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RESEARCH ARTICLE

A new *Actinella* species (Eunotiaceae, Bacillariophyta) from the Yangambi Biosphere Reserve, Democratic Republic of the Congo

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Abstract

Background and aims – *Actinella* species from Central Africa are only rarely reported. The acid waters from many stream and small rivers in the Congo Basin form a potential environment for species of this genus. We studied several samples from the region of Yangambi to confirm whether species belonging to this genus are present.
Material and methods – Samples for diatom investigation were collected in several streams and small rivers (Moni, Ngima, and Libongo), in the tropical rainforest in the Yangambi Biosphere Reserve, Tshopo Province, Democratic Republic of the Congo. The samples were prepared to obtain permanent microscope slides for light microscopy studies using standard methods and cleaned material for scanning electron microscopy.
Key results – A novel *Actinella* was observed in the studied samples. The taxon was compared to other species such as *A. disjuncta*, *A. lange-bertalotii*, *A. modesta*, *A. pereunotioides*, and *A. pseudohantzschia*, although the new species morphologically resembles most *A. eunotioides*, a species discovered in the Amazon basin in Brazil, and *A. eunotioides* var. *minor*, described in 1966 from the Central African Republic. The taxon observed in our material differs from *A. eunotioides* mainly in the valve dimensions, being much smaller, and the higher density of the marginal spines. Stria density also tends to be coarser. The valve length of *A. eunotioides* var. *minor*, on the other hand, corresponds with the smallest valves of our taxon but the valves are narrower, with a higher stria density. The ventral margin is slightly convex, while in the new taxon it is slightly concave. Moreover, in the original description of *A. eunotioides* var. *minor*, the absence of spines is mentioned, while they are present on the valves in the materials we investigated. Based on the observed morphological differences, we consider the taxon recorded in the streams and small rivers in the Yangambi Biosphere Reserve distinct from *A. eunotioides* and its var. *minor* and subsequently, we describe *Actinella kufferathiana* as a species new to science.

Keywords

Actinella, Africa, diatoms, D.R. Congo, new species, taxonomy

INTRODUCTION

Within the genus *Actinella* F.W.Lewis, 71 taxa have been reported, including 57 taxonomically accepted species names and 3 accepted varieties. In addition, there are 5 species names that are currently of uncertain status and 6 names that have not been verified to date (Guiry and Guiry 2023; Kociolek et al. 2023). Synonymy seems to be quite rare in this genus. Following Algaebase (Guiry and Guiry 2023), only 2 species names and 1 variety name were considered as synonyms.

From the African continent and Indian Ocean islands, new species of the genus *Actinella* were described from

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Madagascar and reported from Mauritius, e.g. *Actinella candelabrum* (Manguin) Kociolek & K.Rhode (as *Asterionella candelabrum* Manguin), its form *baculata* Manguin, *A. madagascariensis* (Manguin) Kociolek & K.Rhode (as *Asterionella madagascariensis* Manguin), and *A. bourrellyi* Kociolek & K.Rhode (synonym of *Asterionella madagascariensis* var. *minor* Manguin) (Manguin 1949; Kociolek and Rhode 1998). Several other *Actinella* taxa have been reported from these two islands, such as *A. australis* (Manguin) Kociolek, K.Rhode & D.M.Williams (as *A. punctata* var. *australis* Manguin) (Manguin 1949).

Reports on *Actinella* from sub-Saharan continental Africa are rather limited. *Actinella brasiliensis* Grunow and *A. pliocenica* Hérib. & Perag. were reported from Sierra Leone (Carter and Denny 1982 and Woodhead and Tweed 1956, respectively). From the same country, Woodhead and Tweed (1957) described *Actinella africana* Woodhead & Tweed. Fusey (1964, 1966) mentioned the presence of *Actinella eunotioides* Hust., a species discovered in the Amazon basin in Brazil (Hustedt 1952), in the Central African Republic and described a new variety, *A. eunotioides* var. *minor* Fusey, and a new species, *A. undulata* Fusey. Other *Actinella* reported by Fusey (1964, 1966) from the Central African Republic, a country located in the northern part of the Congo Basin, include *A. australis* (as *A. punctata* var. *australis* Manguin) and *A. fuseyi* Schoeman (as *A. robusta* Fusey nom. illeg.). All these taxa were rare except for *A. undulata* which was "dispersed without being rare". *Actinella punctata* F.W.Lewis was reported by Stoyneva-Gärtner and Descy (2020) in samples from the Congo River, although the identity of this tropical African taxon needs to be verified, as *A. punctata* was described from North America (Lewis 1864). Okito et al. (2021) also reported an *Actinella* sp. on herbarium material kept at the National Herbarium of the Congo at the Institut National pour l'Étude et la Recherche Agronomique (INERA), Yangambi (YBI), Democratic Republic of the Congo (D.R. Congo). More information on Okito's observations of this taxon on *Nymphaea lotus* L. can be found in his master's thesis (Okito 2019).

