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RESEARCH ARTICLE

Tropical African diatoms from the *Eunotia asterionelloides* (Bacillariophyta) species complex, with descriptions of new species

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Abstract

Background and aims – Diatoms from tropical Central Africa are rarely studied and documented. Waters of the Congo Basin are often acidic and thus may be dominated by diatoms from the genus *Eunotia*, usually found in the benthos and periphyton, but rarely in the plankton. We investigated planktonic *Eunotia* species from the *Eunotia asterionelloides* complex to determine their identity and distribution.
Material and methods – Samples were collected in fishponds in the vicinity of Kisangani, D.R. Congo, from herbarium material collected from the Bonkele River, D.R. Congo, and in the Kouilou River, Republic of the Congo. These samples were prepared for diatom analysis, examined using standard methods, and documented using both light and scanning electron microscopy.
Key results – Several taxa belonging to the *Eunotia asterionelloides* complex were observed. Taxa from this complex are recorded in literature as either *E. asterionelloides* or *E. zasuminensis*. The taxon from fishponds in the D.R. Congo was identified as *E. tukanorum*, previously described from Brazil. The structure of the valve outline and raphe appear similar, particularly the raphe position and length. A second species was found on aquatic macrophyte (*Utricularia* and *Nymphaea*) herbarium specimens from the D.R. Congo and a third species from the Kouilou River in the Republic of the Congo. These latter two taxa superficially resemble *E. zasuminensis*. Although similar in valve outline, the taxa differ morphologically. The shape of the apices in larger cells is indented to a larger degree, and the raphe differs in its structure, particularly the shape of the terminal endings. The structure and position of the rimoportula also differ. Also, one of these taxa is sometimes strongly flexed around the apical axis. Based on morphology, we consider these taxa distinct from *E. zasuminensis* and from each other, and thus describe *Eunotia globicephala* sp. nov. and *Eunotia magnaparva* sp. nov.

Keywords

diatoms, D.R. Congo, Congo, *Eunotia zasuminensis*, *Eunotia tukanorum*, *Eunotia magnaparva*, *Eunotia globicephala*, new species, taxonomy, tropical Africa

INTRODUCTION

Diatom studies, and those encompassing many other groups of the aquatic tropical fauna and flora in Central Africa, are sporadic and rather rare given the scale of the territory and the number and extent of water bodies found in this region. In 2010, a large expedition carried out extensive surveys along sections of the Congo River to study the biodiversity in non-protected areas. Known

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as the Belgian-Congolese Boyekoli Ebale Congo 2010 expedition, 250 km of the Congo River and some of its tributaries between Kisangani and Bumba were surveyed (Nagy et al. 2011; Virgilio et al. 2011; Cocquyt et al. 2013). This initial expedition gave the impetus for various other studies that followed in the Democratic Republic of the Congo (D.R. Congo) and the results are gradually appearing in the scientific record. Moreover, a biodiversity centre ("Centre de surveillance de la biodiversité") was established at the University of Kisangani (https://centresurveillancebiodiversite.org) to continue this work.

Of particular importance is the study of the eunotioid diatoms from the region. The waters of the Republic of the Congo (Congo) and D.R. Congo are generally acidic "black waters" coloured by humic substances (except for the Congo River and its major tributaries). These waters are often dystrophic but may range in quality from oligotrophic to eutrophic. Acidic water bodies, as in other regions in the world, are often dominated by *Eunotia* Ehrenb. and related taxa, which are adapted for survival under these low pH conditions. Investigations of material from the D.R. Congo have already led to the description of new species within this genus, e.g. *Eunotia leonardii* J.C.Taylor & Cocquyt (Taylor et al. 2016), *E. pierrefuseyi* J.C.Taylor & Cocquyt (synonym of *E. papilio* var. *africana* (Fusey) Fusey nom. inval. and *E. fuseyi* J.C.Taylor & Cocquyt nom. illeg.) (Taylor et al. 2016), and *E. rudis* Cocquyt & M.de Haan (Cocquyt et al. 2016), and to a detailed discussion on the morphological variability within *E. zygodon* Ehrenb. (Taylor et al. 2016). Moreover, *Eunotia enigmatica* L.F.Costa & C.E.Wetzel, recently described from the Amazon Basin in South America was observed in D.R. Congo and documented by Cocquyt et al. (2019).

