



## ***Eunotia* (Bacillariophyceae) from a subtropical stream adjacent to Iguaçu National Park, Brazil, with the proposition of a new species**

***Cinthia Coutinho Rosa Favaretto*<sup>1</sup>, *Priscila Izabel Tremarin*<sup>2</sup>, *Gabriela Medeiros*<sup>3</sup>, *Thelma Alvim Veiga Ludwig*<sup>4</sup>\* & *Norma Catarina Bueno*<sup>1</sup>**

<sup>1</sup>Universidade Estadual do Oeste do Paraná, Programa de Pós-Graduação em Conservação e Manejo de Recursos Naturais, Cascavel, PR, Brasil.

<sup>2</sup>Acqua Diagnósticos Ambientais Ltda., Rua Lourenço Volpi, 82640-440, Curitiba, PR, Brasil.

<sup>3</sup>Universidade Estadual do Oeste do Paraná, Programa de Pós-Graduação em Engenharia Agrícola, Cascavel, PR, Brasil.

<sup>4</sup>Universidade Federal do Paraná, Departamento de Botânica, Curitiba, PR, Brasil.

\*Corresponding author: Thelma Alvim Veiga Ludwig, e-mail: [veiga13ufpr@gmail.com](mailto:veiga13ufpr@gmail.com)

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**Abstract:** *Eunotia* species from a stream located in the surrounding area of the Iguaçu National Park conservation unit were analyzed. Samples were monthly collected from September 2012 to August 2013. The periphytic material, manually obtained by squeezing aquatic macrophytes, was oxidized and analyzed under light and scanning electron microscopy. Twenty-one infrageneric taxa of *Eunotia* were identified, measured, and illustrated. *Eunotia caniculoides* sp. nov. is proposed as a new species, and eight taxa are new records for Paraná state.

**Keywords:** Bacillariophyta; diatoms; periphyton; southern Brazil; taxonomy.

## ***Eunotia* (Bacillariophyceae) de um riacho subtropical adjacente ao Parque Nacional do Iguaçu, Brasil, com a proposta de uma espécie nova**

**Resumo:** Espécies de *Eunotia* Ehrenberg de um ambiente lótico localizado em área adjacente à unidade de conservação Parque Nacional do Iguaçu foram analisadas. As coletas foram realizadas mensalmente de setembro de 2012 a agosto de 2013. O material perifítico obtido do espremido manual de macrófitas aquáticas foi oxidado e analisado sob microscopia óptica e eletrônica de varredura. Vinte e um táxons de *Eunotia* foram identificados, medidos e ilustrados. *Eunotia caniculoides* sp. nov. é proposta como uma nova espécie e oito táxons constituem novos registros para o estado do Paraná.

**Palavras-chave:** Bacillariophyta; diatomáceas; perifíton; sul do Brasil; taxonomia.

## Introduction

The genus *Eunotia* Ehrenberg is characterized by the valve isopolarity, apical axis asymmetry, uniseriate striae, short raphe system, usually restricted to the valve apices and the presence of rimoportulae (Kocielek & Spaulding 2003, Furey 2011). The extant species are found in freshwater environments (Round et al. 1990) but some fossil *Eunotia* have already been described from marine sediments in New Zealand (Novitski & Kocielek 2005). The genus includes species with ecological optimum in the epiphyton and metaphyton of dystrophic to oligotrophic waters, being valuable indicators of acidic and low-conductivity conditions (Round et al. 1990, Van Dam et al. 1994, Cantonati & Lange-Bertalot, 2011, Cox 2015).

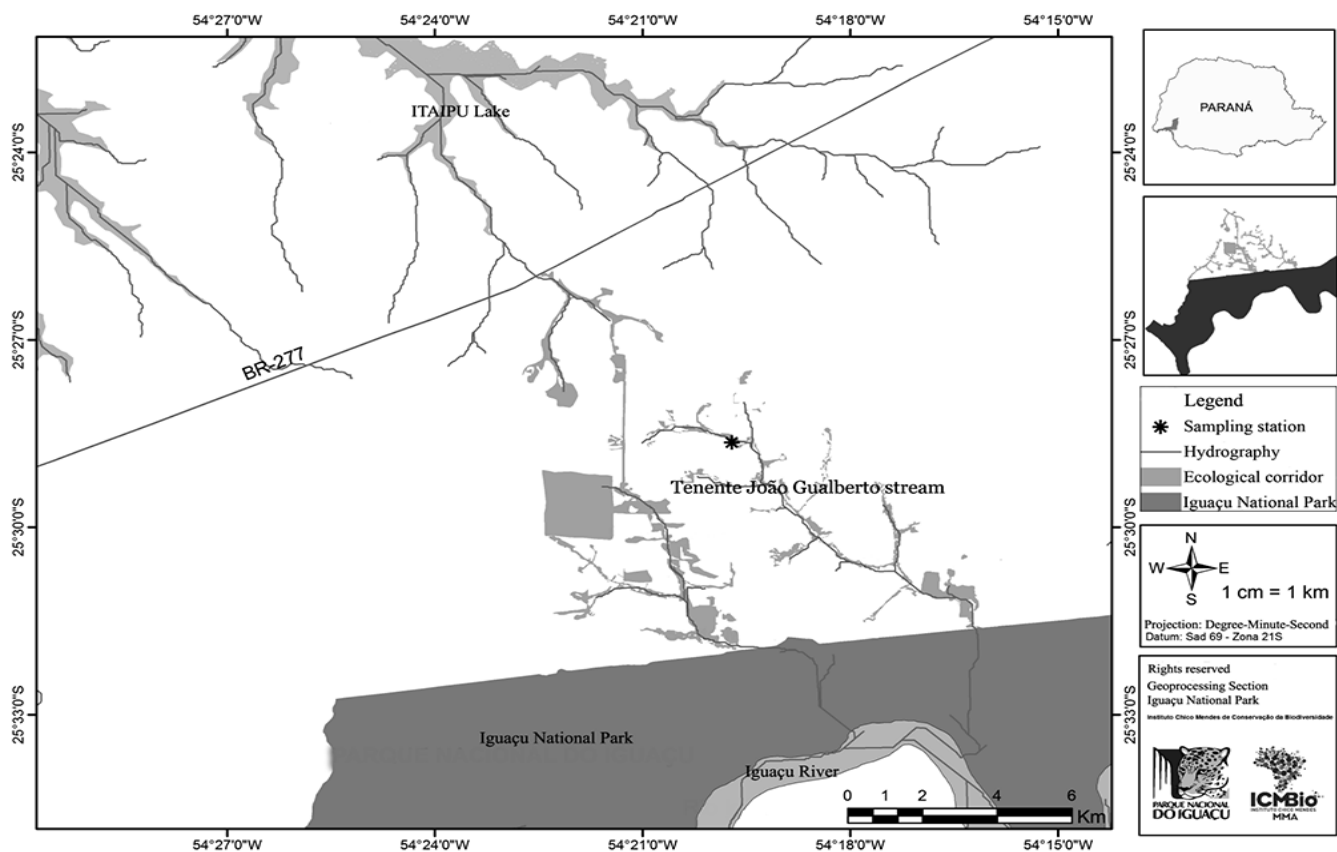
*Eunotia* is one of the most common and species-rich diatom genera in the Neotropics (Patrick 1940a, 1940b, Hustedt 1952a, 1952b, 1965, Metzeltin & Lange-Bertalot 1998, 2007, Sala et al. 2002a, 2002b, Dunck et al. 2016, Faustino et al. 2016, Costa et al. 2017), with remarkable diversity in Brazilian acidic environments (Ferrari et al. 2007, Bicca et al. 2011, Canani & Torgan 2013, Dunck et al. 2013a, 2013b, 2016, Cavalcante et al. 2014). In the state of Paraná, the genus were reported by Ludwig & Valente-Moreira (1989), Fürstenberger & Valente-Moreira (2000), Tremarin et al. (2008), Faria et al. (2010), Bertolli et al. (2010), Santos et al. (2011), Bartozek et al. (2013), Marra et al. (2016), Silva-Lehmkuhl et al. (2019), and Bartozek et al. (2020) recently proposed a new species. Based on a bibliographic check-list of freshwater diatom

studies developed in the state of Paraná from 1954 to 2009, Tremarin et al. (2009) listed 110 *Eunotia* infrageneric taxa, and Cavalcante et al. (2014) mention about 245 taxa registered to Brazil.

The purpose of the present publication was to investigate the species diversity of *Eunotia* from a stream located in the surrounding area of the Iguazu National Park conservation unit. A new species, *Eunotia caniculoides*, was formally described. The distinctive characters between similar taxa were discussed and taxonomical notes were provided. Distribution in the state of Paraná and ecological informations were also added.

## Material and Methods

The present study was carried out on the Tenente João Gualberto stream, located in a conservation unit area adjacent to the Iguazu National Park, in the municipality of São Miguel de Iguazu (25°28'36.3"S; 54°19'40.9"W), the western region of the state of Paraná. According to Horton-Strahler criteria (Horton 1945, Strahler 1952), the stream is a major tributary of the Baixo Iguazu Basin, having its source and part of its course among agricultural fields (Figure 1). The regional climate is Cfa type, subtropical humid mesotherm, with well-defined summer and winter periods, and rains distributed during the year (Alvares et al. 2013).