Our own observations of material collected in tropical Africa, including samples collected during the Boyekoli Ebale Congo 2010 expedition (e.g. Cocquyt and Taylor 2015) and in the framework of several projects thereafter (e.g. Cocquyt et al. 2016; Karthick et al. 2016), confirmed the presence of representatives of the genus *Actinella* in the Congo Basin. Besides valves resembling *A. punctata*, another taxon was observed that morphologically resembles *A. eunotioides* var. *minor*. Based on light microscope (LM) and scanning electron microscope (SEM) investigations, this taxon is here described as a species new to science: *Actinella kufferathiana* Cocquyt & J.C.Taylor, sp. nov.

MATERIAL AND METHODS

Materials studied for the present study were collected between 2013 and 2017 in small rivers and streams in the Yangambi Biosphere Reserve, in the Tshopo Province, D.R. Congo.

CCA 3282: D.R. Congo, Libongo River, tropical rain forest, benthos (sand?) in the stream bed, 0°48'11.59"N and 24°31'53.80"E, collected by Myriam de Haan on 14 Nov. 2013.

CCA 4378: D.R. Congo, Moni River, just downstream from the campsite in the tropical rain forest, sand from the stream bed, 0°50'47.29"N and 24°32'36.63"E, collected by Nils Bourland on 5 Jul. 2017.

CCA 4379: D.R. Congo, Moni River, just upstream from the campsite in the tropical rain forest, sand from the stream bed, 0°50'47.76"N and 24°32'8.34"E, collected by Nils Bourland on 5 Jul. 2017.

CCA 4385: D.R. Congo, Ngima River, stream flowing through fields just outside the tropical rain forest, sand from the stream bed, 0°50'34.55"N and 24°29'15.14"E, collected by Nils Bourland on 5 Jul. 2017.

CCA 4386: D.R. Congo, Moni River, just downstream from the campsite in the tropical rain forest, sand from the stream bed, 0°50'47.29"N and 24°32'36.63"E, collected by Andreas Popelier on 18 Jul. 2017.

The samples were fixed in situ using a 20% v/v final concentration ethanol solution (Taylor and Cocquyt 2016). Part of these materials were cleaned with hydrogen peroxide (30%), rinsed four times with distilled water and mounted in Naphrax®. Light microscopy (LM) was carried out with an Olympus BX51 microscope equipped with Nomarski differential interference contrast optics (DIC) and an Olympus UC30 digital camera. For scanning electron microscopy (SEM), aliquots of the oxidized suspensions were filtered through 1 µm isopore® polycarbonate membrane filters (Millipore). Filters were air-dried, mounted on aluminium stubs using carbon tape, and sputter coated with a platinum layer of 20 nm and studied with a JEOL JSM-7100FLV Field Emission Scanning Electron Microscope (SEM) operating at 1 kV and 6.9 mm working distance.

Samples, cleaned materials, permanent microscope slides and SEM stubs are housed in the herbarium of Meise Botanic Garden (BR), Belgium.

Diatom composition for each sample was studied in LM by counting the first 500 valves observed. This allowed us to calculate the relative abundance of each taxon, including the new *Actinella* species.

RESULTS

Actinella kufferathiana Cocquyt & J.C.Taylor, **sp. nov.**

Figs 1–4

Type. D.R. CONGO • Tshopo Province, Moni River; 0°50'47.76"N, 24°32'8.34"E; sample CCA 4379 collected

by Nils Bourland on 5 Jul. 2017; holotype: slide BR 4757 (BR); the valve representing the type is illustrated here in figure 1I; isotype: slide SANDC 24-079 (SANDC).