Most of the recent work on the diatoms of Central Africa has focussed on benthic diatom taxa; this is in contrast to many of the studies on large lakes in Africa, which focussed on the phytoplankton. In the present work, we examined samples containing planktonic *Eunotia* that form colonies in order to maintain their position in the water column. Several cells link at the corners forming irregular colonies that provide some resistance to sinking. This complex, broadly referred to as the *Eunotia asterionelloides* Hust. complex, has been discussed at length by Wetzel et al. (2010).

Wetzel et al. (2010) provided a systematic review of the *E. asterionelloides* complex and discussed the various challenges encountered while studying this complex. They provided illustrations of a population of *E. zasuminensis* (Cabejsz.) Körner from Finland, an important contribution to augment the illustrations given by Körner (1970) from a population of Lillsjön in Sweden, as the original slide and samples from Lake Zasuminskie in Poland have been lost. Wetzel et al. (2010) also briefly discussed the taxa in this complex occurring in Africa (e.g. Cholnoky 1958), expressing some doubt about the fact that Körner (1970) had synonymised *Asterionella africana* Cholnoky with *E. zasuminensis*. Given the morphology of the illustrated cells, both taxa seem to represent distinct taxa. Unfortunately, both the material and slides of Cholnoky have been lost, making it impossible to determine the identity of the taxon he observed. Wetzel et al. (2010) also expressed some doubt regarding the distribution records for *E. zasuminensis* within Africa, which the present work bears out. Records of the African distribution of the taxa in the *Eunotia asterionelloides* complex are given in Table 1. All records remain doubtful until the material is further examined/resampled.

Diatom material from herbarium sheets held at Meise Botanic Garden (BR) was investigated as part of a study to infer past water quality in the region (Okito et al. 2021). During this study, a number of cells similar in morphology to *Eunotia asterionelloides* were noted and it was decided to study them further. Sampling in the fishponds of Ngene-Ngene, Kisangani (D.R. Congo) was done for a preliminary study of their diatom community and diversity to determine the suitability of these habitats for bachelor and/or master thesis topics at the University of Kisangani (UNIKIS). These fishponds are owned by UNIKIS and are used for experiments to study fish productivity.

The broader Kouilou Plain and neighbouring Noumbi Basin in Congo were surveyed as part of an environmental impact assessment to record a freshwater baseline for the area in 2012. This area shows a high freshwater habitat heterogeneity, ranging from littoral forest and mangroves along the coast, to swamp forests, forest and savannah streams, lakes and large rivers inland. Epiphytic diatom material was collected at freshwater sites to compliment traditional water quality sampling, with the intention to use diatoms as a tool in future monitoring and evaluation studies of the area. These samples were sent to the South African National Diatom Collection (SANDC) after the studies were completed.

Based on our observations of the material from D.R. Congo and Congo, we conclude that the specimens from the Ngene-Ngene fishponds in D.R. Congo have a morphology consistent with that of *E. tukanorum* C.E.Wetzel & D.C.Bicudo. We however consider that the valves observed in the material from D.R. Congo on the herbarium material to have morphological characteristics that distinguish it from *E. zasuminensis*, including the shape of the apices, structure and position of the raphe, and the position of the rimoportula, and it is therefore described here as *Eunotia magnaparva* J.C.Taylor & Cocquyt sp. nov. Similarly, the species observed in the lake outflow in Congo can be distinguished from both *E. zasuminensis* and *E. magnaparva* and is described here as *Eunotia globicephala* J.C.Taylor, Cocquyt & G.Walsh sp. nov.

MATERIAL AND METHODS

Material used in this project was collected from Central Africa from both the D.R. Congo and the Republic of

Table 1. Distribution records within Africa for the *Eunotia asterionelloides* species complex including *Eunotia zasuminensis* and known synonyms. Figure number given if illustrations were included in the publication. These records remain unconfirmed.

Country	Publication
Botswana	Marazzi (2014)
Cameroon	Stager (1999)
Chad	Compère (1975: fig. 55)
Ethiopia	Kebede and Belay (1994)
Ghana	Foged (1966: pl. III fig. 13); Smith et al. (2015)
Ivory Coast	Iltis (1984)
Mali	Maillard (1977)
Mozambique	Compère (1975)
Namibia	Cholnoky (1966: pl. I figs 19–23)
Nigeria	Ziller and Economou-Amilli (1998); Ekhator et al. (2014)
Sierra Leone	Carter and Denny (1982: figs 54–55)
South Africa	Cholnoky (1958: pl. I figs 2–5)
Tanzania	Barker et al. (2003)
Zambia	Thomasson (1966)

the Congo. samples were collected from a variety of habitats including fishponds and the outflow of a lake. In addition, samples originally collected in 1936 and 1946 from standing waters in the D.R Congo were taken from herbarium sheets with preserved aquatic macrophyte plants. These herbarium sheets are housed at Meise Botanic Garden (BR). Details of accession numbers and sampling localities are given below.