**Figure 1.** Location of the sampling station in the Tenente João Gualberto stream, São Miguel do Iguazu, PR, Brazil.

Diatom samples were collected monthly, from September 2012 to August 2013. Limnological variables as dissolved oxygen ( $\text{mg L}^{-1}$ ), pH, water temperature ( $^{\circ}\text{C}$ ), conductivity ( $\text{mS cm}^{-1}$ ), and turbidity (NTU) were measured in the field using a Horiba U-5000 multiparameter probe. Nutrient analysis of organic nitrogen –  $\text{N}_{\text{org}}$  ( $\text{mg L}^{-1}$ ), ammoniac nitrogen –  $\text{NH}_4^+$  ( $\text{mg L}^{-1}$ ), nitrate –  $\text{NO}_3^-$  ( $\text{mg L}^{-1}$ ), nitrite –  $\text{NO}_2^-$  ( $\text{mg L}^{-1}$ ), orthophosphate –  $\text{PO}_4^{3-}$  ( $\text{mg L}^{-1}$ ) e total phosphorous – TP ( $\text{mg L}^{-1}$ ), were carried out at the Limnological Laboratory of the Research Group on Fishery Resources and Limnology (GERPEL), from UNIOESTE, campus Toledo, according to American Public Health Association (2005). Weekly precipitation (mm) data were provided by Paraná Meteorological System (SIMEPAR). Minimum and maximum values of limnological data were described for each identified *Eunotia* species.

Periphytic samples were removed by manually squeezing the aquatic macrophyte *Eleocharis minima* Kunth (Cyperaceae) and were preserved in Transeau solution, in 1:1 proportion (Bicudo & Menezes 2017). The organic matter was eliminated by oxidation with  $\text{KMnO}_4$  and HCl according to Simonsen (1974) technique modified by Moreira-Filho & Valente-Moreira (1981). Cleaned material was dried onto glass coverslips and mounted on slides with Naphrax® (IR= 1.74). The taxonomic analysis was performed using an Olympus BX60 light microscope equipped with a DP71 image capture camera. Subsamples from cleaned samples were mounted on aluminum stubs and coated with gold. Micrographs were taken with JEOL JSM 6360 scanning electron microscope (SEM), operated at 15 kV and 8mm working distance, housed in the Electron Microscopy Center from the Federal University of Paraná. Slides and samples were deposited in the UNOPA Herbarium, UNIOESTE, campus Cascavel and registered as shown in Table 1.

Morphological terminology follows Barber & Haworth (1981) and Round et al. (1990). The classification system was based on Cox (2015).

Every *Eunotia* species was measured at the valve apical length, transapical width and striae density in the central part of the valve. The ecological information cited for identified taxa corresponds to the abiotic parameters measured in the samples collected during this study.

Constancy is a measure of species occurrence (C) and was expressed as follows: constant ( $C \geq 70\%$ ), common ( $30\% \leq C \leq 70\%$ ), sporadic ( $10\% \leq C \leq 30\%$ ) and rare ( $C \leq 10\%$ ) (Dajoz 2005).

## Results

Twenty-one taxa were measured and illustrated. Eight *Eunotia* taxa are new records for the state of Paraná: *E. georgii* Metzeltin & Lange-Bertalot, *E. karenae* Metzeltin & Lange-Bertalot, *E. guianensis* (Ehrenberg) De Toni, *E. juettnerae* Lange-Bertalot, *E. pileus* Ehrenberg, *E. pseudosudetica* var. *rotundata* Cavalcante, Tremarin & T.Ludwig, *E. sedina* Lange-Bertalot, Bak & Witkowski, and *E. tropico-arcus* Metzeltin & Lange-Bertalot. *Eunotia caniculoides* sp. nov. is formally proposed as a new species and are compared with close taxa (Table 2). The occurrence of species in the samples as well as ecological and environmental data from the sampling are listed in Table 3.

Bacillariophyceae Haeckel

Eunotiophycidae D.G. Mann

Eunotiales P.C. Silva

Eunotiaceae Kützing

*Eunotia* Ehrenberg

**Table 1.** Herbarium vouchers (UNOPA), date, geographic coordinates, and collector of samples

UNOPA	Sampling date	Geographic coordinates	Collector
3943	20/IX/2012	25°28'36.3"S 54°19'40.9"W	Servat, L.C
3962	29/X/2012	25°28'36.3"S 54°19'40.9"W	Servat, L.C
3976	13/XI/2012	25°28'36.3"S 54°19'40.9"W	Servat, L.C
4035	18/XII/2012	25°28'36.3"S 54°19'40.9"W	Servat, L.C
4044	15/I/2013	25°28'36.3"S 54°19'40.9"W	Servat, L.C
4057	06/II/2013	25°28'36.3"S 54°19'40.9"W	Servat, L.C
4081	11/III/2013	25°28'36.3"S 54°19'40.9"W	Servat, L.C
4093	04/IV/2013	25°28'36.3"S 54°19'40.9"W	Servat, L.C
4102	09/V/2013	25°28'36.3"S 54°19'40.9"W	Servat, L.C
4116	06/VI/2013	25°28'36.3"S 54°19'40.9"W	Servat, L.C
4126	16/VII/2013	25°28'36.3"S 54°19'40.9"W	Servat, L.C
4136	12/VIII/2013	25°28'36.3"S 54°19'40.9"W	Servat, L.C

*Eunotia ambivalens* Lange-Bertalot & Tagliaventi. In: Lange-Bertalot (ed.), Diatoms of Europe 6, p. 49, pl. 11, figs 1–11, pl. 12, figs 1–6, pl. 13, figs. 1–11, 2011.

Figures 2a–2d, 4a.

Valves slightly arched, 77.6–177.3  $\mu\text{m}$  long and 4.2–5.6  $\mu\text{m}$  wide. Dorsal margin convex, ventral margin concave. Apices rounded. Terminal nodules near the apices. Striae parallel, 11–12 in 10  $\mu\text{m}$  in the middle region of the valve. Rimoportula and areolae not visible in LM. In SEM: presence of short marginal spines, ca. 41 areolae in 10  $\mu\text{m}$ .

**Frequency of occurrence:** common (Table 3).

**Paraná State citations:** cited as *E. bilunaris* var. *linearis* (Okuno) Lange-Bertalot & Nörpel-Schempp; Tremarin et al. (2009), Faria et al. (2010), Santos et al. (2011)

**Taxonomic remarks:** *Eunotia ambivalens* distinguishes from *E. bilunaris* by slightly arched valves, almost parallel margins, ends not protracted, lower striae density (11.5–13/10  $\mu\text{m}$  vs 13–17/10  $\mu\text{m}$ ), and by the presence of marginal spines (Figure 3a) only discernible in SEM (Lange-Bertalot et al. 2011). The species “locus typicus” is Albania, Flower Lake, Lura Mountains, but it was also observed in oligotrophic alkaline or slightly acidic waters with low to moderate conductivity (Lange-Bertalot 2011).

*Eunotia bilunaris* (Ehrenberg) Schaarschmidt. In: Kanitz, Magyar Novenytani Lapok 5: 159, 1880.

Basionym: *Synedra bilunaris* Ehrenberg. Abh. Königl. Akad. Wiss. Berl. 1831: 87, 1832.

Figures 2e–2j, 4b–c.

**Table 2.** Comparison of morphometric data and morphological features of *E. caniculoides* sp. nov., *E. canicula*, *E. intricans* and *E. sioliopsis*

Feature	<i>E. caniculoides</i>	<i>E. canicula</i>	<i>E. intricans</i>	<i>E. sioliopsis</i>
Length (µm)	30–58.5	20–35 <sup>2</sup> 23.2–25 <sup>3</sup>	17–48 <sup>1</sup>	20–40 <sup>4</sup>
Width (µm)	3.5–4.9	3.5–4.0 <sup>2</sup> 3.5–5.2 <sup>3</sup>	4–5 <sup>1</sup>	3.5–4.5 <sup>4</sup>
Striae in 10 µm	10–14 (center) 13–14 (ends)	14–15 <sup>2</sup> (center) 16–22 <sup>3</sup> (center)	11–13 (center) <sup>1</sup> 18 (ends) <sup>1</sup>	16–17 (center) <sup>4</sup> 18 (ends) <sup>1</sup>
Areolar density in the central area in 10 µm	38–40	> 35 <sup>2</sup>	39–42 <sup>1</sup>	Up to 30 <sup>4</sup>
Valve symmetry	Dorsal margin slightly convex, almost straight in the larger valves; ventral margin straight to subtly concave	Dorsal margin convex, ventral margin straight to slightly concave, with valve mantle thickened at the proximal raphe ends <sup>2</sup> Dorsal margin convex, ventral margin concave <sup>3</sup>	Dorsal margin moderately convex to almost straight in the longer valves; ventral margin straight or almost straight <sup>1</sup>	Dorsal margin convex; ventral margin straight to slightly concave, with valve mantle thickened at the proximal raphe end <sup>4</sup>
Valve apices	Apices acutely rounded, nose-like, quite protracted, differentiated from the main body and deflected to the ventral side	Apices acutely rounded, tapered, subrostrate, slightly delimited by a change in the slope of the dorsal margin, appearing nose-like <sup>2</sup>	Apices rounded, shortly nose-like, not flexed to the ventral side <sup>1</sup>	Apices rounded, nose-like, deflected to the ventral side <sup>4</sup>
Rimoportula	At one end, at the center of the apex	At one end, at the center of the apex <sup>2</sup>	At one end, at the center of the apex <sup>1</sup>	No described

Lange-Bertalot & Metzeltin (2009)<sup>1</sup>, Furey et al. (2011)<sup>2</sup>, Fontana & Bicudo (2012)<sup>3</sup>, Moser et al. (1998)<sup>4</sup>

Valves arched tapering toward apices, 16.2–98.7 µm long and 3.1–4.6 µm wide. Dorsal margin convex, ventral margin concave sometimes with median swelling (Figure 2e-2j). Apices rounded to acutely rounded. Terminal nodules near the apices. Distal raphe end strongly curved onto the valve face. One rimoportula per valve, near the apex. Striae parallel, denser near the apices, 12–15 in 10 µm in the middle region of the valve. Areolae rounded, not visible in LM. Cingulum composed of four open bands perforated by a linear row of pores.

