–47.00 mg L^{-1} ; Temp: 19.10–20.91 °C; Turb: 2.82–3.32; Flow: 0.06–0.61 $\text{m}^3 \text{s}^{-1}$; Average depth: 0.03–0.10 m; Prec: 3.48–9.96 mm.

Metal Concentrations: Co: 16.73–47.67 mg L⁻¹; Cr: 25.08–85.31 mg L⁻¹; Cu: 206.13–238.31 mg L⁻¹; Mn: 347.86–406.12 mg L⁻¹; Ni: 28.58–70.77 mg L⁻¹; Pb: 26.51–43.45 mg L⁻¹; Zn: 81.88–364.96 mg L⁻¹.

Family PINNULARIACEAE

Pinnularia divergens (Fig 8AJ)

Deformed valve outline, with bent of the apex (n = 1).

Ecological characteristics: Cond: 60.00 µS cm⁻¹; BOD: 13.63 mg L⁻¹; COD: 25.30 mg L⁻¹; N-NH₃: 200 µg L⁻¹; NO₃: 11.80 mg L⁻¹; DO: 9.50 mg L⁻¹; pH: 6.78; PO₄⁻: 0.002 mg L⁻¹; TP: 0.01 mg L⁻¹; TS: 44.00 mg L⁻¹; Temp: 19.10 °C; Turb: 2.82; Flow: 0.06 m³ s; Average depth: 0.03 m; Prec: 4.96 mm.

Metal Concentrations: Co: 30.30 mg L⁻¹; Cr: 59.68 mg L⁻¹; Cu: 263.28 mg L⁻¹; Mn: 410.09 mg L⁻¹; Ni: 45.89 mg L⁻¹; Pb: 40.73 mg L⁻¹; Zn: 139.68 mg L⁻¹.

P. graciloides* var. *latecapitata (Fig 9B)

Deformed valve outline, with sigmoid shape (n = 3).

Ecological characteristics: Cond: 65.00 µS cm⁻¹; BOD: 1.00 mg L⁻¹; COD: 2.55 mg L⁻¹; N-NH₃: 200 µg L⁻¹; NO₃: 2.90 mg L⁻¹; DO: 12.12 mg L⁻¹; pH: 6.40; PO₄⁻: 0.05 mg L⁻¹; TP: 0.02 mg L⁻¹; TS: 95.00 mg L⁻¹; Temp: 17.93 °C; Turb: 3.78; Flow: 0.94 m³ s; Average depth: 0.24 m; Prec: 2.37–4.96 mm.

Metal Concentrations: Co: 17.50–57.56 mg L⁻¹; Cr: 24.23–83.01 mg L⁻¹; Cu: 195.31–273.01 mg L⁻¹; Mn: 370.99–745.82 mg L⁻¹; Ni: 33.62–85.63 mg L⁻¹; Pb: 33.49–54.83 mg L⁻¹; Zn: 78.88–258.21 mg L⁻¹.

*****Pinnularia laucensis*** (Figs 9J–9K)

Length: 15.5–26.3 µm; width: 3.6–4.5; striae: 12–15 in 10 µm

This taxon was originally described from high-altitude (4000 m) and acidic (pH = 5) river samples from Chile (Rumrich *et al.* 2000). The individuals found in Cascavel river microbasin are similar to *P. laucensis*, except for being slightly larger than the original description (length: 13–20 µm; width: 3.3–4 µm), but similar striae density (11–14 in 10 µm). *Pinnularia sinistra* Krammer, the most similar taxon, can be differentiated by the subrostrate apices and more radiate striae (Krammer 1992).

Family SELLAPHORACEAE

Sellaphora saugerresii (Figs 9AD–9AF)

Deformed valve outline, usually undulated, with asymmetry of the apical axis (n = 33).

Ecological characteristics: Cond: 1.00–70.00 $\mu\text{S cm}^{-1}$; BOD: 1.00–10.3 mg L^{-1} ; COD: 1.22–27.60 mg L^{-1} ; N-NH₃: 110–500 $\mu\text{g L}^{-1}$; NO₃: 2.00–6.88 mg L^{-1} ; DO: 8.18–15.81 mg L^{-1} ; pH: 5.11–7.42; PO₄⁻: 0.002–0.323 mg L^{-1} ; TP: 0.005–0.350 mg L^{-1} ; TS: 7.00–174.00 mg L^{-1} ; Temp: 18.28–22.27 °C; Turb: 1.93–41.90; Flow: 0.05–6.34 $\text{m}^3 \text{s}$; Average depth: 0.02–0.37 m; Prec: 2.37–9.96 mm.

Metal Concentrations: Co: 17.50–65.84 mg L^{-1} ; Cr: 25.92–85.01 mg L^{-1} ; Cu: 163.45–273.45 mg L^{-1} ; Mn: 329.01–642.36 mg L^{-1} ; Ni: 27.57–72.54 mg L^{-1} ; Pb: 31.05–52.76 mg L^{-1} ; Zn: 78.35–287.28 mg L^{-1} .

Family NAVICULACEAE

Caloneis bacillum (Fig 9AM)

Deformed valve outline, with asymmetry of the apical axis (n = 1).

Ecological characteristics: Cond: 44.00 $\mu\text{S cm}^{-1}$; BOD: 4.59 mg L^{-1} ; COD: 6.79 mg L^{-1} ; N-NH₃: 200 $\mu\text{g L}^{-1}$; NO₃: 1.60 mg L^{-1} ; DO: 26.24 mg L^{-1} ; pH: 6.81; PO₄⁻: 0.05 mg L^{-1} ; TP: 0.02 mg L^{-1} ; TS: 50.00 mg L^{-1} ; Temp: 20.30 °C; Turb: 0.00; Flow: 5.88 $\text{m}^3 \text{s}$; Average depth: 0.34 m; Prec: 2.73 mm.

Metal Concentrations: Co: 60.76 mg L^{-1} ; Cr: 33.25 mg L^{-1} ; Cu: 163.45 mg L^{-1} ; Mn: 538.54 mg L^{-1} ; Ni: 32.81 mg L^{-1} ; Pb: 48.63 mg L^{-1} ; Zn: 83.42 mg L^{-1} .

Navicula cryptocephala (Figs 10G–10I)

Deformed valve outline, with loss of transapical symmetry (n = 3).

Cond: 52.00 $\mu\text{S cm}^{-1}$; BOD: 4.16 mg L^{-1} ; COD: 17.43 mg L^{-1} ; N-NH₃: 316 $\mu\text{g L}^{-1}$; NO₃: 0.32 mg L^{-1} ; DO: 14.62 mg L^{-1} ; pH: 6.04; PO₄⁻: 0.004 mg L^{-1} ; TP: 0.012 mg L^{-1} ; TS: 16.00 mg L^{-1} ; Temp: 17.90 °C; Turb: 9.25; Flow: 0.45 $\text{m}^3 \text{s}$; Average depth: 0.19 m; Prec: 9.96 mm.

Metal Concentrations: Co: 19.28–23.11 mg L^{-1} ; Cr: 25.92–83.62 mg L^{-1} ; Cu: 196.71–241.52 mg L^{-1} ; Mn: 400.50–955.89 mg L^{-1} ; Ni: 33.21–59.59 mg L^{-1} ; Pb: 36.28–38.28 mg L^{-1} ; Zn: 78.35–169.95 mg L^{-1} .

Navicula cryptotenella (Figs 10M)

Deformed valve outline, with loss of transapical symmetry (n = 3).

Ecological characteristics: Cond: 50.0–53.00 $\mu\text{S cm}^{-1}$; BOD: 1.49–2.38 mg L^{-1} ; COD: 8.08–13.60 mg L^{-1} ; N-NH₃: 323–378 mg L^{-1} ; NO₃: 0.34–0.37 mg L^{-1} ; DO: 14.57–14.80 mg L^{-1} ; pH: 6.19–6.26; PO₄⁻: 0.005–0.006 mg L^{-1} ; TP: 0.010–0.016 mg L^{-1} ; TS: 10.00–38.00 mg L^{-1} ; Temp: 17.37–17.68 °C; Turb: 10.40–10.70; Flow: 0.47–0.62 $\text{m}^3 \text{s}$; Average depth: 0.19–0.24 m; Prec: 9.96–11.17 mm.

Metal Concentrations: Co: 22.67–33.57 mg L⁻¹; Cr: 23.67–78.86 mg L⁻¹; Cu: 197.55–235.49 mg L⁻¹; Mn: 291.12–444.52 mg L⁻¹; Ni: 31.80–69.89; Pb: 35.58–46.56 mg L⁻¹; Zn: 93.81–167.31 mg L⁻¹.

Family STAURONEIDACEAE

Craticula riparia (Figs 10Z)

Deformed valve outline, slightly undulated (n = 4).

Ecological characteristics: Cond: 53.00 µS cm⁻¹; BOD: 1.49–3.27 mg L⁻¹; COD: 7.23–8.08 mg L⁻¹; N-NH₃: 316–378 mg L⁻¹; NO₃: 0.37 mg L⁻¹; DO: 11.42–14.57 mg L⁻¹; pH: 5.79–6.19; PO₄⁻: 0.006–0.008 mg L⁻¹; TP: 0.010–0.017 mg L⁻¹; TS: 38.00–66.00 mg L⁻¹; Temp: 17.37–17.50 °C; Turb: 7.03–10.70; Flow: 0.47 m³ s; Average depth: 0.19–0.23 m; Prec: 9.96 mm.

Metal Concentrations: Co: 23.73–47.24 mg L⁻¹; Cr: 27.33–80.86 mg L⁻¹; Cu: 160.64–235.49 mg L⁻¹; Mn: 398.05–444.52 mg L⁻¹; Ni: 27.57–69.89 mg L⁻¹; Pb: 35.58–66.21 mg L⁻¹; Zn: 78.97–167.31 mg L⁻¹.

C. submolesta (Figs 10W)

Deformed valve outline, slightly undulated in one side of the valve (n = 1).

Ecological characteristics: Cond: 53.00 µS cm⁻¹; BOD: 1.49 mg L⁻¹; COD: 8.08 mg L⁻¹; N-NH₃: 378 µg L⁻¹; NO₃: 0.37 mg L⁻¹; DO: 14.57 mg L⁻¹; pH: 6.19; PO₄⁻: 0.006 mg L⁻¹; TP: 0.010 mg L⁻¹; TS: 38.00 mg L⁻¹; Temp: 17.37 °C; Turb: 10.70; Flow: 0.47 m³ s; Average depth: 0.19 m; Prec: 4.96–9.96 mm.

Metal Concentrations: Co: 23.91–37.79 mg L⁻¹; Cr: 27.33–85.16 mg L⁻¹; Cu: 197.55–259.07 mg L⁻¹; Mn: 398.50–465.70 mg L⁻¹; Ni: 31.80–69.89; Pb: 35.58–49.66 mg L⁻¹; Zn: 93.81–167.31 mg L⁻¹.

Family BACILLARIACEAE

Nitzschia palea (Figs 11D–11F)

Deformed valve outline, with asymmetry of the apical axis, or constriction near the apex (n = 3).

Ecological characteristics: Cond: 50.00–52.00 µS cm⁻¹; BOD: 0.77–9.90 mg L⁻¹; COD: 1.70–24.32 mg L⁻¹; N-NH₃: 200–540 µg L⁻¹; NO₃: 1.00–9.69 mg L⁻¹; DO: 8.19–8.98 mg L⁻¹; pH: 6.80–6.93; PO₄⁻: 0.007–0.010 mg L⁻¹; TP: 0.010–0.015 mg L⁻¹; TS: 41.00–48.00 mg L⁻¹; Temp: 20.35–23.62 °C; Turb: 8.40–10.6; Flow: 0.66–2.78 m³ s; Average depth: 0.25–0.29 m; Prec: 4.96–9.96 mm.

Metal Concentrations: Co: 23.73–47.24 mg L⁻¹; Cr: 28.46–85.16 mg L⁻¹; Cu: 160.64–259.07 mg L⁻¹; Mn: 419.46–465.70 mg L⁻¹; Ni: 27.57–69.89 mg L⁻¹; Pb: 35.93–66.21 mg L⁻¹; Zn: 78.97–151.46 mg L⁻¹.

