

***Actinella guianensis* Grunow (Eunotiaceae): analysis of the type material and the description of *Actinella cordiformis* sp. nov.**

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**Abstract:** The taxonomic concept of *Actinella guianensis* Grunow is well-established in the literature. However, during the analysis of recent samples from two Amazon blackwater habitats, we found an uncommon and oddly shaped *Actinella* taxon, which corresponds to what is reported in Grunow's drawings in Van Heurck's Atlas as the type species of *A. guianensis*. To understand the identity of the uncommonly shaped *Actinella*, we revisited the type materials and the literature citing *A. guianensis* and compared them with the population found in samples recently collected. Two distinct morphologies were found in the original material. These morphologies differed mainly in the shape of the headpoles, with one being cordiform (heart-shaped) and the other bulbous. Furthermore, differences were observed in the shape of the footpoles, with one being characterized by enlarged and cuneated to protracted apices, while the other presents a spoon-like shape. Following the analysis of the original material, modern samples, and the literature, we propose *Actinella cordiformis* Coêlho, Silva–Lehmkuhl, Kociolek, Lehmkuhl et Ludwig sp. nov. for the species having a cordiform headpole.

**Key words:** *Actinella*, Amazonas, Bacillariophyta, blackwater, Brazil, Grunow

**INTRODUCTION**

The genus *Actinella* F. W. LEWIS was described in 1864 as having linear arcuate valves, with one end larger than the other, and visible terminal nodules, forming stellar groups by adherence of the smaller ends (LEWIS 1864). ROUND et al. (1990) detailed its morphology in SEM, showing a row of prominent spines along the valve edge. The uniseriate striae are composed of small, round areolae. The raphe slits are short, and the single or double rimoportulae can be present at one or both valve ends (METZELTIN & LANGE–BERTALOT 1998; KOCIOLEK et al. 2001). Currently, 71 infrageneric taxa of *Actinella* are known (GUIRY & GUIRY 2023). While this genus is recorded on all continents except Antarctica, the acidic tropical waters of the Amazon basin are particularly notable for their high *Actinella* diversity (SABBE et al. 2001; KOCIOLEK et al. 2001; METZELTIN

& LANGE–BERTALOT 2007; CANANI et al. 2018).

Grunow in VAN HEURCK (1881) provided the first Brazilian records of *Actinella* species. His proposals of three new species lacked descriptions and were presented only on drawings: *Actinella brasiliensis* Grunow, *Actinella guianensis* Grunow and *Actinella mirabilis* Grunow. Particularly, the presentation of *A. guianensis* included two drawings, figures 17 and 20 (VAN HEURCK 1881, plate XXXV (35)). The legend for figure 17 reads “*A. guianensis* Grun. \*Brésil. Guyane”, and the figure 20 legend reads “Même figure que 17 à 300/1\*<sup>o</sup>”. The specimen depicted in figure 17 displays a noticeable cordiform headpole and provides detailed views of the striation, spines, and nodules. Grunow's fig. 20 represents only the valve outline of a slightly arcuate valve, with a broadly rounded headpole and a cuneate footpole.

During the intervening 141 years that have passed since Grunow first published his new species, the widely accepted

concept of *A. guianensis* in the literature corresponds to the specimen represented in Grunow's fig. 20. The mentioned morphotype is well known in South America, including Brazil (CLEVE 1881; HUSTEDT in SCHMIDT 1913; SOUZA & MOREIRA-FILHO 1999; DÍAZ-CASTRO et al. 2003; METZELTIN & LANGE-BERTALOT 1998; MELO et al. 2010; BICCA et al. 2011; DUNCK et al. 2012, 2016; CANANI et al. 2018; SILVA et al. 2021), Argentina (FRENGUELLI 1933), Ecuador (OLIVEIRA & STEINITZ-KANNAN 1992), Colombia (SALA et al. 2002; NÚÑEZ-AVELLANEDA 2008), and Guyana (METZELTIN & LANGE-BERTALOT 1998). On the other hand, reports of specimens resembling Grunow's Fig. 17 emerged a century after its initial description, in two Amazonian studies conducted in Brazil (UHERKOVICH & FRANKEN 1980, pl. VI, figs 12–13; SOUZA-MOSIMANN et al. 1997, fig. 7), and they were represented by drawings only. Despite *A. guianensis* being widely reported in the literature, the type materials of this species have never been revisited.

Recently, we found representative populations in a blackwater river in the state of Amazonas, Brazil, which exhibit morphological similarities to both of Grunow's drawings of *A. guianensis*. This raised the question: What is the real *Actinella guianensis* Grunow? In our pursuit of an answer to this question, we conducted an analysis of Grunow's type slide of *A. guianensis* using light microscopy, comparing it with populations from the Amazon river's blackwater region. We provide the first light microscopy observations of *A. guianensis* based on the type slide, along with a species description that encompasses both the type and other populations. Additionally, we propose a new species, *Actinella cordiformis* Coêlho, Silva-Lehmkühl, Kociolek, Lehmkühl et Ludwig sp. nov., based on recently studied material from samples collected in the Brazilian Amazon, thereby accommodating the second, less frequently reported species of Grunow.

## MATERIAL AND METHODS

**Study Areas.** Samples for this study were collected in two Amazonian aquatic ecosystems, Baixa Grande creek and Uaicurapá river, in the physiographic zone of the Middle Amazon River (AB'SÁBER 2010), in the municipalities of Barreirinha and Parintins, Amazonas, Brazil. The climate of the region is classified as Amw type (tropical humid with temperatures above 18°C) (KÖPPEN 1936), characterized by a rainy period from December to May, with average annual temperatures ranging from 22.4 °C to 33°C (ANA 2023).

The Baixa Grande creek (2°52'26.71"S, 57°24'14.63"W) (Fig. 1) is situated in the Andirá Basin, within the Cametá community in the municipality of Barreirinha. It runs through a primary forest, 4.5 km from the community center. This small creek follows a sinuous course, featuring black and slow-flowing water, weaving its way through vegetation, rocks, and a sandy bottom, reaching a depth of two meters. The blackwater Uaicurapá River (03°10'32.2"S, 56°50'29.8"W) is located in the municipality of Parintins. In the surroundings, small villages carry out family farming, notably with cassava, pineapple, and banana cultivation. This river has black and calm waters during the different phases of the regional hydrological cycle: full water – with maximum water level; drying – with falling water level; lower water – minimum water level; flooding – with a rising water level. According to the season, the water may inundate the

surrounding forest during the full water phases or may reveal extensive margins with white sand beaches during lower water phases. From the Amazon River, the upstream section of the Uaicurapá stretches for approximately 60 km, initially with a relatively narrow width of around 25 meters, which widens downstream to approximately 4 km.

**Field Collections.** In the Baixa Grande, the collection occurred during the flooding phase in April 2014. In the Uaicurapá River, samples were collected from five sections of the river, moving from upstream to downstream: Itatuba stream, Ponta do Paca, São Tomé, Cajual, and Maranhão (Fig. 1). This collection took place over one regional hydrological cycle: flooding – May 2021, full water – June 2021, drying – October 2021, lower water – November 2021, and flooding – February 2022.

Planktonic samples were collected using a plankton net (20 µm mesh) and preserved with TRANSEAU solution (BICUDO & MENEZES 2017). In the Uaicurapá River, measurements of temperature (°C), pH, and electrical conductivity (µS.cm<sup>-1</sup>) were taken using a portable probe (HANNA HI 98194).

**Laboratory Sample Treatment.** The samples were oxidized using concentrated hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 37%) according to BATTARBEE et al. (2001) and were then mounted in Naphrax® (R.I. = 1.74). Species observations, morphometric measurements, and images were taken at a magnification of 1000× using an OPTON light microscope (LM) equipped with a 10MP digital camera and an Axiolab 5 LM equipped with a Zeiss Axiocam 208 color digital camera. For scanning electron microscopy (SEM), cleaned samples were dried on aluminum stubs and coated with gold using a Jeol Smarter Coater. The material was analyzed using a Jeol JSM IT500–HR operated at 5kV voltage and a working distance of 9.6 mm. LM and SEM plates were created using Photoshop Elements edition 2021®. Morphological terminology follows Round et al. (1990). Samples and holotype's slide are kept at the Herbarium of Universidade Federal do Amazonas (HUAM), Brazil.

## Taxonomic Analysis

**Grunow's collection.** The Grunow Diatom Collection is housed in the Department of Botany (Herbarium W) at the Natural History Museum in Vienna. This collection contains original samples, labeled with Grunow's sample number, and in many cases, collection localities. Additionally, the collection includes supplementary materials, such as Grunow's microscope slides (e.g. Fig. 2), a catalog (e.g. Fig. 3) listing all of his diatom sample numbers, and drawings (e.g. Figs 7–10), all of which are detailed in SCHUSTER et al. (2022). *Actinella guianensis* was illustrated from Grunow sample 1849, as indicated by Grunow in a copy of Van Heurck's Atlas plate XXXV, where he also notes "S.[an] Carlos" as the type locality. The single, fragile slide of Grunow sample 1849 (W0164897) was analyzed using an Olympus BX53 microscope at 1000× magnification (N.A. 1.30), equipped with Differential Interference Contrast (Nomarski) optics and the Olympus UC30 Imaging System.

We also analyzed scans of the map of drawings by Grunow for *A. guianensis*. Grunow's drawings at W are organized according to "de Toni numbers", a system used in Giovanni Battista de Toni's (1891, 1892, 1894) "Sylloge Algarum" (SCHUSTER et al. 2022). The specific de Toni number assigned to *Actinella guianensis* is 3742 (Fig. 5), which contains the drawings and photographs depicted in Figs 4–10. Seeing the drawings was helpful, to confirm the Grunow sample number for the material he looked at and its collection locality (Fig 7). Additional details from the drawings and photographs gave us a better idea of what Grunow had in mind when he described the species.

**Cleve's collections.** In addition, we analyzed LM slide no. 212 of the Cleve & Möller exsiccate series, collected from a *Sphagnum* vegetation near Minas Gerais. CLEVE (1881) recorded *A. guianensis* in this sample, which was collected by Dr. Hj. Mosén in Caldas, Brazil.