Registration. http://phycobank.org/104059

LM description. Valves dorsiventral, heteropolar, the broadest part located in the upper halve of the valve. Valve length 23.5–75.5 µm, width 5.0–7.0 µm at the broadest part (n = 40). Dorsal margin convex, ventral margin slightly concave except for the smaller valves where three quarters of the margin, starting from the footpole, is almost straight. Headpole rounded and protracted in the larger valves, in the smaller valves the ventral side is slightly expanded. Footpole slightly protracted and not tumescent. Stria density 8–13 in 10 µm in the centre of the valve, becoming denser near the apices and up to 12–14(–16) in 10 µm (n = 40). Striae parallel mid-valve becoming

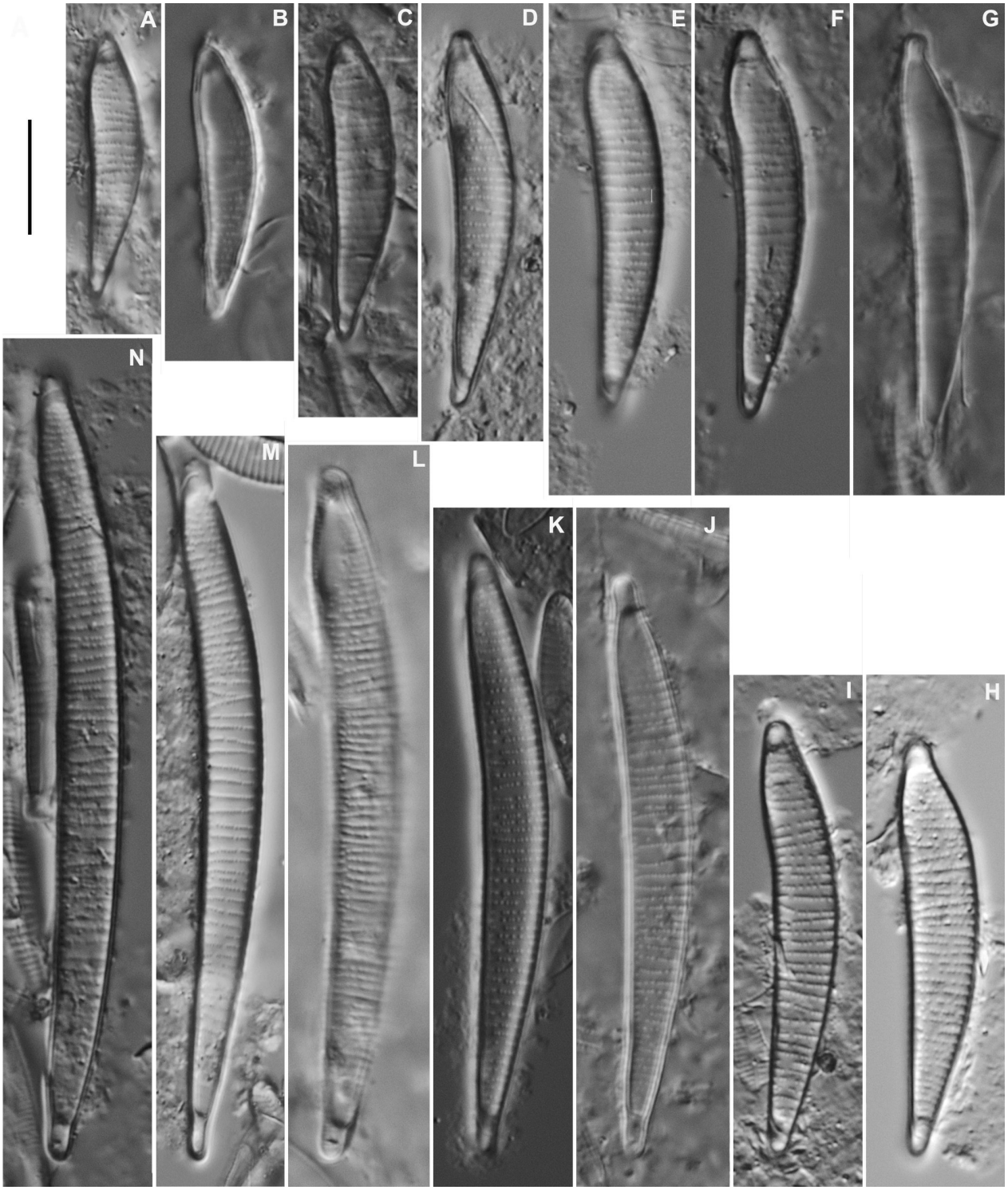

Figure 1. Light microscope micrographs of valves of *Actinella kufferathiana* sp. nov. showing the size diminution series. Valves from type material CCA 4379 from the Moni River. Type represented by Fig. 1I. Scale bar = 10 µm.