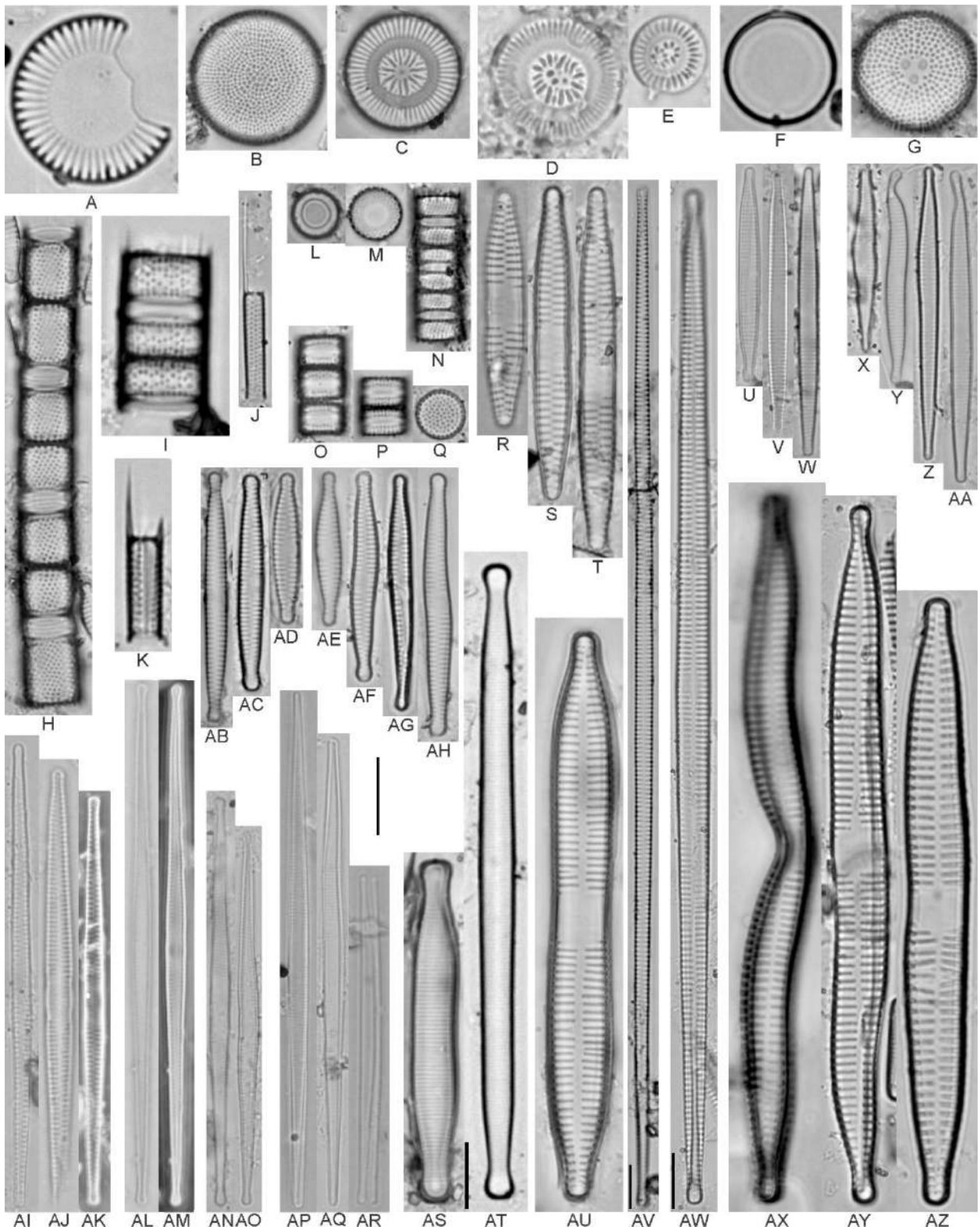


FIGURE 3. Epilithic diatoms from Cascavel River microbasin in LM. A. *Cyclotella distinguenda*; B. *C. kingstonii*; C–E. *Discostella stelligera*. D–E. Teratological valves; F. *Melosira varians*; G. *Orthoseira rooseana*; H. *Aulacoseira ambigua*; I. *A. brasiliensis*; J. *A. granulata* var. *angustissima*; K. *A. granulata*; L–N. *A. pusilla*; O–Q. *A. tenella*. R–T. *Fragilaria fragilarioides*; U–AA. *F. gracilis*. X–AA. Teratological valves; AB–AH. *F. pectinalis*. AE–AH. Teratological valves; AI–AK. *F. tenera* var. *tenera*; AL–AO. *F. tenera* var. *nanana*. AN–AO. Teratological valves; AP–AR. *F. spectra*; AS–AT. *Fragilariforma javanica*; AU. *Ulnaria contracta*; AV. *U. delicatissima*; AW–AZ. *U. ulna*; AX–AZ. Teratological valves. Scale bars represent 10 µm.

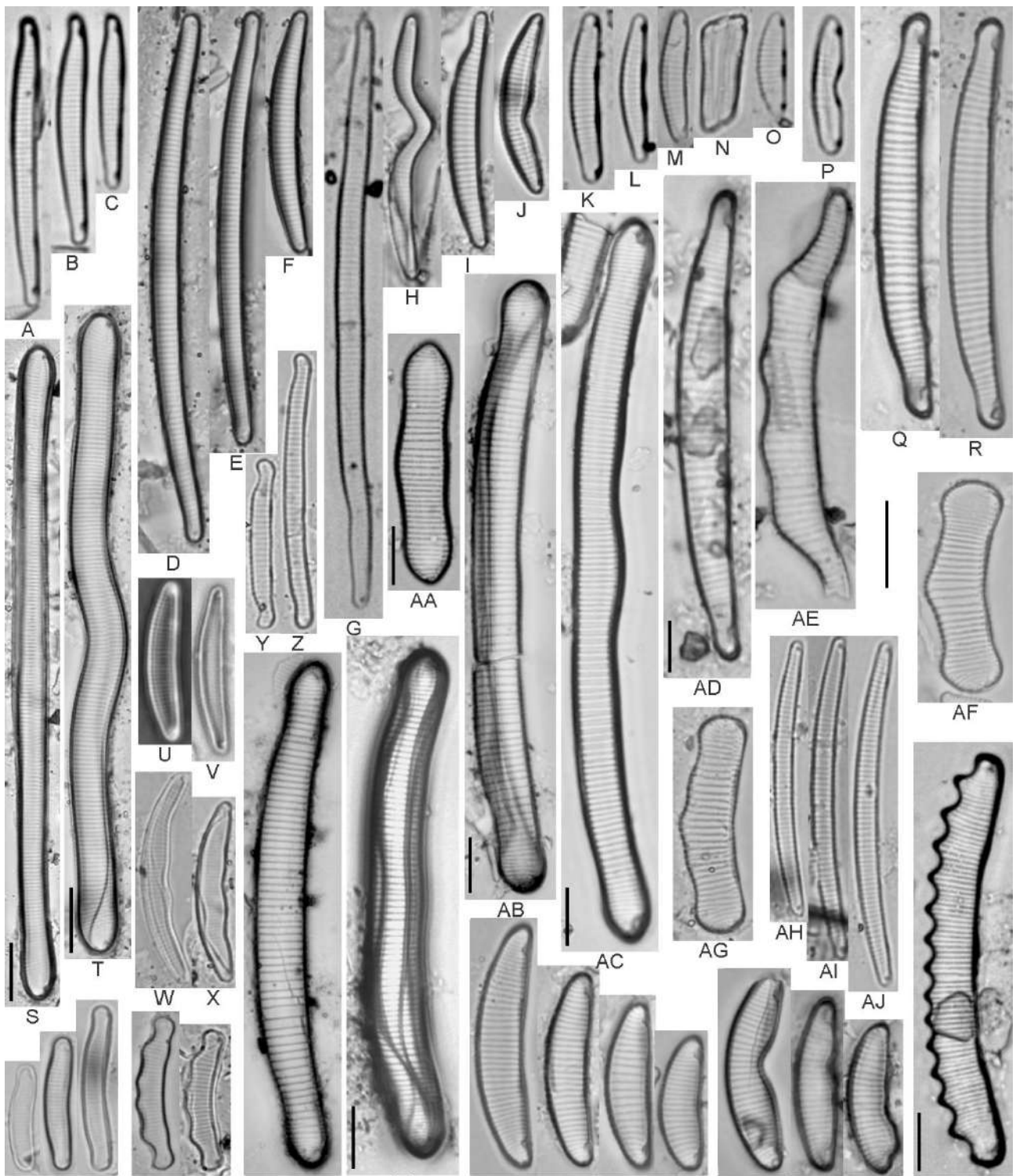


FIGURE 4. Epilithic diatoms from Cascavel River microbasin in LM. A–C. *Actinella hermes-moreirae*; D–J. *Eunotia bilunaris*. G–J. Teratological valves; K–P. *E. botulitropica*. P. Teratological valve; Q–R. *E. canicula*; S–T. *E. desmogonioides*. T. Teratological valve; U–X. *E. subarcuatooides*; W–X. Teratological valves; Y–Z. *E. fallax*; AA. *E. formica*; AB. *E. formicina*; AC. *E. karenae*; AD. *E. intricans*; AE. *E. longicamelus*; AF. *E. rabenhorstii* var. *monodon*; AG. *E. rabenhorstii* var. *triodon*; AH–AJ. *E. juettnerae*; AK–AM. *E. kruegeri*; AN–AO. *E. muscicola*; AP–AQ. *E. monodon*. AQ. Teratological valve; AR–AX. *E. meridiana*. AV–AX. Teratological valves; AY. *E. georgii*. Scale bars represent 10 μm .

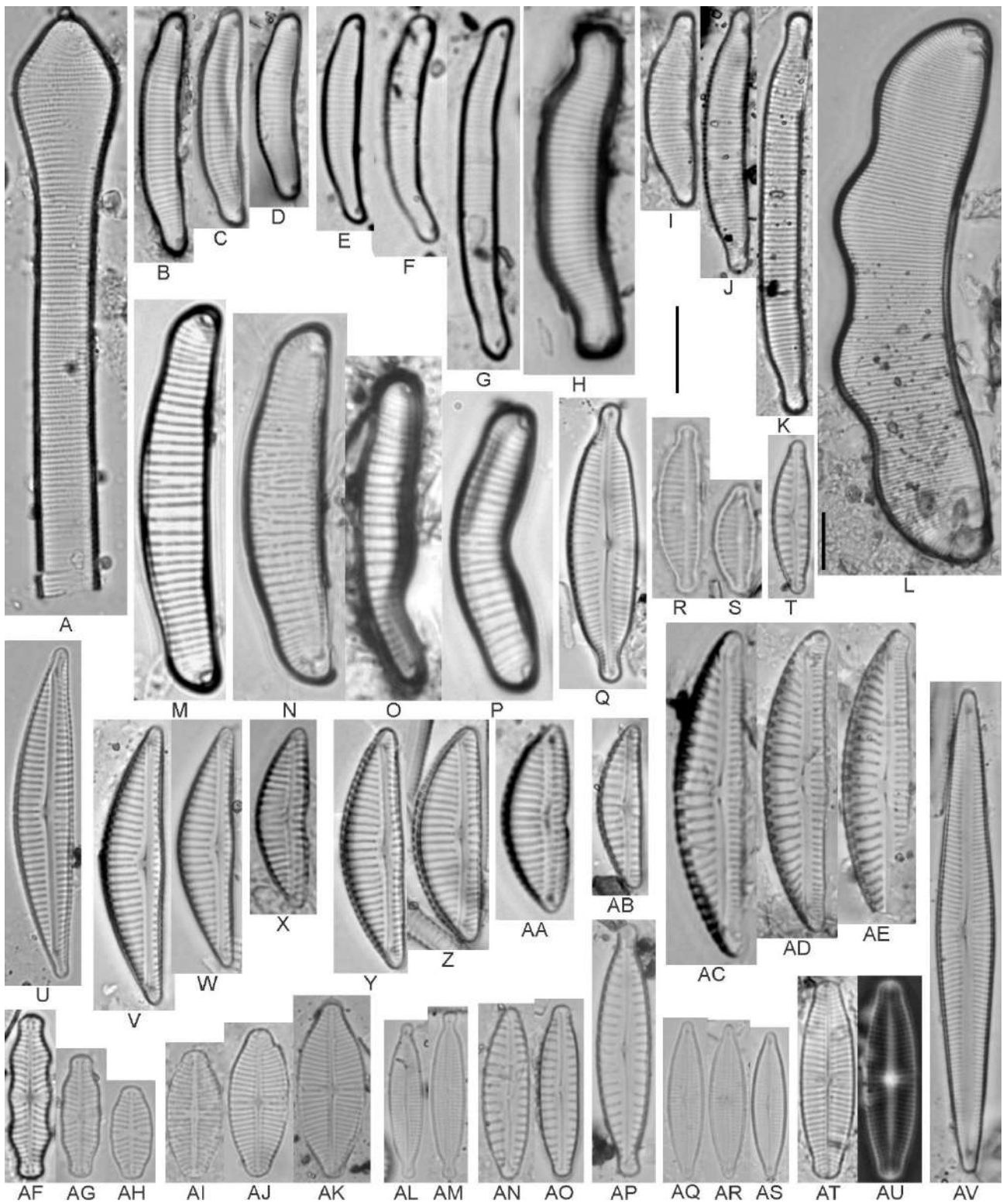


FIGURE 5. Epilithic diatoms from Cascavel River microbasin in LM. A. *Eunotia ossicula*; B–G. *E. rhomboidea*. E–G. Teratological valves; H. *E. tropico-arcus*; I–K. *E. veneris*; L. *E. yanomami*; M–P. *E. yberai*. N–P. Teratological valves; Q. *Cymbopleura naviculiformis*; R–S. *Encyonema angustecapitatum*; T. *E. perpusillum*; U. *E. neogracile*; V–X. *E. neomesianum*. X. Teratological valve; Y–AA. *E. silesiacum*. AA. Teratological valve; AB. *E. minutiforme*; AC–AE. *E. exuberans*; AF–AH. *Navigeia ignota*; AI–AK. *Placogeia kriegeri*; AL–AM. *Encyonopsis subminuta*; AN–AO. *E. difficilis*; AP. *E. schubartii*; AQ–AS. *E. thienemannii*; AT–AU. *Geissleria punctifera*; AV. *Kurtkammeria spicula*. Scale bars represent 10 μm .

