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Achnanthes Bory Sensu Stricto (Bacillariophyta) from Terrestrial Habitats of Rio de Janeiro (Brazil), with Description of *Achnanthes pseudoinflata* sp. nov.

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Abstract: The aim of the present work was to present the ecological and morphological characteristics of species from the genus *Achnanthes* Bory *sensu stricto*, which develops in terrestrial mosses near the Rio de Janeiro Botanic Garden, Brazil. A literature comparison was made with other similar species, including the LM and SEM analysis of original material bearing *Achnanthes inflata* (Kützing) Grunow housed at the Grunow Collection in Vienna, and data from the available literature. Samples were collected from clumps of moss growing on tree trunks, and from a concrete wall within the botanic garden. Four taxa from the genus *Achnanthes* were recorded: *A. coarctata*, *A. inflata* var. *gibba*, *A. mauiensis* and *Achnanthes pseudoinflata* sp. nov. The main morphological differences between these taxa were cell dimensions (length and width), striae and areolae density. The most common diatom species found in these samples were *Humidophila* sp. (90%), *Humidophila contenta* (74.8%), *Luticola moreirae* (17.9%), and *Achnanthes pseudoinflata* sp. nov. (7.4%).

Keywords: terrestrial environment; taxonomy; mosses; SEM description; tropical biodiversity

1. Introduction

The genus *Achnanthes* Bory [1] *sensu stricto* includes monoraphid diatoms, with mainly linear-to-lanceolate valves. Under light microscopy observation, they are often viewed in a girdle position and are clearly bent. Raphe valves with fascia reach the valve margins, while longitudinal raphe sternum run along the apical axis to the center of the valve. A rapheless valve without fascia have a sternum which are often shifted to the valve margin. Areolae are covered by perforate cribra. Cells may grow alone or create colonies. Living cells are usually attached to the substratum via mucilage stalks but are also motile. Cells of this genus can contain either two large or many small chloroplasts [2–6].

The type of species for this genus is *Achnanthes adnata* Bory [1]. This genus currently contains around 150 species, which mostly develop in marine and brackish conditions. Only a few species of *Achnanthes* are known from freshwater and terrestrial environments [7–10].

C.G. Ehrenberg described three *Achnanthes* species from Brazil, all of which are currently considered invalid: *Achnanthes brasiliensis* Ehrenberg *nom. inval.*, *Achnanthes incrassata* Ehrenberg *nom. inval.* and *Achnanthes ventricosa* Ehrenberg *nom. inval.* [11]. Here, we reproduced the figure from the Clara Ehrenberg Index.

Additional records are from the J.D. Möller collection. According to the image database of algae at Charles University in Prague (https://botany.natur.cuni.cz/algo/database/, accessed 10 March 2020), *Achnanthes ventricosa* Ehrenberg was presented on slide A118 which originated from Brazil and was collected in 1869.

Zimmermann [12] mentioned for the first time *Achnanthes mesogongyla* Grunow and *Achnanthes brasiliensis* Ehrenberg from the state of Santa Catarina growing on Hypnos. Subsequently, Zimmermann [13] also mentioned *Achnanthes inflata* in the Jaburú river at the Itaparica Island (Bahia) while Zimmermann [14] observed *Achnanhtes coarctata* from samples collected at the Rio de Janeiro Botanical Garden. Zimmermann [15] mentioned *Achnanthes longipes* and *Achnanthes brevipes* from the Santos and Rio de Janeiro states, respectively. Zimmermann [16] described *Achnanthes ambigua* C. Zimmermann as a new species from Itaparica [17]. Zimmermann [18] has described *Achnanthes solea* C. Zimmermann from marine samples from Santos (São Paulo).

In recent years, intensive diatomological research has been carried out in Brazil, focusing both on whole assemblages and on single genera [19–29]. However, research on terrestrial diatoms from this area is extremely scarce (limited to the diatom *Coenogonium linkii* Ehrenberg [30] living inside the thalli of lichens).

The aim of this work was to describe the ecological and morphological characteristics of a new *Achnanthes* species and compare it with other co-occurring species. Additionally, the comparison with the original material from the Grunow collection was made with one original material from the Grunow collection (Naturhistorisches Museum Wien, Austria).

2. Materials and Methods

Study Area

Research was conducted in the Rio de Janeiro region of south-eastern Brazil, at three sampling sites (Table 1). Two sites were located in the city of Rio de Janeiro itself (in the botanical garden and its immediate vicinity), in an area with a tropical urban climate [31]. The third site was located in the vicinity of Tijuca Park, the world's largest urban forest. This area is part of the Atlantic Rainforest, and has a humid, subtropical climate [32].

Table 1. Sampling site characteristics.

Sample Signature	2015/1	2015/2	2015/3
Sampling date	29 March 2015	29 March 2015	3 April 2015
Geographical coordinates	22°57′09″ S 43°13′40″ W	22°57′09″ S 43°13′40″ W	22°57′39″ S 43°16′21″ W
Altitude (m a.s.l.)	60	60	340
Habitat	Mosses collected from concrete wall in Rio de Janeiro city	Lichens and mosses collected from *Roystonea regia* L. trunk, 1.5 m above ground level	Mosses collected from palm trunk growing in rainforest

Samples were collected in March and April 2015, both from clumps of moss growing on tree trunks at a height of 150 cm, and from a concrete wall, about 15 cm above the surface of the ground in Rio de Janeiro city, Brazil (Table 1). Three samples were taken in total. Additionally, the original material of *Achnanthes inflata* (Kützing) Grunow [33] was observed under light microscopy (LM) and scanning electron microscopy (SEM). The sample originated from the Grunow collection slide/material n° 788,

from freshwater diatomaceous earth, collected by Hochstetter at Cabbage Tree Swamp, Auckland, New Zealand.