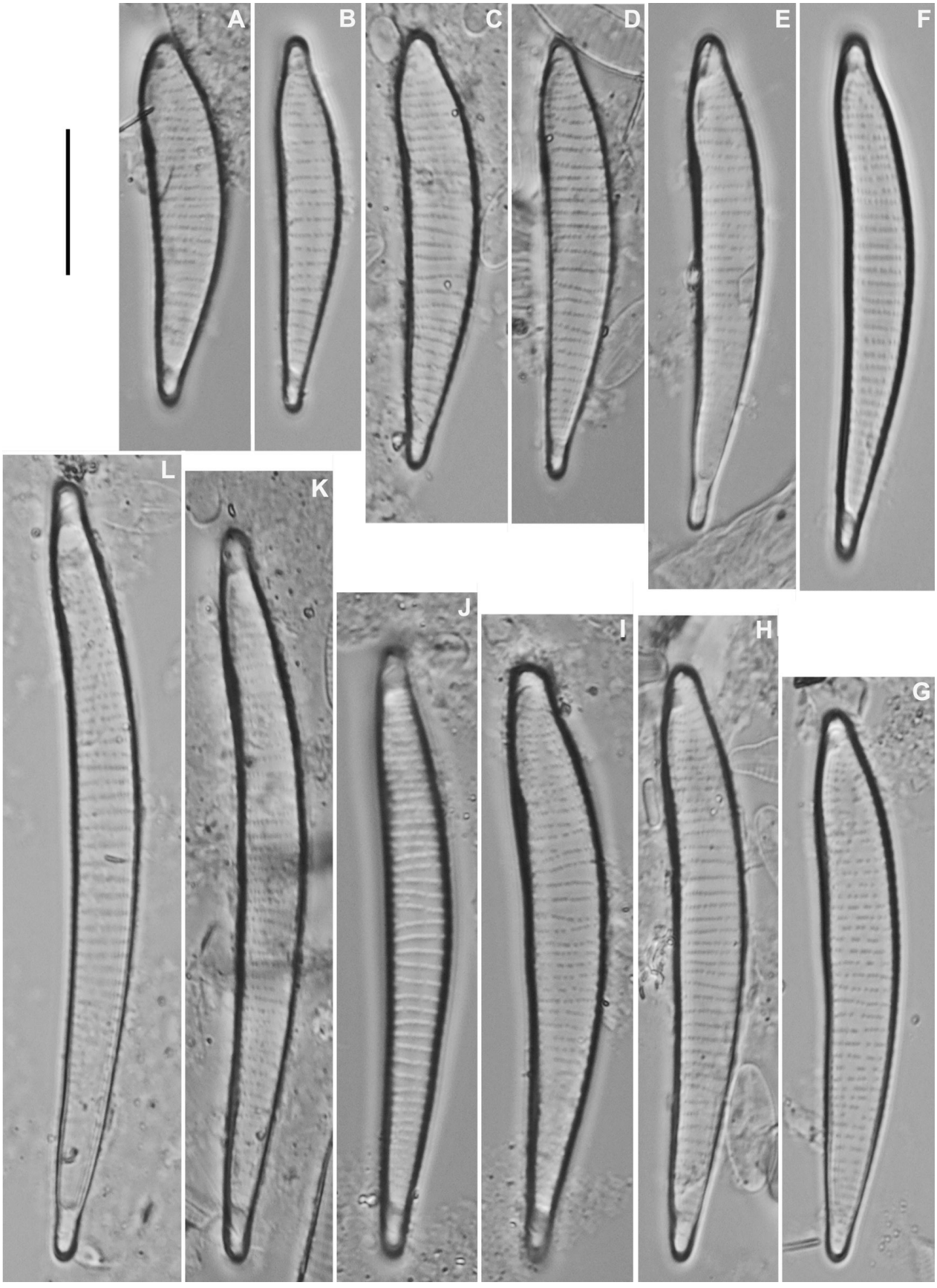

Figure 2. Light microscope micrographs of valves of *Actinella kufferathiana* sp. nov. showing the size diminution series. Valves from material CCA 4386 from the Moni River close to the type locality. Scale bar = 10 µm.