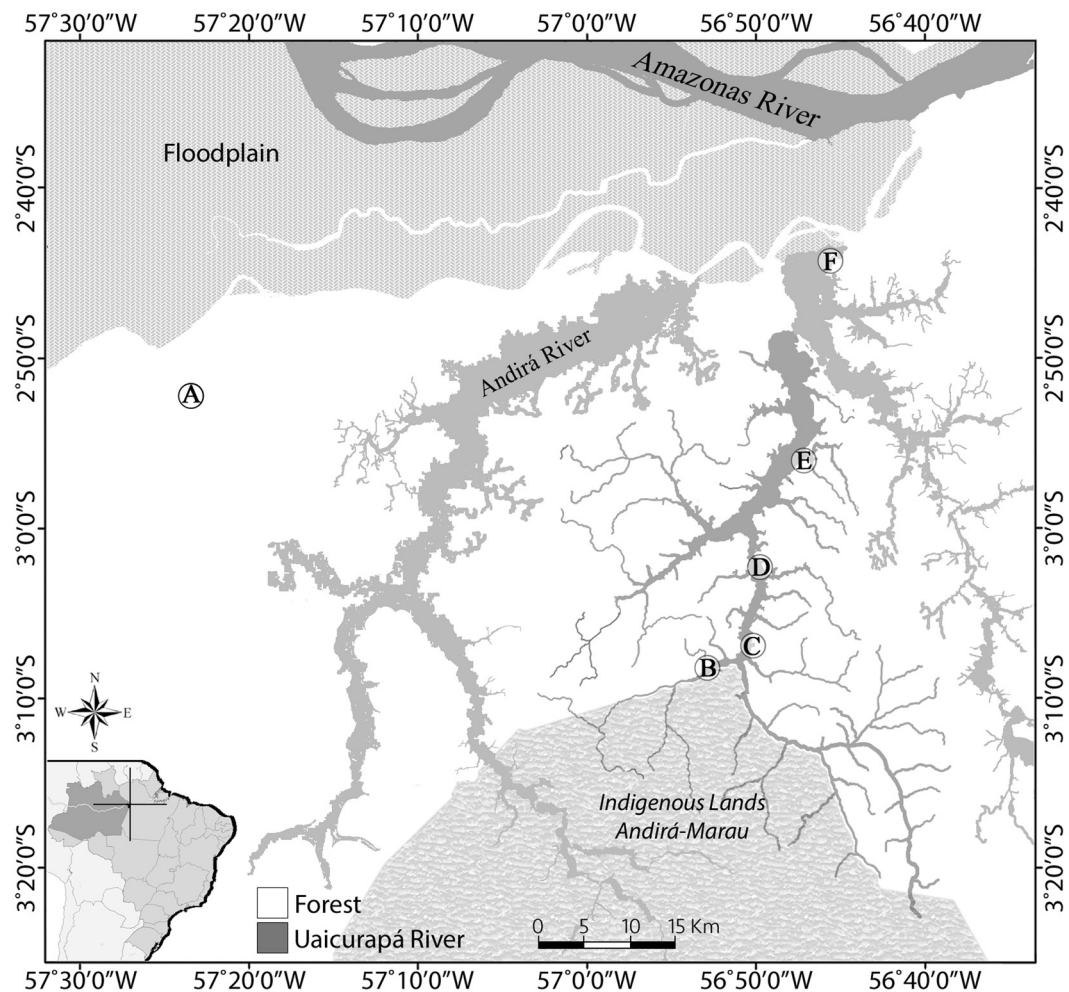


Fig. 1. Map of the Uaicurapá River and Baixa Grande creek showing the sampling points where recent samples of *Actinella guianensis* and *A. cordiformis* sp. nov. were collected. Sampling points: (A) Baixa Grande; (B) Itatuba; (C) Ponta do Paca; (D) São Tomé; (E) Cajual; (F) Maranhão.

## RESULTS

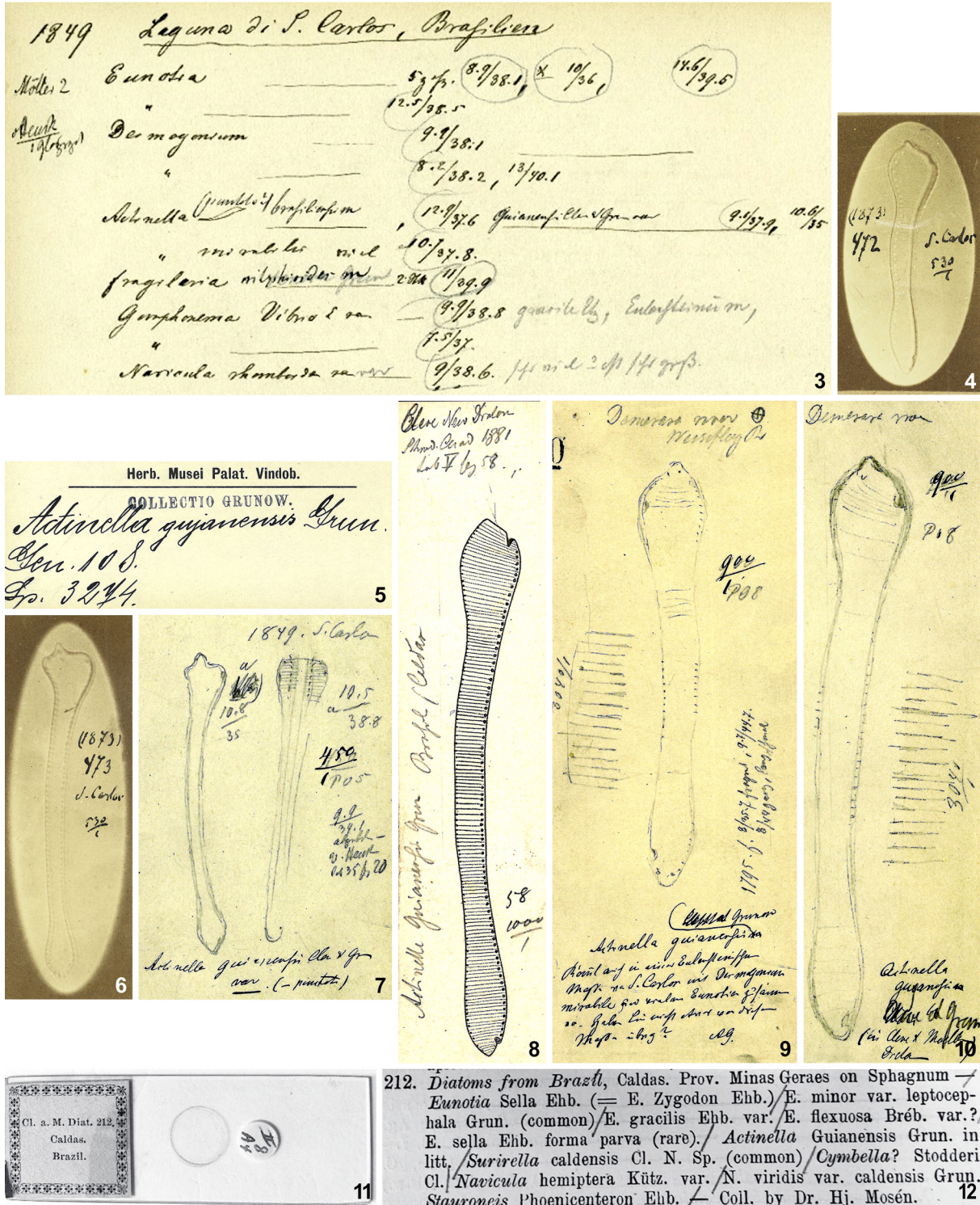
*Actinella cordiformis* Coêlho, Silva–Lehmkuhl, Kociolek, Lehmkuhl et Ludwig sp. nov. (Figs 13–47)

### Description

**LM (Figs 13–30):** Valves moderately arcuate. Headpole wide, cordiform, with a pronounced apical point displaced to valve convex margin. Apical point slightly displaced to the median region in smaller specimens. Footpole widened, ending in a weakly cuneate to protracted apex in larger specimens. Spines visible along valve margin, with a prominent single apical spine at headpole point, and a pair at the footpole end. Conspicuous terminal nodules. Terminal raphe fissures curving subterminally onto valve face. Striae are parallel and evenly distributed along the valve face, becoming uneven near the apices. At the apices, striation pattern denser than at the center of the valve and interrupted near ventral margin by a narrow sternum. Valve dimensions (n=40): Length: 40.0–147.5 µm; center of the valve width: 5–8 µm; headpole width: 13.5–18.0 µm; footpole width: 5–8 µm; striae: 12–17 in 10 µm; spines: 5–8 in 10 µm.

**SEM (Figs 31–47):** Externally, valve margins lined with canine tooth-shaped spines (Fig. 31). At the headpole apical point, a single large spine present (Fig. 32), rarely, two spines observed (Fig. 33). Cingulum areolate (Fig. 34). Two to three robust spines present around the apical point at the footpole (Figs 36–37). Uniseriate, striae linear composed of rounded areolae (24–30 in 10 µm) (Figs 31–37) extending to the mantle (Fig. 32), becoming radiate at the apices (Figs 35–37), and this same pattern observed internally (Fig. 46). Thin and straight sternum present at ventral margin of the poles, being more evident at the footpole (Figs 36–37) than at the headpole (Fig. 32). Raphe on the valve mantle (Figs 35–36) having simple terminal fissures curved onto the valve face near the apices (Figs 35, 37). Internally, helictoglossae present at both apices (Figs 38–39, 43). A single rimoportula occasionally present within the headpole apical point (Figs 41–42), or on the footpole located posterior to the helictoglossa displaced of the apical point (Fig. 44). Footpole rimoportulae larger than the one present on the headpole (Fig. 45). Hyaline line interrupting the striae from the terminal raphe ends, continuing along the ventral margin and terminating before the valve central area (Figs 40, 42, 44, 47).





Figs 2–12. (2–10) Original materials of Grunow's Collection, (2) slide of sample 1849 from Laguna di S. Carlos, Brasilien, (3) scan of Grunow's catalog for that sample, (4–10) Grunow's drawings and two photographs for *Actinella guianensis* Grunow; (11) Cleve's slide of sample 212 from Caldas, Brazil; (12) list of diatom species from sample 212 by Cleve & Möller, indicating the presence of *A. guianensis*.



**Holotype:** HUAM Naphrax slide HUAM12400! (Herbarium of Universidade Federal do Amazonas – HUAM), Manaus municipality, Brazil. Holotype specimen illustrated as LM Figs 3–5. Illustrated by Grunow in Van Heurck (1881, Plate 35, fig. 17).

**Type locality:** Brazil, State of Amazonas, Parintins municipality, Uaicurapá River, 03°10'32.2"S, 56°50'29.8"W, collected by J.M.S. Coêlho et al. on 02/12/2022 (Table 1).

