



## *Achnantheidium neotropicum* sp. nov., a new freshwater diatom from Lake Apastepeque in El Salvador (Central America)

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### Abstract

A new freshwater diatom recovered from modern and subfossil sediments of Lake Apastepeque in El Salvador, *Achnantheidium neotropicum* sp. nov., is described based on light and scanning electron microscopy observations. The species is characterized by valves with linear, sometimes centrally constricted (mainly in large cells), outlines, broadly rounded to subrostrate apices, and often rectangular fascia. Striae are composed of 3–4 rounded to slit-like areolae. It can be separated from similar species by valve outline, together with striae density, and number of areolae per stria. Based on the straight distal raphe endings this species can be assigned to the *A. minutissimum* complex. The new species is compared to morphologically resembling species from the genus *Achnantheidium* Kützing.

**Key words:** Diatoms, taxonomy, *Achnantheidium*, Bacillariophyceae, El Salvador, Neotropics, new species

### Introduction

The diatom genus *Achnantheidium* (Kützing 1844: 75) is common in different climatic zones all over the world. Species belonging to this genus are frequently found in various freshwater habitats ranging from oligotrophic to eutrophic (Krammer & Lange-Bertalot 1991). However, because of their small size, fine striation and limited number of clear morphological characteristics visible in light microscopy, taxa were often lumped together into species complexes in the past, thereby obscuring biogeographical patterns and ecological boundaries of specific species (Van de Vijver *et al.* 2011, Novais *et al.* 2015). Especially for the accurate application of taxa as bioindicators in water quality assessment, this knowledge is important though (Novais *et al.* 2015).

In the genus *Achnantheidium* two main complexes are distinguished: the *Achnantheidium minutissimum* (Kützing 1833: 578) Czarnecki (1994: 157) complex, characterized by straight terminal raphe endings, and the complex including *A. pyrenaicum* (Hustedt 1939: 554–555) H.Kobayasi (1997: 148), distinguishable by a raphe with unilaterally deflected or hooked distal external raphe fissures (Round & Bukhtiyarova 1996, Kobayasi 1997, Potapova & Hamilton 2007). Recently, a thorough revision, especially regarding taxa belonging to the *Achnantheidium minutissimum* complex, was carried out and shed light on this difficult complex (e.g. Potapova & Hamilton 2007, Compère & Van de Vijver 2011, Hlúbiková *et al.* 2011, Van de Vijver *et al.* 2011, 2018, Wojtal *et al.* 2011, Witkowski *et al.* 2012, Pérès *et al.* 2014, Van de Vijver & Kopalová 2014, Novais *et al.* 2015, Pinseel *et al.* 2017). The identification, however, still remains difficult, and the use of scanning electron microscopy (SEM) is often necessary to clarify taxonomic uncertainties.

In general, the number of publications dealing with freshwater diatoms from Central America is still low. Some paleoecological studies have been conducted in Costa Rica (e.g. Chávez & Haberyan 1996, Haberyan & Horn 1999, 2005), Guatemala (e.g. Rosenmeier *et al.* 2004, Cohuo *et al.* 2018), Nicaragua (e.g. Slate *et al.* 2013) and Panama (e.g. Temoltzin-Loranca *et al.* 2018). Other studies investigated the ecology and distribution of modern diatoms in different parts of Central America (among others Haberyan *et al.* 1995, 1997, Silva-Benavides 1996a, b, Michels 1998a, b, Wydrzycka & Lange-Bertalot 2001, Lakatos *et al.* 2004, Michels *et al.* 2006, Silva-Benavides *et al.* 2008, Pérez *et al.* 2013, Céspedes-Vargas *et al.* 2016, Flores-Stulzer *et al.* 2017). New taxonomic works were recently published for species from e.g. Panama (Lange-Bertalot & Metzeltin 2009), El Salvador (Wetzel & Ector 2014) and

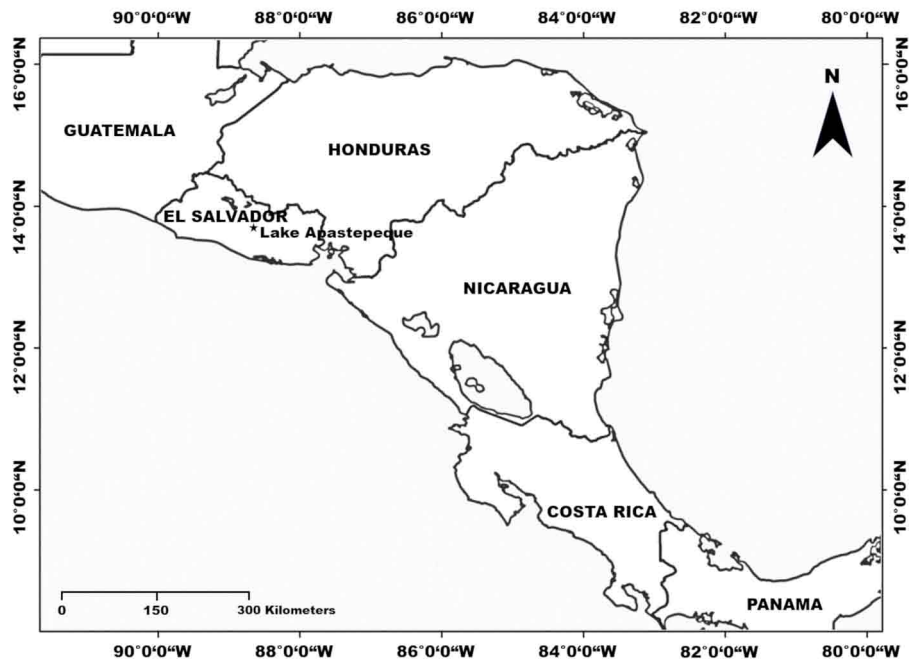
Guatemala (Paillès *et al.* 2018). Many species belonging to *Achnanthydium* are described in Europe, but comparatively little floristic information on this genus is available for Central America (e.g. Hustedt 1953, Michels-Estrada 2003), although overall biological diversity is believed to increase from northern latitudes to the equator (Mittelbach *et al.* 2007). Here, we present both a detailed description of the morphology and ecology of the new species *A. neotropicum* from El Salvador and a comparison with similar species. Additionally, restudied type material from *A. lineare* W.Smith (1855: 8) was compared to this newly described species.