Samples

CCA 1842. Ngene-Ngene, Kisangani, D.R. Congo, 00°35'31.1"N, 25°17'52.4"E. Pond, mixed benthic and periphytic sample collected by C. Cocquyt on 10 Feb. 2013.

CCA 1847. Ngene-Ngene, Kisangani, DR Congo, 00°35'25.5"N, 25°17'52.0"E. Pond, phytoplankton collected with a phytoplankton net (mesh width of 10 µm) by C. Cocquyt on 10 Feb. 2013.

CCA 1849. Ngene-Ngene, Kisangani, D.R. Congo, 00°35'25.5"N, 25°17'52.0"E. Pond (same pond where CCA 1847 was sampled but in another corner), phytoplankton collected with a phytoplankton net (mesh width of 10 µm) by C. Cocquyt on 10 Feb. 2013.

CCA 4510. Material collected from herbarium sheet (*Nymphaea lotus* L.) stored at Meise Botanic Garden (BR [BR0000016930063]) (https://www.botanicalcollections.be/specimen/BR0000016930063). In the water. Bamanya, near Eala, D.R. Congo, 0°00'06.0"N, 18°20'57.0"E. Collector J. Léonard on 27 Aug. 1946. Material subsampled from herbarium by C. Cocquyt.

CCA 4511. Material collected from herbarium sheet *Nymphaea lotus* L.) stored at Meise Botanic Garden (BR [BR0000016930094]) (https://www.botanicalcollections.be/specimen/BR0000016930094). Ruki river, creek with slow running water. Lolifa in the south of Eala, D.R. Congo, 0°00'06.0"N, 18°20'57.0"E. Collector J. Louis on 31 May 1936. Material subsampled from herbarium by C. Cocquyt.

CCA 4521. Material collected from herbarium sheet (*Utricularia foliosa* L.) stored at Meise Botanic Garden (BR [BR0000017402477]) (https://www.botanicalcollections.be/specimen/BR0000017402477). Large mats in front of water meadow with stands of *Cyperus pectinatus* Vahl and *Panicum parvifolium* Lam., sometimes mixed. Bonkele River between Bamania and Ilenge near Eala, D.R. Congo, 0°00'06.0"N, 18°20'57.0"E. Collector J. Léonard on 6 Nov. 1946. Material subsampled from herbarium by C. Cocquyt.

13-335 (Aqua 32). Confluence of outflow from Lake Nanga and the Kouilou River, Republic of the Congo, 4°19'37.9"S, 11°52'01.0"E. Kouilou River, periphytic sample from aquatic macrophytes, sample collected by M. Jonker on 24 Jul. 2012.

Sample processing and observation

Samples from the Ngene-Ngene ponds were fixed in situ in a 20% v/v final concentration ethanol solution. Small parts of the macrophytes from the herbarium sheets were removed without damaging the integrity of the specimen. Material from these samples was cleaned using hydrogen peroxide (30%), rinsed three times with distilled water, and mounted in Naphrax®.

Samples from the Kouilou River, Congo, were digested with potassium permanganate for 24 hours, then cleared with boiling hydrochloric acid. Clean samples were rinsed 3–4 times with distilled water and aliquots were mounted on slides using Pleurax.

Light microscopy (LM) was carried out both at Meise Botanic Garden and the North-West University with an Olympus BX51 microscope and a Nikon 80i respectively, both equipped with Normaski differential interference contrast optics (DIC) and a 100× objective

with a numerical aperture of 1.40. Light micrographs were taken with an Olympus UC30 digital camera and Nikon DSF1. Aliquots of the cleaned material were filtered through 1 µm isopore polycarbonate membrane filters (Millipore®) and mounted on aluminium stubs. After air drying, the material on the stubs were sputter-coated with 50 nm of gold (Balzers Unions SCD 020) and studied with a JEOL JSM-71000 FLV Field Emission Scanning Electron Microscope (SEM) operating at 1 kV and 6.9 mm working distance. Additional SEM images were obtained using a FEI QUANTA 200 Field Emission Scanning Electron Microscope operating at 10 kV and 10 mm working distance. For *Eunotia magnaparva*, 132 valves were measured using LM and 14 using SEM, while for *E. globicephala*, we measured 18 valves using LM and 13 using SEM. Stria density was determined both near the apices and in the mid-valve. Terminology specific to *Eunotia* followed that used by Mayama (2001).