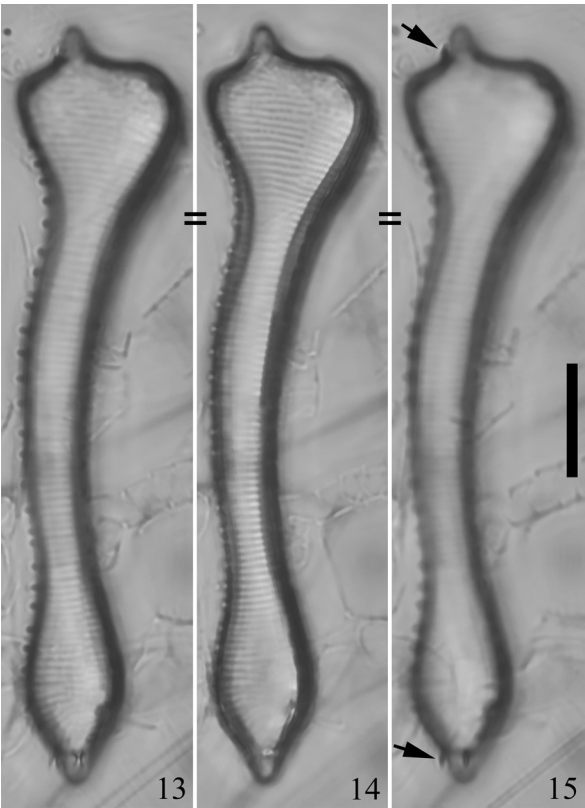
**Habitat:** planktonic.

**Etymology:** The species is named for the heart-shaped headpole.

**Ecology:** *Actinella cordiformis* sp. nov. was found exclusively in the planktonic samples from Baixa Grande, which is located in the primary Amazon Forest and has black waters. In the Uaicurapá River, this species co-occurred with *Actinella guianensis* in the acid (pH= 4.0 to 5.1) blackwaters, where there is low conductivity (8 to 25  $\mu\text{S}.\text{cm}^{-1}$ ), high temperature (29.5 to 30.7 °C), and a maximum depth of 15.9 meters during the full water phase (Table 1).

**Distribution:** The species has been found so far in the type locality and in Baixa Grande, Amazonas state, Brazil.

**Samples occurrence:** HUAM12095, HUAM12283, HUAM12284, HUAM12293, HUAM12303, HUAM12304, HUAM12305, HUAM12313, HUAM12314.



Figs 13–15. *Actinella cordiformis* Coêlho, Silva–Lehmkuhl, Kociolek, Lehmkuhl et Ludwig sp. nov. type specimen (HUAM 12400). LM – holotype population, Uaicurapá River, views at different focal planes: (13) showing spines along the valve edge; (14) sternum at headpole; (15) pronounced spines at headpole and footpole (black arrows). Scale bar 10  $\mu\text{m}$ .

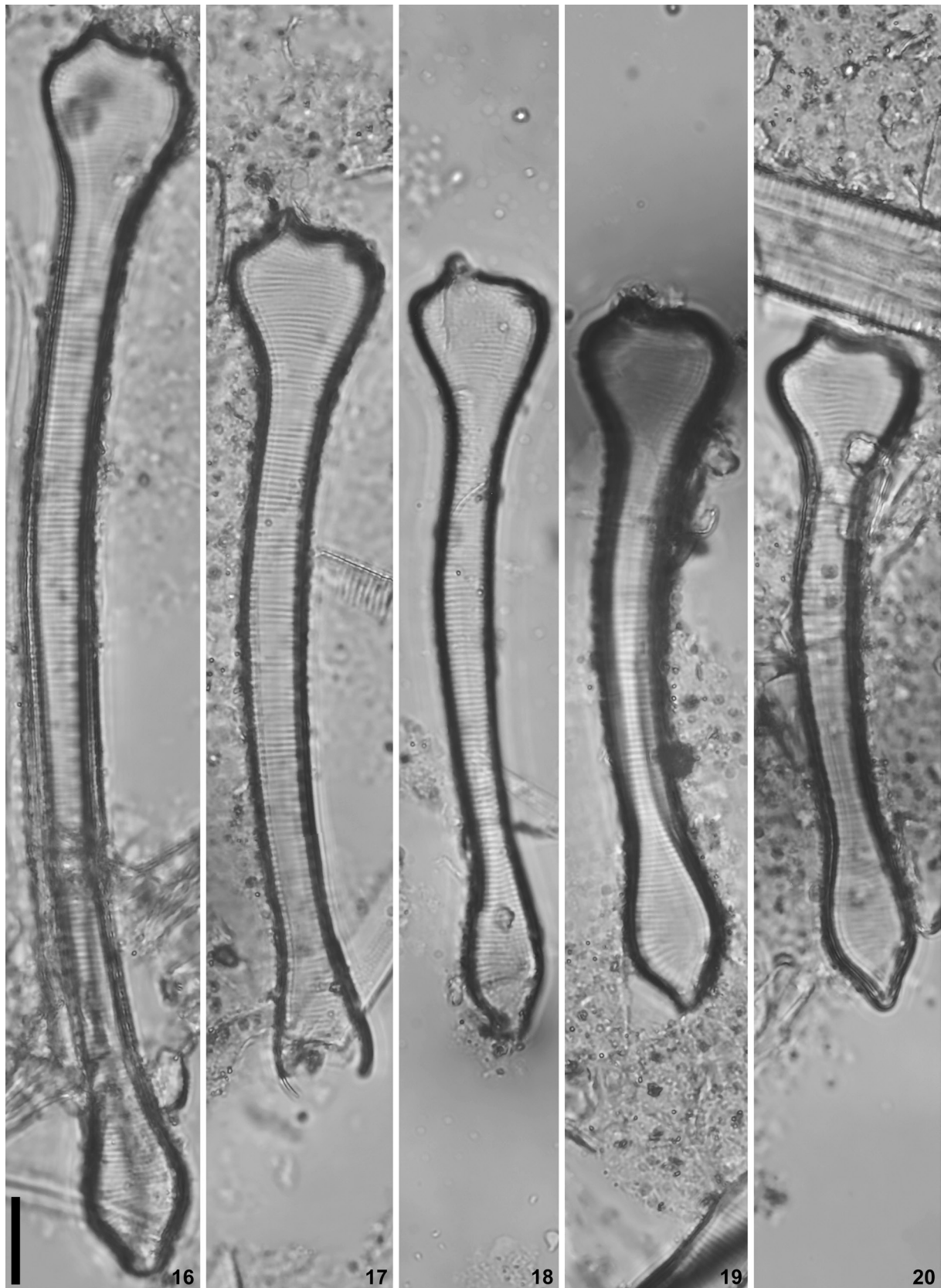
Table 1—Occurrence of *Actinella cordiformis* sp. nov. and *Actinella guianensis* Grunow in the samples analyzed in this study. Values of Temperature (Temp. °C), pH and electrical conductivity (EC  $\mu\text{S}.\text{cm}^{-1}$ ) corresponding to the unit samples of the Uaicurapá river, Amazonas state, Brazil.

Taxa	Locality	Accession number	Date	Hydrological cycle	Temp	pH	EC
<i>Actinella cordiformis</i> sp. nov. and <i>Actinella guianensis</i> Grunow	Laguna Di San Carlos – Brasilien	Grunow sample 1849, slide (W0164897)	1881	–	–	–	–
<i>Actinella guianensis</i> Grunow	Caldas – Brazil	Cleve & Möller 212 (ANSP)	1881	–	–	–	–
<i>Actinella cordiformis</i> sp. nov.	Baixa Grande 02°50'15.73"S 57°25'0.83"W	HUAM1214 HUAM1216	12/04/2014	flooding	–	–	–
<i>Actinella guianensis</i> Grunow	Itatuba 03°10'32.2"S 56°54'46.1"W	HUAM12267 HUAM12095	15/05/2021 29/06/2021	flooding full water	28.1 26.8	4.2 4.2	22 13
<i>Actinella cordiformis</i> sp. nov.		HUAM12283	02/10/2021	drying	25.5	4.7	14
<i>Actinella cordiformis</i> sp. nov. and <i>Actinella guianensis</i> Grunow		HUAM12293	27/11/2021	lower water	25.9	4.3	22
<i>Actinella cordiformis</i> sp. nov. and <i>Actinella guianensis</i> Grunow		HUAM12303	12/02/2022	flooding	25.1	4.0	25

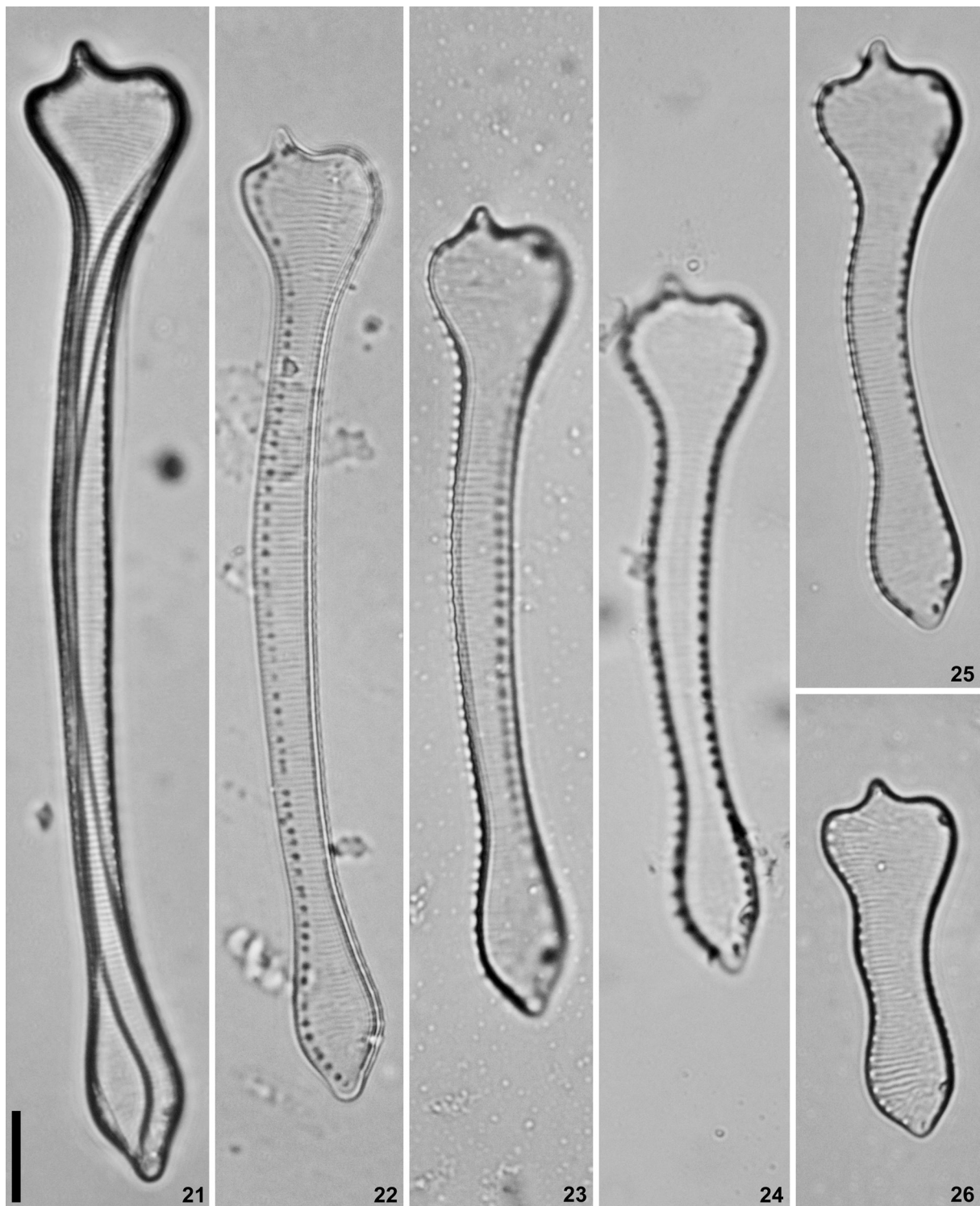
Table 1 Cont.