**Frequency of occurrence:** constant (Table 3).

**Paraná State citations:** Tremarin et al. (2009), Faria et al. (2010), Silva et al. (2010), Santos et al. (2011), Marra et al. (2016), Bartozek et al. (2013). Sometimes cited as *Eunotia curvata* (Kützing) Lagerstedt var. *curvata* and as *Eunotia lunaris* (Ehrenberg) Grunow var. *lunaris* (Tremarin et al. 2009).

**Taxonomic remarks:** *Eunotia bilunaris* is a very common periphytic species, broadly distributed in the state of Paraná, and frequently registered in Europe, Africa, Asia, North and South America (Costa et al. 2017). According to Lange-Bertalot et al. (2011), the species have a heterogeneous concept and a confusing taxonomy due to their wide morphological variability, mostly related to shape and dimensions. Specimens with uncommon morphology are common and have already been formally described as infraspecific taxa. The material analysed showed a wide morphological and metric variation of the valves, corroborating with the observations of Krammer & Lange-Bertalot (1991) and Tavares & Valente-Moreira (2000).

*Eunotia botulitropica* C.E. Wetzel & L.F. Costa. In: Costa et al., *Bibliotheca Diatomologica* 64: 14, pl. 58, figs 11–46, pl. 59, figs 1–3, pl. 61, figs 1–3, 2017.

Figures 2p–2t, 4d–4f.

Frustule rhomboid in lateral view. Valves dorsiventral, sometimes heteropolar, 11.1–22.5 µm long and 2.5–3.7 µm wide. Dorsal margin convex, ventral margin straight to slightly concave. Apices rounded to obtusely rounded. Terminal nodules near the apices. Distal raphe end curved onto the valve face. One small rimoportula per valve, near the apex. Striae parallel, 14–19 in 10 µm in the middle region of the valve. Areolae rounded, not visible in LM. Cingulum composed of four open bands perforated by one row of pores.

**Frequency of occurrence:** common (Table 3).

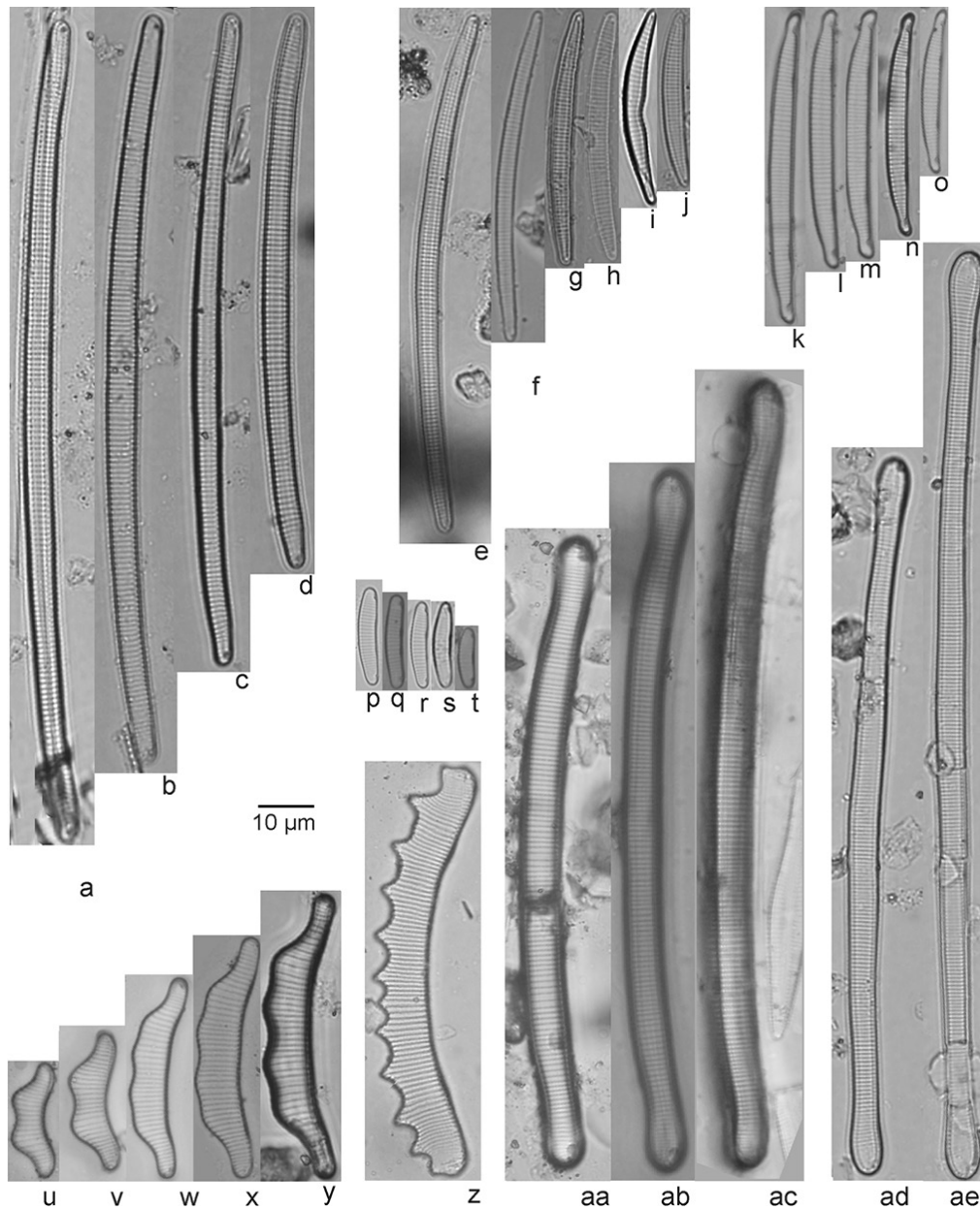
**Paraná State citations:** cited as *E. intermedia* (Krasske ex Hustedt) Nörpel & Lange-Bertalot in Tremarin et al. (2008, fig. 28).

**Taxonomic remarks:** *Eunotia botulitropica* was recently described based on samples collected in reservoirs from São Paulo state. According to Costa et al. (2017), *E. botulitropica* differs from *E. botuliformis* Wild, Nörpel & Lange-Bertalot by its “narrower valve, lower striae density, rounded and more tapered ends” (Lange-Bertalot et al. 2011). However, the measurements and number of striae overlap, and *E. botuliformis* can be separated by the parallel valvar margins and the regular distribution of the striae along the valve. *Eunotia botulitropica* shows rhomboid girdle view, resembling *E. rhomboidea* Hustedt, but the latter is clearly a heteropolar species and presents larger valves (Costa et al. 2017).

*Eunotia caniculoides* Favaretto, Tremarin, T.Ludwig & Bueno sp. nov.

**Table 3.** Occurrence of *Eunotia* species, ecological data and environmental data by sampling in Tenente João Gualberto stream, Paraná, Brazil

	UNOPA	3943	3962	3976	4035	4044	4057	4081	4093	4102	4116	4126	4136
	Sampling date	20/ IX/2012	29/ X/2012	13/ XI/2012	18/ XII/2012	15/ I/2013	06/ II/2013	11/ III/2013	04/ IV/2013	09/ V/2013	06/ VI/2013	16/ VII/2013	12/ VIII/2013
<b>Occurrence in samples</b>													
<i>Eunotia ambivalens</i>			x		x				x	x	x	x	x
<i>Eunotia bilunaris</i>	x	x						x	x		x	x	x
<i>Eunotia botulitropica</i>	x	x						x	x		x	x	x
<i>Eunotia caniculoides</i>	x	x				x		x	x	x	x	x	x
<i>Eunotia desmogonioides</i>				x						x	x		x
<i>Eunotia georgii</i>		x											
<i>Eunotia guianensis</i>	x			x	x				x				
<i>Eunotia juettnerae</i>	x			x	x				x		x	x	
<i>Eunotia karenae</i>	x	x					x		x	x		x	
<i>Eunotia longicamelus</i>	x	x	x	x	x	x			x	x	x	x	x
<i>Eunotia meridiana</i>	x	x				x			x		x		x
<i>Eunotia minor</i>	x	x			x				x				
<i>Eunotia monodon</i>	x					x		x	x	x		x	
<i>Eunotia pileus</i>	x	x	x			x	x			x	x	x	
<i>Eunotia pseudosudetica</i> var. <i>pseudosudetica</i>	x	x			x				x		x		
<i>Eunotia pseudosudetica</i> var. <i>rotundata</i>	x	x	x						x		x		x
<i>Eunotia rabenhorstii</i> var. <i>monodon</i>	x	x	x				x	x			x	x	x
<i>Eunotia rabenhorstii</i> var. <i>triodon</i>	x	x	x				x	x	x		x	x	x
<i>Eunotia sedina</i>	x	x						x	x	x	x	x	x
<i>Eunotia tropico-arcus</i>									x		x	x	
<i>Eunotia yanomami</i>							x			x		x	
Riches of <i>Eunotia</i> species	16	15	8	6	5	5	5	7	16	9	15	14	11
<b>Ecological data</b>													
Water Temperature (°C)		19.39	22.46	23.58	25.38	24.07	23.26	23.32	22.12	15.39	16.93	17.07	13.46
Conductivity (ms.cm <sup>-1</sup> )		0.025	0.023	0.031	0.03	0.012	0.025	0.022	0.018	0.017	0.018	0.019	0.020
pH		6.14	5.41	5.6	6.5	6.21	5.08	6.56	6.33	8.1	6.73	7.69	5.74
Turbidity (NTU)		3.74	4.29	14.5	9.36	7.59	5.77	1.42	2.61	0.76	2.11	1.37	1.17
Dissolved oxygen (mg.L <sup>-1</sup> )		11.05	0.09	1.49	1.44	4.43	2.56	3.29	11.88	16.49	22.89	9.83	5.85
Ammoniac nitrogen (mg.L <sup>-1</sup> )		0.1405	0.183	0.138	0.218	0.178	0.3105	0.373	0.223	0.018	0.0755	0.058	0.0605
Nitrite (mg.L <sup>-1</sup> )		0.0028	0.0026	0.0117	0.0026	0.0096	0.0053	0.0001	0.0001	0.0008	0.0005	0.0008	0.00001
Nitrate (mg.L <sup>-1</sup> )		0.3775	0.365	0.22125	0.12125	0.115	0.15875	0.1025	0.07125	0.09	0.05875	0.1025	0.115
Total phosphorous (mg.L <sup>-1</sup> )		0.0198	0.0523	0.019	0.0048	0.0157	0.0157	0.0123	0.0115	0.009	0.0065	0.004	0.0057
Orthophosphate (mg.L <sup>-1</sup> )		0.0057	0.054	0.004	0.0073	0.0065	0.0115	0.0082	0.0057	0.0065	0.0057	0.009	0.0032
<b>Environmental data</b>													
Depth (m)		0.16	0.21	0.17	0.18	0.24	0.22	0.22	0.22	0.20	0.22	0.27	0.23
Flow (m <sup>3</sup> .s <sup>-1</sup> )		0.16	0.20	0.19	0.18	0.37	0.26	0.35	0.27	0.24	0.24	0.37	0.23
Weekly precipitation (mm)		21.4	77.6	15.8	63	0	64.2	84.4	32.2	53.2	23.4	0	0