## Material and Methods

### *Study area*

Lake Apastepeque is a volcanic crater lake with a maximum depth of 54 m. It is located in the department of San Vicente in Central El Salvador (13°41'32.84"N and 88°44'42.41"W) at an elevation of 509 m a.s.l. (Fig. 1). A 30 cm long sediment core was taken in October 2013 from 47 m water depth using a gravity corer. Physicochemical parameters were measured in the field during sample collection with a WTW Multi Set 350i multiparameter probe.

El Salvador has a tropical climate with little temperature variation throughout the year and a pronounced rainfall seasonality (average annual rainfall ~190 cm) (Sayre & Taylor 1951). During the rainy season, from May to October, heavy rainfall occurs while its relatively dry from November to April.



**FIGURE 1.** Map of Central America with location of Lake Apastepeque (star) in El Salvador.

### *Sample preparation and analysis*

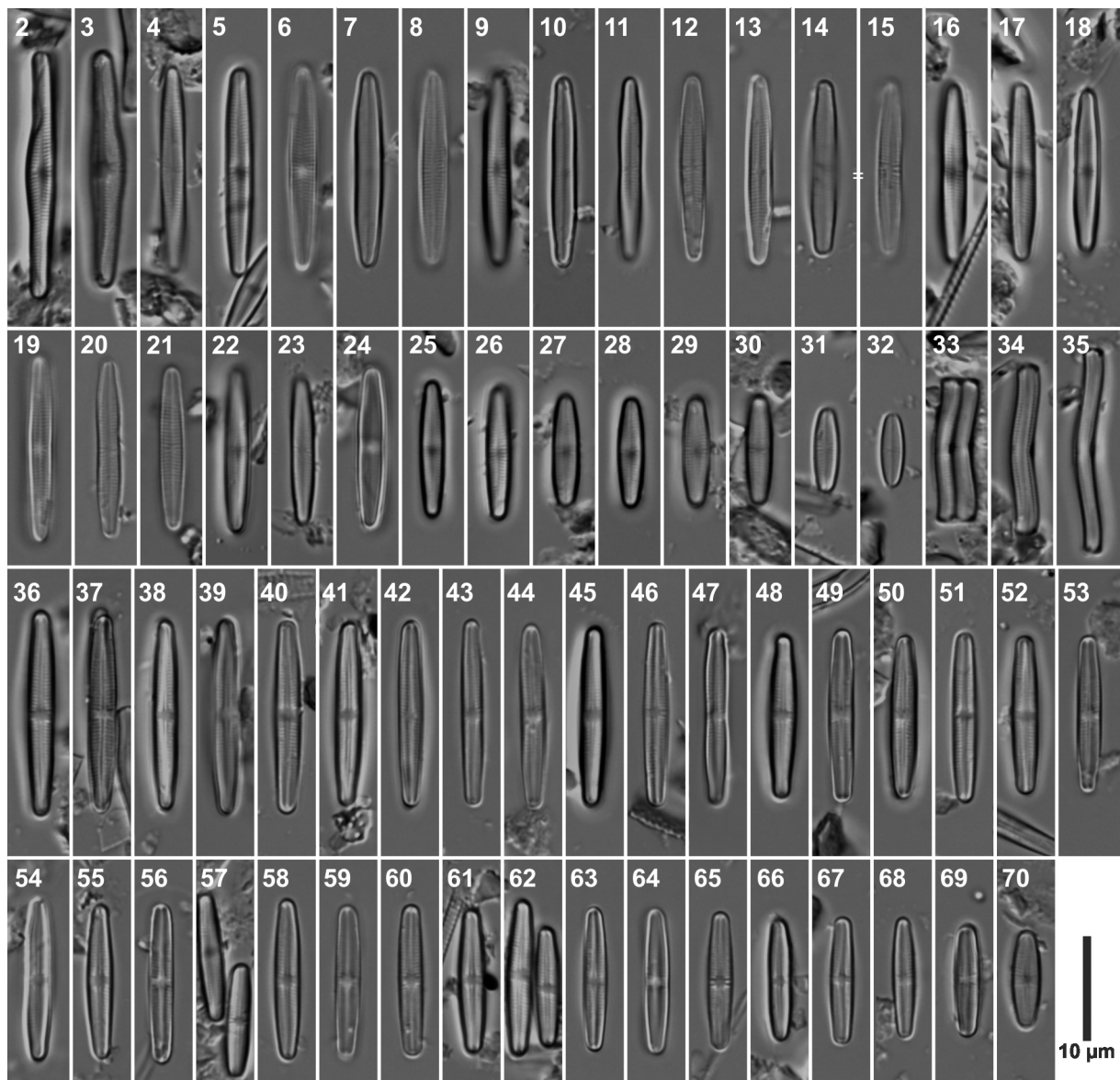
Procedure for slide preparation was adapted from standard methods (Battarbee *et al.* 2001): 0.4 g wet sediment was treated with 37% HCl and 30% H<sub>2</sub>O<sub>2</sub> and heated at 70 °C to remove carbonates and to oxidize organics, respectively. Samples were washed with distilled water during preparation to dilute acids and peroxide remnants. Permanent slides for light microscope (LM) analyses were prepared using Naphrax® as a mountant. Slides were analyzed using a Leica DM 5000 B LM with Differential Interference Contrast under oil immersion at ×1000 magnification equipped with a Leica® DC500 camera. Processed samples for scanning electron microscopy were filtered through a 3 µm pore diameter polycarbonate membrane, put on adhesive carbon tabs and fixed on aluminum pin stubs. The stubs were sputter-coated with platinum using a BAL–TEC MED 020 Modular High Vacuum Coating System for 30 s at 100 mA. An ultra-high-resolution analytical field emission (FE) SEM Hitachi SU–70 (Hitachi High-Technologies Corporation, Tokyo, Japan) was used for the analysis. No changes or trends in species morphology were observed throughout the core, so