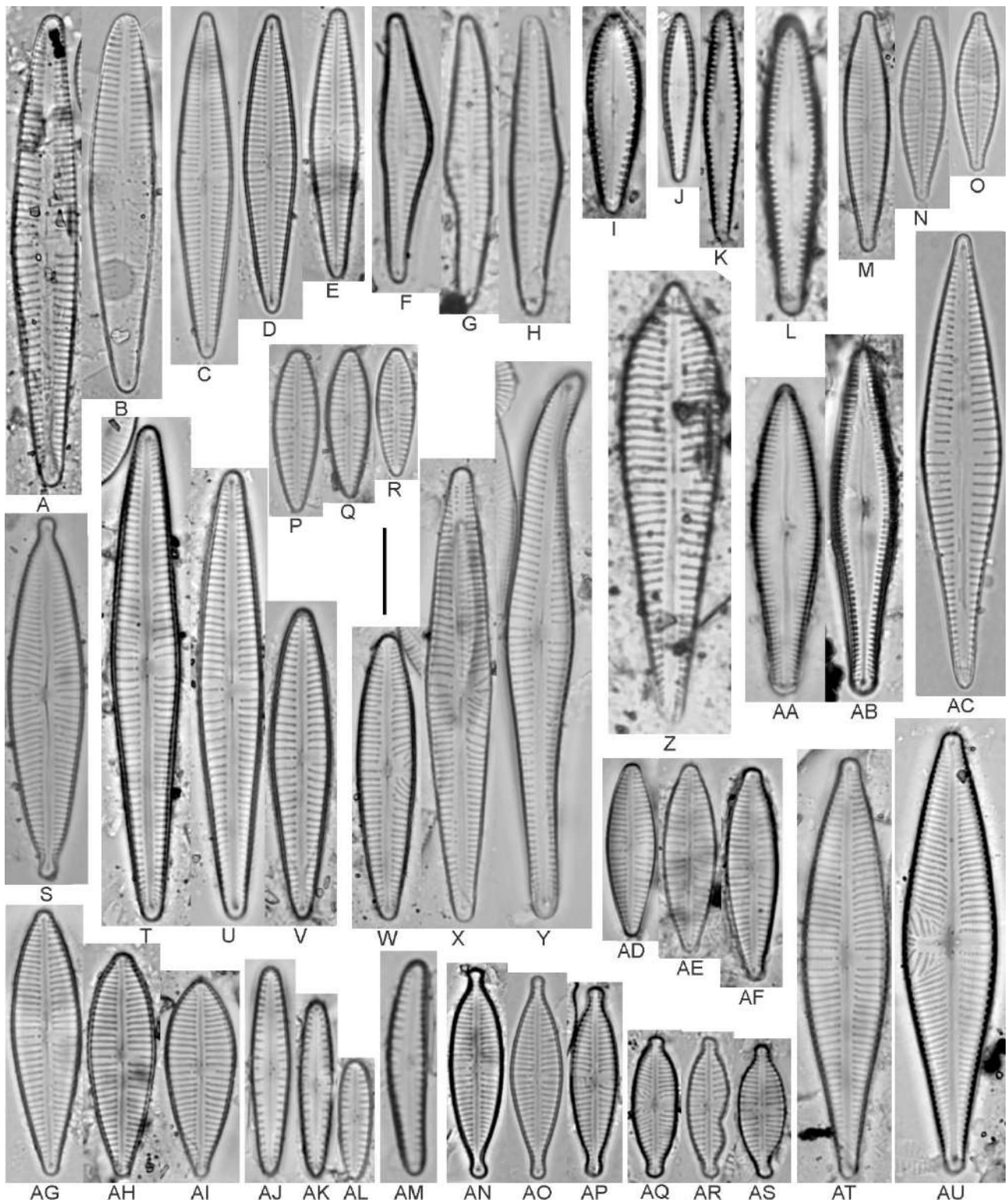


FIGURE 6. Epilithic diatoms from Cascavel River microbasin in LM. A–B. *Gomphonema affine*; C–H. *G. angustatum*. F–H. Teratological valves; I. *G. brasiliense*; J–K. *G. brasiliense* subsp. *pacificum*; L. *G. brasiliense* subsp. *pacificum*; M–O. *G. exilissimum*; P–R. *G. parvulum*; S. *G. frequentiformis*; T–Y. *G. graciledictum*. W–Y. Teratological valves; Z. *G. perapicatum*; AA–AB. *G. hawaiiense*; AC. *G. guaraniarum*; AD–AF. *G. saprophilum*; AG–AI. *G. pseudoaugur*; AJ–AM. *G. pumilum*. AM. Teratological valve; AN–AS. *G. lagenula*. AQ–AS. Teratological valves; AT–AU. *G. naviculoides*. AU. Teratological valve. Scale bars represent 10 μm .

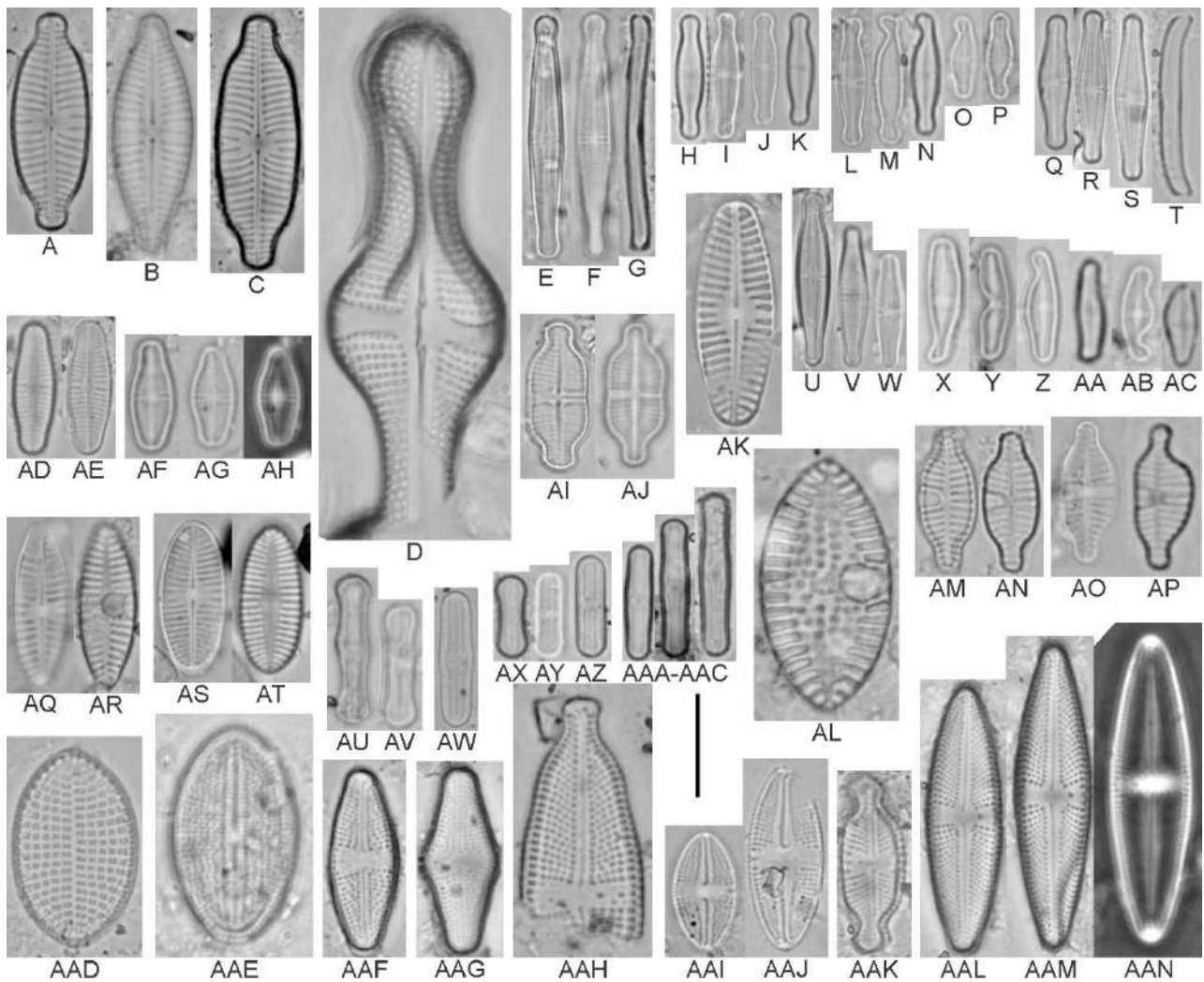


FIGURE 7. Epilithic diatoms from Cascavel River microbasin in LM. A. *Placoneis elginensis*; B. *P. hambergii*; C. *P. undulata*; D. *Achnanthes inflata*; E–G. *Achnantheidium caledonicum*; H–P. *A. macrocephalum*. L–P. Teratological valves; Q–T. *A. tropicocatenatum*; U–AC. *A. minutissimum*. X–AC. Teratological valves; AD–AH. *A. modestiforme*. AF–AH. Teratological valves; AI–AJ. *Gogorevia constricta*; AK. *Planothidium bagualensis*; AL. *P. laghereimii*; AM–AP. *P. rostratum*. AO–AP. Teratological valves; AQ–AR. *P. frequentissimum*; AS–AT. *Platessa hustedtii*; AU–AV. *Humidophila arcuatoides*; AW. *H. implicata*; AX–AZ. *H. contenta*; AAA–AAC. *H. subtropica*; AAD. *Cocconeis fluviatilis*; AAE. *C. lineata*; AAF. *Luticola ectorii*; AAG. *L. hustedtii*; AAH. *L. charlatii*; AAI–AAJ. *L. permuticoides*; AAK. *L. ventricosa*; AAL–AAN. *L. goeppertiana*. Scale bars represent 10 μm .

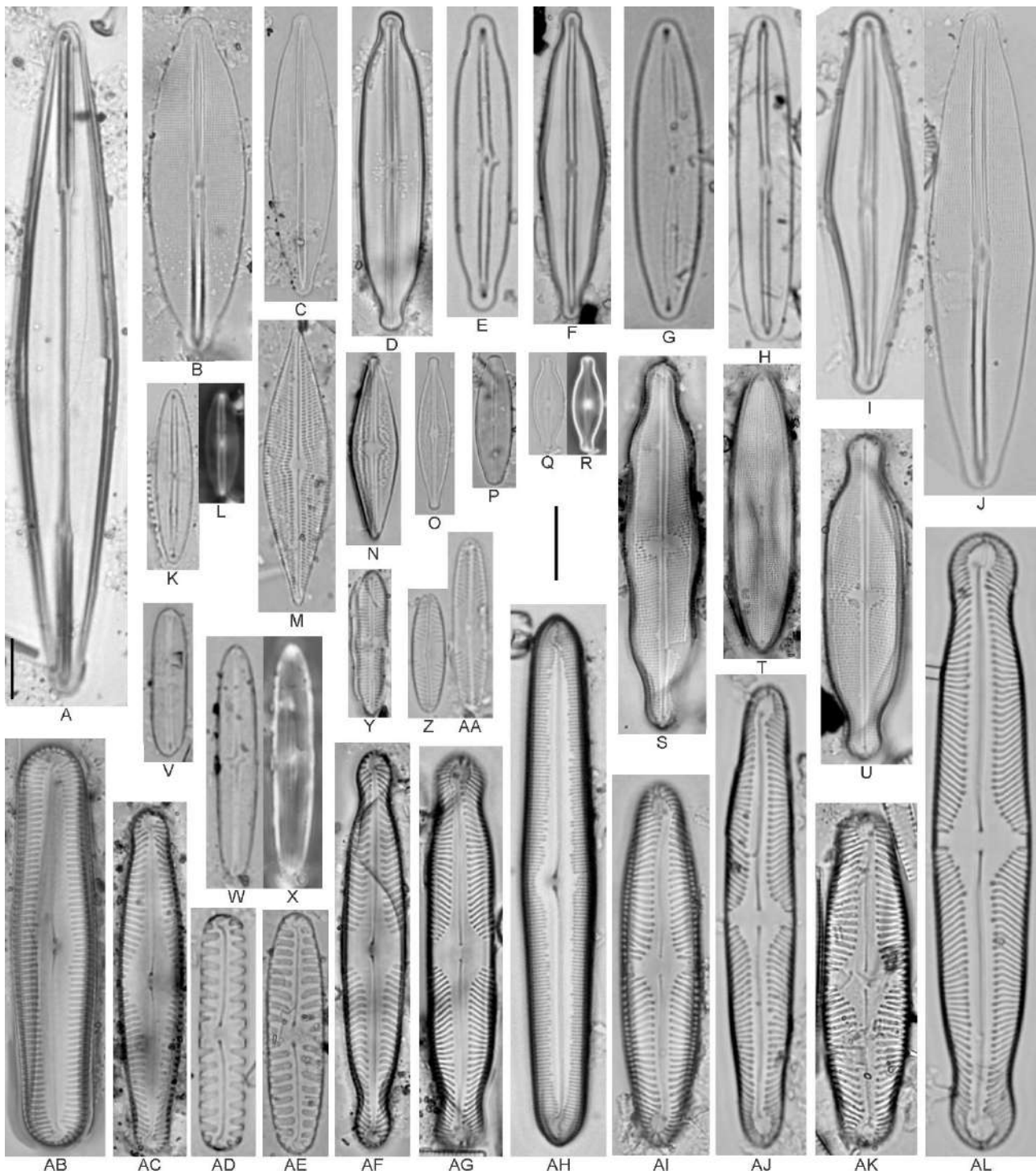


FIGURE 8. Epilithic diatoms from Cascavel River microbasin in LM. A. *Amphipleura lindheimeri*; B. *Frustulia acidophilissima*; C. *F. australocrassinervia*; D. *F. guayanensis*; E. *F. neomundana*; F. *F. undosa*; G. *F. weinholdii*; H. *F. vulgaris*; I–J. *F. saxonica*. I. Teratological valve; K–L. *F. pumilio*; M. *Brachysira serians* var. *acuta*; N. *B. brebissonii*; O. *B. microcephala*; P. *Nupela praecipuoides*; Q–R. *N. semifasciata*; S. *Neidium gracile* f. *aequale*; T. *N. iridis*; U. *N. affine* var. *amphirhynchus*; V. *N. tenuissimum*; W–X. *N. essequiboanum*; Y. *N. catarinense*; Z–AA. *Pinnularia acoricola*; AB. *P. acrosphaeria*; AC. *P. gaiserae*; AD. *P. borealis* var. *rectangularis*; AE. *P. borealis* var. *sublinearis*; AF. *P. brauniana*; AG. *P. divergens* var. *media*; AH. *P. butantanum*; AI–AJ. *P. divergens*. AJ. Teratological valve; AK. *P. decrescens* var. *ignorata*; AL. *P. divergens* var. *mesoleptiformis*. Scale bars represent 10 μm .