**Figures 2.** *Eunotia* of a subtropical stream adjacent to Iguacu National Park, Brazil. LM. a–d. *Eunotia ambivalens*. e–j. *Eunotia bilunaris*. k–o. *Eunotia caniculoides* sp. nov. p–t. *Eunotia botulitropica*. u–y. *Eunotia longicamelus*. z. *Eunotia georgii*. aa–ac. *Eunotia karenae*. ad–ae. *Eunotia desmogonioides*. Scale: 10 µm.

Figures 2k–2o, 5a–5d.

Valves dorsiventral, 30.0–58.5 µm long and 3.5–4.9 µm wide. Dorsal margin slightly convex, to almost straight in the larger valves; ventral margin straight to subtly concave. Apices acutely rounded, nose-like, quite protracted from the main body, deflected to ventral side. Pseudosepta in the valve ends. Terminal nodules ventral, somewhat apart from the apices. Distal raphe ends simple, not reaching the valve center. Raphe fissure obliquely curved toward ventral mantle, with proximal ends dilated in pore. One small rimoportula per valve, at the center of the apex. Striae parallel to radiate, denser near the apices, 10–14 in 10 µm in the middle region of the valve. Areolae round, 38–40 in 10 µm, not visible in LM. Cingulum composed of four open bands perforated by a single linear row of pores. On the mantle, at the middle of raphe

branch, 3 areolae between valve face margin and raphe fissure; 2 to 3 areolae between the raphe branch and lower mantle margin.

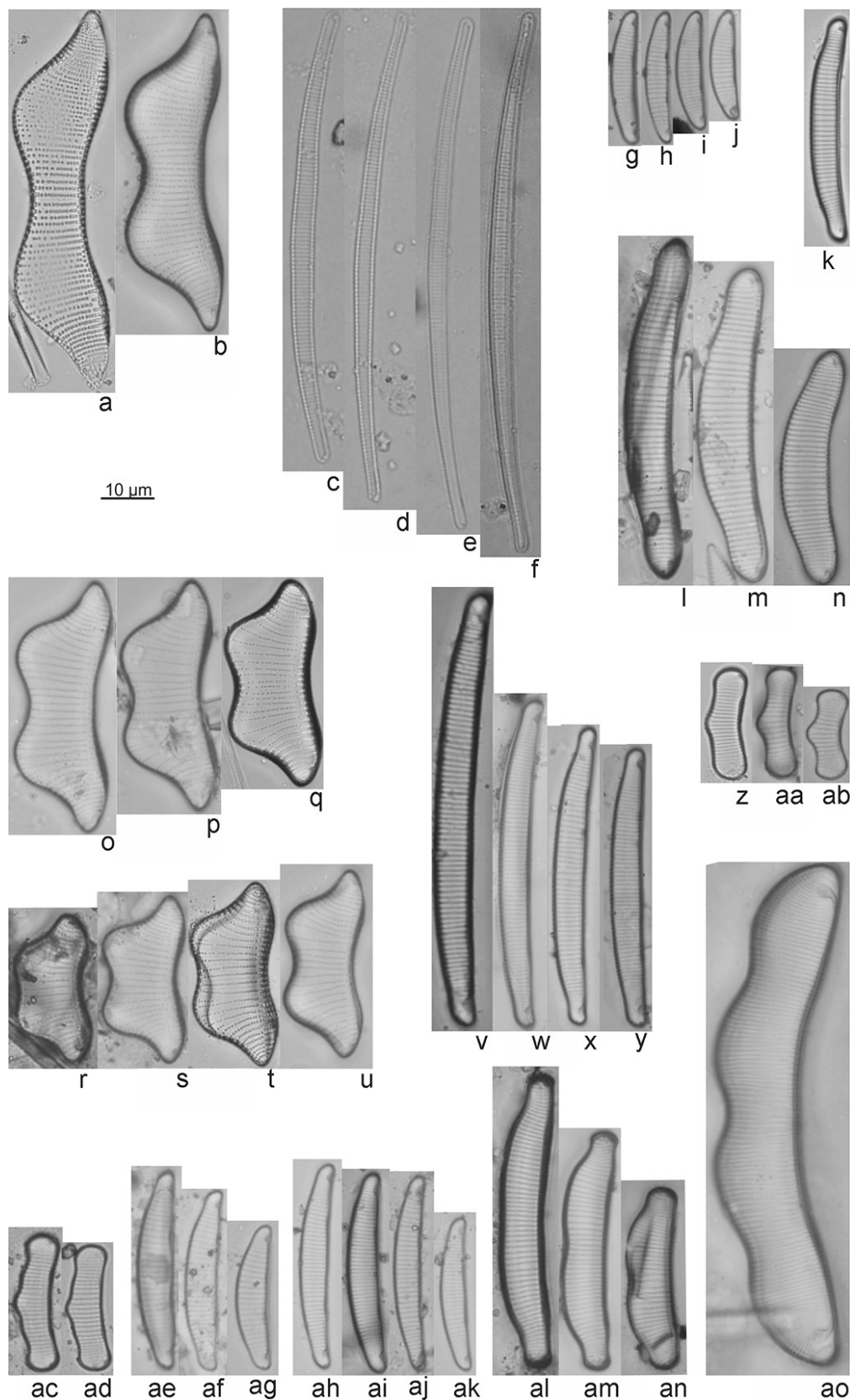
**Holotype (here designated):** BRAZIL, Paraná: São Miguel do Iguacu, Tenente Gualberto stream, 20 september 2012, L.C. Servat (holotype UNOPA 3943!, depicted in Figure 2n).

**Etymology:** The epithet refers to the general similarities to *Eunotia canicula* Furey, Lowe & Johansen (2011: 57, pl. 30, figs. 39–46).

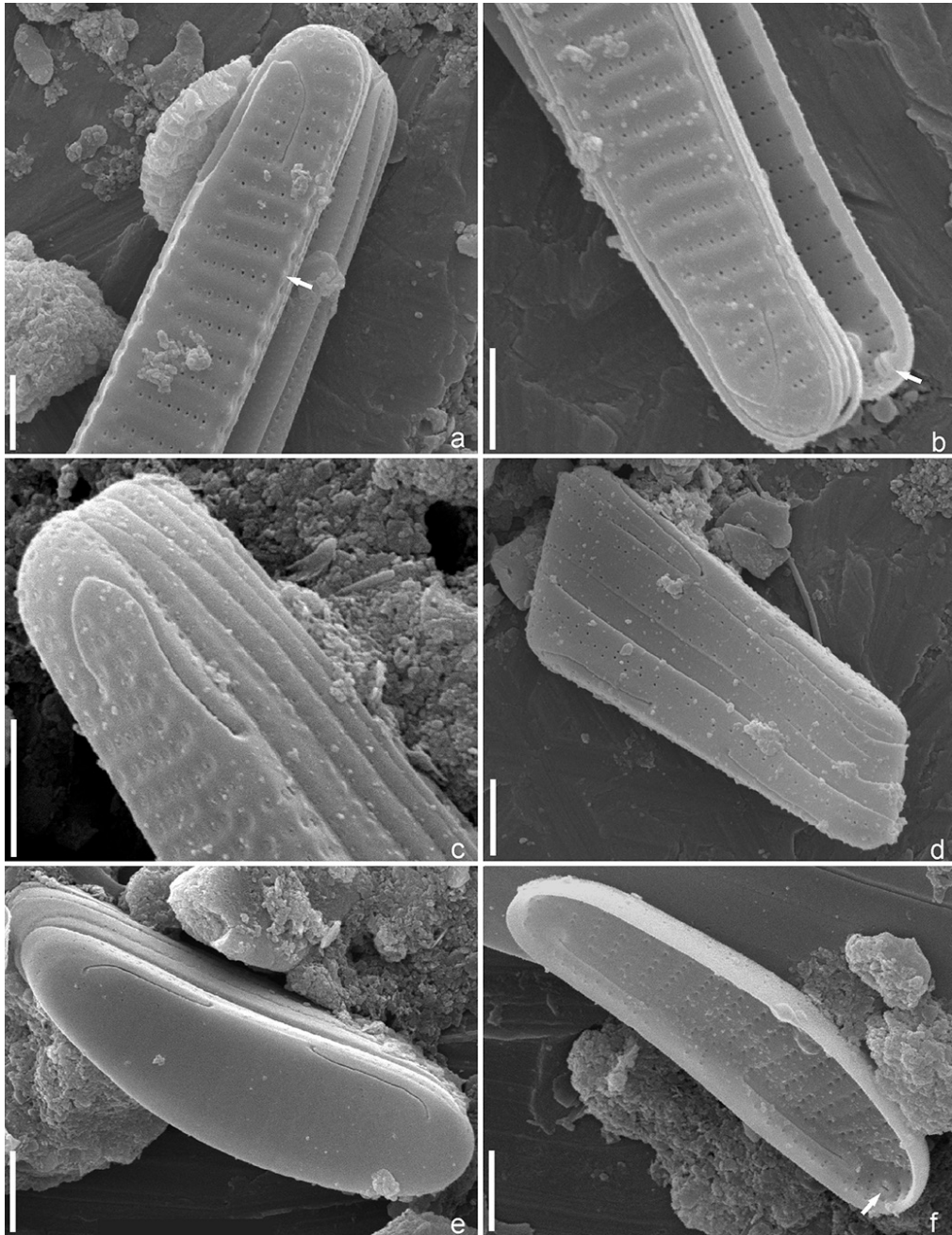
**Frequency of occurrence:** constant (Table 3).