one sample (APA\_D\_2–4cm) from the younger part of the sediment core with very high abundances of *Achnanthydium* was chosen for description of the new species. Measurements were taken from 50 valves. Additionally, the original material of *Achnanthydium lineare* W.Smith (Lasswade near Edinburgh, Scotland, UK, coll. date 03/07/1854) was re-investigated under SEM (Figs 89–99). The material housed in the Van Heurck Collection in Meise correspond to the slides deposited at the Botanic Garden Meise (BR), slide IV-2-C7, and to the type slide **BM-445** deposited at the British Museum (London, UK), complementing the information published by Van de Vijver *et al.* (2011). Photomicrographic plates displaying light and scanning electron microscopy images were created using CorelDraw X8®.

## Results

*Achnanthydium neotropicum* K.J.Krahn et C.E.Wetzel *sp. nov.*

(Figs 2–70 (LM), 71–88 (SEM))



**FIGURES 2–70.** *Achnanthydium neotropicum* *sp. nov.*, LM. 2–32. Rapheless valve. 33–35. Frustules in girdle view. 36–70. Raphe valve.

## Light microscopical observations

Frustules bent in girdle view with recurved apices (Figs 33–35). Valves ( $n = 50$ ) linear to narrow linear-lanceolate with almost parallel margins and broadly rounded to slightly subrostrate apices (Figs 2–32, 36–70). Rapheless valves tend to be slightly more lanceolate than raphe valves. Longer valves partly centrally constricted (Fig. 47). Valves 6.7–23.4  $\mu\text{m}$  in length and 2.0–3.0  $\mu\text{m}$  in width. Transapical striae weakly radiate to radiate with 30–36 in 10  $\mu\text{m}$ , areolae not discernible in LM. Raphe valve (Figs 36–70) concave with a narrow axial area, slightly widening towards the central area. Central area often forming a broad rectangular fascia or sometimes with shortened, more widely spaced striae, which becomes more often in shorter valves (e.g. Figs 36, 65). Rapheless valve (Figs 2–32) convex with a narrow and very weakly lanceolate axial area. Central area almost absent or with irregularly shortened and wider spaced striae.

## Scanning electron microscopical observations

### *Rapheless valve*

Mantle areolae slit-like, parallel to the transapical axis (Figs 80, 81). Sternum linear to weakly lanceolate and slightly depressed below the valve surface (Figs 71, 72). Central area almost absent or irregularly developed due to shortened, more widely spaced striae (Figs 71–76). Striae slightly more closely spaced towards the apices and composed of 3–4 areolae, becoming 2–3 near the apices (Fig. 73). Areolae rounded, often becoming transapically elongated to slit-like near the valve margin and directly at the apices (Figs 71–76). Internally, the rapheless valve interstriae are elevated and well visible, and areolae openings are occluded (Fig. 81).

### *Raphe valve*

The mantle bears a single row of slit-like areolae parallel to the transapical axis (Figs 87, 88). External raphe filiform and straight to weakly undulate, distal raphe endings almost straight, small and drop shaped, not continuing onto the valve mantle (Figs 82–85). Proximal raphe endings small, distant and straight or very slightly curved to the same site (Figs 82–85). Internally, proximal endings weakly deflected in opposite directions, distal raphe endings terminating in helictoglossae (Fig. 86). Transapical striae weakly radiate to radiate throughout the valve, composed of 3–4 (rarely 2, in the central striae of large constricted cells) areolae near the valve center and becoming less numerous at the apices (Figs 82–85). Areolae near the valve margin in the middle of the valve and next to the central area often transapically elongated or slit-like and only rounded near the apices (Fig. 82). Striae becoming slightly denser towards the apices (Fig. 83).