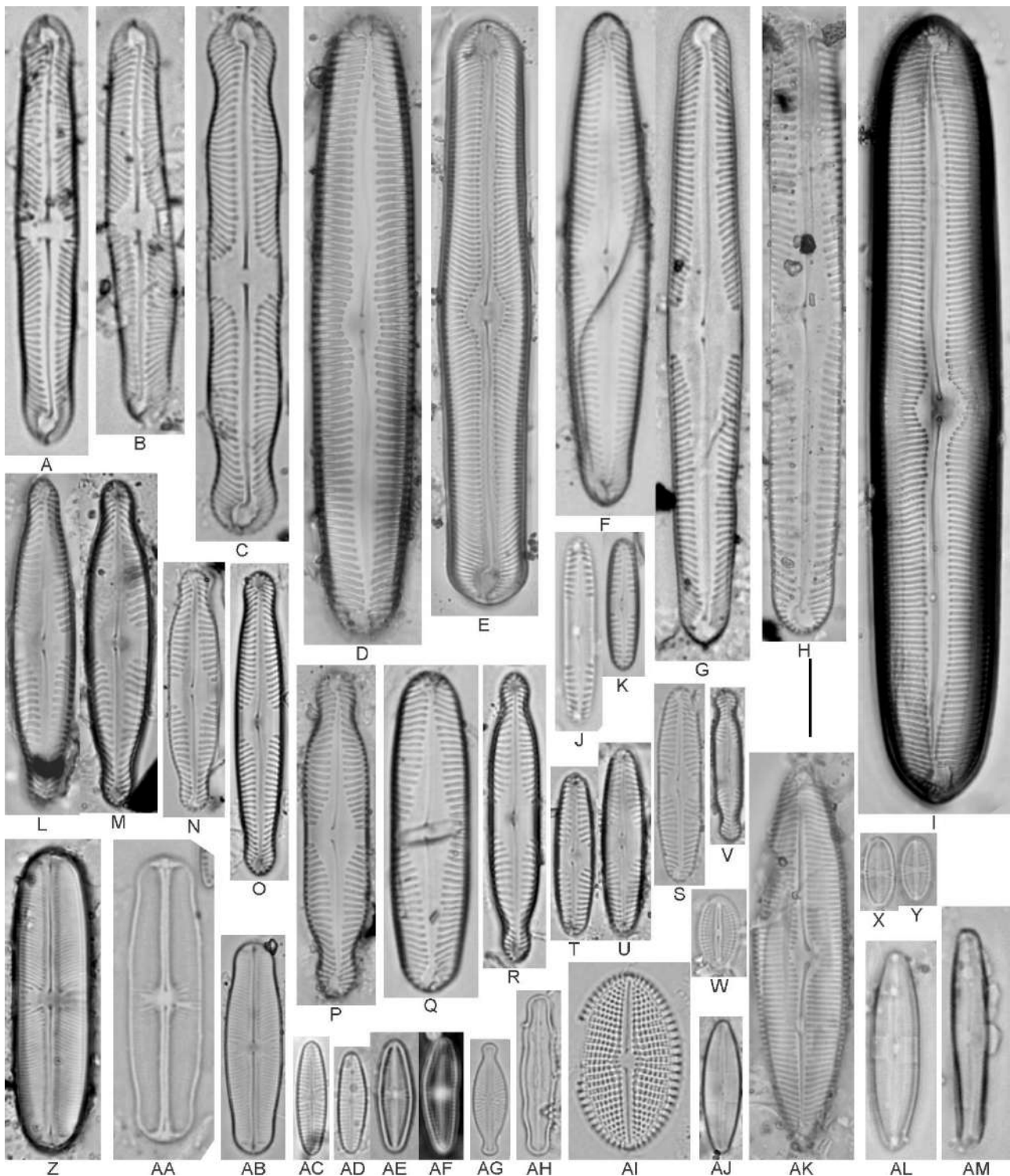


FIGURE 9. Epilithic diatoms from Cascavel River microbasin in LM. A–B. *Pinnularia graciloides* var. *latecapitata*. B. Teratological valve; C. *P. graciloides* var. *triundulata*; D. *P. viridiformis*; E. *P. tabellaria*; F–G. *P. subgibba* var. *undulata*; H. *P. subgibba*; I. *Pinnularia stidolphii*; J–K. *P. laucensis*; L–M. *P. certa*; N. *P. microstauron* var. *rostrata*; O. *P. subcapitata*; P. *P. subanglica*; Q. *P. meridiana*; R. *P. rumrichae*; S–T. *P. obscura*; U. *P. schoenfelderi*; V. *P. jocolata*; W. *Fallacia insociabilis*; X–Y. *Sellaphora nigri*; Z. *S. laevissima*; AA. *S. rhombicarea*; AB. *S. pupula*; AC–AF. *S. saugerresii*. AD–AF. Teratological valves; AG. *S. sassiana*; AH. *S. tridentula*; AI. *Diploneis subovalis*; AJ. *Caloneis hyalina*; AK. *C. westii*; AL–AM. *C. bacillum*. AM. Teratological valve. Scale bars represent 10 μm .

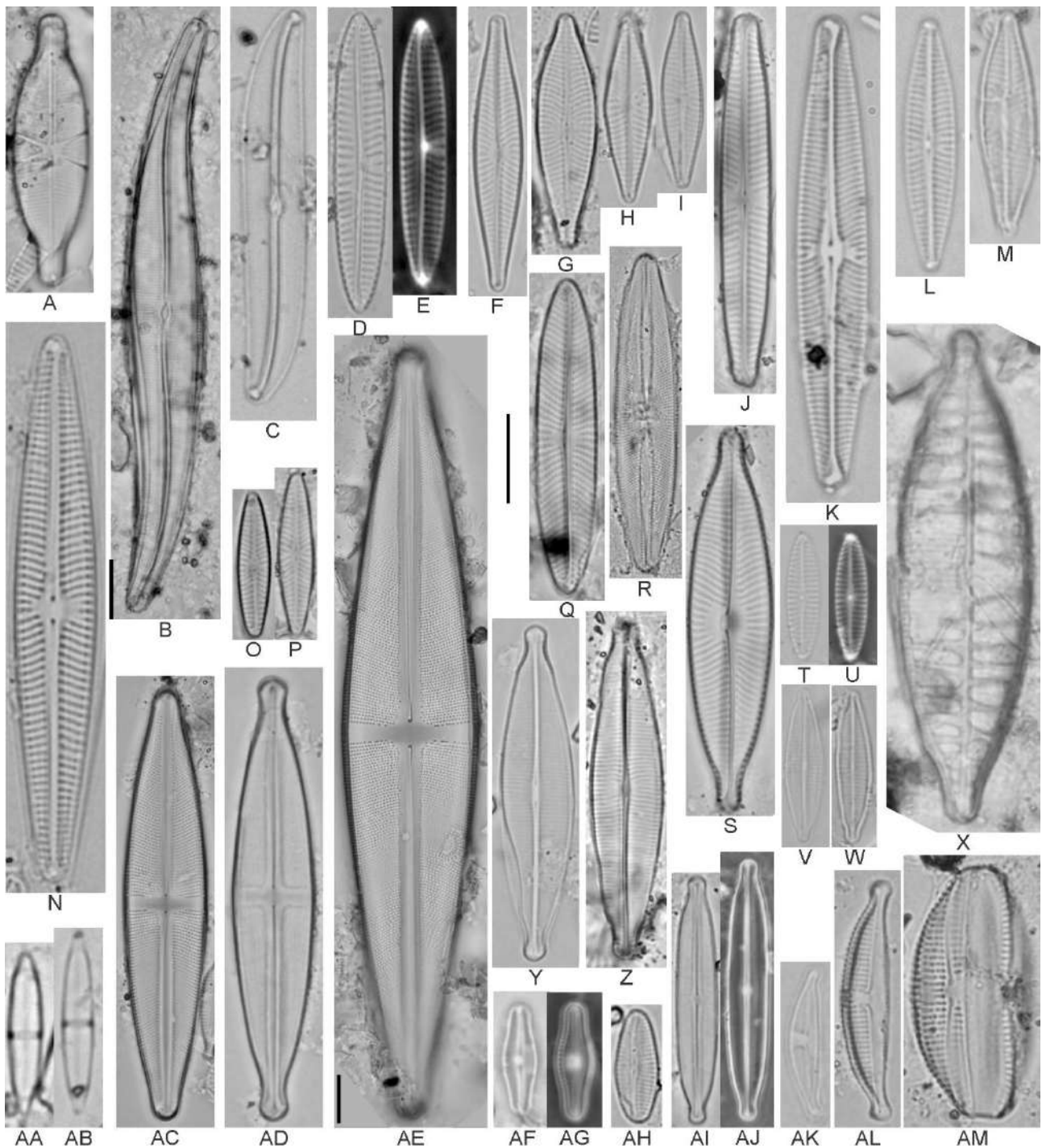


FIGURE 10. Epilitic diatoms from Cascavel River microbasin in LM. A. *Capartogramma crucicula*; B. *Gyrosigma acuminatum*; C. *G. obtusatum*; D–E. *Navicula eichhorniaephila*; F–I. *N. cryptocephala*. G–I. Teratological valves; J. *N. angusta*; K. *N. leptostriata*; L–M. *N. cryptotenella*. M. Teratological valve; N. *N. radiosa*; O–P. *N. tenelloides*; Q. *N. symmetrica*; R. *N. schwabei*; S. *N. rostellata*; T–U. *N. salinicola*; V–W. *Craticula submolesta*. W. Teratological valve; X. *C. ambigua*; Y–Z. *C. riparia*. Z. Teratological valve; AA–AB. *Stauroneis smithii*; AC. *S. gracilis*; AD. *S. neohyalina*; AE. *S. phoenicenteron*; AF–AG. *Chammaepinnularia brasilianopsis*; AH. *Pseudofallacia monoculata*; AI–AJ. *Kobayasiella parasubtilissima*; AK. *Halamphora montana*; AL. *H. normanii*; AM. *Amphora copulata*. Scale bars represent 10 μm .

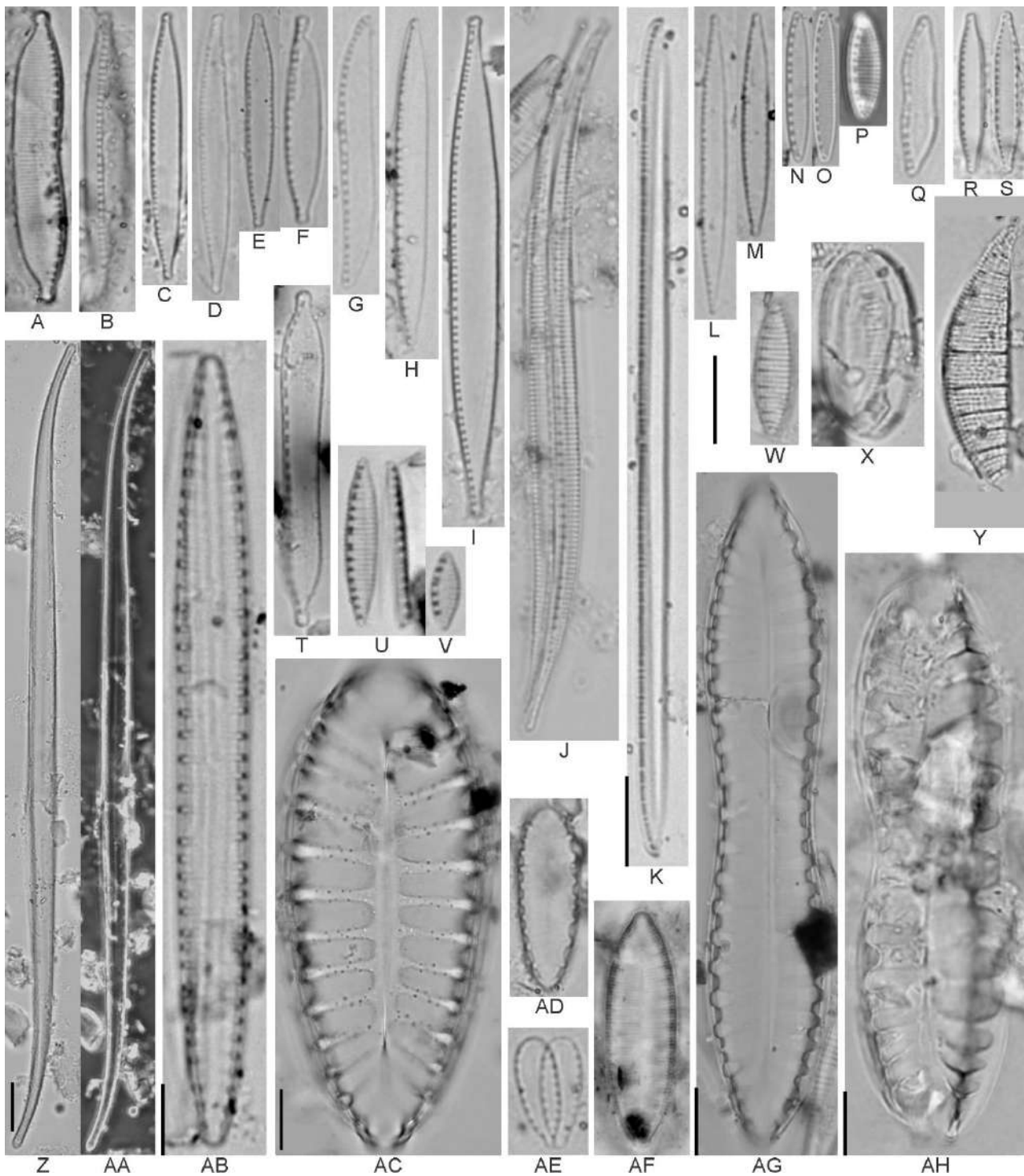


FIGURE 11. Epilithic diatoms from Cascavel River microbasin in LM. A. *Hantzschia amphioxys*; B. *Nitzschia dissipata*; C–F. *N. palea*. D–F. Teratological valves; G. *N. clausii*; H. *N. recta*; I. *N. intermedia*; J. *N. lorenziana*; K. *N. linearis*; L–M. *N. palea* var. *debilis*; N–P. *N. inconspicua*; Q. *N. brevissima*; R–S. *N. perminuta*; T. *N. terrestris*; U–V. *N. amphibia*; W. *N. semirobusta*; X. *Tryblionella debilis*; Y. *Epithemia gibberula*; Z–AA. *Iconella curvula*; AB. *I. delicatissima*; AC. *I. splendida*; AD. *I. tenuissima*; AE. *Surirella stalagma*; AF. *S. angusta*; AG. *S. biseriata* var. *constricta*; AH. *S. grunowii*. Scale bars represent 10 μm .