The samples were digested in sulfochromic mixture (a mixture of concentrated sulfuric acid (VI) and potassium dichromate) in a laboratory, in order to obtain clean diatom frustules. To remove the sulfochromic mixture, the material was then centrifuged at 2500 rpm with distilled water. The cleaned diatom valves were then enclosed in synthetic Pleurax resin, ZBE Kraków, Poland (refractive index 1.75).

Diatoms were identified and counted under an LM Carl Zeiss Axio Imager A2, equipped with a 100× Plan Apochromatic objective with differential interference contrast (DIC) for oil immersion. Diatom images were captured with a Zeiss AxioCam ICc5 camera (Jena, Germany). In total, 18 slides (6 per sample) were examined. Diatoms were identified according to the following resources: Krammer and Lange-Bertalot [34], Metzeltin and Lange-Bertalot [35,36], Rumrich et al. [37], Metzeltin et al. [38].

For the SEM observations, the samples were applied to a polycarbonate membrane filter with a 3 µm mesh, attached to aluminum stubs, and sputtered with 20 nm of gold using a turbo-pumped Quorum Q 150T ES coater. Diatoms were observed using a Hitachi SU 8010 SEM. The Grunow original material was analyzed using a Leica® DMRX bright field microscope (Wetzlar, Germany) with 100× oil immersion objective. An ultrahigh-resolution analytical field emission (FE) scanning electron microscope Hitachi SU-70 (Hitachi High-Technologies Corporation, Tokyo, Japan) operated at 5 kV and 10 mm distance was used for the SEM analysis. Diatom terminology follows Round et al. [2] and Cox [4]. Species composition and the relative abundance of taxa were determined by counting 500 specimens.

3. Results

Achnanthes pseudoinflata M. Rybak, Peszek, Skoczylas, Ector & C.E. Wetzel, sp. nov.
Figure 1 (LM), Figures 2 and 3 (SEM) here.

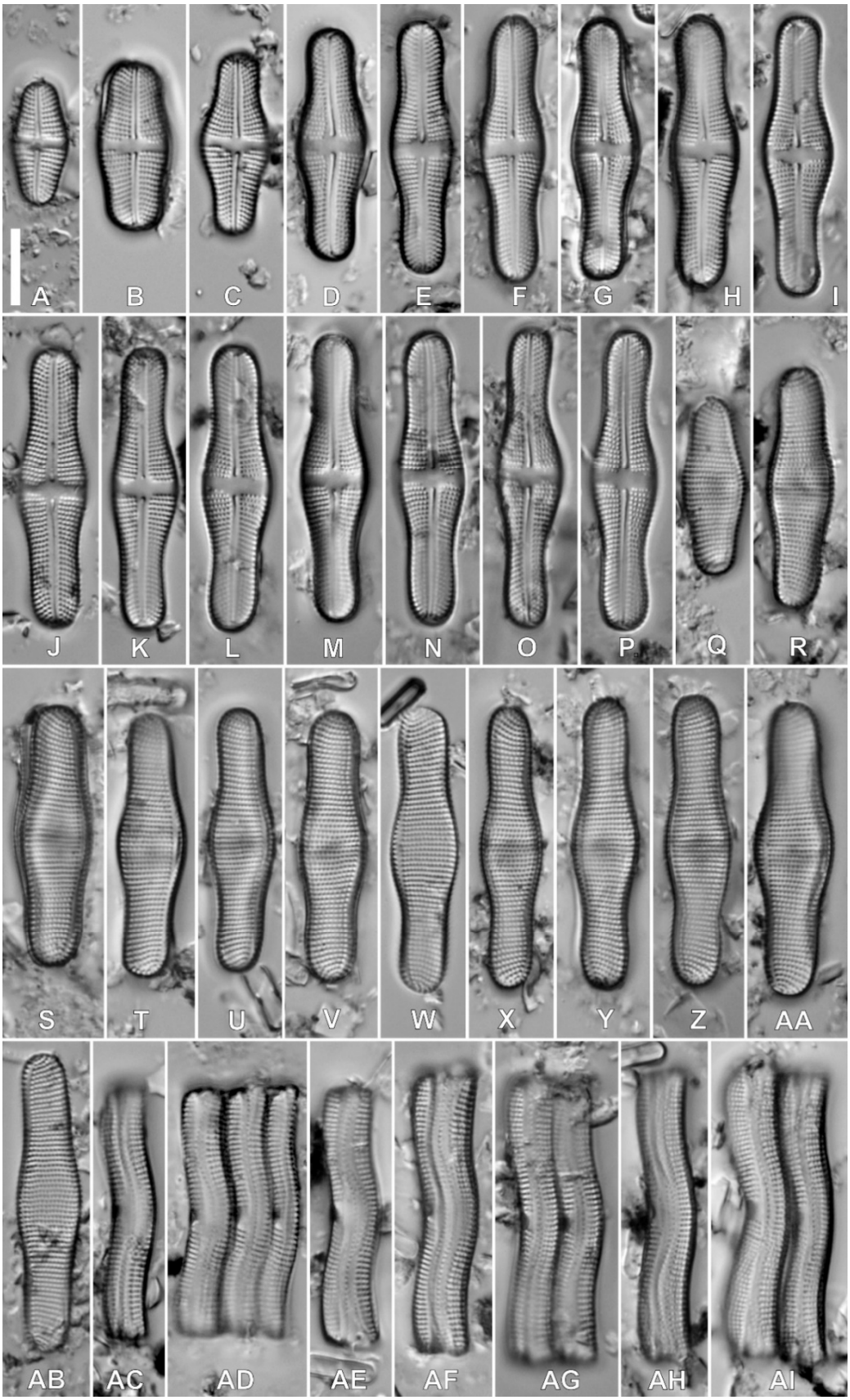

Figure 1. *Achnanthes pseudoinflata* sp. nov. Light micrographs. Scale bar = 10 μm. (**A**–**P**) Raphe valve. (**Q**–**AB**) Rapheless valve. (**AC**–**AI**) Girdle view.