**Taxonomic remarks:** *Eunotia canicula* Furey, Lowe & Johansen is similar to *E. caniculoides*, but the valve outline and ends shape are quite different. *Eunotia canicula* has dorsal margin more convex and higher striae density (14–15 in 10 µm). *E. caniculoides* shows longer protracted ends when compared to *E. canicula* (Furey et al. 2011).

*Eunotia* from a subtropical Brazilian stream



**Figures 3.** *Eunotia* of a subtropical stream adjacent to Iguacu National Park, Brazil. LM. a–b. *Eunotia guianensis*. c–f. *Eunotia juettnerae*. g–j. *Eunotia meridiana*. k. *Eunotia minor*. l–n. *Eunotia monodon*. o–u. *Eunotia pileus*. v–y. *Eunotia pseudosudetica* var. *rotundata*. z–ab. *Eunotia rabenhorstii* var. *monodon*. ac–ad. *Eunotia rabenhorstii* var. *tridon*. ae–ag. *Eunotia pseudosudetica* var. *pseudosudetica*. ah–ak. *Eunotia sedina*. al–an. *Eunotia tropico-arcus*. ao. *Eunotia yanomami*. Scale: 10 µm.



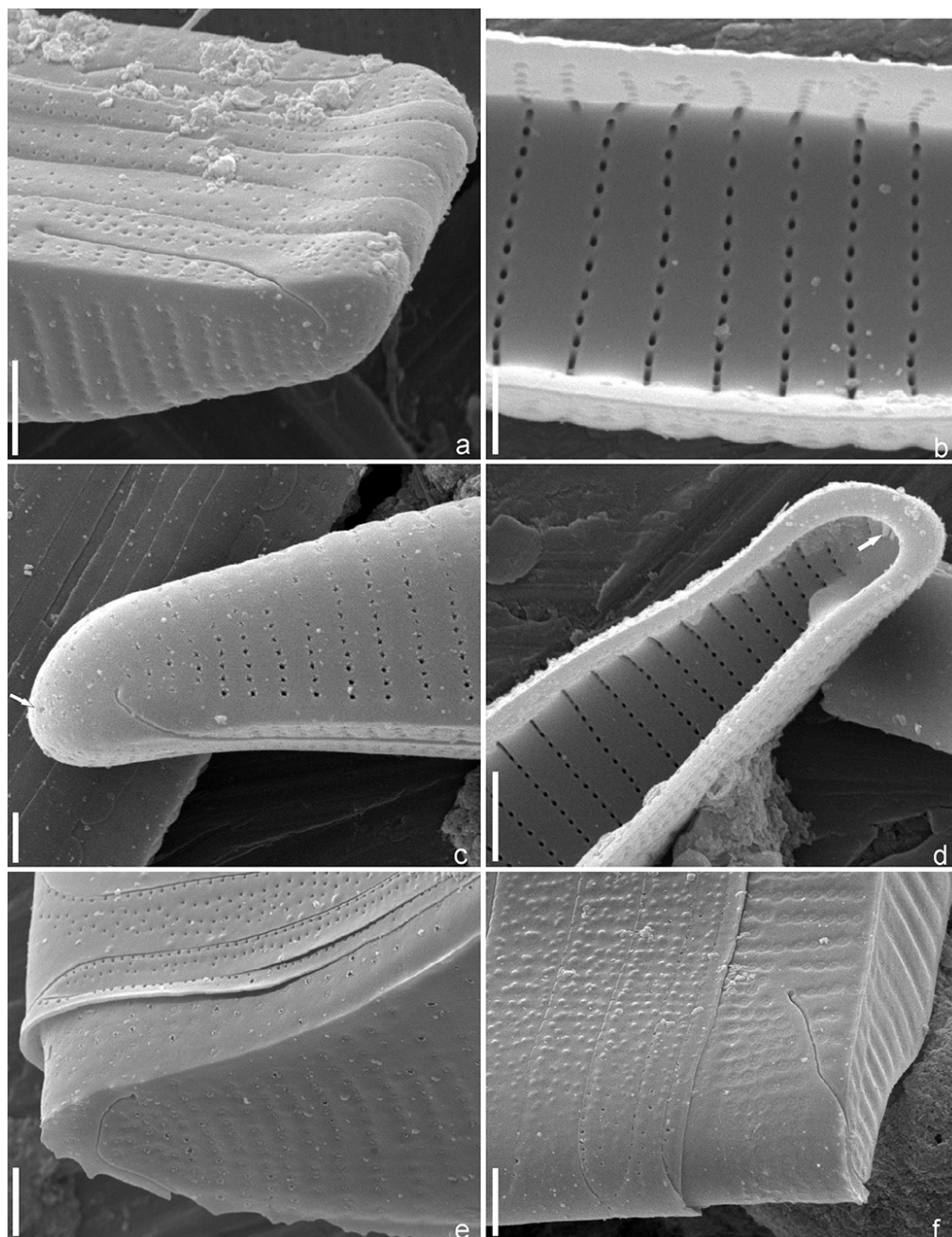
**Figures 4.** *Eunotia* of a subtropical stream adjacent to Iguaçu National Park, Brazil. SEM. a. *E. ambivalens* - external view of valve apex showing the raphe and striae pattern. Note the marginal spines (arrow). b. *E. bilunaris* - external and internal view of valve apex showing the raphe, striae pattern and rimoportula (arrow). c. *E. bilunaris* - tilted frustule showing the raphe and girdle bands. d. *E. botulitropica* - girdle view of frustule. e. *E. botulitropica* - external view of valve showing the raphe fissures. f. *E. botulitropica* - internal view of valve. Note the rimoportula (arrow). Scales: 2  $\mu\text{m}$ .

Fontana & Bicudo (2012) found *E. canicula* in the Paranapanema river watershed, state of São Paulo, but the population is clearly distinct from *E. caniculoides*, and the striae density are even much higher (16–22/10  $\mu\text{m}$ ).

*Eunotia caniculoides* is similar by the valvar contour and nose-like apices to *E. intricans* Lange-Bertalot & Metzeltin, described from a lake in Panamá (Lange-Bertalot & Metzeltin 2009), and to *E. sioliopsis* Moser, Lange-Bertalot & Metzeltin, proposed based on samples collected in New Caledonia. *E. intricans* has similar dimensions

(length 17–48  $\mu\text{m}$ , width 4.0–5.0  $\mu\text{m}$ ) and striae density (11–13 in 10  $\mu\text{m}$ ) (Table 2) but the apices are more rounded, less protracted, and not flexed to ventral side as in *E. caniculoides* (Lange-Bertalot & Metzeltin 2009). *E. sioliopsis* shows similar dimensions and apices shape, but striae and areolae densities are lower and dorsal margins are more convex (see Table 2). Raphe distal ends in *E. sioliopsis* and in *E. intricans* are shorter in valve face and longer in the mantle; and the terminal nodules are less prominent (Lange-Bertalot & Metzeltin 2009, Moser et al. 1998), when compared to *E. caniculoides*. Also, in



*Eunotia* from a subtropical Brazilian stream

**Figures 5.** *Eunotia* of a subtropical stream adjacent to Iguaçú National Park, Brazil. SEM. a. *E. caniculoides* - tilted frustule showing the raphe and girdle bands. b. *E. caniculoides* - striae in the median region of valve in internal view. c. *E. caniculoides* - external view of apex. Note the rimoportula opening (arrow). d. *E. caniculoides* - internal view of apex showing the rimoportula (arrow). e. *E. guianensis* - external view of apex showing the raphe. f. *E. longicamelus* - girdle view of frustule showing the raphe and bands. Scales: 1  $\mu\text{m}$  (Figs 5b, 5c), 2  $\mu\text{m}$  (Figs 5a, 5d–5f).