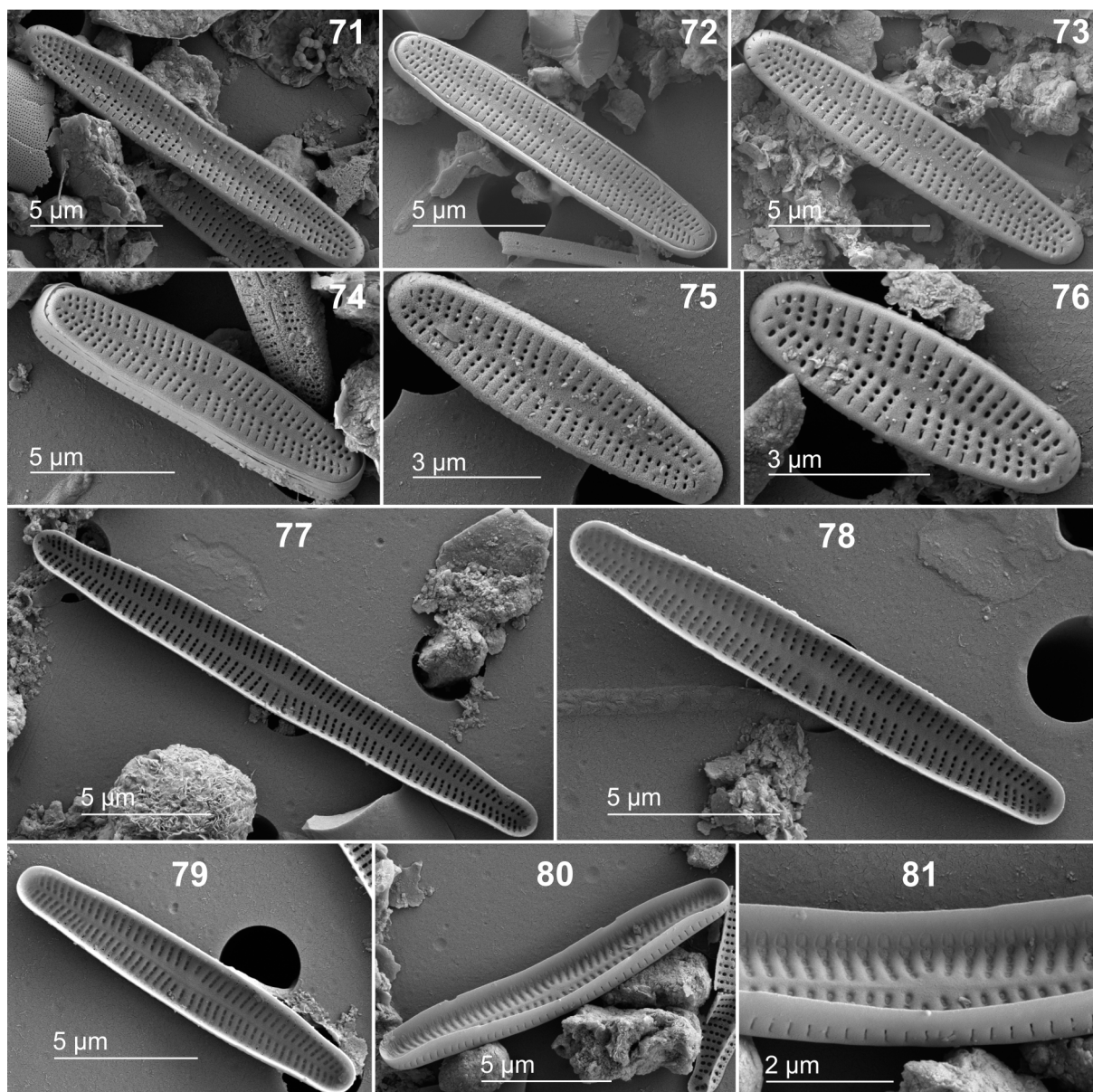
**Type locality:**—EL SALVADOR. Department of San Vicente: Lake Apastepeque, volcanic crater lake, 509 m a.s.l., 13°41'32.84"N, 88°44'42.41"W, sediment core sample (APA\_D\_2–4cm), 47 m water depth, collection: L. Macario-González and S. Cohuo-Durán, 09.10.2013. Holotype: slide BR-4526 (National Botanic Garden, Meise, Belgium).

**Etymology:**—The specific epithet refers to the geographical area where the new species was found: Neotropics.

## Ecology and associated diatom flora

The type population of *Achnantheidium neotropicum* was found in modern and subfossil sediments of Lake Apastepeque, El Salvador. The lake had in October 2013 alkaline pH (8.57), high water temperature (29.5 °C) and very low dissolved oxygen content (2.82 mg/L). Electric conductivity was 100  $\mu\text{S}/\text{cm}$ . Water analyses determined  $\text{Ca}^{2+}$  (17.86 mg/L) and  $\text{Mg}^{2+}$  (15.21 mg/L) as the dominant cations and  $\text{HCO}_3^-$  (145.18 mg/L) as important anion (Table 1). Therefore, *A. neotropicum* can be considered characteristic of calcium-(magnesium)-bicarbonate-rich freshwaters.

*Achnantheidium neotropicum*, clearly dominating three-quarter of the sediment core samples (~90 % relative abundance), is associated among others with *Aulacoseira granulata* var. *granulata* (Ehrenberg 1843: 415) Simonsen (1979: 58), *Aulacoseira granulata* var. *angustissima* (O.Müller 1899: 315) Simonsen (1979: 58), *Achnantheidium minutissimum*, *Fragilaria* cf. *tenera* (W.Smith 1856: 98) Lange-Bertalot (1980: 746), *Encyonopsis* spp. and *Ulnaria grunowii* (Lange-Bertalot & S.Ulrich 2014: 22) Cantonati et Lange-Bertalot in Kusber *et al.* (2017: 92). No diatom plankton and benthos samples were taken during the sampling campaign, therefore no specific information about substrate preferences of *A. neotropicum* can be given so far.