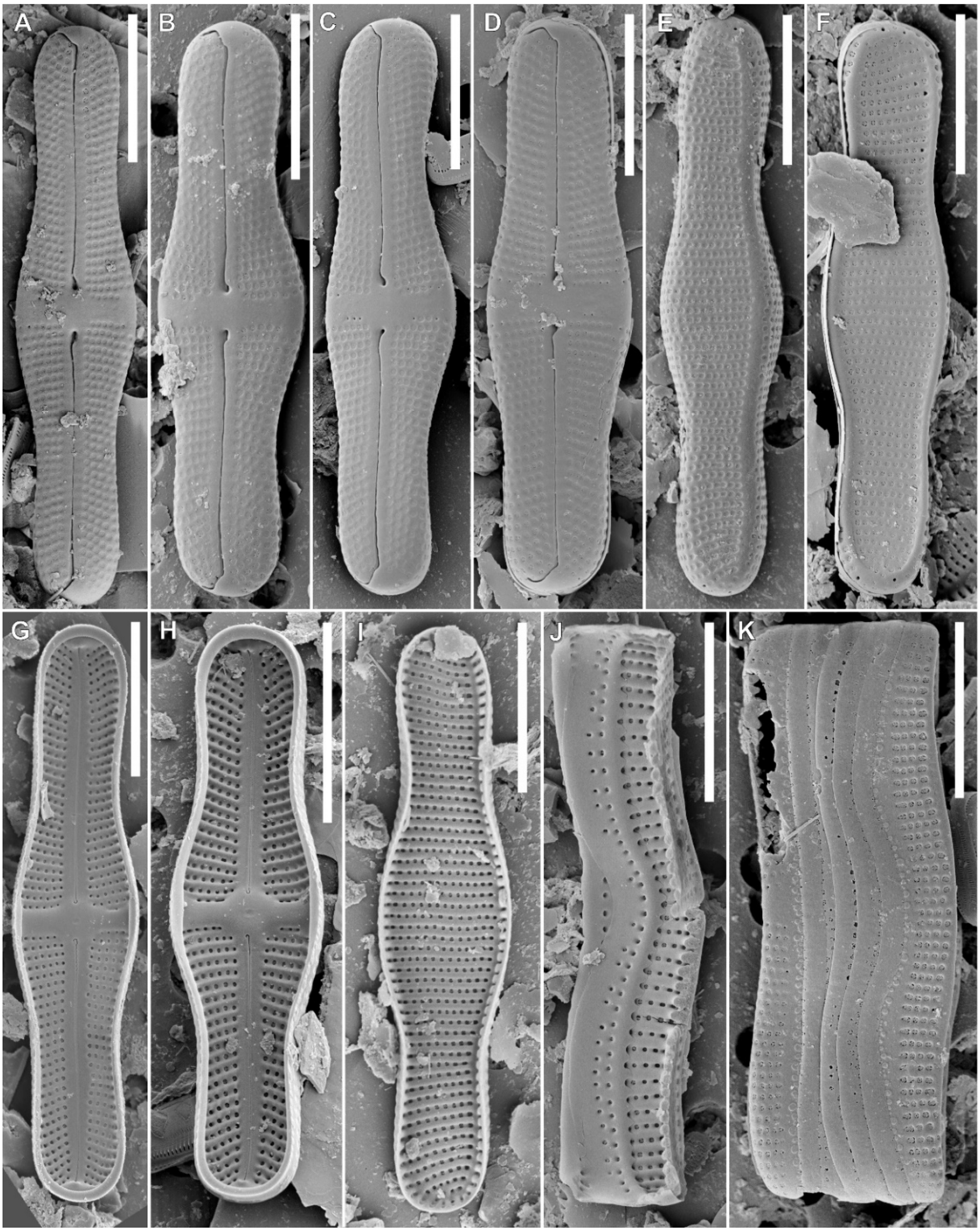

Figure 2. *Achnanthes pseudoinflata* sp. nov. Scanning electron microscope micrographs. Scale bar =10 µm. (**A**–**D**) External view of the raphe valve. (**E**,**F**) External view of the rapheless valve. (**G**,**H**) Internal view of the raphe valve. (**I**) Internal view of the rapheless valve. (**J**) Internal girdle view of the band and rapheless valve. (**K**) Girdle view.