slightly radiate towards the apices but often irregular and sometimes even divergent (Fig. 1E–G). Hyaline area present near the terminal raphe fissures, less obvious on the footpole. Striae clearly punctate. Raphe located on the ventral valve mantle except for the terminal fissures, located on the valve face near the apices. Terminal raphe fissures on both apices almost reaching the dorsal margin. Apical spine on the headpole sometimes visible. Valves of the initial cell showing an irregular valve outline with a slight swelling at the mid-valve, often a characteristic of

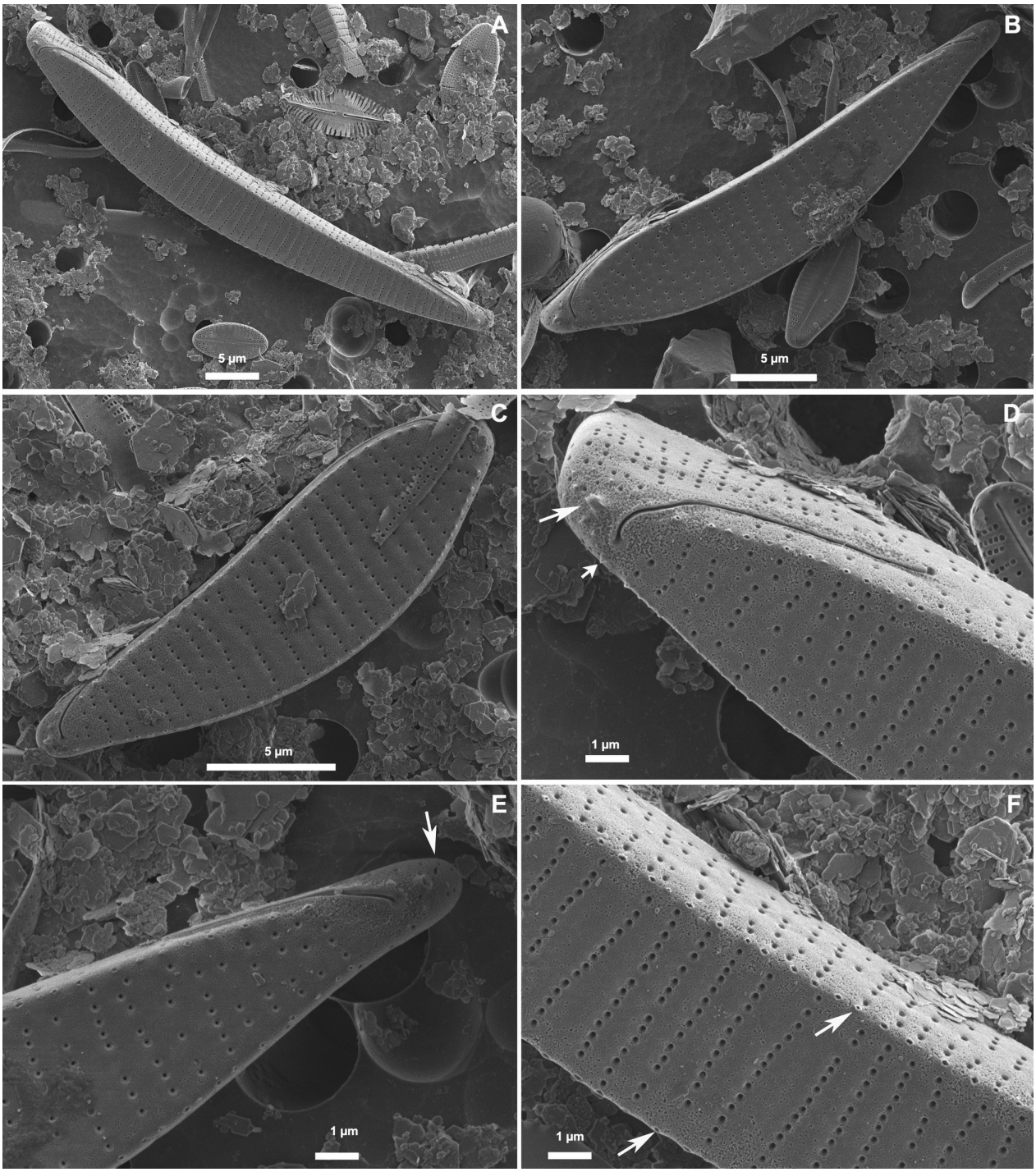

Figure 3. SEM external views of valves of *Actinella kufferathiana* sp. nov. from type material CCA 4379. **A**. Oblique view of an entire valve showing the largest part of the raphe on the mantle. **B**–**C**. Entire valve with the irregular placed areolae composing the striae. **D**. Detail of the head pole with one large spine (arrow), and short spines at the junction of the valve face and mantle showing those that are not broken (short arrow). **E**. Detail of the foot pole with a simple opening of the rimoportula on the pole valve mantle (arrow). **F**. Detail of the junction of the valve face and mantle showing the remnants of broken spines (arrows). Scale bars: A–C = 5 µm, D–F = 1 µm.

post-auxospore initial cells, and a short raphe, entirely located on the valve face (Fig. 1N). Frustules in girdle view not observed.