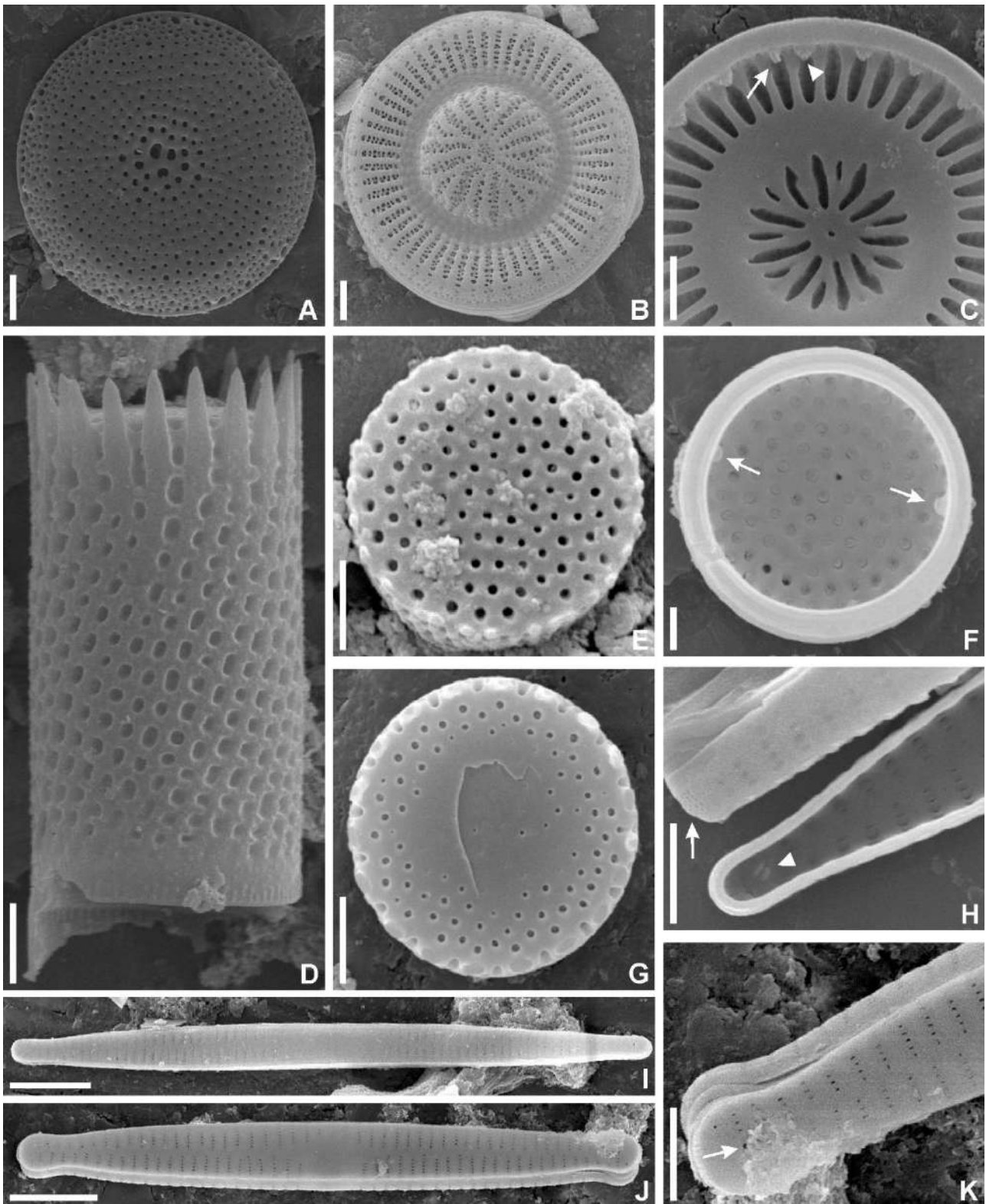


FIGURE 12. Epilithic diatoms from Cascavel River microbasin in SEM. A. *Cyclotella kingstonii*; B–C. *Discostella stelligera*. C. Internal view showing rimoportula (arrow) and fultoportula (arrowhead); D. *Aulacoseira ambigua*; E–F. *A. tenella*. F. Internal view showing rimoportulae (arrows); G. *A. pusilla*; H–I. *Fragilaria gracilis*. H. Internal valve view showing apical pore field (arrow) and rimoportula (arrowhead); J–K. *F. pectinalis*. K. External view showing rimoportula (arrow). Scale bars represent 2 μm ; 5 μm (I, J).

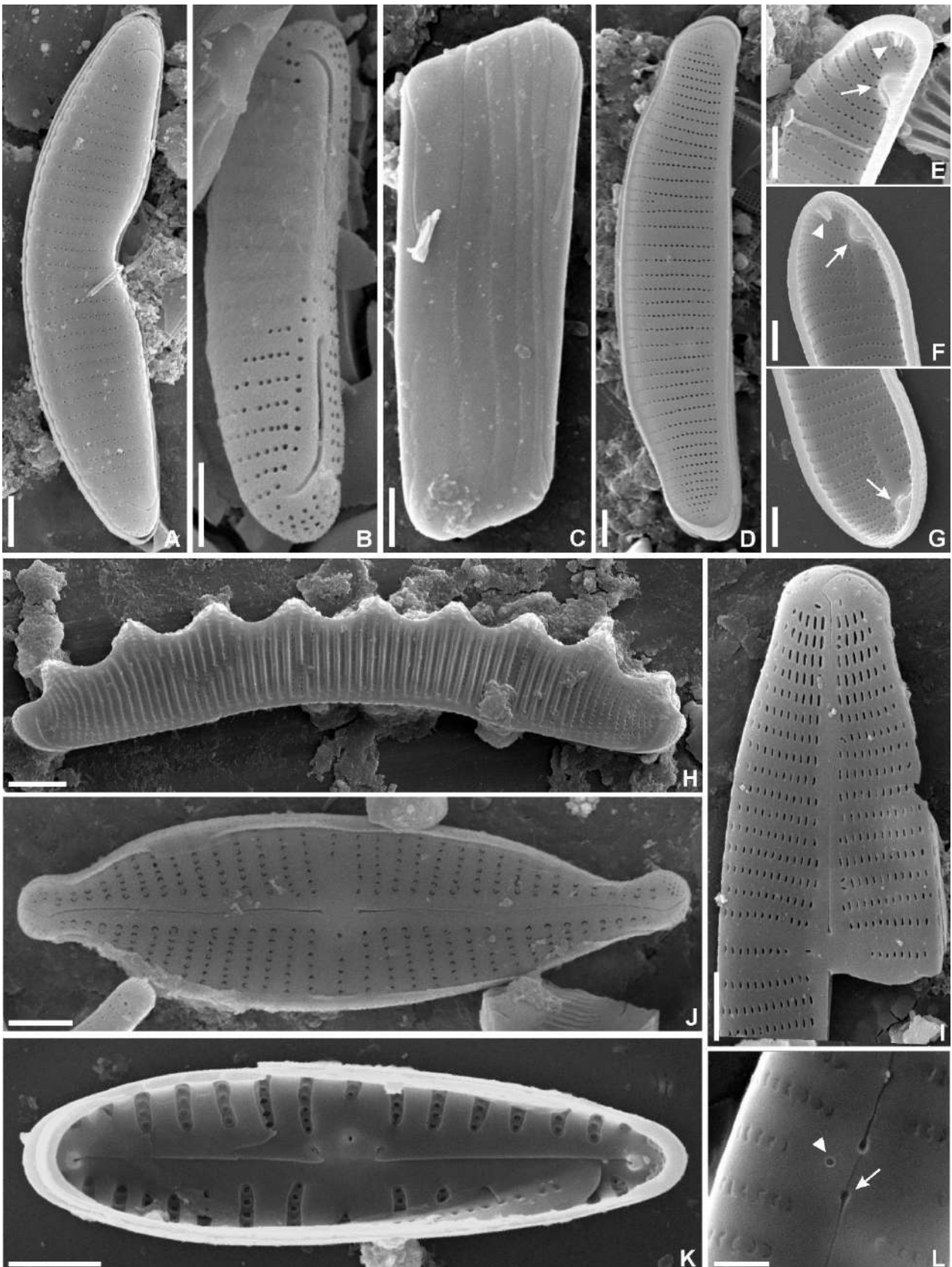


FIGURE 13. Epilithic diatoms from Cascavel River microbasin in SEM. A. *Eunotia bilunaris*, teratological valve; B–C. *E. botulitropica*. C. Girdle view; D–E. *E. rhomboidea*. E. Internal view showing helictoglossa (arrow) and rimoportula (arrowhead); F–G. *E. desmogonioides*. Internal view showing rimoportula (arrow) and helictoglossa (arrowhead); H. *E. georgii*; I. *Geissleria punctifera*; J. *G. lagenula*, teratological valve; K–L. *G. pumilum*. L. External view showing pore-like stigma (arrow), proximal raphe ends (arrow) and C-like areolae. Scale bars represent 2 μm , 5 μm (H), 1 μm (L).

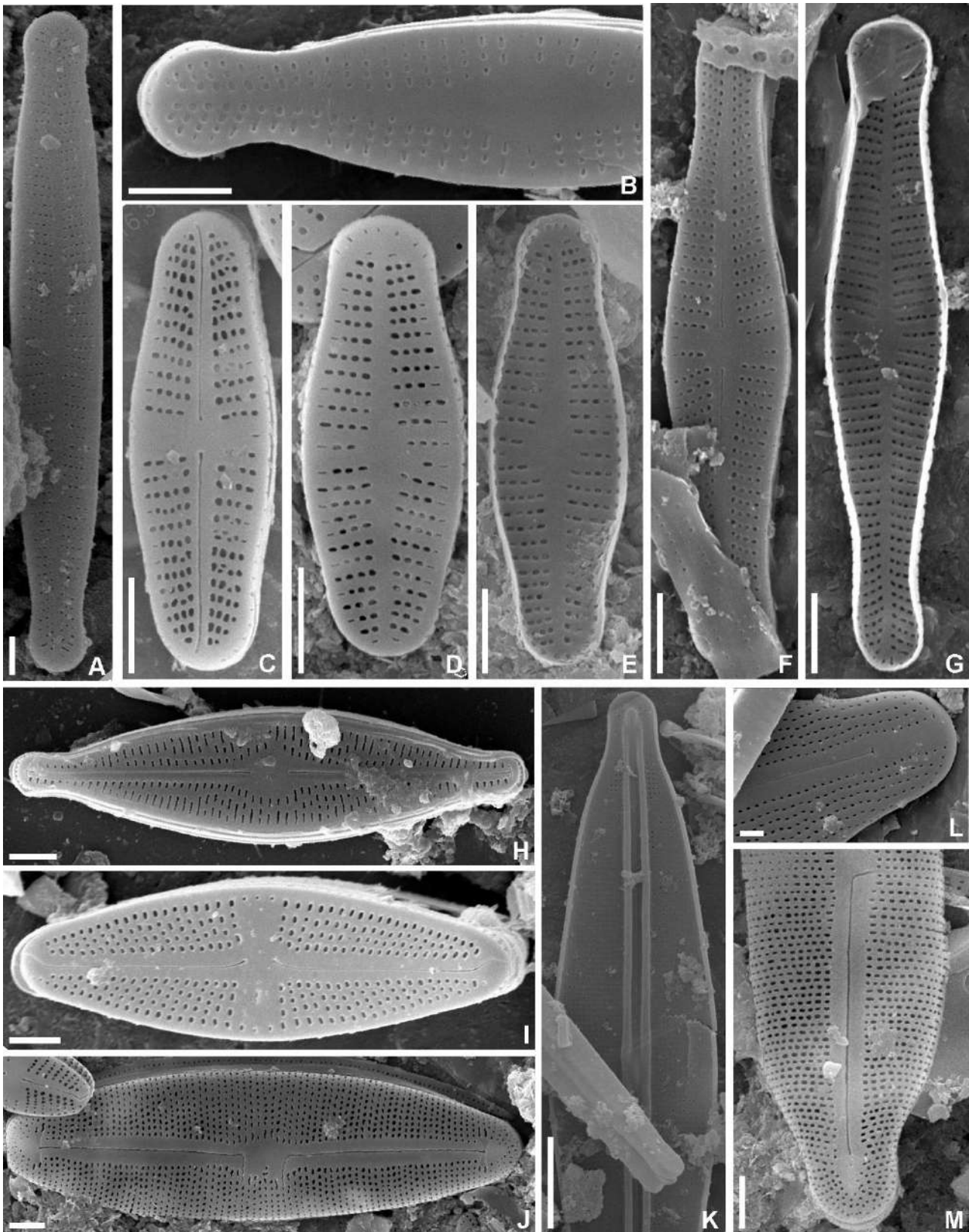


FIGURE 14. Epilithic diatoms from Cascavel River microbasin in SEM. A. *Achnanthidium caledonicum*, rafeless valve; B. *A. macrocephalum*, rafeless valve; C–E. *A. minutissimum*. C–D. Raphid and rafeless valve in external view; E. Internal view of rafeless valve; F–G. *A. tropicocatenatum*, raphid and rafeless valve; H. *Brachysira microcephala*; I. *Luticola ectorii*; J. *Frustulia weinholdii*. K–L. *Frustulia australocrassinervia*. L. External view showing proximal raphe ends T-shaped; M. *F. neomundana*. Scale bars represent 2 μm , 5 μm (K), 1 μm (L).