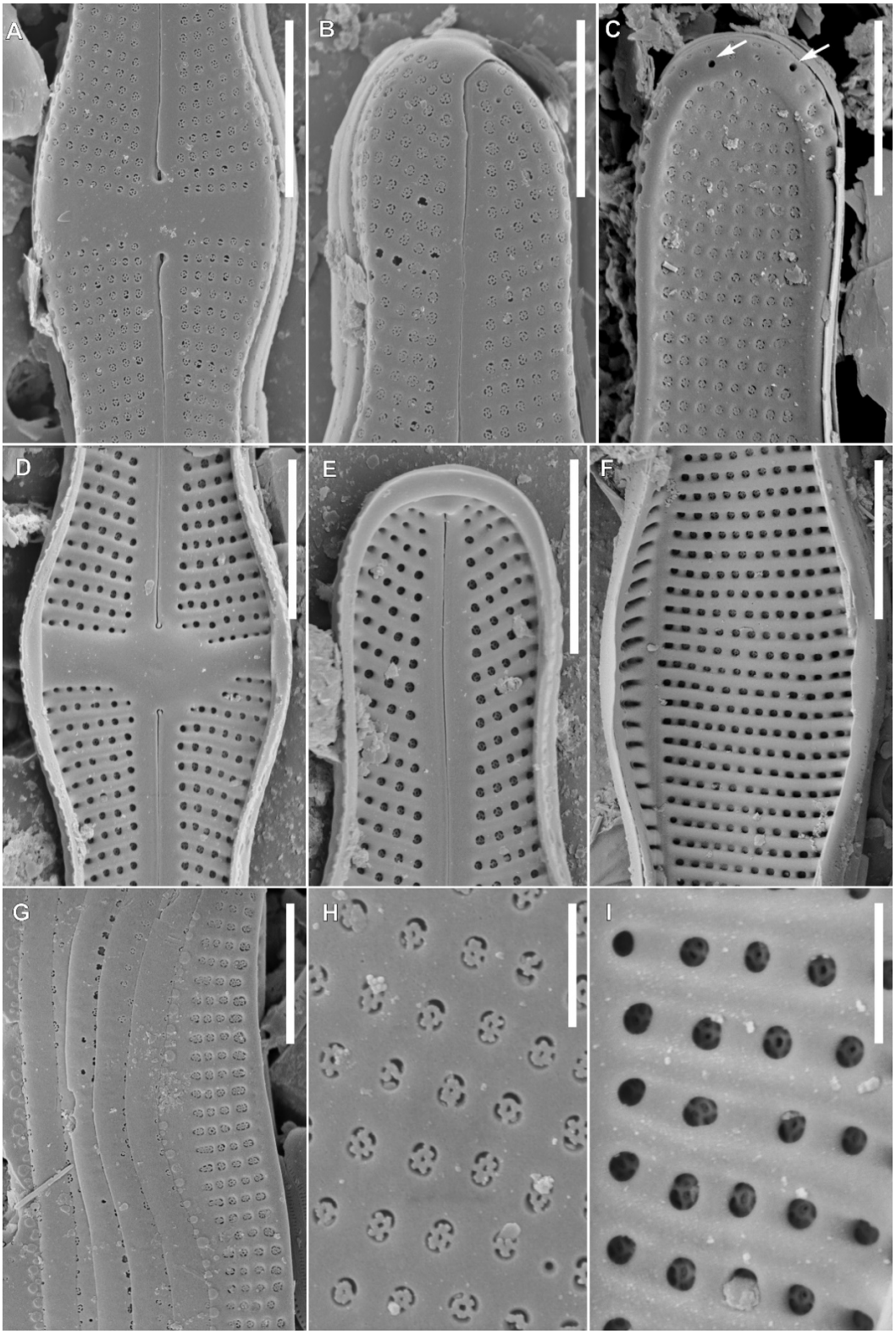

Figure 3. *Achnanthes pseudoinflata* sp. nov. Scanning electron microscope micrographs. Scale bar: A–G = 5 µm; H, I = 1 µm. (**A**) External view of the central part of the raphe valve. (**B**) External view of the raphe valve apex. (**C**) External view of the rapheless valve apex with two non-occluded areolae (see arrows). (**D**) Internal view of the central part of the raphe valve. (**E**) Internal view of the raphe valve apex. (**F**) Internal view of the central part of the rapheless valve. (**G**) Details of the external girdle view. (**H**) External view of the areolae. (**I**) Internal view of the areolae.

Description: Valves with rounded capitate ends. Central part bulged—wider than apices. Frustules are often connected to each other—visible in girdle view (Figure 1AC–AI and Figure 2J,K). Observed range of valve dimensions (n = 300): 15.1–38.5 µm in length and 6.9–9.6 µm in width (Table 2). Both valves with 15–17 striae per 10 µm. Fascia on the raphe valve variable, from rectangular to bow shaped (Figure 1A–P and Figure 3A,D). Axial area narrow and linear, shifted towards the cell margin on rapheless valve (Figure 1Q–AP and Figure 2E,F). Striae parallel mid-valve, becoming curvate with respect to the axial area in apices (Figure 1). Areolae clearly distinct, 18–22 per 10 µm on both valves. Areolae are also present on the valve mantle. Distal raphe endings are strongly deflected in the same direction. Proximal raphe endings are slightly deflected and teardrop shaped (Figure 2A–C and Figure 3A). Internally, the striae are separated by thickened virgae. Externally, areolae are occluded by cribra. The cribrum is attached to the valve mostly via 3 (and rarely, 2 or 4) struts (Figure 3H). Internally, areolae have round openings with recessed cribra (Figure 3I). Orbiculi are absent on the apices of rapheless valves, and few areolae (1–3) with different structures are present (Figure 3C). In the internal view, distal raphe endings end in small helictoglossae (Figure 2E). In the internal view, proximal raphe endings are strongly hooked (Figure 3D).

Holotype: slide 26943 at the Szczecin Diatom Collection hosted by the University of Szczecin (SZCZ). The holotype specimen is illustrated in Figure 1I.

Isotypes: slide 2015/1/1 and cleaned material, University of Rzeszów.

Etymology: The species epithet is used to show a similarity to *Achnanthes inflata*.

Locality: Brazil, Rio de Janeiro region: Rio de Janeiro, moss samples from the concrete wall, geographic coordinates: 22°57′09″ S, 43°13′40″ W.

Similar species: *Achnanthes inflata* (Kützing) Grunow [33] (Figure 4A–L and Figure 5F–J), *A. mauiensis* R.L. Lowe and A.R. Sherwood [8], *A. inflata* var. *gibba* Gandhi [39], *A. inflata* var. *javanica* Gandhi [40], *A. inflatagrandis* Metzeltin, Lange-Bertalot and García-Rodríguez [38], *A. elata* (Leuduger-Fortmorel) Gandhi [39], *A. elata* var. *curvula* Gandhi [40], *A. subelata* Metzeltin, Lange-Bertalot and García-Rodríguez [38] and *A. longboardia* A.R. Sherwood and R.L. Lowe [8].