SEM description. Striae uniseriate, composed of 28–35 round areolae in 10 µm. Striae and areolae irregularly spaced (Fig. 3). Near the terminal raphe fissures, striae absent leaving a hyaline area on the terminal nodules. On the valve mantle, stria density similar as on the valve face except beneath the raphe slit and on the apices where stria density is much higher, reaching up to 24 striae in 10 µm (Fig. 3D). Well-pronounced spine present at both apices (Fig. 3C, D); short spines on the junction of the valve face and the valve mantle, placed at irregular distances from each other, about 12–15 in 10 µm, often broken off leaving a round hollow opening, not perforating the cell wall, smaller in diameter at the base than an areola (Fig. 3F). Raphe short, mostly located on the valve mantle. Terminal raphe fissures simple, curved at the headpole, almost reaching the entire valve width (Fig. 3A, D). Raphe slit at the footpole only slightly inflected on the valve face in smaller valves (Fig. 3B, C, E). Internally, terminal raphe endings terminating onto small, elongated helictoglossae (Fig. 4B). One rimoportula present at the head- or footpole, located on the valve mantle close to the valve face (Fig. 4B), the spine and the apex (Fig. 3E). Slightly thickened base of the mantle at the headpole but not at the footpole (Fig. 4A, B).

Distribution. This taxon is, up to now, only found in the Libongo, Moni, and Ngima rivers in the Yangambi Biosphere Reserve, D.R. Congo. Some valves were sporadically observed in the Isalowe River, close to one of its sources (samples CCA 4335, CCA 4337, CCA 4339, CCA 4340 in BR, Meise Botanic Garden) which of comparable ecology as the type locality (sandy beds, tropical rainforest of the Yangambi Biosphere Reserve).

Ecology. This species was found in pristine streams and small rivers with sandy beds in the tropical rain forest. No further ecological data were collected at the time of sampling.

Etymology. The epithet *kufferathiana* was given in honour of Dr Hubert Kufferath (1882–1957), who conducted algal research on material collected in the former Belgian Congo (van Oye 1958; Van Meel 1959).

DISCUSSION

A morphologically closely related species to *Actinella kufferathiana* sp. nov. is the tropical South American taxon *Actinella eunotioides*. Described from Jurucui Lake in Brazil, *A. eunotioides* was observed in the plankton (Hustedt 1952). The species is mentioned in the catalogue of plants and fungi from Brazil (Eskinazi-Leça et al. 2010) with a distribution of "native, not endemic; Southeast (RJ); Atlantic forest; epicontinental". The taxon observed in our material from D.R. Congo differs from *A. eunotioides* mainly in that the dimensions of the valves are much smaller, and in that *A. eunotioides* has well-developed spines on the junction of the valve face and valve mantle, which are also of higher density (Table 1). The stria density is also sightly coarser. Moreover, the raphe endings are comma-shaped in *A. eunotioides* (Kociolek et al. 2001), while only curved at the head pole and slightly inflected on the valve face in *A. kufferathiana.* The valve length of *A. eunotioides* var. *minor*, a taxon described from the Central African Republic (Fusey 1964, 1966) on the other hand, corresponds with the smallest valves observed of the new taxon but the valves of this variety are narrower and the striae are denser. The ventral margin of *A. kufferathiana* is slightly concave while the illustration of *A. eunotioides* var. *minor* given by Fusey (1966) shows a slightly convex ventral side. Moreover, in the original description of the var. *minor* the absence of spines is mentioned. Although spines are present in *A. kufferathiana*, these are small and not always discernible in LM.