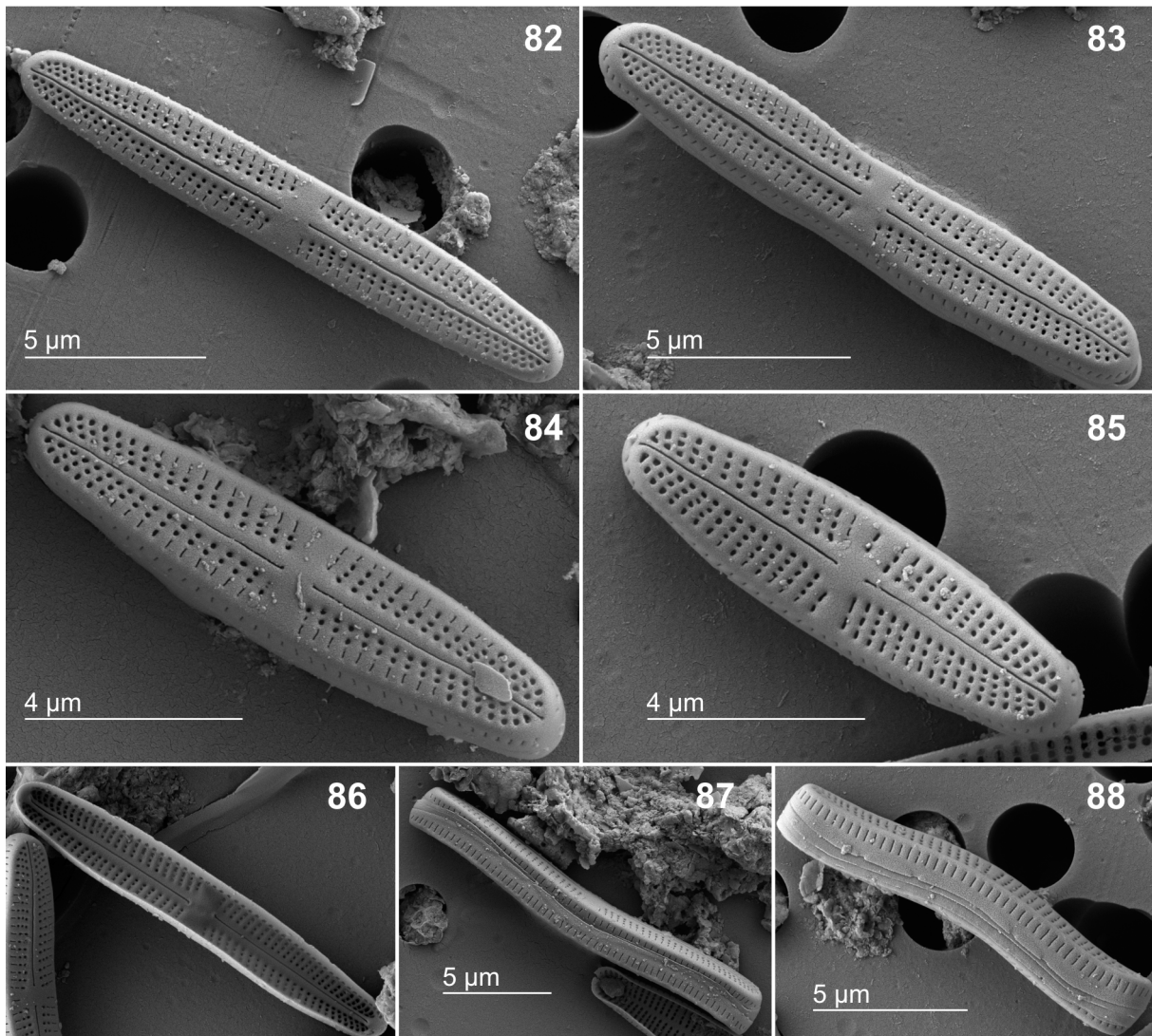
**FIGURES 71–81.** *Achnanthydium neotropicum* sp. nov., SEM. 71–76. External view of rapheless valve. 77–81. Internal view of rapheless valve. 81. Internal detail of central part of a rapheless valve showing the occluded areolae.

**TABLE 1.** Main physical and chemical variables and water chemistry data from Lake Apastepeque in October 2013. Abbreviations and units: T=Water temperature [°C], DO=Dissolved oxygen [mg/L], Cond=Electrical conductivity [ $\mu\text{S}/\text{cm}$ ], Sal=Salinity [‰], Major ions [mg/L].

T	DO	pH	Cond	Sal	$\text{HCO}_3^-$	$\text{SO}_4^{2-}$	$\text{Cl}^-$	$\text{Na}^+$	$\text{K}^+$	$\text{Ca}^{2+}$	$\text{Mg}^{2+}$
29.5	2.82	8.57	100	0	145.18	7.59	10.5	10.8	7.54	17.86	15.21

### Discussion and Taxonomical remarks

Based on its rather straight than clearly deflected or hooked terminal raphe fissures, *Achnanthydium neotropicum* can be grouped within the *A. minutissimum* complex. It can be distinguished from *A. minutissimum* by its linear to linear-lanceolate, sometimes constricted outline, while *A. minutissimum* has a broader lanceolate to elliptic-lanceolate outline. Moreover, *A. neotropicum* has a higher striae density (30–36 striae per 10  $\mu\text{m}$ ) compared to *A. minutissimum* (around 30 striae per 10  $\mu\text{m}$  in raphe valve). In the middle of the raphe valves, striae of *A. minutissimum* are sometimes composed of only two areolae. In *A. neotropicum*, this has only been observed in constricted valves, otherwise the striae consist always of 3–4 areolae.



**FIGURES 82–88.** *Achnantheidium neotropicum* sp. nov., SEM. 82–85. External view of raphe valve showing the linear to weakly lanceolate axial area and the central area with rectangular fascia (Figs 82, 83) or shortened, more widely spaced striae (Fig. 85). 86. Internal view of a raphe valve showing the oppositely deflected proximal raphe endings and helictoglossae. 87, 88. Frustules in girdle view with slit-like areolae on the mantle.