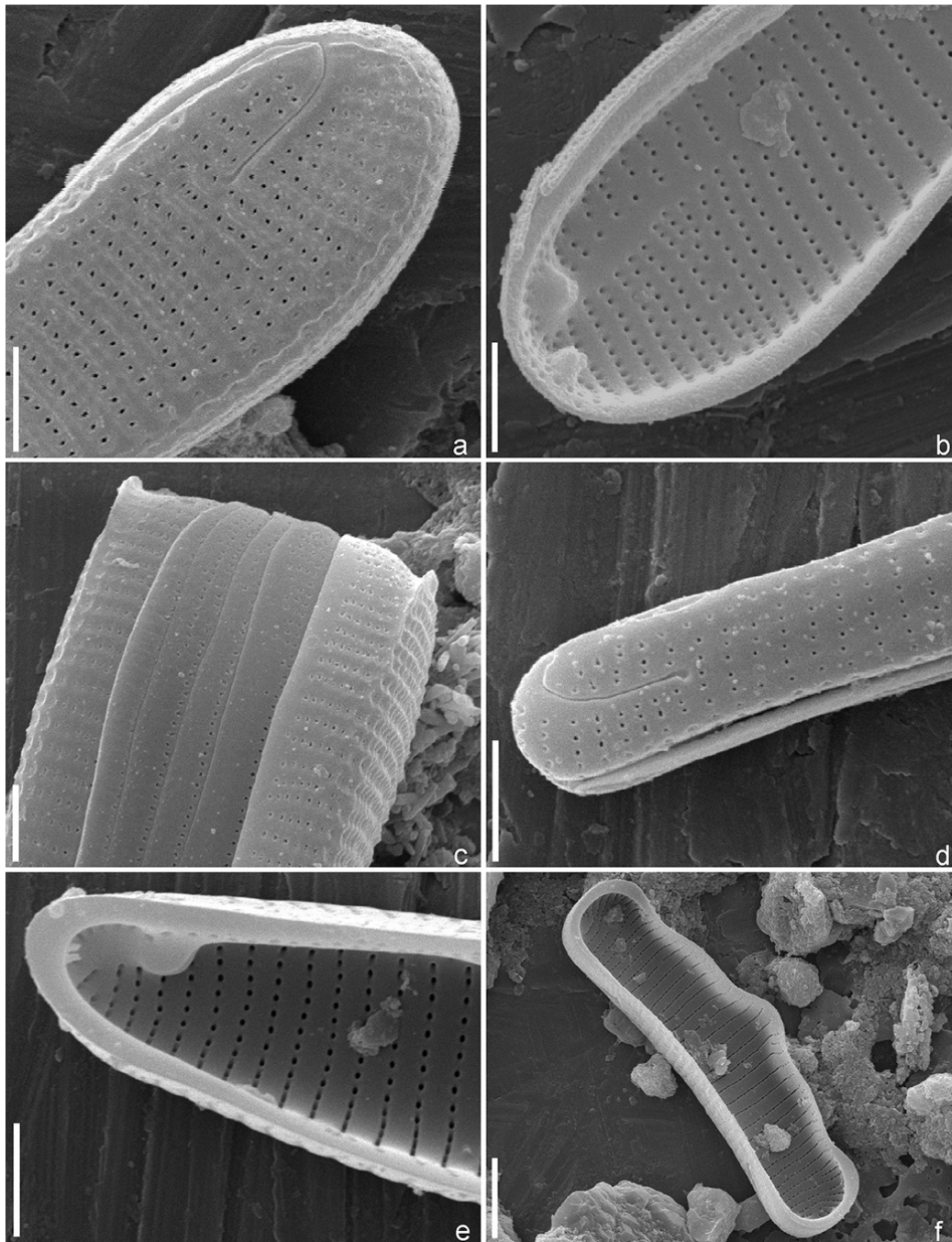
*E. sioliopsis*, the areola number in the middle of the raphe branch in the mantle, between the raphe and the mantle margin is higher (4 to 5) (Moser et al. 1998), and the stability of this character in the epitheca had been reported to the genus by Mayama (2001).

Morphometric data and morphological features of closer species are compared in Table 2. *Eunotia caniculoides* occurred regularly along the sampling period, except in February and November.

*Eunotia desmogonioides* Metzeltin & Lange-Bertalot. In: Lange-Bertalot, H. (ed.), *Iconographia Diatomologica* 11: pl. 5, figs 1–7, pl. 6: figs 1–4, 2002.

Figures 2ad–2ae, 6a–6c.

Valves linear, sometimes sinuous, 129.9–177.7  $\mu\text{m}$  long and 4.7–6.5  $\mu\text{m}$  wide. Dorsal margin slightly convex, ventral margin almost straight. Apices capitate-rounded. Terminal nodules in the apices. Distal raphe



**Figures 6.** *Eunotia* of a subtropical stream adjacent to Iguaçú National Park, Brazil. SEM. a. *E. desmogonioides* - external view of apex showing the raphe. b. *E. desmogonioides* - internal view of apex showing the rimoportula and helictoglossa. c. *E. desmogonioides* - detail of apex of frustule showing the valvar mantle and girdle bands. d. *E. juettnerae* - external view of apex showing the raphe. e. *E. pseudosudetica* var. *pseudosudetica* - internal view of apex showing the rimoportula and helictoglossa. f. *E. rabenhorstii* var. *triodon* - internal view of valve. Scales: 5  $\mu\text{m}$  (Fig. 6f), 2  $\mu\text{m}$  (Figs 6a–6e).

end strongly curved onto the valve face. One rimoportula per valve, near the apex. Striae parallel, 14–15 in 10  $\mu\text{m}$  in the middle region of the valve. Areolae rounded, not visible in LM. Cingulum composed of four to five open bands perforated by rows of pores.

**Frequency of occurrence:** common (Table 3).

**Paraná State citations:** Marra et al. (2016), Silva-Lehmkühl et al. (2019); cited as *Eunotia rabenhorstiana* (Grunow) Hustedt): Tremarin et al. (2009).

**Taxonomic remarks:** *Eunotia rabenhorstiana* (Grunow) Hustedt resembles *E. desmogonioides*, but differs by the terminal nodules more distant from the apices, by the shorter raphe fissures in the valve face, by the presence of rimoportula at two valve poles, and marginal spines (Metzeltin & Lange-Bertalot 1998, 2002).

*Eunotia georgii* Metzeltin & Lange-Bertalot. In: Lange-Bertalot, H. (ed.), *Iconographia Diatomologica* 5, p. 61–62; pl. 41, fig. 1–7, pl. 42, fig. 7–8, 1998.

## Figure 2z.

Valves arched, 76.2  $\mu\text{m}$  long and 7.7  $\mu\text{m}$  wide. Dorsal margin convex with twelve undulations, ventral margin concave. Apices obtusely rounded. Terminal nodules near the apices. Striae parallel, denser near the apices, 12 in 10  $\mu\text{m}$  in the middle region of the valve. Rimoportula and areolae not visible in LM.

**Frequency of occurrence:** rare (Table 3).

**Paraná State citation:** first record.

**Taxonomic remarks:** *E. georgii* resembles *E. muelleri* Hustedt, but the latter distinguishes by the wider and more rounded ends, nodules closer to the ends and lower number of dorsal undulations. Metzeltin & Lange-Bertalot (1998) stated that the terminal raphe fissure of *E. muelleri* extends along the valvar face reaching the dorsal margin and might be an important feature to analyse.

*Eunotia guianensis* (Ehrenberg) De Toni, Syll. Alg. 2: 792, 1892.

Basionym: *Himantidium guianense* Ehrenberg, Abh. Königl. Akad. Wiss. Berlin: 417, 2/1, fig. 4, (1841) 1843.

Figures 3a–3b, 5e.

Valves subtly arched, 31.5–73.1  $\mu\text{m}$  long and 10.2–12.7  $\mu\text{m}$  wide. Dorsal margin convex, with two undulations; ventral margin concave. Apices attenuate to acutely rounded, slightly deflected to the dorsal side. Terminal nodules near the apices. Distal raphe end curved onto the valve face, almost reaching the dorsal margin. Rimoportula not visible in LM. Striae parallel to radiate, not equidistant, denser near the apices, 7–12 in 10  $\mu\text{m}$  in the middle region of the valve. Areolae rounded, 15–18 in 10  $\mu\text{m}$ . Cingulum composed of six open bands perforated by several rows of pores.

**Frequency of occurrence:** common (Table 3).

**Paraná State citation:** first record.

**Taxonomic remarks:** the smaller valves of *Eunotia guianensis* resemble *E. pileus* Ehrenberg and *E. ventriosa* var. *brevis* (Patrick) Metzeltin & Lange-Bertalot. *E. pileus* has more rounded ends and terminal raphe fissures straight. The population analyzed here is similar to *Eunotia guianensis* registered by Metzeltin & Lange-Bertalot (1998:286, fig.26:1-7) in “Brasilien, Lago Calado”. Registered before as part of *E. didyma* Hustedt ex Zimmermann population illustrated by Bicudo et al. (1995, fig. 24).

*Eunotia juettnerae* Lange-Bertalot. In: Lange-Bertalot (ed.), Diatoms of Europe 6: 127, pl. 26, figs 1–17, pl. 27, figs 1–6, 2011

Figures 3c–3f, 6d.

Valves arcuate, gradually tapering towards the apices, 51.7–121.9  $\mu\text{m}$  long and 2.4–3.7  $\mu\text{m}$  wide. Dorsal margin convex, ventral margin concave. Apices rounded, slightly deflected to dorsal side. Distal raphe fissure curved extending to the middle of the valve. Striae parallel, denser near the apices, 15–19 in 10  $\mu\text{m}$  in the middle region of the valve. Areolae rounded, ca. 50 in 10  $\mu\text{m}$ . Rimoportula and areolae not visible in LM.

**Frequency of occurrence:** common (Table 3).

**Paraná State citation:** first record.

**Taxonomic remarks:** The studied population of *Eunotia juettnerae* has morphology and measurements (20.9–99.7  $\mu\text{m}$  long, 2.5–3.1  $\mu\text{m}$  wide, 17–20 striae in 10  $\mu\text{m}$ ) similar to those recorded in specimens from southeastern Brazil observed by Costa et al. (2017). *Eunotia naegelii* has narrower valves (1.5–3.0  $\mu\text{m}$ ) and higher striae density (17–27 in 10  $\mu\text{m}$ ) and *E. bilunaris* differs on broader valves (3.5–5.5  $\mu\text{m}$ ) and lower striae density (13–17 in 10  $\mu\text{m}$ ) (Lange-Bertalot et al. 2011).