Taxa	Locality	Accession number	Date	Hydrological cycle	Temp	pH	EC
<i>Actinella guianensis</i> Grunow	Ponta do Paca 03°08'08"S, 56°50'18"W	HUAM12094	15/05/2021	flooding	31.2	5.0	7
<i>Actinella cordiformis</i> sp. nov. and <i>Actinella guianensis</i> Grunow		HUAM12284	02/10/2021	drying	30.7	5.1	8
<i>Actinella cordiformis</i> sp. nov. and <i>Actinella guianensis</i> Grunow		HUAM12294	27/11/2021	lower water	30.9	4.5	9
<i>Actinella guianensis</i> Grunow	São Tomé 03°03'13"S, 56°50'07"W	HUAM12304	12/02/2022	flooding	29.5	4.8	11
<i>Actinella guianensis</i> Grunow		HUAM12268	15/05/2021	flooding	31.3	5.0	7
<i>Actinella guianensis</i> Grunow		HUAM12276	29/06/2021	full water	30.0	5.0	6
<i>Actinella guianensis</i> Grunow		HUAM12285	02/10/2021	drying	30.9	5.2	6
<i>Actinella guianensis</i> Grunow		HUAM12295	27/11/2021	lower water	30.8	5.1	8
<i>Actinella cordiformis</i> sp. nov. and <i>Actinella guianensis</i> Grunow		HUAM12305	12/02/2022	flooding	29.8	5.1	9
<i>Actinella guianensis</i> Grunow	Cajual 02°56'39"S, 56°47'12"W	HUAM12269	15/05/2021	flooding	30.7	5.0	7
<i>Actinella guianensis</i> Grunow		HUAM12277	29/06/2021	full water	30.3	5.1	7
<i>Actinella guianensis</i> Grunow		HUAM12286	02/10/2021	drying	31.1	5.3	6
<i>Actinella guianensis</i> Grunow		HUAM12296	27/11/2021	lower water	30.9	5.2	7
<i>Actinella guianensis</i> Grunow		HUAM12306	12/02/2022	flooding	30.1	5.2	8
<i>Actinella guianensis</i> Grunow	Maranhão 02°45'03"S, 56°45'02"W	HUAM12270	15/05/2021	flooding	29.5	6.0	16
<i>Actinella guianensis</i> Grunow		HUAM12287	02/10/2021	drying	30.3	5.3	6
<i>Actinella guianensis</i> Grunow		HUAM12307	12/02/2022	flooding	29.5	5.2	10



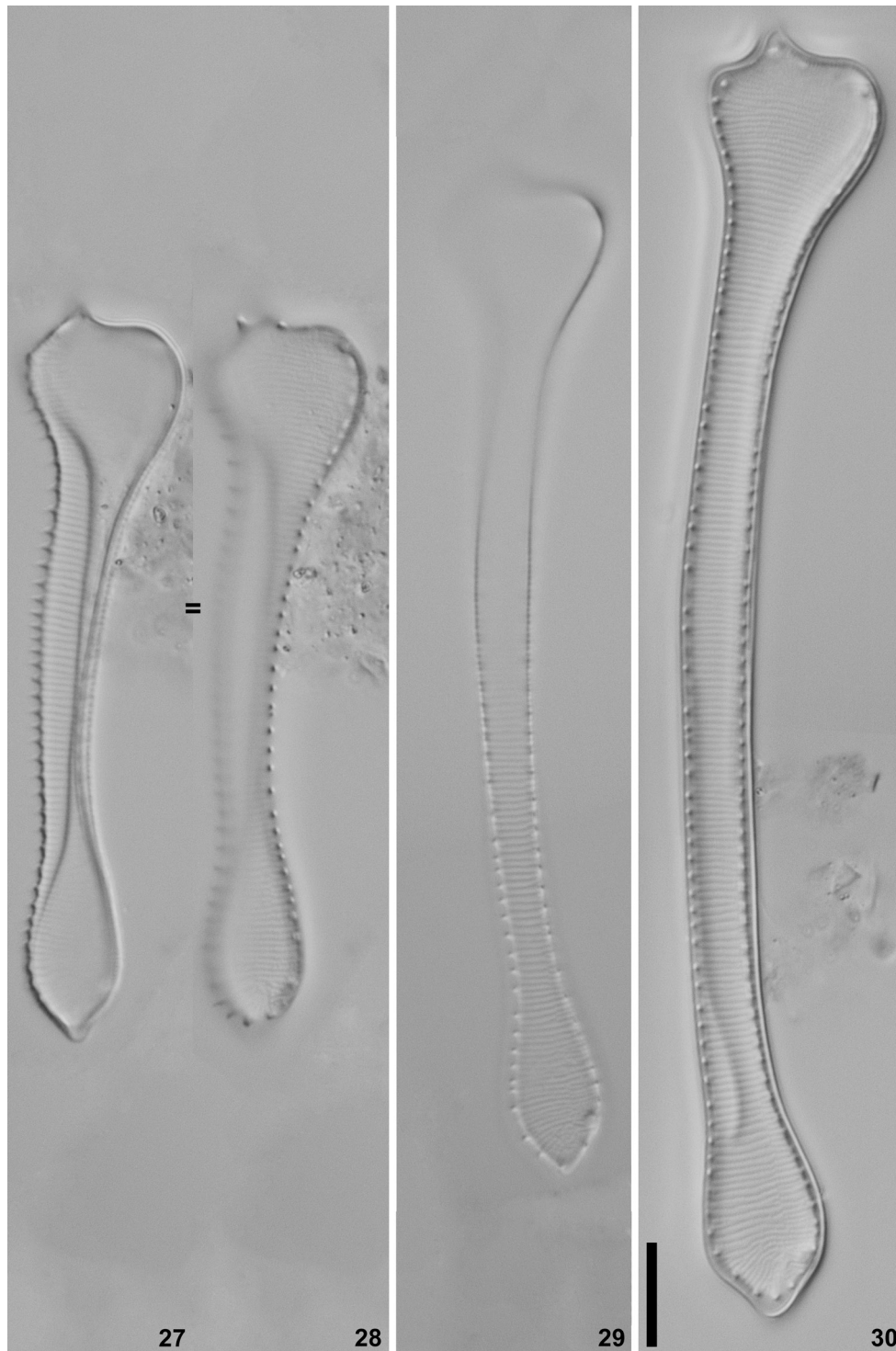


Figs 16–20. *Actinella cordiformis* Coêlho, Silva–Lehmkuhl, Kociolek, Lehmkuhl et Ludwig sp. nov., LM – Uaicurapá River. Scale bar 10  $\mu$ m.

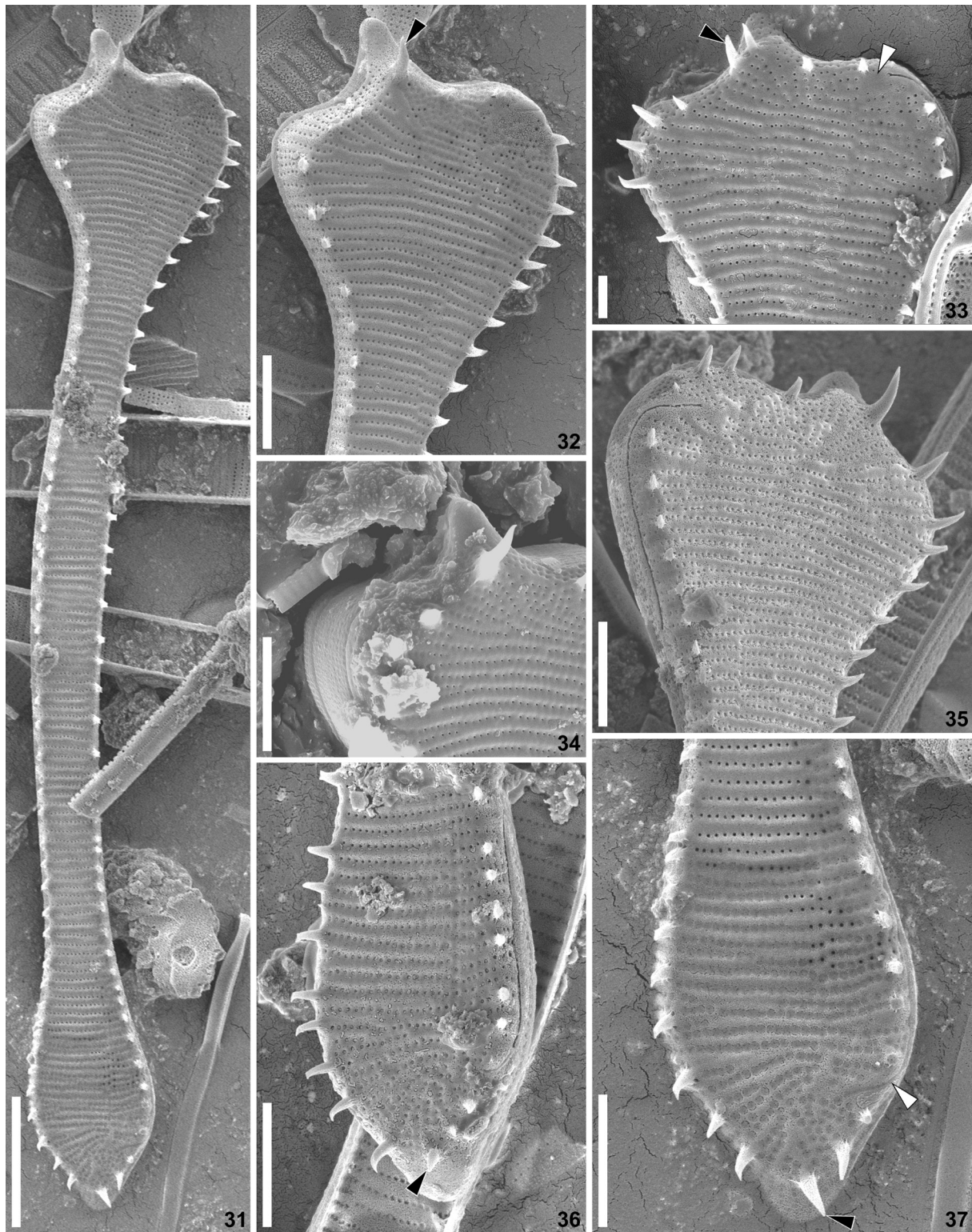


Figs 21–26. *Actinella cordiformis* Coêlho, Silva–Lehmkuhl, Kociolek, Lehmkuhl et Ludwig sp. nov., LM – Baixa Grande population. Scale bar 10 μm.