Several other taxa also show some morphological similarities to *A. neotropicum*. Among those are *A. digitatum* Pinseel, Vanormelingen, P.B.Hamilton et Van de Vijver in Pinseel *et al.* (2017: 271), *Achnantheidium ennediense* (Compère) Compère et Van de Vijver (2011: 7), *A. jackii* Rabenhorst (1861: no. 1003), *A. lailae* Van de Vijver in Zidarova *et al.* (2009: 297), *A. lineare*, *A. maritimo-antarcticum* Van de Vijver et Kopalová (2014: 6) and *A. pseudolineare* Van de Vijver, Novais et Ector in Van de Vijver *et al.* (2011: 186) (see Table 2).

*Achnantheidium digitatum* clearly resembles *A. neotropicum*. Both taxa have a relatively similar striae density. However, valves of *A. neotropicum* are sometimes constricted in the center, especially in longer species, which has not been observed in *A. digitatum*. Moreover, *A. digitatum* exhibits two, rarely three areolae per stria on the raphe valve, whilst *A. neotropicum* has three to four areolae per stria.

*Achnantheidium ennediense* has lanceolate to rhombic-lanceolate valves, never linear as observed in *A. neotropicum*. Another distinguishing feature is the central area. Raphe valves of *A. neotropicum* often possess a rectangular fascia, whilst the central area of *A. ennediense* is rounded to transapically elongated and never forms a fascia. Ultimately, the striae on the raphe valves of *A. ennediense* are usually composed of two to three areolae. This contrasts with *A. neotropicum*, which usually has three to four areolae per stria.

*Achnantheidium jackii* differs from *A. neotropicum* in having a more lanceolate outline and centrally wider spaced striae (25–30/10 μm to 30–36/10 μm, respectively) on the raphe valve. Valves of *A. jackii* generally exhibit a greater width (3.0–3.9 μm) as those of *A. neotropicum* (2.0–3.0 μm). Furthermore, striae of *A. jackii* are composed of 4–5 areolae per stria in the valve center, whereas in valves of *A. neotropicum* only 3–4 areolae per stria are found.

**TABLE 2.** Comparison between *Achnanthydium neotropicum* and the most morphologically comparable *Achnanthydium* species.

<i>Achnanthydium</i>	<i>A. neotropicum</i> sp. nov.	<i>A. digitatum</i> Pinseel, Vanormelingen, P.B.Hamilton et Van de Vijver	<i>A. ennediense</i> (Compère) Compère et Van de Vijver	<i>A. jackii</i> Rabenhorst	<i>A. lailae</i> Van de Vijver	<i>A. lineare</i> W.Smith	<i>A. maritimo- antarcticum</i> Van de Vijver et Kopalová	<i>A. minutissimum</i> (Kützing) Czarnecki	<i>A. pseudolineare</i> Van de Vijver, Novais et Ector
Valve length (µm)	6.7–23.4	8.6–19.1	15.5–26.5	8.0–17.0	10–12.5(14)	9.0–13.5	12.0–15.0	8.8–17.4	7.5–26
Valve width (µm)	2.0–3.0	1.8–2.3	2.3–3.3	3.0–3.9	(1.8)2–2.5	2.2–2.8	2.3–2.7	2.2–4.1	2.1–3.3
<b>Raphe valve</b>									
Valve outline	linear to linear-lanceolate, parallel margins, partially constricted	linear to slightly linear-lanceolate with parallel margins	lanceolate to rhombic-lanceolate	linear-lanceolate to weakly lanceolate	linear to very slightly linear-lanceolate	linear, almost parallel margins	linear lanceolate	linear-elliptic to linear-lanceolate	linear, parallel margins
Valve apices	broadly rounded, subrostrate	protracted, subrostrate, becoming broadly rounded, non-protracted in smaller species	broadly rounded, non-protracted	broadly rounded, protracted, rostrate	non-protracted, broadly rounded, never rostrate or capitate	broadly rounded, weakly rostrate	clearly protracted, rostrate, occasionally subcapitate	protracted, rostrate to subcapitate	broadly rounded, non-protracted
Central area	rectangular fascia or shortened, more widely spaced striae	small, rounded or forming a clear rectangular fascia	small, oval to very slightly apically elongated, no fascia, central striae more distant	clear, often asymmetrical fascia	rectangular fascia, occasionally shortened striae	rectangular fascia	small, irregular, more widely spaced striae	irregular, almost absent, slightly rounded, rarely stauros-like	rectangular fascia, isolated striae
Striae (in 10 µm)	30–36	31–36	30–32	25–30, up to 35 near the apices	30–33	28–32	30–33, up to 40–42 near the apices	around 30, up to 35 near the apices	35–40
Number of areolae (per stria)	3–4, rarely 2 in constricted valves	2, rarely 3	2–3, rarely 4	4–5, 2–4 near apices	2–3	2–3	4–5, 2–3 near apices	3–4, rarely 2	3–5
Striation pattern	weakly radiate to radiate	moderately radiate, more radiate near apices	radiate throughout	weakly radiate to almost parallel	weakly radiate near the valve center, becoming more radiate near the apices	radiate to weakly radiate, more densely spaced near apices	slightly radiate throughout, more radiate towards the apices	radiate throughout, strongly radiate near apices	clearly more densely spaced near the apices, radiate to strongly radiate near the apices