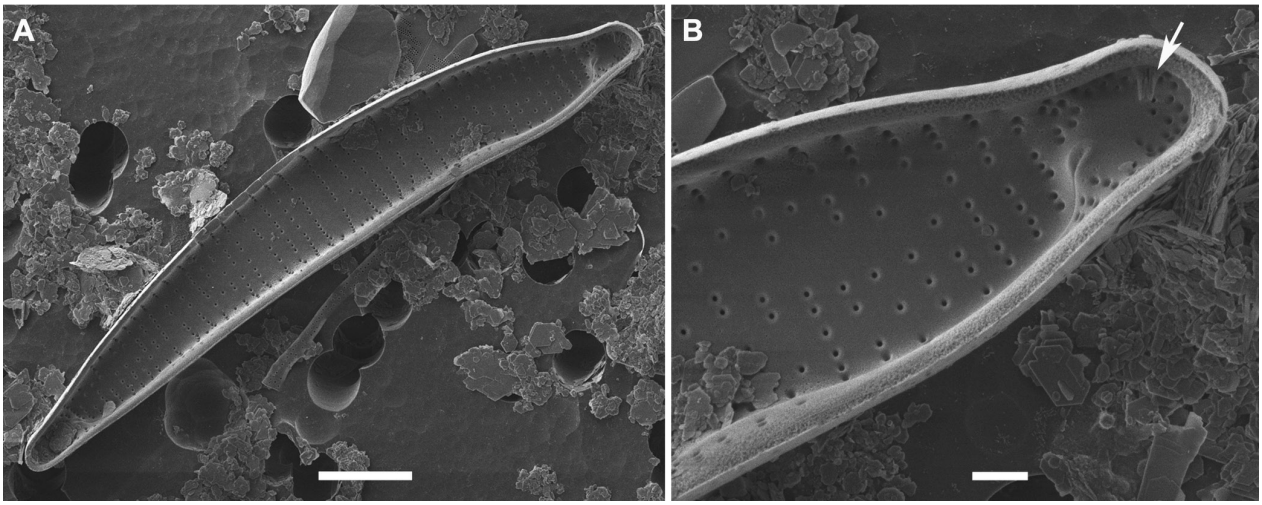

Figure 4. SEM internal views of valves of *Actinella kufferathiana* sp. nov. from type material CCA 4379. **A**. Entire valve. **B**. Detail of the head pole with small helictoglossa at the end of the terminal raphe ending and the lipped inner aperture of the rimoportula (arrow). Scale bars: A = 5 µm, B = 1 µm

Table 1. Comparison of some characteristics of *A. kufferathiana* and morphologically related taxa.

Species	Length (µm)	Width (µm)	Striae /10 µm	Spines /10 µm	Areolae /10 µm	Country type	Literature
A. kufferathiana Cocquyt & J.C.Taylor sp. nov.	23.5–75.5	5.0–7.0	8–11(–13) 12–14(–16)	12–15	13–14	D.R. Congo	This study
A. eunotioides Hust.	60–120	4.0–5.0	10–15	± 7		Brazil	Hustedt (1952)
A. eunotioides* var. *minor Fusey	30	2.5–4.5	15–16	not present		Central African Republic	Fusey (1966)
A. pereunotioides Metzeltin & Lange-Bert.	66–113	4–5	13.5–15			Venezuela	Metzeltin and Lange-Bertalot (2007)
A. disjuncta Metzeltin & Lange-Bert.	22–73	3.5–5.0	14–17			Brazil	Metzeltin and Lange-Bertalot (2007)
A. lange-bertalotii Kociolek	19–87	3.5–4.5	15–16			Suriname	Kociolek et al. (2001)
A. modesta Gerd Moser, Lange-Bert. & Metzeltin	50–90	3.0 –4.0	16–18			New Caledonia	Moser et al. (1998)
A. pseudohantzschia Metzeltin & Lange-Bert.	36–60	7.0–9.0	10–11	4–6	± 24	Brazil	Metzeltin and Lange-Bertalot (1998)