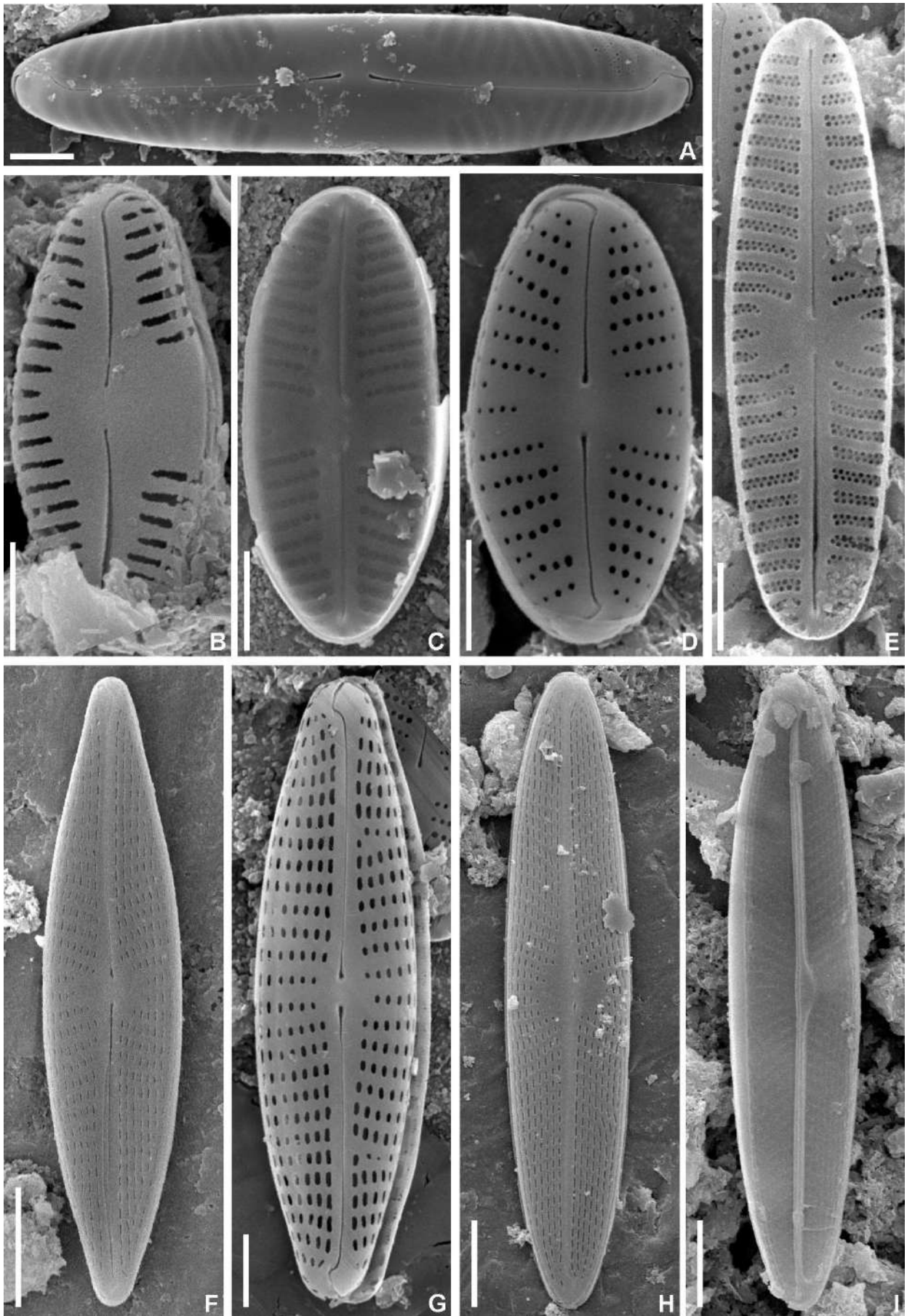


FIGURE 15. Epilithic diatoms from Cascavel River microbasin in SEM. A. *Pinnularia acricola*; B. *Chamaepinnularia brasilianopsis*; C–D. *Sellaphora nigri*, internal and external views; E. *S. saugerresii*; F. *Navicula cryptocephala*; G. *N. salinicola*; H–I. *N. symmetrica*, external and internal views. Scale bars represent 2 μm , 5 μm (F, H, I).

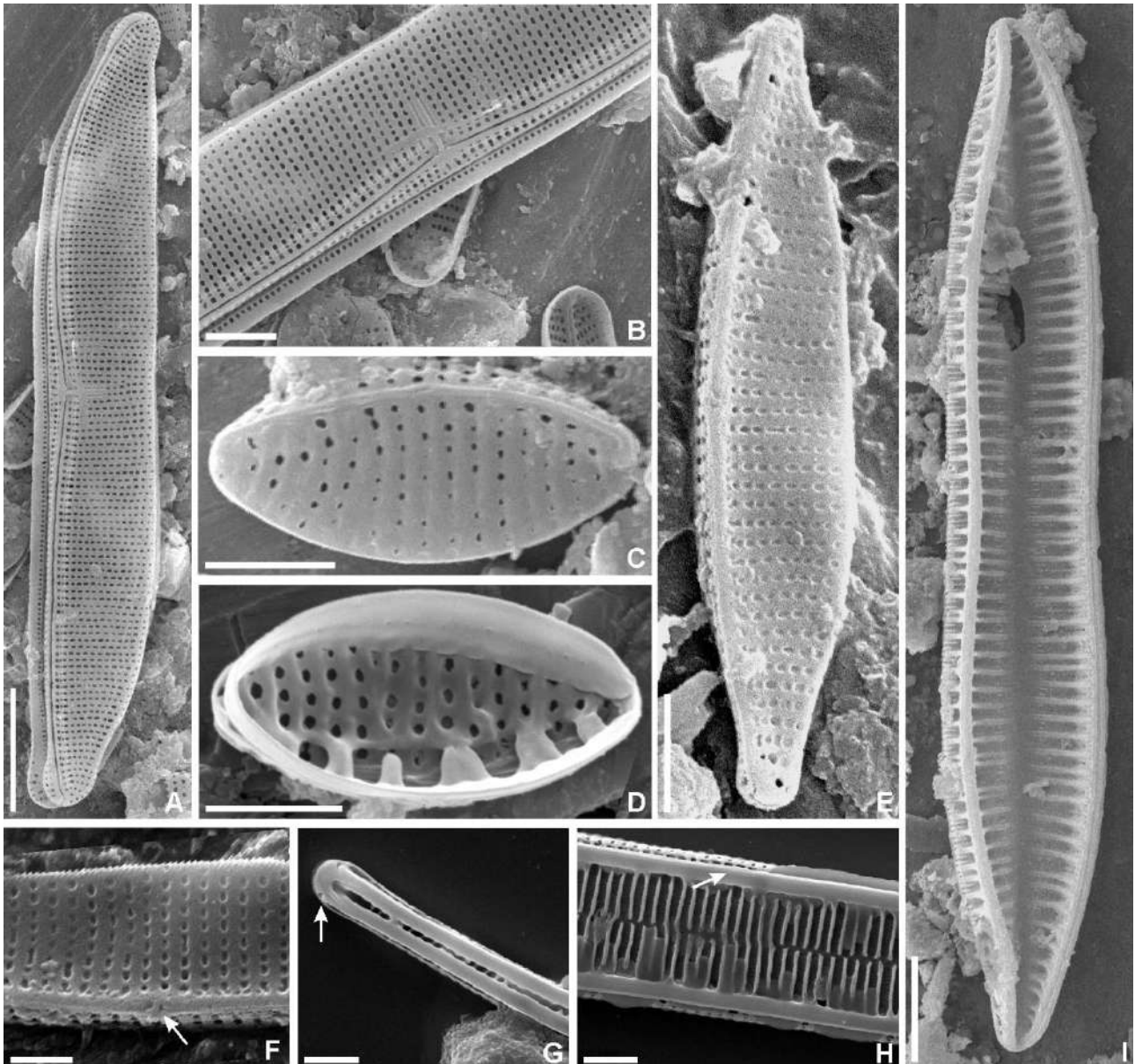


FIGURE 16. Epilithic diatoms from Cascavel River microbasin in SEM. A–B. *Nitzschia clausii*. B. External view showing the raphe and mantle ornamentation. C–D. *N. inconspicua*. D. Internal view showing fibulae; E–F. *N. perminuta*. F. External view showing proximal raphe ends (arrow); G–H. *Iconella curvula*. G. External view showing distal raphe ends (arrow). H. External view showing raphe (arrow) and striae. I. *I. delicatissima*. Scale bars represent 2 μm , 5 μm (A), 10 μm (I).

Discussion

Our taxonomic work provided morphological and meristic data on 221 epilithic taxa found in subtropical streams in Brazil. Three species were recorded for the first time in Paraná State, and one represented a new record for Brazil.

Belonging to a predominantly urban microbasin, and located in a region of red dystrophic latosol (rich in Fe and Al), the studied streams were characterized by distinct metals, predominantly acidic pH, and high conductivity (dos Santos *et al.* 2018). Sampling sites connected to urban headwaters showed higher metal concentrations than sites with considerable riparian vegetation,

such as S5 and S6, for example (Corbi *et al.* 2018, Khan *et al.* 2021). Further, the absence of riparian vegetation generates silting in several stretches of the basin, which favors the accumulation of sediment and increases the concentration of metals (Martins *et al.* 2021). Despite the conservation unit in the headwaters of the watershed, the region suffers from diffuse pollution sources from the urban center, as it is an open visitation area with uncontrolled capybara populations, contributing to high nitrate and conductivity values (Figure 2a) (Remor *et al.* 2018, Medeiros *et al.* 2020).

The slightly acid pH found in the Cascavel River microbasin could explain the high species richness of *Eunotia* and *Pinnularia*, although these genera are associated with streams with oligotrophic conditions and low conductivity (Round *et al.* 1990, Metzeltin & Lange-Bertalot 1998, Silva-Lehmkuhl *et al.* 2019). Furthermore, the Cascavel River microbasin was composed of many small diatom species of the genera *Achnanthisidium* Kützing, *Brachysira* Kützing, *Humidophila* (Lange-Bertalot & Werum) Lowe *et al.*, *Nupela* Vyverman and Compère, *Planothidium* Round & Bukhtiyarova, and *Sellaphora* Mereschowsky, which may be related to the high availability of rocky substrates (Richards *et al.* 2020), or indicate selection of adnate species, which are tolerant to carryover by water flow and also to metal pollution (Morin *et al.* 2008, 2012, Barral-Fraga *et al.* 2016, Pandey *et al.* 2018a).

Polluted urban and rural streams also tend to suffer a reduction in the number of diatom species (Fernández *et al.* 2018), highlighting acidified and high conductivity environments (Luís *et al.* 2009, Moresco *et al.* 2011). However, the number of taxa found in our study (221 spp.) is higher when compared to other floras in the State (see Bertolli *et al.* 2010, Faria *et al.* 2010, Silva *et al.* 2010, Moresco *et al.* 2011, Santos *et al.* 2011, Marra *et al.* 2016, Silva-Lehmkuhl *et al.* 2019). This might be explained by the higher sampling effort in our study, and the fact that these studies have mostly evaluated reservoir floras, using artificial and epiphytic substrates.

Diatoms exposed to metal-contaminated environments may undergo modifications in community structure, such as alteration in colonization/growth form or turnover from sensitive to tolerant species (da Silva *et al.* 2009, Morin *et al.* 2012, Pandey *et al.* 2018a), or modifications on an individual scale, such as reduction in valve size or emergence of teratologies (Lavoie *et al.* 2012, Su *et al.* 2018, Olenici *et al.* 2017, 2019, Pandey *et al.* 2019). Although naturally high in the Cascavel River microbasin (see Table 1 in Appendix D), morphological abnormalities have previously been associated with Fe (McFarland *et al.* 1997, Dickman 1998, Cattaneo *et al.* 2004, Sienkiewicz & Gasiorowski 2016, Pandey *et al.* 2018b), and Al (Furey *et al.* 2009, Soleimani *et al.* 2020), affecting the valve outline, striae pattern, and areolae shape.

Considering other metals evaluated in our study, Cd, Cu and Zn are recorded in the literature as causing teratology in diatoms (Falasco *et al.* 2009a, 2021), increasing the number of deformed

valves in water bodies (Pandey & Bergey 2016, Pandey *et al.* 2018b). Deformed valve outline (type 1 teratology) is common in diatoms exposed to copper (Pandey *et al.* 2014), which may explain the several species with abnormalities at S3 and S8 (see Table 2) (Moir *et al.* 2022). Centrally bent valves of *Eunotia* and bent apices in *Achnantheidium*, for example, also represent an effect of copper exposure (Cantonati *et al.* 2014, Olenici *et al.* 2017). Cadmium and zinc also contribute to the appearance of teratological valves, causing deformed valve outline in *Achnantheidium*, *Fragilaria*, *Gomphonema* (Morin & Coste 2006, Morin *et al.* 2007, de Jonge *et al.* 2008), abnormal striae pattern in *Encyonema*, *Gomphonema*, *Nitzschia*, *Planothidium*, and *Ulnaria* (Morin & Coste 2006, Morin *et al.* 2007, Falasco *et al.* 2009b), and duplicated central area in *Planothidium* (Morin *et al.* 2007). Anthropized environments, such as S4 and S7, are subject to receiving harmful concentrations of these metals to diatoms (see Table 1 in Appendix B and Figure 2b), suggesting their contribution as drivers of abnormalities in numerous species in our study (Table 2).