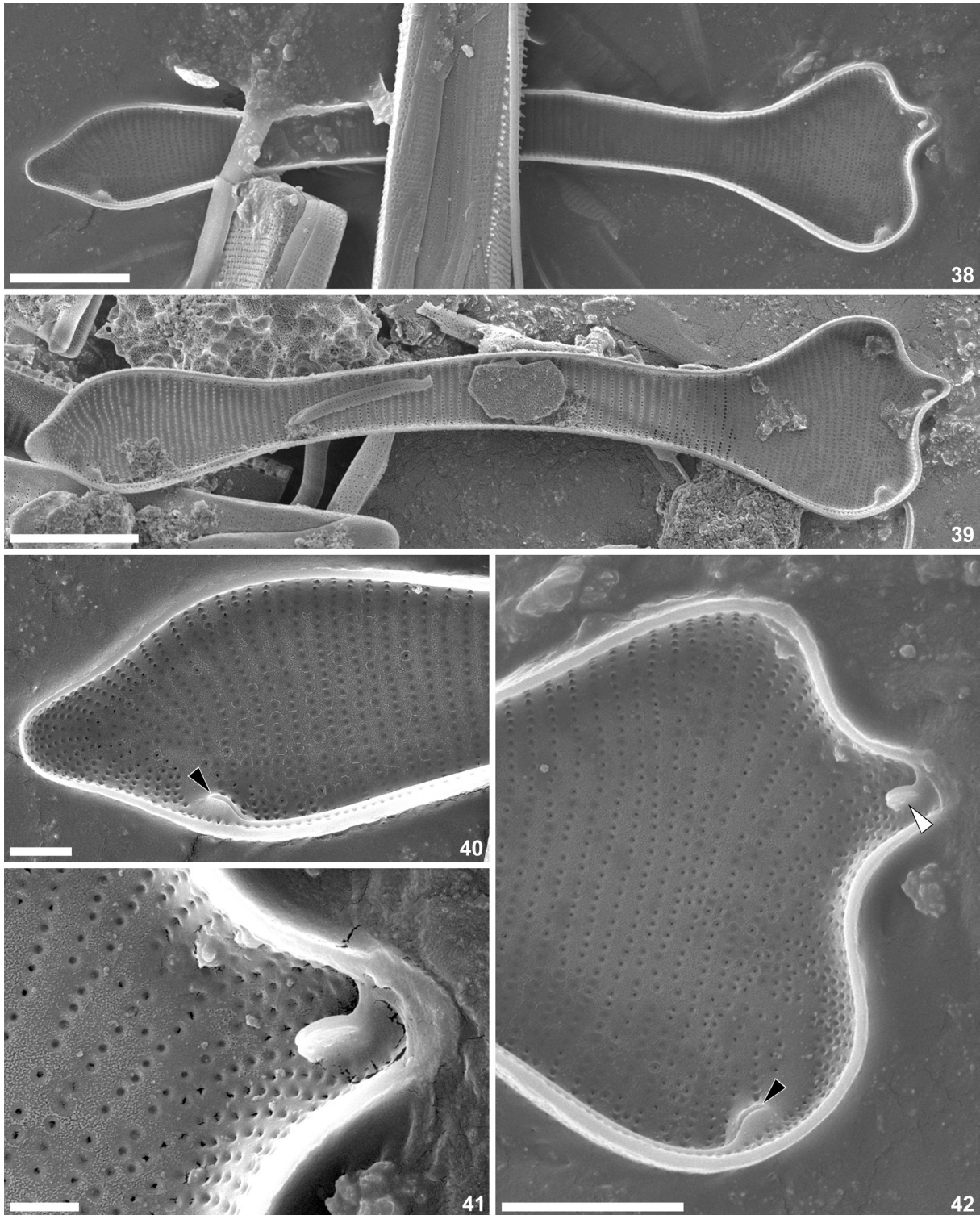


Figs 27–30. *Actinella cordiformis* Coêlho, Silva–Lehmkuhl, Kociolek, Lehmkuhl et Ludwig sp. nov., LM views of the population from Grunow sample 1849 (W0164897). Scale bar 10  $\mu$ m.



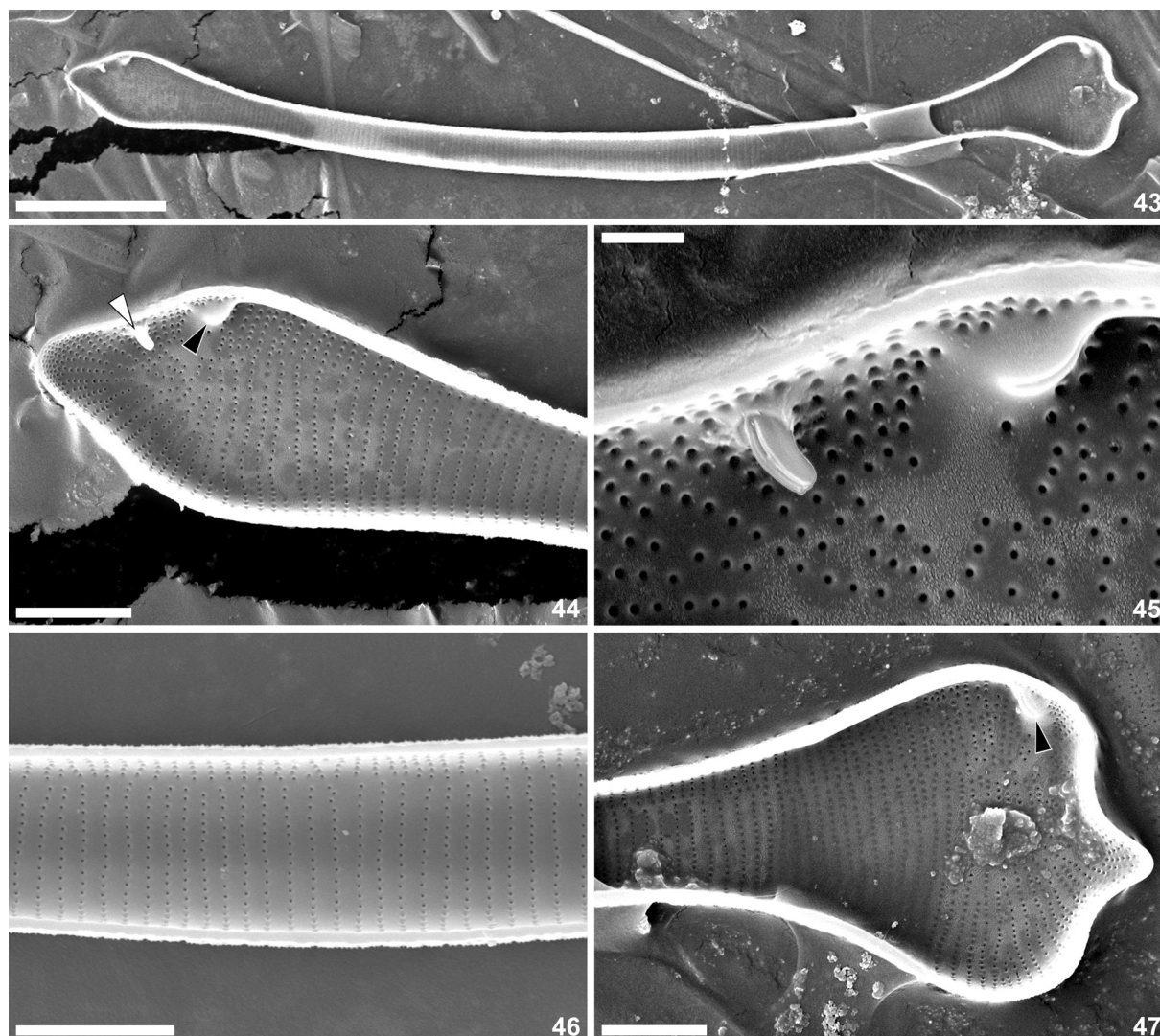
Figs 31–37. SEM – *Actinella cordiformis* Coelho, Silva–Lehmkühl, Kociolek, Lehmkühl et Ludwig sp. nov.: (31) external valve view; (32) detail of the headpole showing mantle areolae, the single spine at the apical point (arrowhead), and striae; (33) shows two spines at the headpole apical point (black arrowhead) and terminal nodule (white arrowhead); (34) shows areolate cingulum; (35) detail of the valve mantle showing the raphe and the distal end on the valve face; (36) detail of the footpole showing spines on the valve edge and a single spine at the apical point (arrowhead); (37) shows a large spine at the footpole apical point (black arrowhead) and the distal raphe end terminating subterminally on the valve face (white arrowhead). Scale bars 10 µm (Fig. 31), 5 µm (Figs 32, 34–37) and 2 µm (Fig. 33).





Figs 38–42. Scanning electron micrographs of *Actinella cordiformis* Coêlho, Silva–Lehmkuhl, Kociolek, Lehmkuhl et Ludwig sp. nov.: (38–39) internal valve view; (40) details of the helictoglossa (black arrowhead) and the thin sternum at the footpole; (41) details of the rimoportula at the headpole apical point; (42) headpole showing helictoglossa (black arrowhead), rimoportula (white arrowhead) and the striation pattern. Scale bars 10 µm (Figs 38–39), 2 µm (Fig. 40) and 1 µm (Fig. 41), 5 µm (Fig. 42).