TAXONOMIC TREAMENT

Eunotia magnaparva J.C.Taylor & Cocquyt, **sp. nov.**
Figs 1–4

Type. D.R. CONGO • Province of Équateur, Bonkele River, between Bamania and Ilenge near Eala; 0°00'06.0"N, 18°20'57.0"E; sample CCA 4521; holotype: BR, slide BR 4755, the valve representing the type is illustrated in Fig. 1Y; isotype: SANDC, slide SANDC–23-154.

LM description. Valves linear, arcuate, with considerable morphological variance over cell cycle. Ventral margins convex, dorsal margins concave. Apices distinctly capitate in large cells, subcapitate in smaller cells, bluntly rounded in the smallest cells. Apices rounded to weakly cordiform, slightly asymmetrical, expanded ventrally, indented forming a cleft in larger cells. Terminal nodule barely visible. In girdle view cells weakly heteropolar, often exhibiting some degree of flexing around the apical axis. Larger cells constricted mid-valve. Valve length 8.5–40.0 µm, width 2.0–4.0 µm in the middle, and 2.5–8.0 µm at the poles. Striae visible under LM, 16–24 in 10 µm. Striae interrupted at various points around the mid region on the apical axis of the valve, especially near to the apices in larger cells but throughout the valve in smaller cells.

SEM description. Striae uniseriate externally and internally, composed of round areolae, occluded externally by hymenes (Fig. 3D). Striae continuing onto valve mantle near the apices becoming discontinuous and offset near the centre of the valve (Fig. 2C). Externally striae