Habitat and associated diatom flora: The described species occurred in each of the studied samples. It was most frequent (up to 7.4%) in the moss material collected from the concrete wall (sample 2015/1), but it occurred rarely in samples 2015/2 and 2015/3—only a few cells per slide. Together with *Achnanthes pseudoinflata* sp. nov., other species from the genus *Achnanthes* were found in the analyzed material (Table 2). The most frequent (not including *A. pseudoinflata* sp. nov.) was *Achnanthes inflata* var. *gibba* (Figure 5A–C and Figure 6A–N), which reached a 2.4% share in the assemblage in the moss sample 2015/1, collected from the concrete wall. The other two taxa occurred in much smaller numbers. *Achnanthes mauiensis* R.L. Lowe and A.R. Sherwood, presented in Figure 5D,E and Figure 6O–Z occurred in sample 2015/3 (up to a few cells per slide) while the only two specimens of *Achnanthes coarctata* (Brébisson) Grunow [33] were found in sample 2015/1. The most frequent co-existing diatoms in the studied material were *Humidophila* sp. (90%) and *Achnanthes pseudoinflata* sp. nov. (7.4%) in the sample 2015/1, and *Humidophila contenta* (Grunow) R.L. Lowe, Kociolek, J.R. Johansen, Van de Vijver, Lange-Bertalot and Kopalová [41] (74.8%) and *Luticola moreirae* Straube, Tremarin and Ludwig [28] (17.9%) in the sample 2015/3. *Achnanthes pseudoinflata* sp. nov. was found in sample 2015/2, but the total number of diatoms was very low. Therefore, the diatom assemblage structure was impossible to assess.

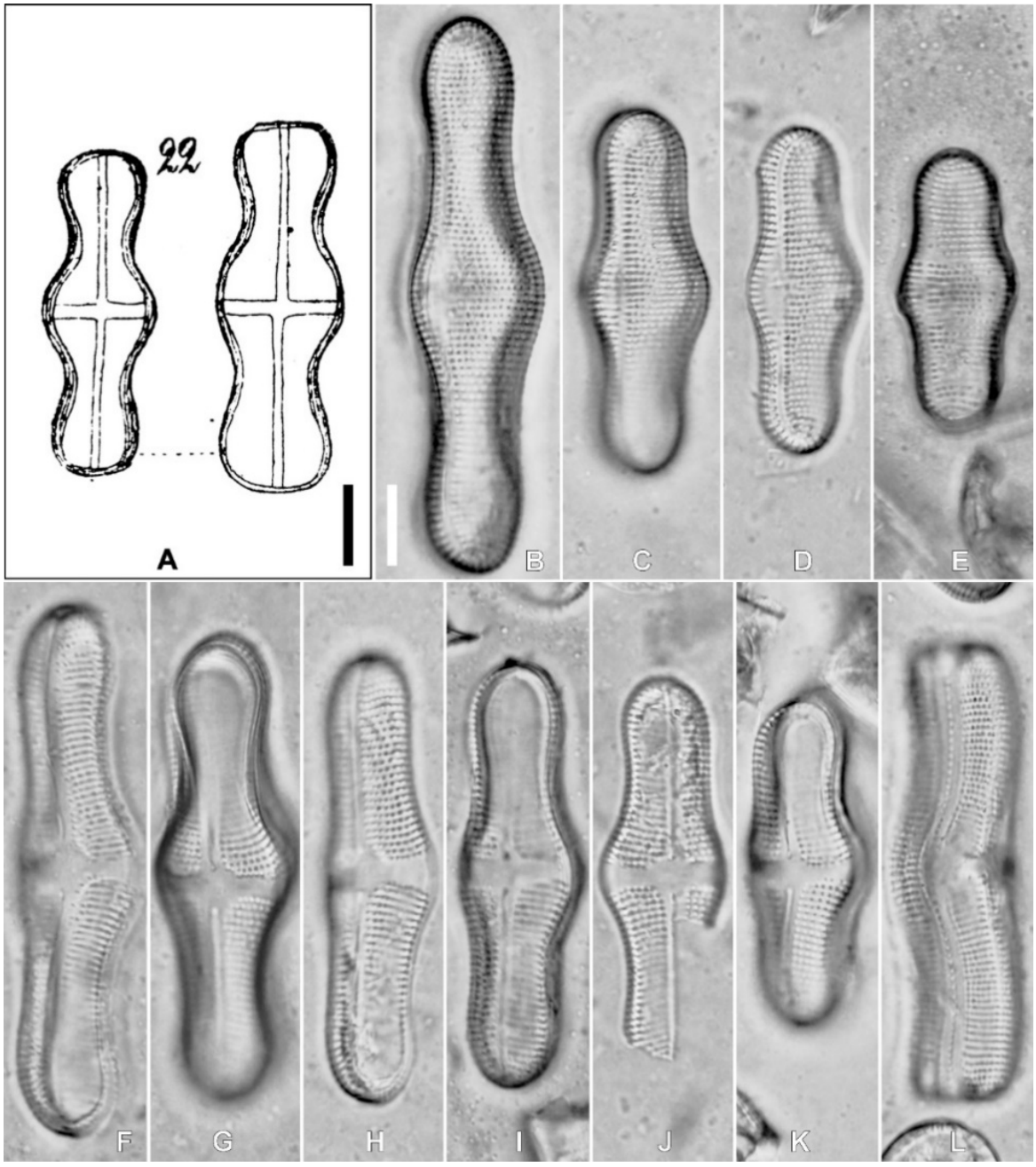

Figure 4. *Achnanthes inflata* Grunow. Photographs from the original material of *Stauroneis inflata* Kützing. Scale bar = 10 µm. (**A**) Original drawing of *Stauroneis inflata* Kützing 1844. (**B**–**L**) Light micrographs of *Achnanthes inflata* Kützing (Grunow) from the original sample. (**B**–**E**) Rapheless valve. (**F**–**K**) Raphe valves. (**L**) Girdle view.