Figs 43–47. Scanning electron micrographs of *Actinella cordiformis* Coêlho, Silva–Lehmkuhl, Kociolek, Lehmkuhl et Ludwig sp. nov.: (43) internal valve view; (44) footpole showing the rimoportula (white arrowhead) and helictoglossa (black arrowhead); (45) shows rimoportula and helictoglossa; (46) linear parallel punctate striae; (47) helictoglossa (black arrowhead) and the thin sternum at the headpole. Scale bars 20  $\mu$ m (Fig. 43), 5  $\mu$ m (Figs 44, 46–47) and 1  $\mu$ m (Fig. 45).

***Actinella guianensis* Grunow in Van Heurck, Synopsis des Diatomées de Belgique, Atlas: pl 35; fig. 20 (1881) (Figs 48–74)**

**Lectotype designated here:** slide (W 0164897) made by Grunow, his sample 1849 Laguna di S. Carlos, Brasilia, in W!

Illustrated by Grunow in Van Heurck (1881, Plate 35, fig. 20), and herein as Figs 48–52.

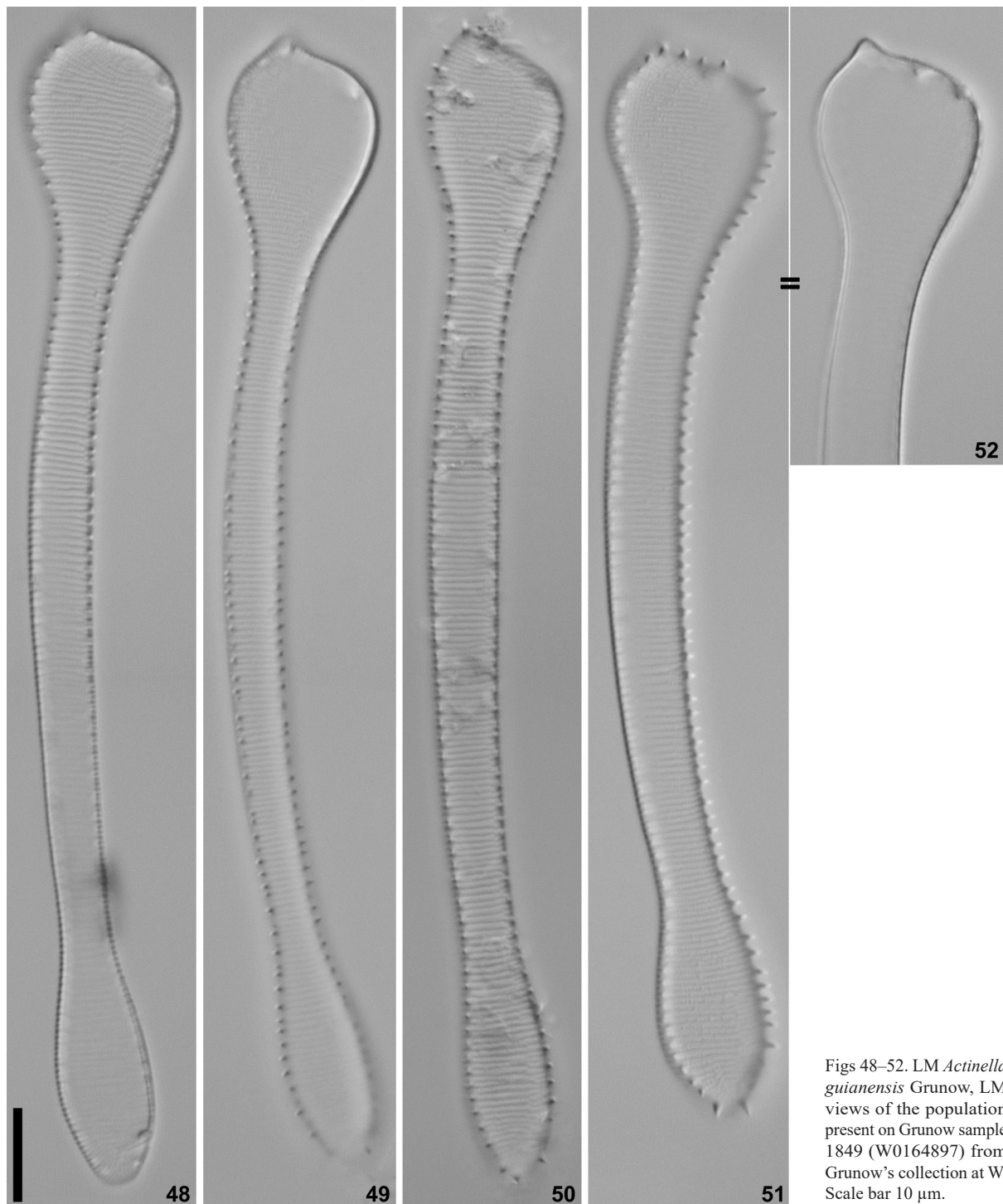
**Description**

**LM (Figs 48–62):** Valves moderately arcuate. Bulbous headpole with an apical point weakly displaced to valve's convex margin, footpole inflated, spatulate to narrowing gradually to rounded to subrostrate apices. Robust spines visible along valve margin, with prominent spines at extremities. Robust terminal nodules. Striae parallel along the valve face, and slightly divergent at the extremities. At apices, striae interrupted near ventral margin by a narrow sternum. Short raphe with terminal raphe fissures curved onto the valve face. Helictoglossae clearly visible.

Valve dimensions (n=75): Length: 47–201  $\mu$ m; center of the valve width: 4–8  $\mu$ m; headpole width: 14–17  $\mu$ m; footpole width: 8–12  $\mu$ m; striae: 12–14(17) in 10  $\mu$ m; spines: 5–8 in 10  $\mu$ m.

**SEM (Figs 63–74):** Externally, frustule in girdle view weakly clavate, with truncate extremities (Fig. 63). Robust areolae present on the valve mantle (Figs 63–65). Girdle bands open, with over four rows of poroids (Figs 64–65). Spines prominent, canine tooth-shaped, distributed regularly around the valve edge (Figs 63, 66). A single external rimoportula aperture occasionally observed at the headpole (Fig. 64) or at the footpole (Fig. 67). Striae linear are composed of round areolae (21–30 in 10  $\mu$ m). Raphe on the valve mantle, short and straight, with drop-like ends (Figs 64–65, 67, 69); terminal raphe fissures curving subterminally onto valve face. Parallel, uniseriate striae, slightly curved near the apical points of the poles (Figs 64–65, 69). On the ventral margin, striae interrupted by a hyaline line at both the headpole and footpole (Fig. 68). Internally, robust helictoglossae present



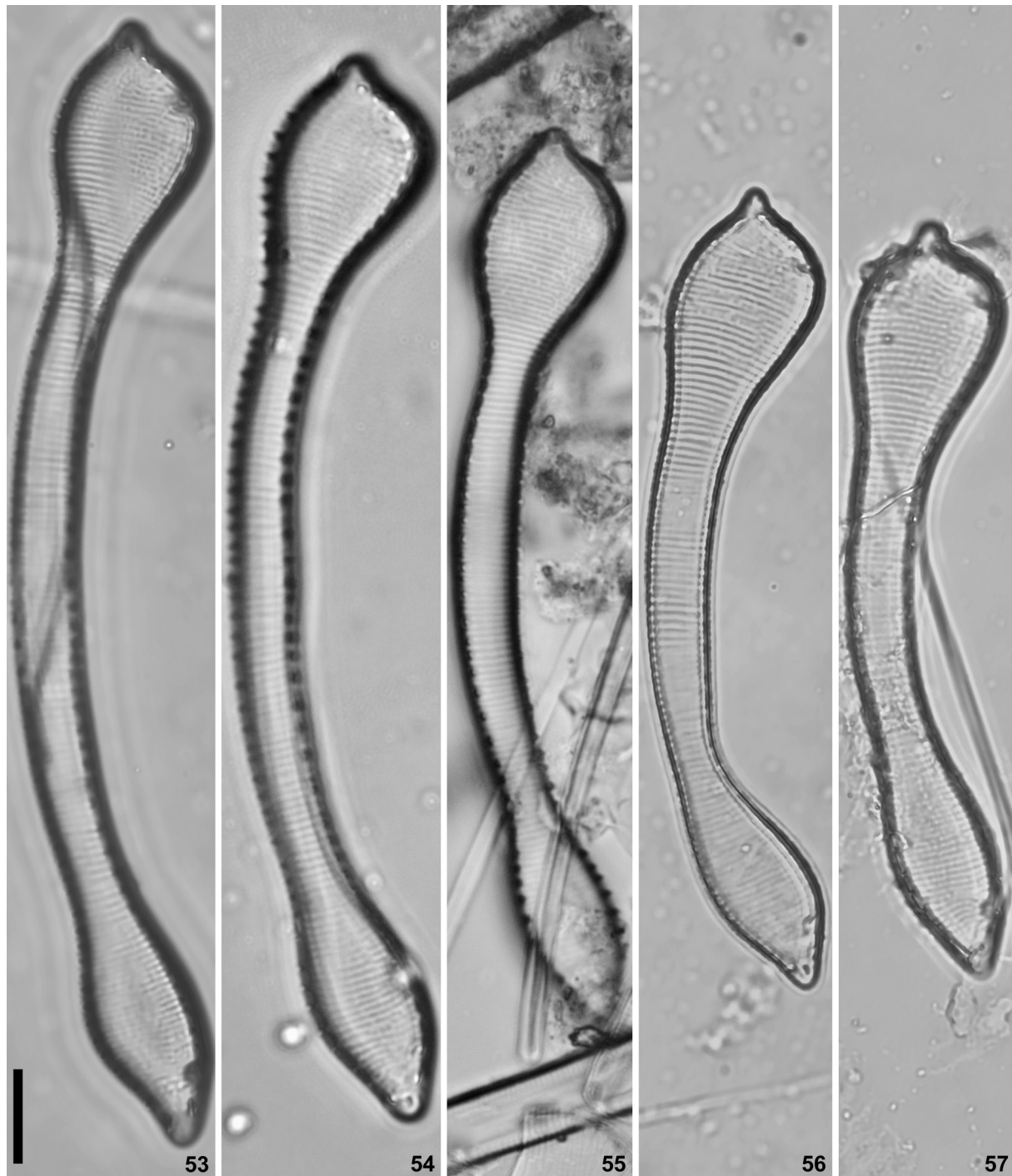


Figs 48–52. LM *Actinella guianensis* Grunow, LM views of the population present on Grunow sample 1849 (W0164897) from Grunow's collection at W. Scale bar 10  $\mu\text{m}$ .

at both ends (Figs 70–74). A single rimoportula occasionally present at the headpole apical point, as discussed by METZELTIN & LANGE-BERTALOT (1998), or at the footpole end (Figs 71–72). Striae composed of punctate areolae, appearing strongly radiate posterior the helictoglossae, at both valve extremities (Figs 72–73).