*Eunotia karenae* Metzeltin & Lange-Bertalot. In: Lange-Bertalot (ed.), Iconographia Diatomologica 18: 105, pl. 48, figs 1–4, 2007.

Figures 2aa–2ac.

Valves arched, 118.3–148.6  $\mu\text{m}$  long and 6.2–7.6  $\mu\text{m}$  wide. Dorsal margin convex, ventral margin concave. Apices subcapitate, slightly deflected to the dorsal side. Terminal nodules thickened, near the apices. One rimoportula per valve, near the apex. Striae parallel, denser near the apices, 10–12 in 10  $\mu\text{m}$  in the middle region of the valve. Areolae visible in LM, 23–26 in 10  $\mu\text{m}$ .

**Frequency of occurrence:** common (Table 3).

**Paraná State citation:** first record.

**Taxonomic remarks:** The overlapped length measure and valve contour of the neotropical *E. karenae* (length: 128–200  $\mu\text{m}$ ) and the similar *E. glacialifalsa* Lange-Bertalot (length: 60–150  $\mu\text{m}$ ) represent a difficulty to separate both. *E. glacialifalsa*, a holartic species, is narrower (5–6  $\mu\text{m}$ ), and has lower striae density (8.5–10 in 10  $\mu\text{m}$ ), according to (Metzeltin & Lange-Bertalot et al. 2007). *Eunotia glacialis* F.Meister has wider valves (7–12  $\mu\text{m}$ ) and *E. glacispinosa* Lange-Bertalot & Cantonati possess polar spines (Lange-Bertalot 2011), characteristics that distinguish them from *E. karenae*.

*Eunotia longicamelus* L.F.Costa, D.C.Bicudo & C.E.Wetzel, Bibliotheca Diatomologica 64: 32, pl. 73, figs 1–17, pl. 74, figs 1–8, pl. 75, figs 1–5, pl. 76, figs 1–4, 2017.

Figures 2u–2y, 5f.

Valves arched, 22.6–73.5  $\mu\text{m}$  long and 5.0–9.6  $\mu\text{m}$  wide. Dorsal margin convex, with two to four undulations; ventral margin concave. Apices rostrate to subcapitate, slightly deflected to the dorsal side. Terminal nodules in the apices. Distal raphe end curved onto the valve face, reaching the dorsal margin. Striae parallel to radiate, denser near the apices, 8–15 in 10  $\mu\text{m}$  in the middle region of the valve. Rimoportula and areolae not visible in LM. Cingulum composed of six open bands perforated by several rows of pores.

**Frequency of occurrence:** constant (Table 3).

**Paraná State citations:** Silva-Lehmkuhl et al. (2019); cited as *E. camelus* var. *denticulata* Grunow in Moreira-Filho & Kutner (1962); cited as *E. camelus* in Tremarin et al. (2009), Bertolli et al. (2010), Faria et al. (2010), Silva et al. (2010), Santos et al. (2011), Bartozek et al. (2013), Marra et al. (2016).

**Taxonomic remarks:** *Eunotia longicamelus* is easily separated from *E. camelus* Ehrenberg by dorsal margin with two to four undulations and less concave ventral margin. *Eunotia camelus* has two evident undulations subdivided into two more smooth ones, and ventral margin more concave (Costa et al. 2017). The specimen shown in Figure 2y slightly differs from the others (figs 2u–2x) in the sub-capitate apices and irregular striae density pattern. Although similar to *Eunotia karveerensis* (Gandhi) Glushchenko & Kulikovskiy (= *E. camelus* var. *karveerensis* Gandhi) (Glushchenko & Kulikovskiy 2017), the valves are much more curved and narrower (6–7  $\mu\text{m}$  and 4.5–5.5  $\mu\text{m}$  in the humps) than *E. longicamelus*. The authors registered a morphologically diverse population occurring in southeast Asia, western India and Ceylon.

*Eunotia meridiana* Metzeltin & Lange-Bertalot. In: Lange-Bertalot (ed.), Iconographia Diatomologica 5: 67, pl. 59, figs 7–10, 1998.

Figures 3g–3j.

Valves subtly arched, 14.7–37.7  $\mu\text{m}$  long and 3.7–5.4  $\mu\text{m}$  wide. Dorsal margin convex, ventral margin linear to slightly concave.

Apices attenuate-rounded. Terminal nodules in the ventral margins. Striae parallel to radiate, denser near the apices, 14–17 in 10 µm in the middle region of the valve. Rimoportula and areolae not visible in LM.

**Frequency of occurrence:** common (Table 3).

**Paraná State citations:** Tremarin et al. (2008, 2009), Santos et al. (2011), Marra et al. (2016).

*Eunotia minor* (Kützing) Grunow in Van Heurck, Syn. Diat. Belg., pl. 33: figs 20, 21, 1881.

Figure 3k.

Valves dorsiventral, 23.5–59.0 µm long and 4.0–5.2 µm wide. Dorsal margin straight, slightly undulate; ventral margin straight to subtly concave. Apices nose-like. Terminal nodules near the apices. Striae parallel to radiate, denser near the apices, 12–17 in 10 µm in the middle region of the valve. Rimoportula and areolae not visible in LM.

**Frequency of occurrence:** common (Table 3).

**Paraná State citations:** Tremarin et al. (2009), Santos et al. (2011), Silva et al. (2010, Marra et al. 2016), Faria et al. (2019), Silva-Lehmkuhl et al. (2019).

**Taxonomic remarks:** it is a species with complex taxonomy and we based the identification on Lange-Bertalot (2011).

*Eunotia monodon* Ehrenberg, Abh. K. Akad. Wiss. Berlin, p. 414, pl. 2, fig. 7, 1841 (1843).

Figures 3l–3n.

Valves arched, 44.5–80.7 µm long and 7.9–10.8 µm wide. Dorsal margin convex, ventral margin concave. Apices rounded, differentiated from the main body. Terminal nodules in the apices. Striae parallel to radiate, 9–12 in 10 µm in the middle region of the valve. Rimoportula and areolae not visible in LM.

**Frequency of occurrence:** common (Table 3).

**Paraná State citations:** Tremarin et al. (2009), Marra et al. (2016); cited as *E. monodon* var. *major* (W. Smith) Hustedt - Tremarin et al. (2009), Procopiak et al. (2006), Faria et al. (2010).

**Taxonomic remarks:** *E. maior* (W. Smith) Rabenhorst is easily distinguished from *E. monodon* by the clearly set off capitate ends and by the more convex dorsal margin, but parallel to the ventral margin. *Eunotia monodon* usually has dorsal margin more strongly convex and ventral concave, but not parallel, rounded apices, slightly differentiated from the main body (Patrick & Reimer 1966).

*Eunotia pileus* Ehrenberg, Ber. K. Akad. Wiss. Berlin 1841: 414, pl. 2/1, fig. 5, 1843.

Figures 3o–3u.

Valves subtly arched, 24.8–43.9 µm long and 11.6–13.1 µm wide. Dorsal margin convex, with two undulations; ventral margin concave. Apices obtusely rounded to cuneate, weakly deflected to the dorsal side. Terminal nodules in the apices. Rimoportula not visible in LM. Striae parallel to radiate, not equidistant, denser near the apices, 7–10 in 10 µm in the middle region of the valve. Areolae rounded, 15–19 in 10 µm.

**Frequency of occurrence:** common (Table 3).

**Paraná State citation:** first record.

**Taxonomic remarks:** see *Eunotia guianensis* for comments.

*Eunotia pseudosudetica* Metzeltin, Lange-Bertalot & García-Rodríguez var. *pseudosudetica*. In: Lange-Bertalot (ed.), Iconographia Diatomologica 15: 57, pl. 24, figs 15–18, 2005.

Figures 3ac–ag, 6e.

Valves dorsiventral, 27.2–38.0 µm long and 4.8–6.6 µm wide. Dorsal margin convex, ventral margin straight to subtly concave. Apices

nose-like, differentiated from the main body. Terminal nodules near the apices. Distal raphe end short, obliquely curved onto the valve face. One rimoportula per valve, near the apex. Striae parallel to radiate, denser near the apices, 11–15 in 10 µm in the middle region of the valve. Areolae rounded, ca. 38 in 10 µm.

**Frequency of occurrence:** common (Table 3).

**Paraná State citations:** Bartozek et al. (2013), Tremarin et al. (2009), Marra et al. (2016), Silva-Lehmkuhl et al. (2019).

**Taxonomic remarks:** The population of *Eunotia pseudosudetica* analyzed here is similar to type material from Uruguay (Metzeltin et al. 2005) and to southern Brazilian specimens described and illustrated by Cavalcante et al. (2014).

*Eunotia pseudosudetica* var. *rotundata* Cavalcante, Tremarin & T. Ludwig. In: Cavalcante et al., Biota Neotropica 14(3): 10, figs 67–74, 80–83, 2014.

Figures 3v–3y.

Valves dorsiventral, 52.0–63.3 µm long and 5.0–6.0 µm wide. Dorsal margin slightly convex, ventral margin subtly concave to almost straight. Apices rounded, subtly nose-like. Terminal nodules near the apices. Striae parallel to radiate, denser near the apices, 11–16 in 10 µm in the middle region of the valve. Rimoportula and areolae not visible in LM.

**Frequency of occurrence:** common (Table 3).

**Paraná State citation:** first record.

**Taxonomic remarks:** *Eunotia pseudosudetica* var. *rotundata* was originally found in macrophyte samples collected in the state of Santa Catarina. The more rounded ends and less pronounced dorsiventrality distinguish this variety from the typical (Cavalcante et al. 2014).