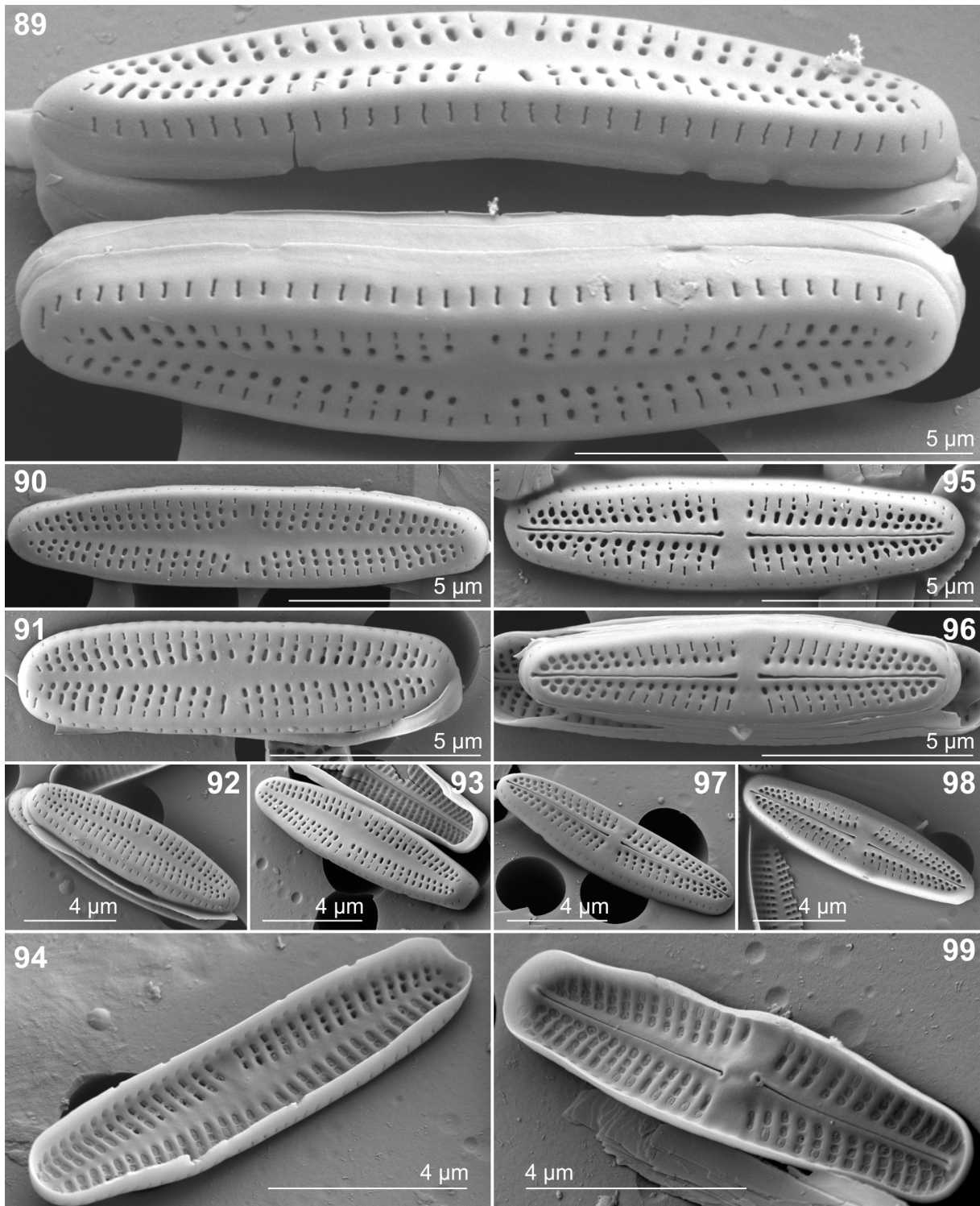
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TABLE 2. (Continued)

<i>Achnanthydium</i>	<i>A. neotropicum</i> sp. nov.	<i>A. digitatum</i> Pinsee, Vanormelingen, P.B. Hamilton et Van de Vijver	<i>A. ennediense</i> (Compère) Compère et Van de Vijver	<i>A. jachii</i> Rabenhorst	<i>A. lailae</i> Van de Vijver	<i>A. lineare</i> W. Smith	<i>A. maritimo-antarcticum</i> Van de Vijver et Kopalová	<i>A. minutissimum</i> (Kützing) Czarnecki	<i>A. pseudolineare</i> Van de Vijver, Novais et Ector
<b>Rapheless valve</b>									
Valve outline	linear to linear-lanceolate, parallel margins, partially constricted	linear to slightly linear-lanceolate with parallel margins	lanceolate to rhombic-lanceolate	linear-lanceolate to weakly lanceolate	linear to very slightly linear lanceolate	more lanceolate, convex margins	linear-lanceolate	linear-elliptic to linear-lanceolate	narrowly lanceolate, convex margins
Valve apices	broadly rounded, subrostrate	protracted, subrostrate, becoming broadly rounded, non-protracted in smaller species	broadly rounded, non-protracted	broadly rounded, protracted, rostrate	non-protracted, broadly rounded, rostrate or capitate	broadly rounded, weakly rostrate	clearly protracted, rostrate, occasionally subcapitate	protracted, rostrate to subcapitate	weakly rostrate, broadly rounded
Central area	almost absent or irregular with shortened, more widely spaced striae	almost non-existent	almost absent or very small, apically elongated	variable: absent, small fascia or asymmetrical	apically elongated, no fascia	almost absent to weakly elliptical	weakly elliptical, never transversally elongated	weakly elliptical and almost absent, 1–2 more widely spaced striae	almost absent or forming a very narrow fascia
Striae (in 10 µm)	30–36	31–36	32–34	28–30, up to 34 near apices	28–30	28–32	30–32	35, up to 40 near apices	32–35
Number of areolae (per stria)	3–4, 2–3 near apices	2, rarely 3	3–5	4–5, 2–4 near apices	2–3	1–3	2–5	3–5	3, sometimes 4
Striation pattern	weakly radiate to radiate	almost parallel to weakly radiate	radiate throughout, more radiate near central area	radiate, more radiate near apices	parallel to weakly radiate near the valve center, more radiate near the apices	weakly radiate to radiate, more densely spaced near apices	slightly radiate throughout	radiate throughout, more strongly radiate near apices	radiate to strongly radiate near the apices
Type locality	Lake Apastepeque, El Salvador, Central America	epiphyton on moss, Mathiessondalen, northern Billefjorden, Central Spitsbergen	Ennedi Mountains, Ouro Gale guelta, Republic of Chad, Africa	Württemberg, southern Germany, Europe	Katia Lake, James Ross Island, Antarctic Peninsula	Lasswade, Scotland, United Kingdom, Europe	Byers Peninsula, Livingston Island, South Shetland Islands, Antarctica	near Aschersleben, Saxony-Anhalt, Germany, Europe	Ribeira da Abelheira, Alto Trás-os-Montes, Vila Real District, Portugal, Europe
References	This study	Pinsee <i>et al.</i> 2017	Compère & Van de Vijver 2011	Van de Vijver <i>et al.</i> 2018	Zidarova <i>et al.</i> 2009, Van de Vijver & Kopalová 2014	Van de Vijver <i>et al.</i> 2011	Van de Vijver & Kopalová 2014	Novais <i>et al.</i> 2015	Van de Vijver <i>et al.</i> 2011