**Sample occurrence:** HUAM– 12094, 12095, 12267, 12268, 12269, 12270, 12276, 12277, 12284, 12285, 12286, 12287, 12293, 12294, 12295, 12296, 12303, 12304, 12305, 12306, 12307.

**Ecology:** In the Uaicurapá River, *A. guianensis* (Table 1) occurs in planktonic samples, waters with pH ranging from 4.0 to 6.0, and low conductivity ( $< 25 \mu\text{S}\cdot\text{cm}^{-1}$ ). The literature indicates that *A. guianensis* is widely distributed in tropical regions, being collected on submerged macrophytes and wood stems, sediment, and plankton samples, in waters with pH ranging from acid to weakly acidic ( $\text{pH} = 4.5\text{--}6.0$ ), low conductivity ( $2.7\text{--}9.6 \mu\text{S}\cdot\text{cm}^{-1}$ ), and temperature ranging from 24 to 29.8 °C (CLEVE 1881; DÍAZ-CASTRO et al. 2003; MELO et al. 2010; CANANI et al. 2018; SILVA et al. 2021).



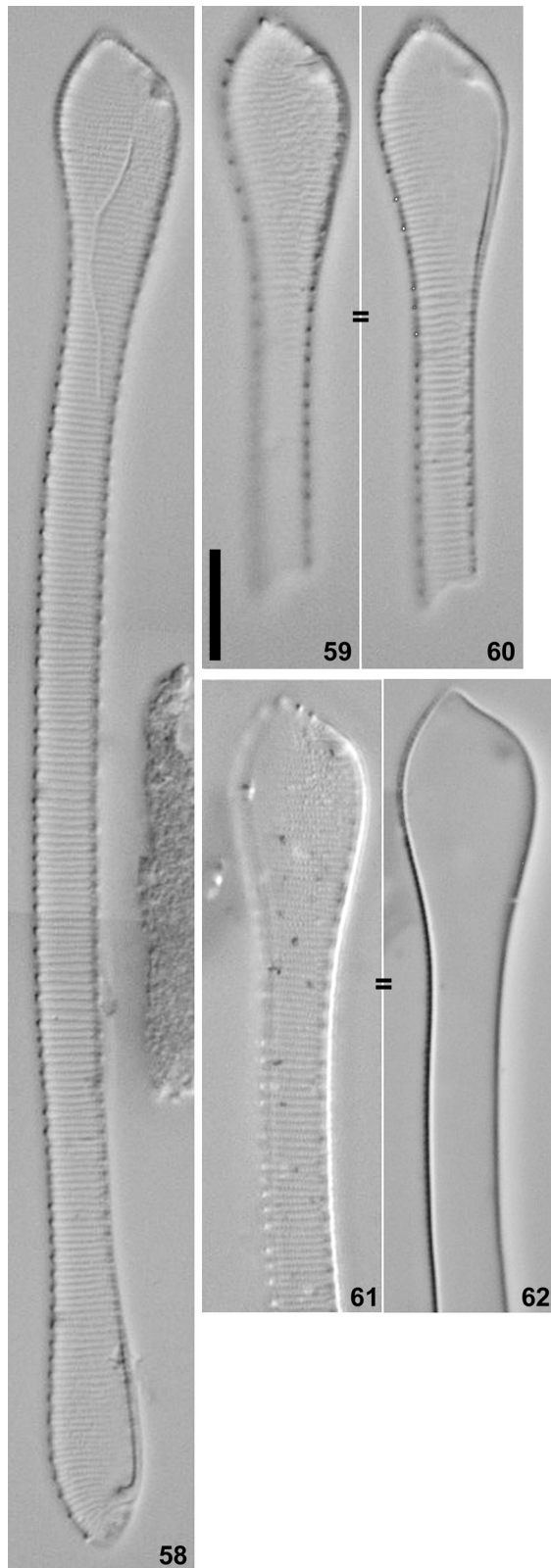
Figs 53–57. *Actinella guianensis* Grunow, LM views of Uaicurapá River population. Scale bar 10  $\mu$ m.

## DISCUSSION

Our analyses of Grunow's original material leads us to the conclusion that two different species were originally included in the taxon described as *Actinella guianensis* Grunow. In addition to examining the type slide of *A. guianensis* (Grunow sample 1849, W0164897), we also studied Grunow's drawings and photographs for this species (de Toni number 3742). These images, which depict two LM images from S. Carlos, bear a striking resemblance to

figures 17 and 20 in VAN HEURCK (1881). However, Grunow did not indicate a sample number for these images (Figs 4, 6), as these are not shown in brackets, as he typically did for publication citations "(1873)" here. Additionally, samples 472 and 473 were attributed to "Dubni" in his catalog (not shown), not S. Carlos, and these numbers are therefore not Grunow's sample numbers. Grunow's sample numbers appear as the topmost numbers on the drawings. Unfortunately, the slides for Grunow samples 472 and 473 no longer exist in the collection to check their species composition. Furthermore, the analysis of





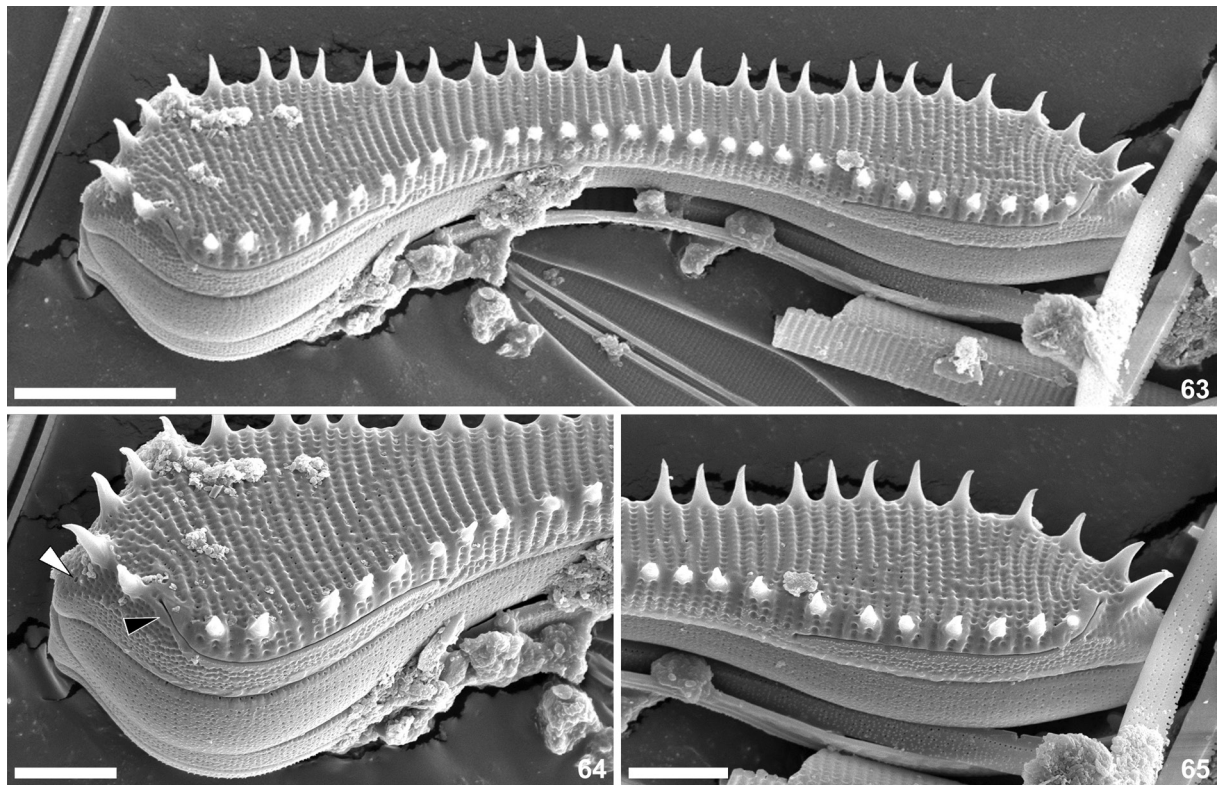
Figs 58–62. *Actinella guianensis* Grunow, LM views of population from Cleve's & Möller's 212 slide (ANSP), Caldas, Brazil. Scale bar 10 µm.

slide No. 212 by Cleve & Möller was important, as it dates back to the same period as the *A. guianensis* type sample and certainly contributed to the taxonomic concept of this species from 1881 to the present day.