*Eunotia rabenhorstii* var. *monodon* Cleve & Grunow. In: Van Heurck, Syn. Diat. Belg., pl. 35, fig. 12B, 1881.

Figures 3z–3ab.

Valves arched, 17.2–32.7 µm long and 6.2–8.5 µm wide. Dorsal margin convex with one undulation in the middle of the valve, ventral margin concave. Apices broadly rounded. Terminal nodules in the apices. Striae parallel to radiate, denser near the apices, 12–15 in 10 µm in the middle region of the valve. Rimoportula and areolae not visible in LM.

**Frequency of occurrence:** common (Table 3).

**Paraná State citations:** Tremarin et al. (2009), Marra et al. (2016), Silva-Lehmkuhl et al. (2019), Faria et al. (2010, 2019).

**Taxonomic remarks:** The pronounced dorsal median undulation distinguishes *E. rabenhorstii* var. *monodon* from *E. rabenhorstii* var. *triodon* characterized by three dorsal undulations (Van Heurck, 1881).

*Eunotia rabenhorstii* var. *triodon* Cleve & Grunow. In: Van Heurck, Syn. Diat. Belg., pl. 35, fig. 12A, 1881.

Figures 3ac–3ad, 6f.

Valves slightly arched, 22.9–32.7 µm long and 6.5–8.5 µm wide. Dorsal margin convex with three undulations being the median undulation more pronounced than the others; ventral margin concave. Apices broadly rounded. Terminal nodules in the apices. Striae parallel to radiate, denser near the apices, 12–14 in 10 µm in the middle region of the valve. Rimoportula and areolae not visible in LM. In SEM, 40 areolae in 10 µm.

**Frequency of occurrence:** constant (Table 3).

**Paraná State citations:** Tremarin et al. (2009), Bartozek et al. (2013).

*Eunotia sedina* Lange-Bertalot, Bak & Witkowski. In: Lange-Bertalot (ed.), *Diatoms of Europe* 6: 215, pl. 47, figs 1–17, 2011.

Figures 3ah–3ak.

Valves dorsiventral, 22.1–40.5 µm long and 4.7–5.5 µm wide. Dorsal margin convex, ventral margin straight to slightly concave. Apices rounded, subtly nose-like, undifferentiated to the main body. Terminal nodules near the apices. Striae parallel to radiate, denser near the apices, 12–14 in 10 µm in the middle region of the valve. Rimoportula and areolae not visible in LM.

**Frequency of occurrence:** common (Table 3).

**Paraná State citation:** first record.

**Taxonomic remarks:** the analyzed population was similar to that described by Lange-Bertalot et al. (2011). *E. sedina* resembles *Eunotia sioliopsis* Moser, Lange-Bertalot & Metzeltin, but the later differs by narrower valves (length 20–40 µm, width 3.5–4.5 µm) and higher striae density (16–17 in 10 µm) (Moser et al. 1998).

*Eunotia tropico-arcus* Metzeltin & Lange-Bertalot. In: Lange-Bertalot (ed.), *Iconographia Diatomologica* 18: 123, pl. 74, figs 1–10, 2007.

Figures 3al–3an.

Valves dorsiventral, 34.3–56.1 µm long and 7.6–8.5 µm wide. Dorsal margin convex with a median depression; ventral margin slightly concave. Apices truncate, differentiated to the main body. Terminal nodules in the apices. Striae parallel to radiate, denser near the apices, 11–12 in 10 µm in the middle region of the valve. Rimoportula and areolae not visible in LM.

**Frequency of occurrence:** sporadic.

**Paraná State citation:** first record.

**Taxonomic remarks:** *Eunotia tropico-arcus* resembles *E. bidens* Ehrenberg, *E. praerupta* Ehrenberg and *E. arcus* Ehrenberg. However, *E. bidens* has more pronounced dorsal median depression and *E. praerupta* has dorsal margin strongly convex and higher valve width (10–18 µm) (Metzeltin & Lange-Bertalot 2007, Lange-Bertalot et al. 2011). Finally, *E. arcus* differs in having more arched valves, concave ventral margin and wider apices (Metzeltin & Lange-Bertalot 2007).

*Eunotia yanomami* Metzeltin & Lange-Bertalot. In: Lange-Bertalot (ed.), *Iconographia Diatomologica* 5: 86, pl. 34, figs 1–6, pl. 35, figs 1–6, pl. 36, figs 1–13, pl. 37, figs 7–8, 1998.

Figure 3ao.

Valves arched, 67.0–95.3 µm long and 10.8–13.2 µm wide. Dorsal margin convex with two undulations; ventral margin concave. Apices cuneate, rounded. Terminal nodules near the apices. Striae parallel to radiate, denser near the apices, 15–16 in 10 µm in the middle region of the valve. Rimoportula and areolae not visible in LM.

**Frequency of occurrence:** sporadic (Table 3).

**Paraná State citations:** Marra et al. (2016), Silva-Lehmkuhl et al. (2019); cited as *Eunotia zygodon* Ehrenberg in Tremarin et al. (2008).

**Taxonomic remarks:** Metzeltin & Lange-Bertalot (1998) described *E. yanomami* based on Brazilian samples. This species resembles *E. zygodon* Ehrenberg, however, *E. zygodon* has a trilobate terminal nodule (Patrick & Reimer 1966) and smaller valves (lectotype: length 53.3 µm and width 10 µm) (Reichardt 1995). Ferrari et al. (2007) commented that further studies would be needed in order to conclude on the best distinguishing features between the two species.

The more common taxa in the studied river, occurring in at least 50% of the samples, were: *E. bilunaris*, *E. ambivalens*, *E. longicamelus*,

*E. karenae*, *E. minor*, *E. pseudosudetica* var. *rotundata*, *E. monodon*, *E. juettnerae*, *E. pileus*, *E. rabenhorstii* var. *monodon*, *E. rabenhorstii* var. *triodon*, *E. botulitropica* and *E. sedina*. 66.6% of identified species were widely distributed in the study area and considered of common occurrence, 19.04% were constant, 9.52% were sporadic and 4.76% were rare. In general, the high frequencies of occurrence of the periphytic *Eunotia* community were in the spring period (76%) and in October (71%).

## Discussion

The number of taxa found in the present study (21) can be considered high when compared to *Eunotia* studies carried out in other Brazilian regions that used a similar methodology, obtaining samples by squeezing an aquatic macrophyte. We highlight Ludwig & Valente-Moreira (1989) that identified 24 *Eunotia* and Santos et al. (2011) with 18 species. Other studies about diatom flora based on samples collected in the state of Paraná included 44 taxa (Tremarin et al. 2008), 16 taxa (Marra et al. 2016), 13 taxa (Bichoff et al. 2016), 10 taxa (Fürstenberger & Valente-Moreira 2000), and 8 taxa (Bertolli et al. 2010). Bicudo et al. (1995) identified 14 taxa on samples from Mato Grosso wetland, Cavalcante et al. (2014) found 12 taxa in the state of Santa Catarina, and Ferrari et al. (2007) registered 23 species in amazonian water bodies.

Despite numerous investigations of diatom diversity in the state of Paraná, the taxonomic study of the *Eunotia* based on 12 samples from the Tenente João Gualberto stream resulted in eight new citations for the State, and *Eunotia caniculoides* was proposed as new. The more common taxa in the studied river, occurring in at least 50% of the samples, were: *E. bilunaris*, *E. ambivalens*, *E. longicamelus*, *E. karenae*, *E. pseudosudetica* var. *rotundata*, *E. monodon*, *E. juettnerae*, *E. pileus*, *E. rabenhorstii* var. *monodon*, *E. rabenhorstii* var. *triodon*, *E. botulitropica* and *E. sedina*. 66.6% of identified species were widely distributed in the study area and considered of common occurrence, 19.04% were constant, 9.52% were sporadic and 4.76% were rare. In general, the high frequencies of occurrence of the periphytic *Eunotia* community were in the spring period (76%) and in October (71%).

Species of Eunotiaceae are influenced by pH and prefer acidic water (Patrick & Reimer 1966, Cantonati & Lange-Bertalot 2011). They are typical components of the diatom flora from tropical and subtropical areas, frequently associated with acid waters and oligotrophic or dystrophic habitats (Metzeltin & Lange Bertalot 1998, Kociolek & Spaulding 2003, Furey et al. 2011, Burliga et al. 2013). During this study period, the annual pH mean value was predominantly acidic (6.3), an environmental condition that may have favored the species richness of *Eunotia*. The months of September and October 2012, April and June 2013, with greater richness of *Eunotia* taxa, showed higher concentrations of dissolved oxygen (11.05 – 22.89 mg.L<sup>-1</sup>). Of the months cited, those of 2012 had higher concentrations of TP and NO<sub>3</sub><sup>-</sup> (0.0198-0.0523 mg.L<sup>-1</sup> and 0.365-0.3775 mg.L<sup>-1</sup> respectively), and lower depth and flow values (0.16-0.21 m and 0.16-0.20 m<sup>3</sup>.s<sup>-1</sup> respectively).

In conclusion, *Eunotia* is of great taxonomic complexity and studies are still needed, leading to more complete knowledge of species diversity and distribution in Brazil. In addition, taxonomic knowledge of *Eunotia* species is essential due to their already known ecological preferences, being useful information for ecological researches aimed at the conservation and management of aquatic environments.

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## Author Contributions

Norma Bueno: contributed in the concept and design of the study.

Cynthia Favaretto: contributed in the data collections.

Cynthia Favaretto, Priscila Tremarin, Thelma Ludwig, Gabriela Medeiros, Norma Bueno: contributed to data analysis and interpretation; critical revision and manuscript preparation, all adding intellectual content.

## Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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