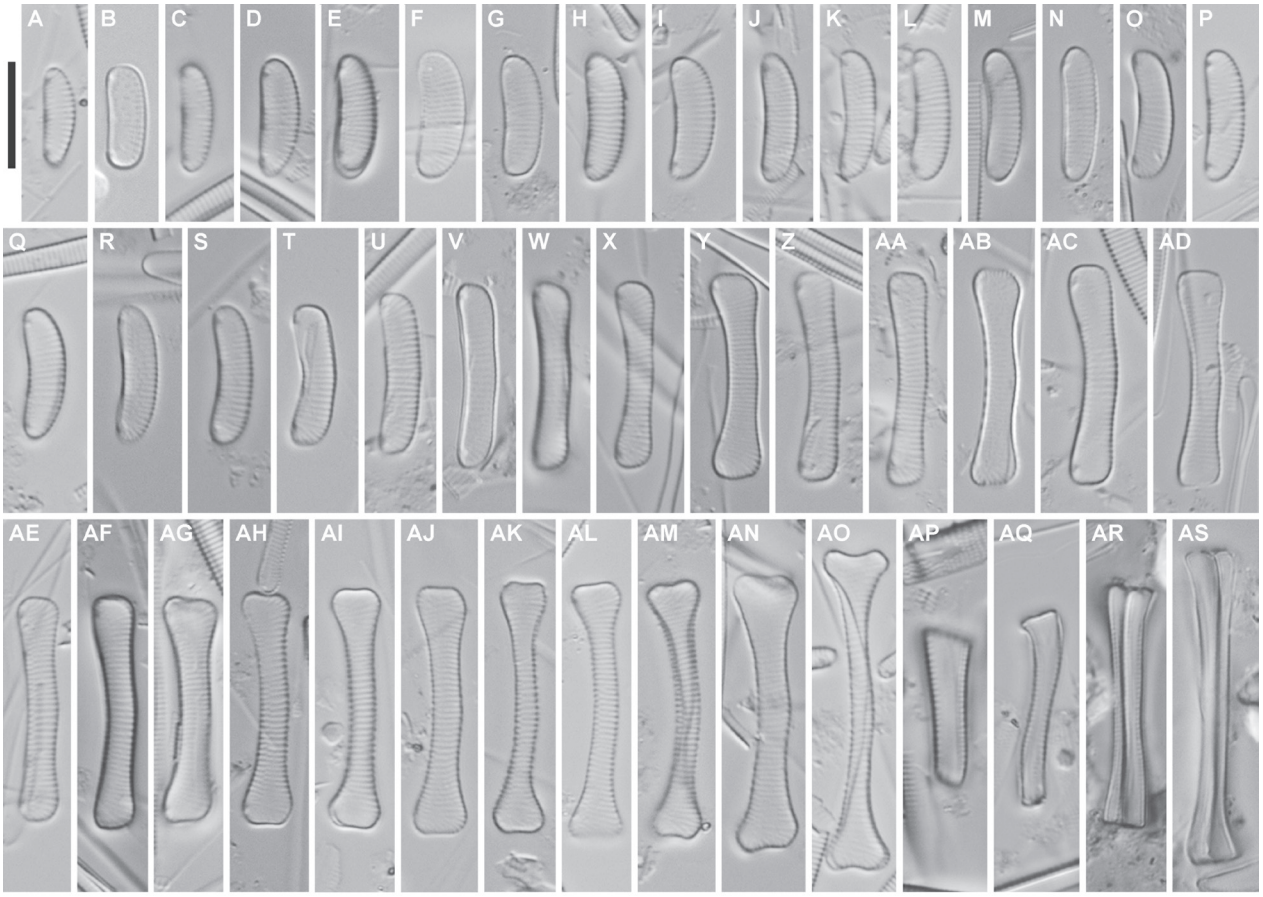


Figure 1. *Eunotia magnaparva* sp. nov., LM from type material, sample CCA 4521. Type represented by Fig. 1Y. **A**–**AO**. Size diminution series, valve view. **AP**–**AS**. Girdle view. Scale bar = 10 µm.

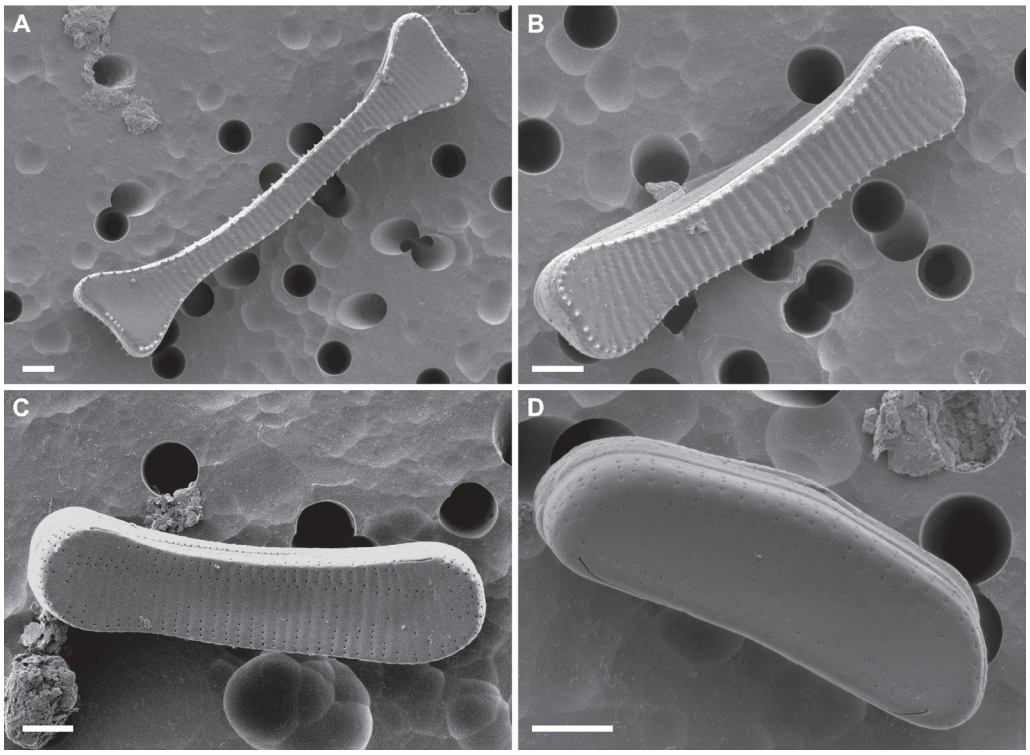


Figure 2. *Eunotia magnaparva* sp. nov., SEM from type material, sample CCA 4521. **A**. External valve view of larger cell, note the cordiform apices with discontinuous striae. **B–C**. External valve view of cells medium size, note the reduced raphe in C and the slightly more pronounced raphe in D. Similarly, valve B has marginal spines while in valve C spines are almost absent. **D**. Cell representing the smallest extremity of the cell cycle, note pronounced raphe endings extending onto the valve face and interrupted striae. Scale bars = 2 μm.