The rapheless valve of *A. lailae* possesses a broad, clearly lanceolate axial area whereas the axial area of *A. neotropicum* is thinner and more linear-lanceolate. The former has 2–3 areolae per stria on both valve while the number is usually 3–4 for the latter. Weakly deflected distal raphe fissures of *A. lailae* also differentiate this species from *A. neotropicum*, with its straighter raphe endings. Areolae close to the axial area are always rounded in *A. neotropicum*, whilst areolae of *A. lailae* are also elongated.



**FIGURES 89–99.** Original material of *Achnanthisdium lineare* from the Van Heurck Collection in Meise (SEM). 89–94. Rapheless valve. 95–99. Raphe valve.

*Achnanthisdium lineare* has two to three areolae per stria on the raphe valve (e.g. Fig. 96), whereas the striae of *A. neotropicum* generally consist of three to four areolae (Figs 83, 84). Even smaller rapheless valves of *A. neotropicum* (e.g. Fig. 76) usually have four areolae per stria near the center, never two as it is common in *A. lineare* (Figs 92, 93).

Additionally, the central area of *A. lineare* always forms a rectangular fascia (Figs 95–97), lacking the shortened striae which occasionally occur in the central area of *A. neotropicum* (Fig. 85). Although there is an overlap in size, valves of *A. lineare* (9.0–13.5 µm) are generally smaller than valves of *A. neotropicum* (6.7–23.4 µm). A further difference exists with regards to the axial area of the rapheless valves. In *A. neotropicum*, the axial area is narrow and linear to only weakly lanceolate (e.g. Figs 72, 76), while in *A. lineare*, it is slightly expanded near the center of the valve (see Figs 90 and 93). Moreover, *A. lineare* never present constricted cells nor subrostrate apices, in contrast to *A. neotropicum* (e.g. Figs 20, 47).

*Achnantheidium neotropicum* also reveals several clear morphological differences to *A. maritimo-antarcticum*. The striae of *A. maritimo-antarcticum* are never composed of slit-like areolae as often found near the valve margin of *A. neotropicum* and *A. maritimo-antarcticum* has up to five areolae per stria, whereas *A. neotropicum* has a maximum of four areolae. Additionally, the former never forms a rectangular fascia and has more clearly protracted and occasionally subcapitate apices, in contrast to *A. neotropicum*.

*Achnantheidium pseudolineare* (35–40/10 µm) can be distinguished from *A. neotropicum* (30–36/10 µm) by a higher striae density on the raphe valve and the clearly more densely spaced and strongly radiate striae near apices in *A. pseudolineare*. *Achnantheidium pseudolineare* can also be separated from *A. neotropicum* based on the apices that are always broadly rounded in *A. pseudolineare*, whereas *A. neotropicum* also shows subrostrate apices. The number of areolae per stria in raphe valves of *A. pseudolineare* is higher (3–5) than in *A. neotropicum* (3–4).

## Conclusion

The unique morphological characters of *Achnantheidium neotropicum* visible in SEM, e.g. number and shape of areolae, clearly separate this taxon from other species belonging to the *A. minutissimum* complex. Identification solely based on light microscopy might be difficult due to overlaps in morphological features and the limited number of distinguishable morphological characteristics. However, the valve outline together with the striae density and form of the central area already distinguish *A. neotropicum* from several other similar taxa in LM, including *A. minutissimum*. Most of the morphological similar *Achnantheidium* species were described from Europe and Antarctica (Table 2). So far, *A. neotropicum* represents one of the few newly described *Achnantheidium* species from Central America and neighboring countries, although an increased biodiversity is proposed for tropical regions compared to higher latitude areas. The continuing discovery of new *Achnantheidium* taxa during the last years furthermore highlights the need for more ecological and biogeographic studies regarding the *Achnantheidium minutissimum* complex, to fully understand species boundaries and preferences.

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