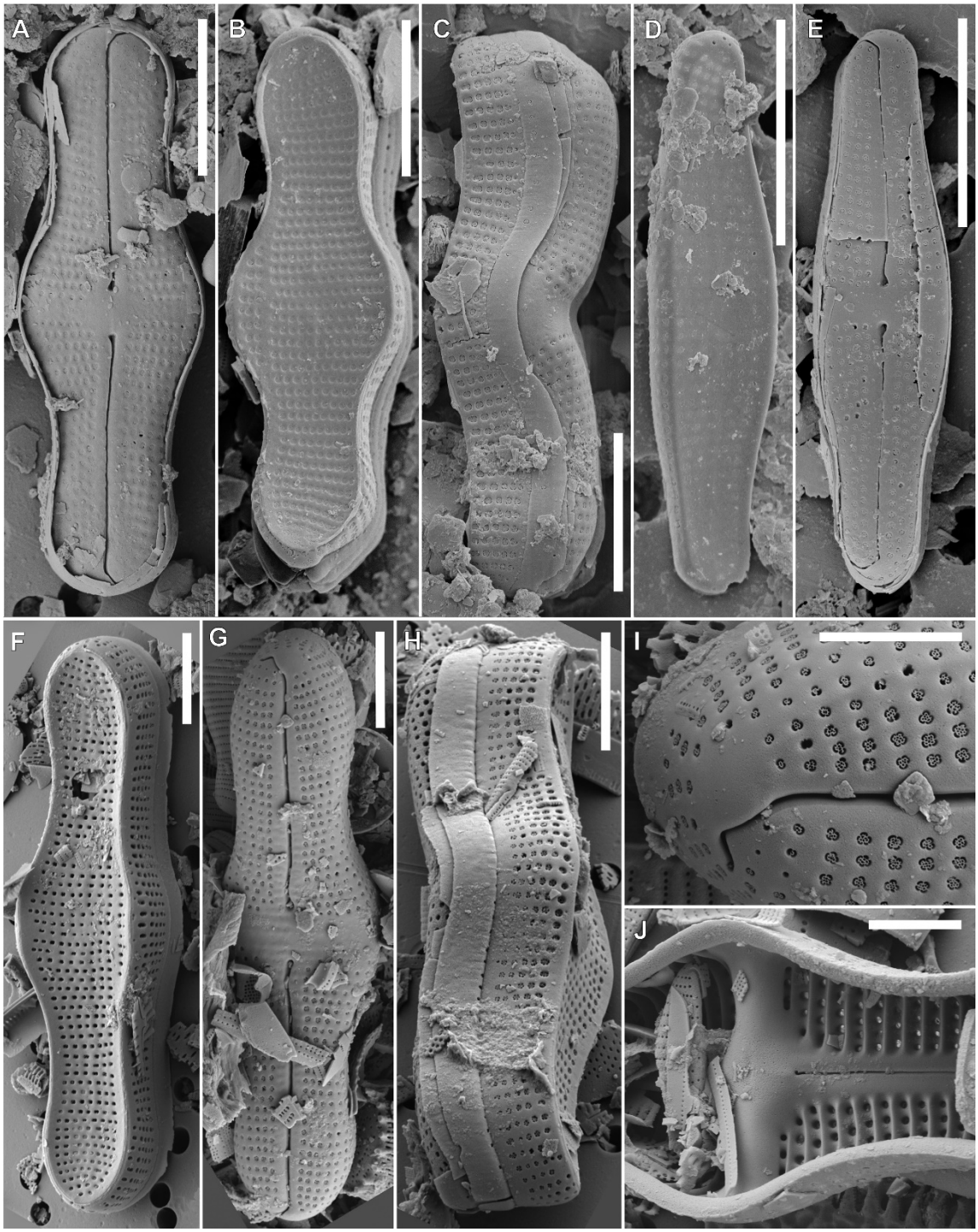

Figure 5. Scanning electron microscope micrographs. Scale bar: A–G = 10 μm; I, J = 5 μm. (**A**–**C**) *Achnanthes inflata* var. *gibba* Gandhi. (**A**) External view of the raphe valve. (**B**) External view of the rapheless valve. (**C**) Girdle view. (**D**,**E**) *Achnanthes mauiensis* R.L. Lowe and A.R. Sherwood. (**D**) External view of the rapheless valve. (**E**) External view of the raphe valve. (**F**–**J**) *Achnanthes inflata* (Kützing) Grunow from the original Grunow's sample. (**F**) External view of the rapheless valve. (**G**) External view of the raphe valve. (**H**) Girdle view. (**I**) External view of the valve apex with distal raphe endings. (**J**) Internal view of the central area with proximal raphe endings.