Other metals evaluated in our study may have contributed to the morphologically abnormal valves, such as Ba and Mn in *Eunotia subarcuatooides* (Furey *et al.* 2009), Cr in *Ulnaria ulna* (Pandey *et al.* 2018b), and Ni in *Achnantheidium minutissimum* and *Nitzschia palea* (Gómez *et al.* 2008, Morin *et al.* 2014, Sienkiewicz & Gasiorowski 2016, Lavoie *et al.* 2018), although not always related to specific sampling sites (see Table 1 in Appendix D and Figure 2b). Other species, however, showed a close relationship with these metals, such as *Gomphonema pumilum*, *Caloneis bacillum* (manganese) *Eunotia yberai*, *Achnantheidium macrocephalum*, *Pinnularia graciloides* var. *latecapitata* (chromium), *Eunotia desmogonioides*, *Achnantheidium modestiforme*, *Sellaphora saugerresii* (nickel) (Table 2), possibly indicating synergistic effects of metals on altered valve formation. The initial portion of the microbasin has a strong influence of Pb, especially S1 and S2, as previously indicated by Remor *et al.* (2018) and in our study (see Figure 2b), suggesting relationships with the occurrence of teratological forms in *Eunotia*, *Encyonema*, *Frustulia*, *Pinnularia*, and *Navicula* (Table 2). Lead is known to cause deformed valves in numerous diatom species, usually in association with other metals (Gómez *et al.* 2008, Pandey *et al.* 2014, Simic *et al.* 2018) or high concentrations of nutrients, especially phosphorus and nitrogen (Mora *et al.* 2015, Pandey *et al.* 2018b). Environmental factors combined with metal pollution also contribute to modify diatom frustules (Millan *et al.* 2019, Olenici *et al.* 2020), such as low pH ((Luís *et al.* 2011, 2016, Leguay *et al.* 2016, Olenici *et al.* 2020), high conductivity (Pandey *et al.* 2018a), low flow (Al-Handal & Abdullah 2010), and high nutrient concentrations (Gómez & Licurs 2003), which can be related to the characteristics found in the Cascavel River microbasin (see Table 1 in Appendix C and Figure 2a). Eutrophic conditions, for example, may have contributed to the duplicate pattern of alveoli in *Discostella stelligera* recorded by Silva *et al.* (2010). Different abiotic conditions lead to teratological forms in different species, such as high temperature, conductivity, total nitrogen, and

coliforms for *U. ulna* (Morin *et al.* 2008, Pandey *et al.* 2018a, 2019), low pH for *E. bilunaris* (Grabowska *et al.* 2014), phosphorus and nitrogen for *A. minutissimum* (Pandey *et al.* 2018b).

These factors are recurrent in the Cascavel River microbasin, especially due to the lack of riparian vegetation and diffuse urban or agricultural pollution (see Table 1 in Appendix A and Figure 2a). Additionally, we point out that teratological valves of *Gomphonema lagenula* were recorded in almost all sampled sites, but there are no specific data on teratologies in this species. In general, teratologies associated with the genus *Gomphonema* arise from high metal concentrations and acidic pH (Gómez *et al.* 2008, Falasco *et al.* 2009b, Fernández *et al.* 2018).

It is interesting to note that some individuals of *Eunotia desmogonioides*, *E. monodon*, *E. subarcuatooides*, *Achnantheidium macrocephalum*, *Pinnularia brauniana*, *P. subgibba* var. *undulata*, and *Craticula riparia* (see Figs 4T, 4W, 4X, 3AQ, 7I, 8AF, 9F, 10Y) possibly presented cingulum teratology (type 8), since this type of abnormality was described in epilithic samples from metal-polluted environments, but further analysis are needed, especially observation in SEM.

Floristic studies are crucial for better understanding the biodiversity of algae, as well as their geographic distribution, ecological preferences, in addition to providing insights into ecosystem dynamics. Further studies including SEM images are needed to verify whether morphological abnormalities occur at a lower scale, such as in the raphe system, areolae pattern, and cingulum bands. The influence of metals on specific taxa and the other factors driving morphological abnormalities in diatom communities (e.g., flow, shading, substrate availability, nutrient content) should be better investigated, especially metal concentrations and their bioavailability.

Conclusion

Our work contributes to the taxonomic knowledge of epilithic diatoms in the region by extending measurements and occurrences. Three species were registered for the first time in the state of Paraná, and one species was recorded for the first time in Brazil, which helps to understand ecological and biogeographical aspects of diatoms. The numerous diatom valves with teratological characteristics suggest that the concentrations of metals in the river sediment, added to the other environmental variables in the microbasin, disturb this periphytic community. In fact, 34 diatom species presented deformed valve outline or unusual striae pattern. In this sense our study also contributes to avoid teratological populations from being described as new taxa. Further studies are needed, increasing the number of sampling sites, SEM analyses, and ecological characteristics, in order to contribute to the taxonomy of the group, as well as to the understanding of the effects of metals on freshwater diatom communities.

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APPENDIX A – TABLE 1. Predominant land use, and physical characteristics of sampling sites.

Sampling stations	Land use	Physical characteristics
S1	Urban	Conservation area, with the presence of <i>Hydrochoerus hydrochaeris</i> Linnaeus populations. Stream with visible silting up process
S2	Urban	Close to a highway, containing ciliary vegetation
S3	Urban	Close to a highway, lacking ciliary vegetation and visible silting up process
S4	Urban	Close to a garbage collection property, with sparse ciliary vegetation. Presence of a bridge over the stream for pedestrian traffic
S5	Rural	Close to temporary croplands and a highway, containing ciliary vegetation. Presence of a bridge over the stream for pedestrian traffic
S6	Rural	Conservation area within a rural property, with goat farming, <i>Pinus</i> sp. plantation, and temporary croplands
S7	Urban	Close to a deactivated abattoir, containing ciliary vegetation. Riverbanks in erosive process and stream with visible silting up process
S8	Rural	Close to temporary croplands and to a basalt mining company using mineral deposits. Presence of a bridge over the stream for vehicle traffic

APPENDIX C - TABLE 1. Limnological variables (mean \pm standard deviation) analyzed from water samples collected in the eight sampling sites from Cascavel River microbasin (Cond: electrical conductivity; CLa: chlorophyll *a*; TC: total coliforms; BOD: biochemical oxygen demand; COD: chemical oxygen demand; *E. coli*: *Escherichia coli*; N-NH₃: ammoniacal nitrogen; NO₃: nitrate; DO: dissolved oxygen; PO₄⁻: orthophosphate; TP: total phosphorus; TS: total solids; temp: temperature; turb: turbidity; Prec: precipitation).

Variables (mean \pm standard deviation)	Cond	DO	BOD	COD	N- NH ₃	NO ₃	PO ₄ -	TP	TS	pH	Temp	Turb	Flow	Depth	Prec	CLa	<i>E. coli</i>	TC	
	mS cm-1	mg L-1	mg L-1	mg L- 1	mg L- 1	mg L-1	mg L- 1	mg L- 1	mg L-1		°C	NTU	m ³ s-1	m	mm	mg L- 1	NMP 100 mL-1	NMP 100 mL-1	
S1	Autumn	0.06 \pm 0.01	15.29 \pm 4.81	2.94 \pm 0.85	5.52 \pm 5.81	0.27 \pm 0.12	2.24 \pm 0.12	0.04 \pm 0.04	0.1 \pm 0.12	55.33 \pm 8.5	6.22 \pm 0.33	18.01 \pm 0.99	4.71 \pm 0.31	0.11 \pm 0.07	0.04 \pm 0.02	5.01 \pm 4.15	0.6 \pm 0.91	9807.78 \pm 7958.08	11572.22 \pm 9715.93
	Winter	0.06 \pm 0	23.12 \pm 23.28	6.76 \pm 6.25	12.11 \pm 11.42	0.2 \pm 0	6.7 \pm 4.71	0.03 \pm 0.03	0.02 \pm 0.01	57 \pm 13.53	6.53 \pm 0.24	18.48 \pm 0.54	3.47 \pm 0.69	0.07 \pm 0.02	0.04 \pm 0.01	3.29 \pm 1.45	1.03 \pm 1.05	2242 \pm 762.03	5213.33 \pm 6384 \pm 63
	Spring	0.06 \pm 0.01	12.28 \pm 5.28	3.24 \pm 2.13	6.29 \pm 5.89	0.2 \pm 0	9.8 \pm 6.74	0.04 \pm 0.02	0.16 \pm 0.24	165.67 \pm 182.73	5.79 \pm 0.79	20.11 \pm 1.41	10.99 \pm 15.09	0.13 \pm 0.13	0.05 \pm 0.03	5.74 \pm 3.67	0.56 \pm 0.53	31276.67 \pm 42614.57	38993.33 \pm 53359.09
	Summer	0.03 \pm 0.03	11.45 \pm 2.95	2.5 \pm 2.1	3.72 \pm 4.62	0.2 \pm 0	8.85 \pm .5	0.04 \pm 0.02	0.026 \pm 0.021	31.67 \pm 13.5	6.22 \pm 0.81	21.42 \pm 0.68	4.53 \pm 0.86	0.12 \pm 0.05	0.05 \pm 0.01	6.51 \pm 4.05	1.45 \pm 2.44	1829.33 \pm 1821.46	4151 \pm 2452.62
S2	Autumn	0.04 \pm 0.04	10.35 \pm 2.29	2.38 \pm 1.3	10.73 \pm 14.66	0.37 \pm 0.21	1.64 \pm 0.62	0.14 \pm 0.16	0.26 \pm 0.14	63 \pm 22.91	5.42 \pm 0.28	18.12 \pm 2.44	2.96 \pm 1.64	0.65 \pm 0.29	0.12 \pm 0.05	5.01 \pm 4.15	0.12 \pm 0.17	36077.78 \pm 55005.17	11572.22 \pm 9715.93
	Winter	0.06 \pm 0.01	13.31 \pm 2.46	5.46 \pm 4.51	9.74 \pm 11	0.2 \pm 0	3.99 \pm 2.61	0.04 \pm 0.01	0.05 \pm 0.05	101.67 \pm 62.29	6.3 \pm 0.28	19.12 \pm 1.39	1.93 \pm 0.54	0.89 \pm 0.58	0.15 \pm 0.07	3.29 \pm 1.45	0.58 \pm 0.52	24809.33 \pm 38103 \pm 95	28170 \pm 41454.65
	Spring	0.08 \pm 0.01	10.6 \pm 3.52	3.83 \pm 2.65	7.4 \pm 8.64	0.2 \pm 0	8.97 \pm 4.05	0.04 \pm 0.02	0.36 \pm 0.49	63 \pm 5.57	5.64 \pm 0.82	20.3 \pm 1.17	4.5 \pm 6.46	1.16 \pm 0.93	0.16 \pm 0.09	5.74 \pm 3.67	0.38 \pm 0.28	38018 \pm 41445.63	75386.67 \pm 4809.3
	Summer	0.08 \pm 0.01	15.18 \pm 6.87	3.15 \pm 3.07	4.31 \pm 3.1	0.2 \pm 0	7.38 \pm 5.1	0.05 \pm 0	0.1 \pm 0.06	43 \pm 20.3	5.91 \pm 0.23	21.13 \pm 0.36	2.34 \pm 1.14	1.02 \pm 0.53	0.17 \pm 0.08	6.51 \pm 4.05	0.37 \pm 0.29	43666.67 \pm 37056.35	64289 \pm 47700.82
S3	Autumn	0.05 \pm 0.02	11.56 \pm 4.59	3.7 \pm 0.75	6.36 \pm 7.95	0.27 \pm 0.12	1.76 \pm 0.72	0.04 \pm 0.02	0.12 \pm 0.1	51.33 \pm 16.07	5.81 \pm 0.23	18.22 \pm 3.18	9.7 \pm 2.37	4.65 \pm 3.96	0.37 \pm 0.23	5.01 \pm 4.15	0.21 \pm 0.28	3220 \pm 3236.85	4546.67 \pm 1955.13
	Winter	0.06 \pm 0	12.23 \pm 3.47	6.3 \pm 3.4	10.9 \pm 11.88	0.2 \pm 0	4.46 \pm 4.53	0.04 \pm 0.02	0.03 \pm 0.02	60.33 \pm 22.23	6.46 \pm 0.31	18.39 \pm 1.74	8.15 \pm 1.93	2.75 \pm 2.16	0.29 \pm 0.17	3.29 \pm 1.45	0.3 \pm 0.22	47640 \pm 80509.7	94606.67 \pm 159010.23
	Spring	0.06 \pm 0	9.55 \pm 5.39	3.86 \pm 2.13	8.16 \pm 8.46	0.2 \pm 0	6.77 \pm 1.54	0.04 \pm 0.01	0.35 \pm 0.5	48.67 \pm 2.52	5.94 \pm 0.12	22.93 \pm 1.46	11.07 \pm 10.01	4.83 \pm 4.06	0.15 \pm 0.27	5.74 \pm 3.67	1.99 \pm 3.37	22147.33 \pm 26441.61	27756.67 \pm 33821.17