A few South American *Actinella* taxa such as *A. pereunotioides* Metzeltin & Lange-Bert., *A. disjuncta* Metzeltin & Lange-Bert., *A. pseudohantzschia* Metzeltin & Lange-Bert., and *A. lange-bertalotii* Kociolek show morphological similarities to *A. kufferathiana*. The valves of *A. pereunotioides*, described from Venezuela, South America (Metzeltin and Lange-Bertalot 2007), are significant larger (66–113 µm vs 23.5–75.5 µm) and more slender with a width of 4–5 µm (Table 1). *Actinella disjuncta* can easily be confused with *A. eunotioides.* The valve length of *A. disjuncta* (Metzeltin and Lange-Bertalot 2007) is similar to *A. kufferathiana* but the width is much smaller, 3.5–5.0 µm vs 5.0–7.0 µm, and the stria density much higher (Table 1). Moreover, the ventral valve margin is almost totally straight, lacking the slight expansion near the headpole as present in *A. kufferathiana*. The raphe endings are comma-shaped in *A. eunotioides* (Kociolek et al. 2001). *Actinella pseudohantzschia*, on the other hand, has broader valves (7.0–9.0 µm vs 5.0–7.0 µm), while the valve length and stria density are rather similar to *A. kufferathiana* (Table 1). The density of the spines is much coarser in *A. pseudohantzschia* compared to the new taxon (4–6 vs 12–15 in 10 µm).

Many other *Actinella* taxa described and reported from South America have a typical broadly rounded headpole and narrowly rounded footpole, e.g. *A. brasiliensis* Grunow, *A. guianensis* Grunow, or an undulating dorsal and ventral margin such as in *A. mirabilis* (Eulenst. ex Grunow) Grunow. These valve outlines are totally different from those of the taxon here described from the Yangambi Biosphere Reserve in the Congo Basin.

Actinella modesta Gerd Moser, Lange-Bert. & Metzeltin, described from New Caledonia (Moser et al. 1998) also shows some morphological resemblance with *A. kufferathiana.* In general, the valves of this taxon are longer (50–90 µm), and there is a marked difference in width (3.0–4.0 µm vs 5.0–7.0 µm) and the striation is denser (Table 1). Other *Actinella* taxa described from the Australian continent (Sabbe et al. 2001) have a different valve outline and are thus morphologically not closely related to *A. kufferathiana*. They differ among others in the valve shape and in the shape of the head pole which can be more clavate with much broadly rounded head pole or sub-rostrate to capitate.

Actinella sp. 2 (Siver et al. 2015: plate 1, figs P–Q) mentioned among the *Actinella* species described from fossil material from North America (Siver et al. 2015), is a small club-shaped species with some morphological resemblance to *A. kufferathiana*. This taxon, however, is much smaller (valve length 10–14 µm, width 2.5–3.5 µm vs 23.5–75.5 µm and 5.0–7.0 µm respectively) and has a higher stria density (20–22 striae in 10 µm mid-valve measured in Siver et al. (2015: plate 1, figs P–Q) as not given in the publication, vs 8–13 in 10 µm). The headpole of this *Actinella* sp. is more broadly rounded than in the described new species, and not protracted. This latter feature can also be the case in the smaller valves of *A. kufferathiana*.

There could be some doubts as to whether the structures at the edge of the valve face and mantle observed on *Actinella kufferathiana* are spines. According to Spaulding et al. (2021) "A spine is a pointed, silica extension of the valve, shorter than a seta. Spines may be solid or hollow, very long or tiny, single or many, and they may arise from different points on the valve in different taxa." We suggest that the structures observed in the new species are all broken spines, except for the ones near the apices. When looking more closely, we can see that some of these structures are not broken and indeed represent spines (Fig. 3D short arrow). The larger apical spine is in most of the observed valves also broken. But why are most of them broken? This species was found in a river with sand bed and friction of the sand on the valves could be a possible explanation. Later erosion during sample storage could also be a possible explanation, but seems less likely.

Actinella kufferathiana was never common in the samples studied. In most cases it was present with less than 1% of the total diatom community which was dominated by species of *Eunotia* Ehrenb., and small naviculoid taxa, such as *Nupela* Vyverman & Compère and *Mayamaea* Lange-Bert., with an abundance of 37.6% and 50% respectively in sample CCA 4386. Among the *Eunotia* species, *E. rudis* Cocquyt & M.de Haan, described earlier from the Libongo river in the Yangambi Biosphere Reserve (Cocquyt et al. 2016) was observed. It has also been observed from the Moni and Loeo Rivers, and rarely some valves in the Isalowe River, close to one of its sources. Both taxa, *A. kufferathiana* and *E. rudis*, as well as *Cavinula lilandae* Cocquyt, M.de Haan & J.C.Taylor (Cocquyt et al. 2013), another species described from the Yangambi Biosphere Reserve, and also observed in the studied samples, are a typical component of the diatom flora of the rather pristine, sandy small rivers and streams of the Yangambi Biosphere Reserve in the Congo Basin.

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