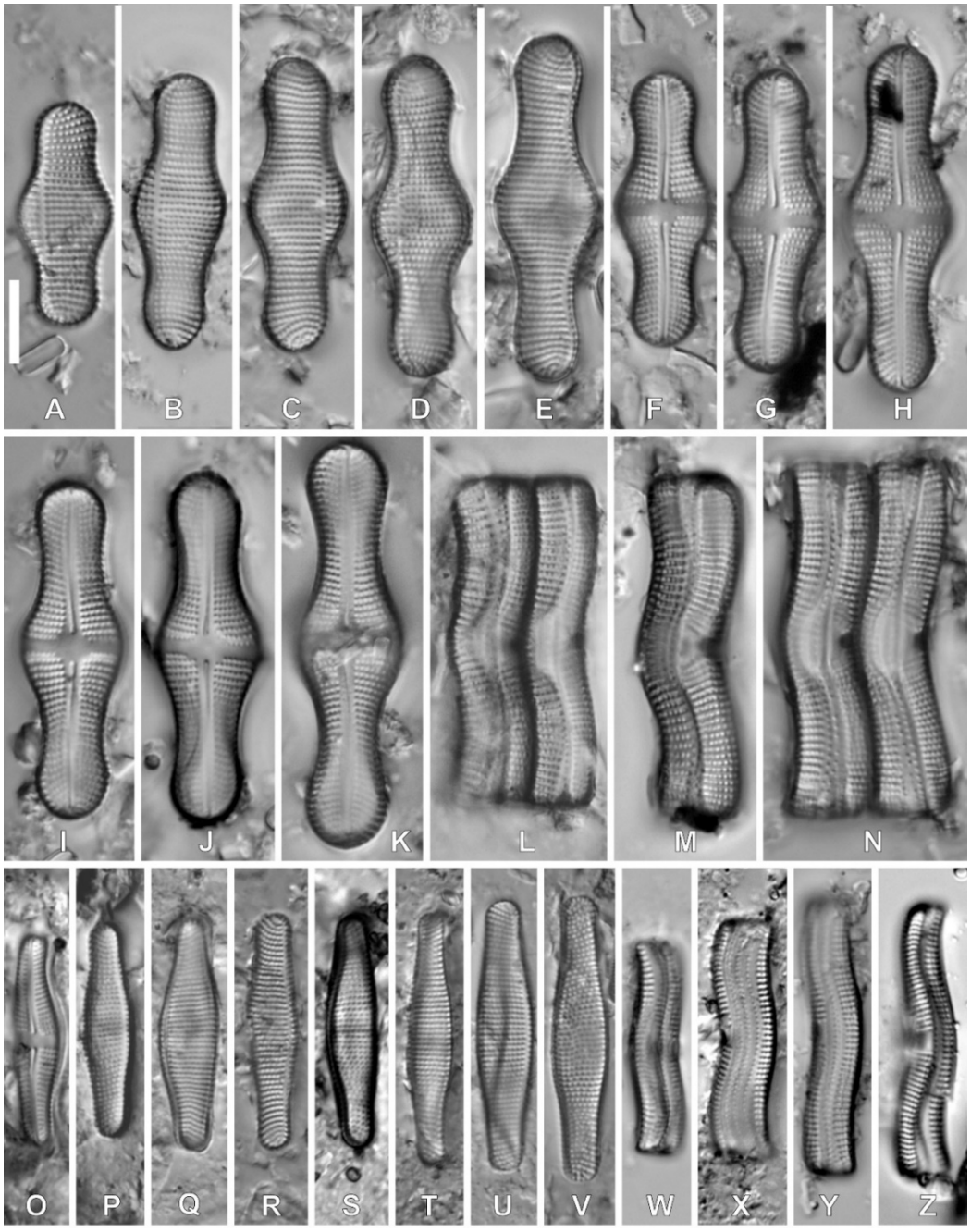

Figure 6. Light micrographs. Scale bar = 10 μm. (**A**–**N**) *Achnanthes inflata* var. *gibba* Gandhi. (**A**–**E**) Rapheless valve. (**F**–**K**) Raphe valve. (**L**–**N**) Girdle view. (**O**–**Z**) *Achnanthes mauiensis* R.L. Lowe and A.R. Sherwood. (**O**) Raphe valve. (**P**–**V**) Rapheless valve. (**W**–**Z**) Girdle view.

Table 2. Dimensions, number of striae and areolae for the taxa from genus *Achnanthes* identified in these studies and the most similar to *A. pseudoinflata* sp. nov. R-valve—raphe valve, *p*-valve—rapheless valve.

Taxa	Length (µm)	Width (µm)	Striae Density (in 10 µm)	Areolae Density (in 10 µm)	References
Taxa from Genus *Achnanthes* Identified in Studied Samples					
Achnanthes pseudoinflata sp. nov.	18.1–38.5	6.9–9.6	15–17	18–22	This study [Supplementary Materials Table S1]
Achnanthes coarctata	31.1–36.4	8.5–9	11	14–15	This study [Table S2]
	17–48	6–15	10–14	14–18	[34]
Achnanthes mauiensis	23.2–32.4	6.3–6.4	15–16	~21	This study [Table S3]
	20–28	5–7.5	15–19	20	[8]
Achnanthes inflata var. *gibba*	24.3–49.5	10–12.7	12–13	18–19 R-valve 16–18 *p*-valve	This study [Table S4]
	34–40	14.3–15	10–12	18–19	[39]
Achnanthes inflata	31–62.4	11.5–17.7	9–12 R-valve 9–13 *p*-valve	12 R-valve 10–11 *p*-valve	Grunow's original material [Table S5] (New Zealand)
	30–96	10–18	8–13	9–12	[34]
	30–65	10–20	10–13	11–12	[38]
	61.5–69	13.5–16.3	9–11	No data	[8]
	39–60	14–15	9–10	10–14	[42]
	30–96	10–21.1	9–12	9–12	[43]
Other Taxa Similar to *Achnanthes pseudoinflata* sp. nov.					
Achnanthes elata	22–86	11–19	8–9 R-valve 9–10 *p*-valve	10–11	[39]
	No data	14–17	~10 R-valve 8–9 *p*-valve	8–9	[38]
Achnanthes elata var. *curvula*	60–68	19–21	8–9 R-valve 9–10 *p*-valve	No data	[39]
	88.9	17.8	8.5 R-valve 8 *p*-Valve	8–9	[40]
Achnanthes inflata var. *javanica*	70–81	22–22.5	8–9 R-valve 9–9.5 *p*-valve	9–10 R-valve 10 *p*-valve	[40]
Achnanthes inflatagrandis	82–100	27–30	7–8	8	[38]
Achnanthes longboardia	19–50.5	8–10.5	13–15 R-valve 12–14 *p*-valve	18–19	[8]
Achnanthes subelata	30–43	9.5–12	11.5–13 R-valve 10–11 *p*-valve	~12	[38]
Achnanthes tumescens	22–36.5	6.5–8.5	15–17	16	[8]
Achnanthes cf. *inflata*	30–40	12–13	No data	No data	[36]
Achnanthes sp. cf. *inflatagrandis*	56–64	16–20	No data	No data	[36]

4. Discussion

The new species is clearly distinct form the material considered here as *Achnanthes inflata*. The analyzed material (Figure 4A–L) and our new species differ in terms of length (36–62.4 µm instead

of 15.1–38.5) and width (11.5–17.7 µm instead of 6.9–9.6). Moreover, differences were also observed regarding the number of striae (9–13 in 10 µm) and the number of areolae per striae (10–12 in 10 µm), see Table 2 and Tables S1–S5. Currently, eight taxa of *Achnanthes* Bory *sensu stricto* have been identified in modern floras from South America (i.e., besides the invalid species mentioned in the introduction by C.G. Ehrenberg). They are *A. brevipes* var. *brevipes*, *A. coarctata*, *A. elata*, *A. inflata* var. *inflata*, *A. inflata* var. *gibba*, *A. inflatagrandis*, *A. kuwaitensis*, and *A. subelata* [19,35,36,38,44,45].