Variables (mean ± standard deviation)	Cond	DO	BOD	COD	N- NH3	NO3	PO4-	TP	TS	pH	Temp	Turb	Flow	Depth	Prec	CLa	<i>E. coli</i>	TC	
	mS cm-1	mg L-1	mg L-1	mg L- 1	mg L- 1	mg L-1	mg L- 1	mg L- 1	mg L-1		°C	NTU	m ³ s-1	m	mm	mg L- 1	NMP 100 mL-1	NMP 100 mL-1	
S4	Summer	0.05 ± 0	13.15 ± 4.76	1.39 ± 0.9	4.07 ± 5.13	0.2 ± 0	5.72 ± 3.13	0.03 ± 0.03	0.03 ± 0.02	35.67 ± 17.56	5.99 ± 0.20	23.60 ± 0.39	19.12 ± 15.99	4 ± 2.17	0.39 ± 0.13	6.51 ± 4.05	1.61 ± 2.32	15240 ± 17234.99	18393.33 ± 19018.1
	Autumn	0.06 ± 0.01	14.01 ± 5.52	2.55 ± 0.79	7.78 ± 9.05	0.13 ± 0.17	2.48 ± 0.3	0.05 ± 0	0.1 ± 0.06	79 ± 22.61	5.96 ± 0.43	17.82 ± 2.56	6.73 ± 2.57	0.51 ± 0.13	0.12 ± 0.04	5.01 ± 4.15	0.08 ± 0.02	5840 ± 9498.46	8360 ± 13555.4
	Winter	0.06 ± 0	10.48 ± 2.4	4.98 ± 4.19	8.84 ± 9.96	0.2 ± 0	3.66 ± 1.24	0.04 ± 0.02	0.02 ± 0.01	76 ± 16.52	6.55 ± 0.18	18.65 ± 1.74	3.4 ± 0.52	0.49 ± 0.44	0.12 ± 0.11	3.29 ± 1.45	1.28 ± 1.67	2253.33 ± 2557.54	3203.33 ± 2352.45
	Spring	0.06 ± 0.02	10.28 ± 3.68	3.37 ± 3.2	7.11 ± 8.39	0.2 ± 0	10.6 ± 6.06	0.04 ± 0.02	0.33 ± 0.53	65.33 ± 2.08	5.81 ± 0.4	20.33 ± 1.38	12.37 ± 18.88	0.48 ± 0.28	0.1 ± 0.03	5.74 ± 3.67	0.4 ± 0.3	206830 ± 345713.98	207763.33 ± 344924.64
S5	Summer	0.06 ± 0.01	15.96 ± 6.65	2.66 ± 2.47	3.73 ± 3.46	0.2 ± 0	57.67 ± 6.11	0.03 ± 0.03	0.03 ± 0.02	39 ± 8.89	6.08 ± 0.34	21.32 ± 0.43	3.1 ± 0.28	0.68 ± 0.52	0.13 ± 0.10	6.51 ± 4.05	0.1 ± 0.13	7202.33 ± 9685.05	7730 ± 9471.05
	Autumn	0.01 ± 0	9.34 ± 3.12	2.39 ± 1.78	15.74 ± 5.14	0.27 ± 0.12	1.06 ± 1.14	0.03 ± 0.03	0.11 ± 0.11	41.33 ± 13.5	6.60 ± 0.29	19.24 ± 1.75	17.66 ± 27.34	0.02 ± 0.02	0.02 ± 0.01	5.01 ± 4.15	0.06 ± 0.04	3333.33 ± 4499.3	6253;33 ± 9342.58
	Winter	0.01 ± 0.01	10.98 ± 1.13	3.96 ± 2.57	6.43 ± 6.03	0.2 ± 0	0.66 ± 0.55	0.03 ± 0.03	0.03 ± 0.04	38.67 ± 10.26	6.88 ± 0.42	20.16 ± 2.41	2.67 ± 0.43	0.05 ± 0.01	0.03 ± 0.01	3.29 ± 1.45	0.58 ± 0.47	67.33 ± 114.89	6067 ± 5139.09
	Spring	0.01 ± 0	12.03 ± 7.82	2.66 ± 2.26	7.30 ± 6.17	0.2 ± 0	1.2 ± 0.92	0.04 ± 0.01	0.27 ± 0.45	27 ± 3.61	5.7 ± 1.27	20.97 ± 0.85	4.26 ± 4.47	0.29 ± 0.34	0.08 ± 0.06	5.74 ± 3.67	0.35 ± 0.28	15444.53 ± 22708.48	23566.67 ± 24964.84
S6	Summer	0.01 ± 0	14.43 ± 5.53	1.57 ± 0.34	3.51 ± 2.22	0.2 ± 0	1.8 ± 2.21	0.03 ± 0.03	0.03 ± 0.04	11.67 ± 5.13	6.44 ± 0.8	21.42 ± 0.71	4.05 ± 4.58	0.05 ± 0.06	0.03 ± 0.02	6.51 ± 4.05	1.63 ± 2.31	17131.33 ± 27127.25	19346.67 ± 30494.72
	Autumn	0.03 ± 0.01	9.88 ± 1.91	3.1 ± 2.91	16.82 ± 19.32	0.23 ± 0.06	0.83 ± 0.2	0.04 ± 0.02	0.07 ± 0.05	49.33 ± 13.5	6.47 ± 0.38	17.83 ± 2.56	7.41 ± 2.63	1.25 ± 0.37	0.14 ± 0.02	5.01 ± 4.15	0.05 ± 0.04	1964.67 ± 3287.52	2504.78 ± 3481.12
	Winter	0.04 ± 0.01	11.72 ± 1	2.86 ± 1.63	6.02 ± 2.79	0.2 ± 0	2.04 ± 1.01	0.03 ± 0.03	0.03 ± 0.04	33.33 ± 12.58	6.59 ± 0.31	19.20 ± 2.3	5.6 ± 1.25	1.19 ± 0.25	0.14 ± 0.02	3.29 ± 1.45	1.01 ± 1.46	2173.33 ± 3660.62	2800 ± 4504.44
Spring	0.03 ± 0.01	11.31 ± 6.36	2.98 ± 2.79	8.8 ± 1.92	0.2 ± 0	2.15 ± 1.24	0.04 ± 0.02	0.28 ± 0.46	35 ± 27.15	6.42 ± 0.17	21.01 ± 1.69	8.86 ± 11.01	1.25 ± 0.49	0.13 ± 0.03	5.74 ± 3.67	0.04 ± 0.04	42476.67 ± 39741.54	47466.67 ± 41812.12	

Variables (mean ± standard deviation)	Cond	DO	BOD	COD	N- NH3	NO3	PO4-	TP	TS	pH	Temp	Turb	Flow	Depth	Prec	CLa	<i>E. coli</i>	TC	
	mS cm-1	mg L-1	mg L-1	mg L- 1	mg L- 1	mg L-1	mg L- 1	mg L- 1	mg L-1		°C	NTU	m ³ s-1	m	mm	mg L- 1	NMP 100 mL-1	NMP 100 mL-1	
S7	Summer	0.04 ± 0.01	14.47 ± 5.32	1.98 ± 1.85	5.66 ±7.48	0.2 ± 0	2.59 ± 1.59	0.03 ± 0.03	0.03 ± 0.02	27 ± 12.29	6.86 ± 0.31	21.80 ± 0.84	7.26 ± 5.36	1.69 ± 1.08	0.17 ± 0.07	6.51 ± 4.05	0.39 ± 0.34	1235.67 ± 869.85	3076.67 ± 2726.29
		Autumn	0.05 ± 0.01	10.37 ± 3.09	3.2 ± 0.74	15.8 ± 19.32	0.27 ± 0.12	0.56 ± 0.16	0.04 ± 0.02	0.06 ± 0.04	53.67 ± 7.51	6.43 ± 0.49	19.41 ± 2.08	0.01 ± 0.01	0.16 ± 0.07	0.06 ± 0.03	5.01 ± 4.15	0.24 ± 0.33	2696.67 ± 3556.27
	Winter		0.04 ± 0.01	13.49 ± 1.7	3.65 ± 2.38	5.61 ± 4.09	0.2 ± 0	2.48 ± 2.74	0.03 ± 0.03	0.01 ± 0.01	41.67 ± 21.55	6.72 ± 0.18	20.25 ± 1.19	0.11 ± 0.15	0.30 ± 0.22	0.1 ± 0.05	3.29 ± 1.45	0.38 ± 0.54	75 ± 65.2
		Spring	0.05 ± 0.01	11.09 ± 5.97	2.78 ± 2.28	8.51 ± 4.63	0.2 ± 0	2.60 ± 1.64	0.04 ± 0.02	0.32 ± 0.48	49.67 ± 27.15	6.41 ± 0.44	21.31 ± 0.62	14.16 ± 24.03	0.11 ± 0.04	0.05 ± 0.02	5.74 ± 3.67	0.21 ± 0.29	171463.67 ± 290692.99
S8	Summer	0.05 ± 0.01	14.99 ± 5.88	1.12 ± 0.68	1.25 ± 0.27	0.2 ± 0	2.43 ± 1.76	0.04 ± 0.03	0.03 ± 0.02	32.67 ± 12.01	6.78 ± 0.18	21.69 ± 0.32	0.84 ± 1.28	0.25 ± 0.12	0.09 ± 0.03	6.51 ± 4.05	0.81 ± 1.15	32768 ± 53833.74	46803.33 ± 76911.24
		Autumn	0.05 ± 0.01	6.45 ± 5.86	2.5 ± 0.88	7.96 ± 9.2	0.3 ± 0.17	1.72 ± 0.57	0.03 ± 0.03	0.09 ± 0.07	48.44 ± 17.44	6.52 ± 0.39	18.75 ± 1.61	4.35 ± 4.17	4.2 ± 1.38	0.3 ± 0.06	5.01 ± 4.15	0.04 ± 0.03	4377.78 ± 2436.88
	Winter		0.05 ± 0	11.44 ± 3.42	3.44 ± 2.11	5.69 ± 4.54	0.2 ± 0	1.65 ± 0.48	0.03 ± 0.03	0.03 ± 0.02	57.67 ± 20.4	6.81 ± 0.28	18.38 ± 0.99	4.56 ± 0.91	3.58 ± 1.67	0.28 ± 0.07	3.29 ± 1.45	0.37 ± 0.6	1776.33 ± 1755.03
		Spring	0.05 ± 0.01	14.35 ± 10.53	3.72 ± 2.5	9.09 ± 6.74	0.2 ± 0	5.35 ± 3.7	0.04 ± 0.01	0.31 ± 0.47	32.67 ± 25.01	6.47 ± 0.40	21.58 ± 1.29	11.26 ± 12.31	5.16 ± 1.67	0.34 ± 0.03	5.74 ± 3.67	0.43 ± 0.56	14318 ± 8167.5
Summer	0.05 ± 0.01	15.27 ± 7.14	1.3 ± 0.74	4.02 ± 4.77	0.2 ± 0	5.07 ± 0.8	0.03 ± 0.03	0.03 ± 0.02	30.33 ± 19.35	6.94 ± 0.22	22.78 ± 0.92	6.63 ± 4.94	6.09 ± 4.38	0.36 ± 0.14	6.51 ± 4.05	0.05 ± 0.04	6020 ± 4966	7416.67 ± 6362.85	
R ²	0.66	0.10	0.21	0.12	0.28	0.49	0.12	0.25	0.23	44.00	0.53	0.14	0.65	0.70	0.17	0.19	0.14	0.127	
F	16.46	0.90	2.24	1.14	3.32	8.00	1.20	2.57	2.56	6.67	9.38	1.38	15.61	19.57	1.47	1.14	1.38	1.235	
p	< 0.0001	0.54	0.02	0.35	0.001	< 0.0001	0.30	0.004	0.01	< 0.0001	< 0.0001	0.20	< 0.0001	< 0.0001	0.17	0.35	0.20	0.28	

APPENDIX D - TABLE 1. Mean concentrations of metals in sediment samples collected from the eight sampling sites in the Cascavel River microbasin.

Metals – sediment		Aluminum	Barium	Cadmium	Chromium	Cobalt	Copper	Iron	Lead	Manganese	Nickel	Zinc
		mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹
S1	Autumn	40,148.52	626.42	22.78	32.12	25.08	210.08	77,564.67	549.67	349.80	471.83	81.88
	Winter	36,411.17	606.53	24.62	23.11	25.93	196.72	82,294.08	549.67	329.01	400.51	78.36
	Spring	38,819.96	735.80	27.91	33.58	27.33	197.56	89,191.63	522.44	398.50	449.39	93.81
	Summer	41,011.46	879.97	43.30	31.83	23.67	222.88	86,451.67	522.44	393.75	543.65	99.55
S2	Autumn	41,346.70	715.91	14.57	36.34	21.98	184.29	86,999.66	544.22	276.04	435.92	73.98
	Winter	38,608.88	760.65	21.55	35.32	21.70	191.39	88,298.16	446.21	500.30	368.59	68.08
	Spring	37,131.33	1,049.01	36.94	46.66	22.26	196.25	88,321.99	465.27	647.00	457.87	93.08
	Summer	35,604.11	1,098.72	41.66	39.39	28.18	170.55	88,059.90	567.36	606.73	489.79	79.00
S3	Autumn	39,633.24	934.66	24.62	40.85	39.73	292.31	88,762.76	789.24	423.57	484.80	112.59
	Winter	40,862.46	919.74	26.27	37.07	24.24	195.31	86,737.58	601.39	397.55	511.23	78.89
	Spring	41,222.54	1,193.18	35.09	37.79	28.46	211.76	91,931.59	508.83	465.71	583.06	94.80
	Summer	34,399.71	1,441.76	72.23	44.92	27.05	231.94	86,523.15	583.70	523.82	917.23	127.17
S4	Autumn	36,634.67	969.46	24.01	45.50	23.39	172.51	78,684.48	474.80	266.77	409.49	84.88
	Winter	37,441.74	1,098.72	30.57	50.59	23.11	173.92	85,045.95	458.46	622.77	428.94	71.01
	Spring	41,309.46	1,556.11	65.87	57.56	29.87	225.59	98,233.49	621.81	745.83	729.69	124.82
	Summer	34,225.88	1,252.84	46.79	55.53	24.52	170.46	84,438.39	484.33	639.87	489.29	123.32
S5	Autumn	40,011.94	1,213.07	34.68	49.28	32.69	189.05	87,416.61	627.26	370.35	693.28	113.26
	Winter	42,203.44	1,133.52	27.70	47.68	31.28	206.14	89,608.58	639.51	373.56	465.87	97.71
	Spring	35,225.41	1,342.33	47.81	47.24	28.46	160.65	79,756.64	459.82	431.41	579.07	78.98
	Summer	40,074.02	1,580.97	51.92	55.09	35.23	228.86	89,644.32	678.98	490.80	638.92	119.34
S6	Autumn	36,572.59	1,103.69	22.98	51.60	19.44	149.34	75,432.27	499.30	234.22	420.96	68.52
	Winter	36,479.47	1,297.59	35.70	58.29	26.49	153.45	83,378.15	502.02	470.60	422.95	73.15
	Spring	37,180.99	1,392.05	43.30	65.85	28.74	186.90	87,309.39	491.13	642.37	460.36	112.75
	Summer	36,715.38	1,491.48	47.81	60.76	33.25	163.45	84,807.69	542.86	538.55	469.34	83.43
S7	Autumn	42,215.85	546.88	25.86	21.08	26.49	244.28	87,595.30	567.36	393.75	547.15	108.37
	Winter	42,085.48	447.44	26.88	22.68	23.67	231.38	87,237.92	610.92	291.12	548.14	99.43
	Spring	41,793.70	611.51	30.99	26.31	21.98	214.38	90,561.61	523.80	387.57	476.32	96.46
	Summer	42,066.86	700.99	40.42	26.60	24.24	268.85	93,694.69	593.23	468.94	596.52	103.05
S8	Autumn	41,464.66	377.84	14.98	23.55	21.98	192.51	85,724.98	574.17	289.70	384.05	76.38
	Winter	40,223.02	412.64	22.78	28.35	16.91	195.50	92,789.31	473.44	458.49	477.82	78.27
	Spring	38,689.59	820.31	36.11	48.41	28.46	231.94	94,576.24	519.72	809.38	429.44	100.19
	Summer	40,154.73	760.65	41.66	37.79	23.95	199.43	94,004.42	615.00	552.09	584.55	91.99