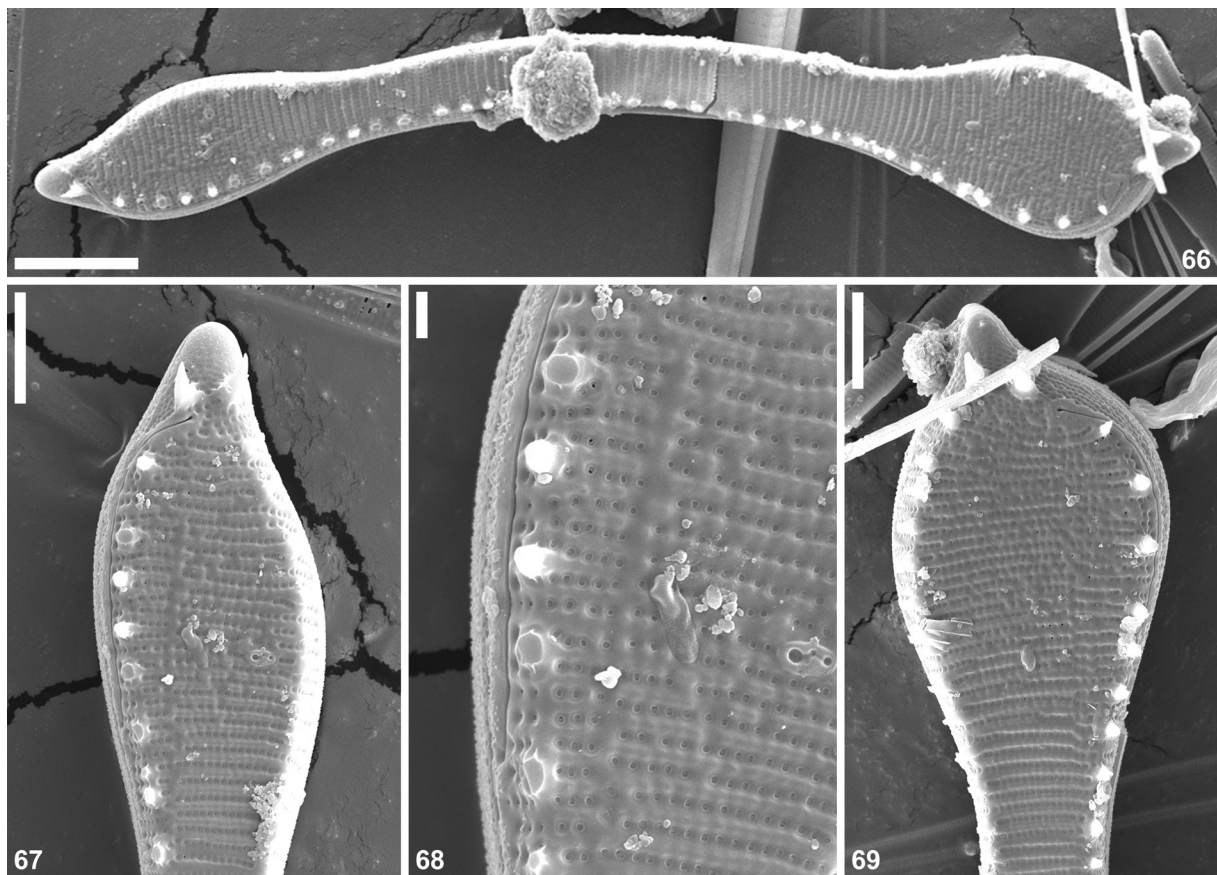
The presence of both shapes of *A. guianensis* in Grunow's materials does not provide a clear indication of his intended type species for *A. guianensis*. Nevertheless, it is evident in the literature what researchers have come to recognize as *A. guianensis*. Morphologically, *A. guianensis* has a bulbous headpole with an apical point medially positioned, enlarged footpole, and spines prominent around the periphery of the valve; these characteristics are well documented in LM and SEM in METZELTIN & LANGE-BERTALOT (1998, pl. 4, figs 6–11, pl. 5, figs 1–7, pl. 8, figs 1–2, pl. 63, fig. 6) and KOCIOLEK et al. (2001, figs 20–24, 29, 30). In terms of distribution, *A. guianensis* has been reported from South America, from tropical (CLEVE 1881; SCHMIDT 1913; OLIVEIRA & STEINITZ-KANNAN 1992; METZELTIN & LANGE-BERTALOT 1998; SOUZA & MOREIRA-FILHO 1999; KOCIOLEK et al. 2001; SALA et al. 2002; DÍAZ-CASTRO et al. 2003; NÚÑEZ-AVELLANEDA 2008; MELO et al. 2010; DUNCK et al. 2012, 2016; CANANI et al. 2018; SILVA et al. 2021) to subtropical regions (FRENGUELLI 1933; BICCA et al. 2011), on submerged substrates, sediment, and plankton samples from acid waters, with low conductivity and high temperatures (CLEVE 1881; DÍAZ-CASTRO et al. 2003; MELO et al. 2010; CANANI et al. 2018; SILVA et al. 2021). *Actinella cordiformis* sp. nov. can be rather easily separated from *A. guianensis* and other species of *Actinella* by the headpole being markedly cordiform; there are no other species of *Actinella* with a similar shape. *Actinella cordiformis* sp. nov. has a pronounced apical point displaced to the dorsal margin, whereas other species show as continuum of the dorsal margin, such as *A. amazoniana* Kociolek, *A. crawfordii* Kociolek, *A. fourtanierae* Metzeltin et Lange-Bertalot, *A. guianensis*, *A. guianensoides* Metzeltin et Lange-Bertalot, and *A. kociolekii* Metzeltin et Lange-Bertalot. The footpole in *A. cordiformis* sp. nov. is enlarged with weakly cuneate to protracted apices, while *A. guianensis* has a spoon-like footpole with rounded apices.

Internally, *A. cordiformis* sp. nov. shows parallel striae regularly distributed along the valve and radiate, while the striae are irregularly distributed in the headpole and footpole. In *A. guianensis*, striae are radiate at the headpole and footpole apices. Both species possess rimoportulae, either at the headpole or the footpole, however, not simultaneously. METZELTIN and LANGE-BERTALOT (1998) reported the rare presence of two rimoportulae at the headpole apical point (plate 63, figure 6) of *A. guianensis*. They also concluded that the variability of the rimoportulae position limits *Actinella* species differentiation by using this feature. *Actinella cordiformis* sp. nov. has the rimoportula at the footpole displaced to the ventral margin, closer to the helictoglossa, differing from *A. guianensis*, which has rimoportulae at the apices of the footpole, as seen in this study (Figs 70–74) and in the literature (METZELTIN & LANGE-BERTALOT 1998, pl. 5, figs 2–5; KOCIOLEK et al. 2001, figs 29–30).

Ecologically, *Actinella* occurs predominantly in acid waters (ROUND et al. 1990). In this study, *A. cordiformis* sp. nov. occurred only in the plankton of Amazonian

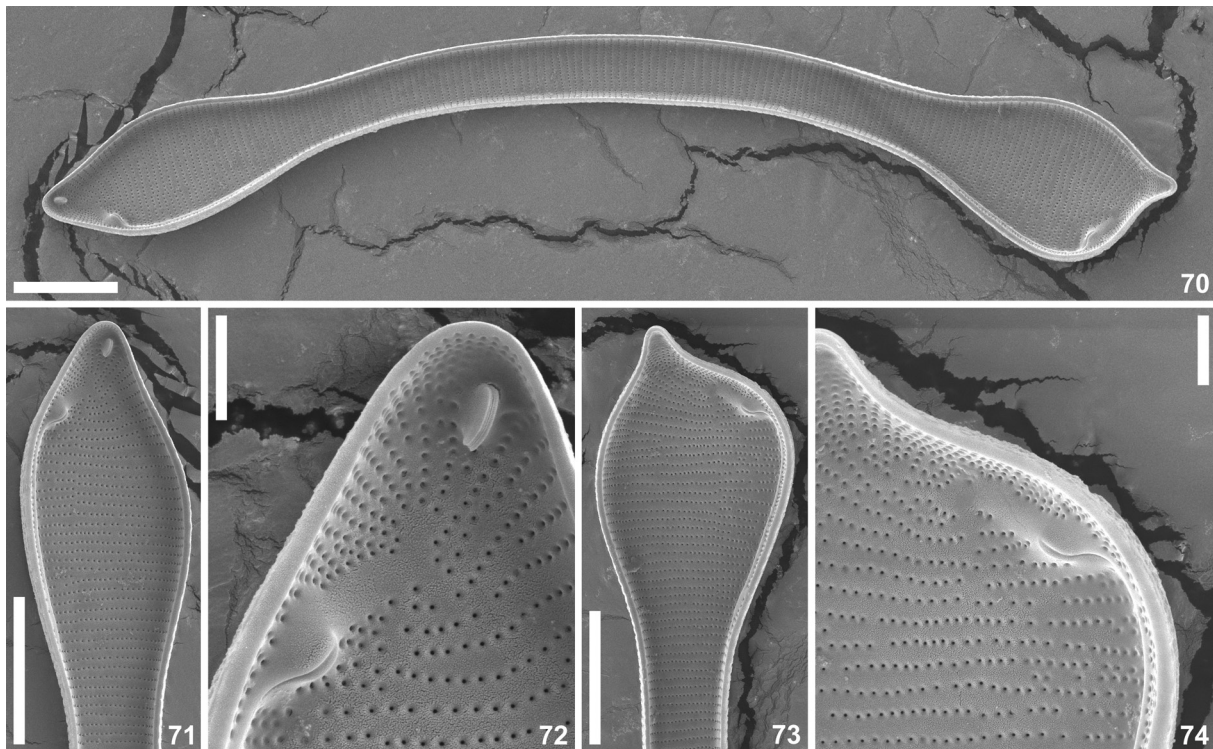


Figs 63–65. SEM views of *Actinella guianensis* Grunow from Uaicurapá River, Parintins, Amazonas, Brazil: (63) frustule in girdle view showing the areolate cingulum, and the robust spines at the poles; (64) shows open band, rimoportula aperture (white arrowhead), terminal nodule (black arrowhead), and raphe; (65) detail of the footpole spines and the raphe. Scale bars 10  $\mu$ m (Fig. 63) and 5  $\mu$ m (Figs 64–65).



Figs 66–69. SEM of *Actinella guianensis* Grunow from Uaicurapá River, Parintins, Amazonas, Brazil: (66) external valve view; (67) details of the robust spines at footpole and the aperture of the rimoportula; (68) striae interrupted by hyaline line; (69) detail of the headpole spines and the distal ends of the raphe. Scale bars 10  $\mu$ m (Fig. 66), 5  $\mu$ m (Figs 67, 69) and 1  $\mu$ m (Fig. 68).





Figs 70–74. SEM of *Actinella guianensis* Grunow from Uaicurapá River, Parintins, Amazonas, Brazil: (70) internal valve view; (71) footpole; (72) details of the rimoportula and helictoglossa; (73) headpole; (74) detail of the helictoglossa. Scale bars 10  $\mu$ m (Figs 70–71, 73) and 2  $\mu$ m (Figs 72, 74).

black waters with acid pH (4–5.1). In the literature, *A. cordiformis* sp. nov. were reported from acid Amazonian samples (pH 4–4.9) from periphyton (UHERKOVICH & FRANKEN 1980, pl. VI, figs 12–13, as *A. guianensis*) and fish stomach contents (SOUZA–MOSIMANN et al. 1997, fig. 7, as *A. guianensis*). *Actinella guianensis* was found in acid to weakly acid pH (4.0–6.1) (DÍAZ–CASTRO et al. 2003; CANANI et al. 2018; SILVA et al. 2021), and both species occur in low conductivity waters.

We believe that in 1881, Grunow may have recognized both shapes as a simple variation in valve morphology for a single species. Considering all the knowledge related to the concept of *A. guianensis*, in this study we propose to conserve this name linked to this widely recognized morphology. This decision is also supported by the Recommendation 9A.4 of the International Code of Nomenclature for algae, fungi, and plants, which recommends that the lectotype should be selected in a way to preserve current usage (TURLAND et al. 2018). Even after tracking the original samples, the type locality of *A. guianensis* is still uncertain, since the site “Laguna de S. Carlos, Brasília” could not be located by us in Brazil using modern and historical gazetteers. Furthermore, the taxonomic delimitation of both species was important to avoid identification errors, as well as to clarify the wide regional distribution of *A. guianensis* in South America. We state that the restricted occurrence of *A. cordiformis* sp. nov. in Amazonian aquatic systems indicates an endemic species and a potential indicator of acidic conditions and little-impacted environments.

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