Achnanthes pseudoinflata sp. nov. occurred in all the analyzed samples. Morphologically similar individuals were reported from New Caledonia by Moser et al. (plate 84, figs 2, 3 as *Achnanthes elata* [46]). However, they did not give the dimensions of their specimens nor did they include a scale bar allowing us to determine the size. A practically identical specimen was also reported from Costa Rica as *Achnanthes* cf. *inflata* by Metzeltin and Lange-Bertalot (plate 108, Figure 12 [36]). The dimensions and the shape of the cell suggest that it may be the same taxon as that described in this paper as new to science. In addition, the specimens corresponding to the species described were also presented on the website of The Florida Coastal Everglades LTER Program (http://fcelter.fiu.edu, accessed date: 30 January 2019), but in this case they were identified as *Achnanthes inflata*.

The most similar species is *A. inflata*, along with its variations. *Achnanthes inflata* was reported from Trinidad Island (South America) as *Stauroneis inflata* by Kützing [47] and was later transferred to *Achnanthes inflata* by Grunow [33]. This transfer was based on specimens recorded from New Zealand. The dimensions and ultrastructure observed in the original material population fully corresponded to those reported in the literature (Table 2, Figure 5A–L and Figure 6F–J). The main difference between these taxa are the cell dimensions (15.1–38.5 µm length and 6.9–9.6 µm width versus 30–65(96) µm length and 10–20.1 µm width). In addition, the striae and areolae densities are different (15–17 striae and 18–22 areolae versus 8–13 striae and 9–14 areolae per 10 µm) [8,34,38,42,43]. Despite the similar cell length and areole density, *Achnanthes inflata* var. *gibba* can be distinguished based on wider valves with a lower striae density [39]. *Achnanthes inflata* var. *javanica* can be easily separated from *A. pseudoinflata* sp. nov. based on a much larger cell with lower striae and areole densities [40].

Taxa with similar valve outlines to *A. pseudoinflata* sp. nov. are *A. inflatagrandis*, *A. elata*, and *A. elata* var. *curvula*. All taxa can be distinguished based on different valve dimensions and striae densities [38–40]. A similar taxon, *Achnanthes subelata*, can be distinguished based on wider valves with lower striae and areolae density. Additionally, *A. subelata* can be differentiated based on rapheless valves with axial areas in the central parts of valves, not shifted to the valve margin as in *A. pseudoinflata* sp. nov. [38]. *Achnanthes tumescens* has similar cell dimensions to *A. pseudoinflata* sp. nov. The taxa described can be distinguished from *A. tumescens* by more rounded and capitated apices, more pronounced narrowing between the central parts and the apices, and a lower areolae density per 10 µm (18–22 versus 16). Additionally, *A. tumescens* has fewer radiate striae than those described in *A. pseudoinflata* sp. nov. [8]. *Achnanthes longboardia* is another taxon with similar dimensions to *A. pseudoinflata* sp. nov. Both species overlap in dimension, but can be easily distinguished based on valve outline, which is lanceolate with rounded ends in *A. longboardia* and undulate with capitate apices and bold central parts in *A. pseudoinflata* sp. nov. [8]. *Achnanthes mauiensis* can be easily differentiated from *A. pseudoinflata* sp. nov., despite similar dimensions, via more lanceolate valves with fewer capitate apices and the difference in width (5–7.5 versus 6.9–9.6 µm). See Table 2 for the valve dimensions concerning *A. pseudoinflata* sp. nov. and other similar taxa from the genus *Achnanthes*.

Achnanthes coarctata is a cosmopolitan species reported from various parts of the world with different climates, mostly from terrestrial and aerophytic habitats [6,9,38,42,48,49]. The specimens observed in this research had valve outlines and dimensions typical for this taxon. *Achnanthes inflata* var. *gibba* is a rare taxon, reported from wet rocks and mosses in India [39] and Uruguay [35]. This taxon occurred only in sample 2015/1 of the analyzed material, where it was found to be common. The specimens studied (n = 50) had a wider range in length, but a narrower range in width. Valve outlines and areole density were typical (Table 2). Until now, *Achnanthes mauiensis* was known only from type locality on the island of Maui in the Hawaii archipelago. With respect to the type locality,

this species occurred in aerophytic habitats [8]. In the analyzed material, this species occurred rarely in samples from the rainforest. Morphologically similar taxa were reported from Sri Lanka (Ceylon) by Foged as *Achnanthes coarctata* (plate 5, figs 18, 19 [50]). Reports from this study and most probably from Sri Lanka suggest that this species could be widely distributed in the terrestrial and aerophytic habitats in tropical regions.

In relation to the numerous other species in the material studied, as well as the preferences of many taxa from the genus *Achnanthes*, it seems that *Achnanthes pseudoinflata* sp. nov. also prefers aerial and terrestrial habitats. These new taxa are probably widespread, but so far it has not been possible to distinguish them from similar species. Species of the genus *Achnanthes* are diatoms rarely identified in algological research. This is the result of their preference for terrestrial and aerophytic environments, which are still poorly investigated.

Supplementary Materials: The following are available online at http://www.mdpi.com/1424-2818/12/10/375/s1, Table S1: Dimensions, number of striae and areolae for *Achnanthes pseudoinflata* sp. nov., Table S2. Dimensions, number of striae and areolae for *Achnanthes coarctata*, Table S3. Dimensions, number of striae and areolae for *Achnanthes mauiensis*, Table S4. Dimensions, number of striae and areolae for *Achnanthes inflata* var. *gibba*, Table S5. Dimensions, number of striae and areolae for *Achnanthes inflata* originated from Grunow's original material.

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