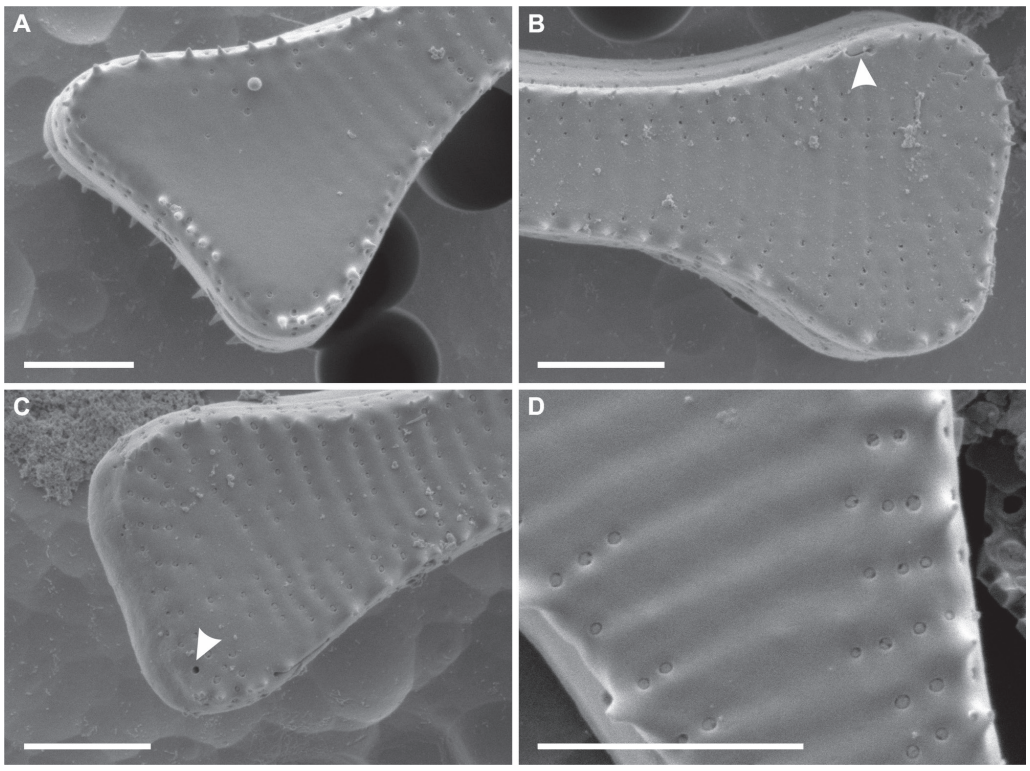


Figure 3. *Eunotia magnaparva* sp. nov., SEM from type material, sample CCA 4521. **A–C.** External view of valve apices showing pattern of striation, the highly reduced raphe structure and the discontinuous striae both at the apices in A and through the cell in B. **B**. External valve view, note the reduced raphe (arrowhead). **C**. Note the external opening of the rimoportula (arrowhead). **D.** External valve view showing areolae occluded by hymenes, note the discontinuous striae. Scale bars = 2 μm.

interrupted at the margin by a slightly thickened silica ridge. Small, conical marginal spines present in some cells (Fig. 2A, B), especially prominent at the valve apex (Fig. 2A), sometimes reduced and barely visible or absent on smaller cells (Fig. 2D). Striae evenly spaced, parallel at the centre of the valve becoming radial at the apices (Fig. 3C). Raphe slits visible externally and internally, sometimes extending onto the valve face and terminating very close to the valve apices (Fig. 3D). Raphe slit often, though not always, greatly reduced externally in larger cells (Fig. 3B). Valve mantle shallow, at 90° to the valve face. Internally, terminal raphe branches terminating onto small, rather elongated but well-defined helictoglossae in smaller cells (Fig. 4D, E), absent in larger cells (Fig. 4F). Girdle bands open with one row of occluded areolae (Fig. 4B, C). One rimoportula present on each valve, always located close to the terminal raphe ending at the ventral part of one of the apices (Fig. 4F).

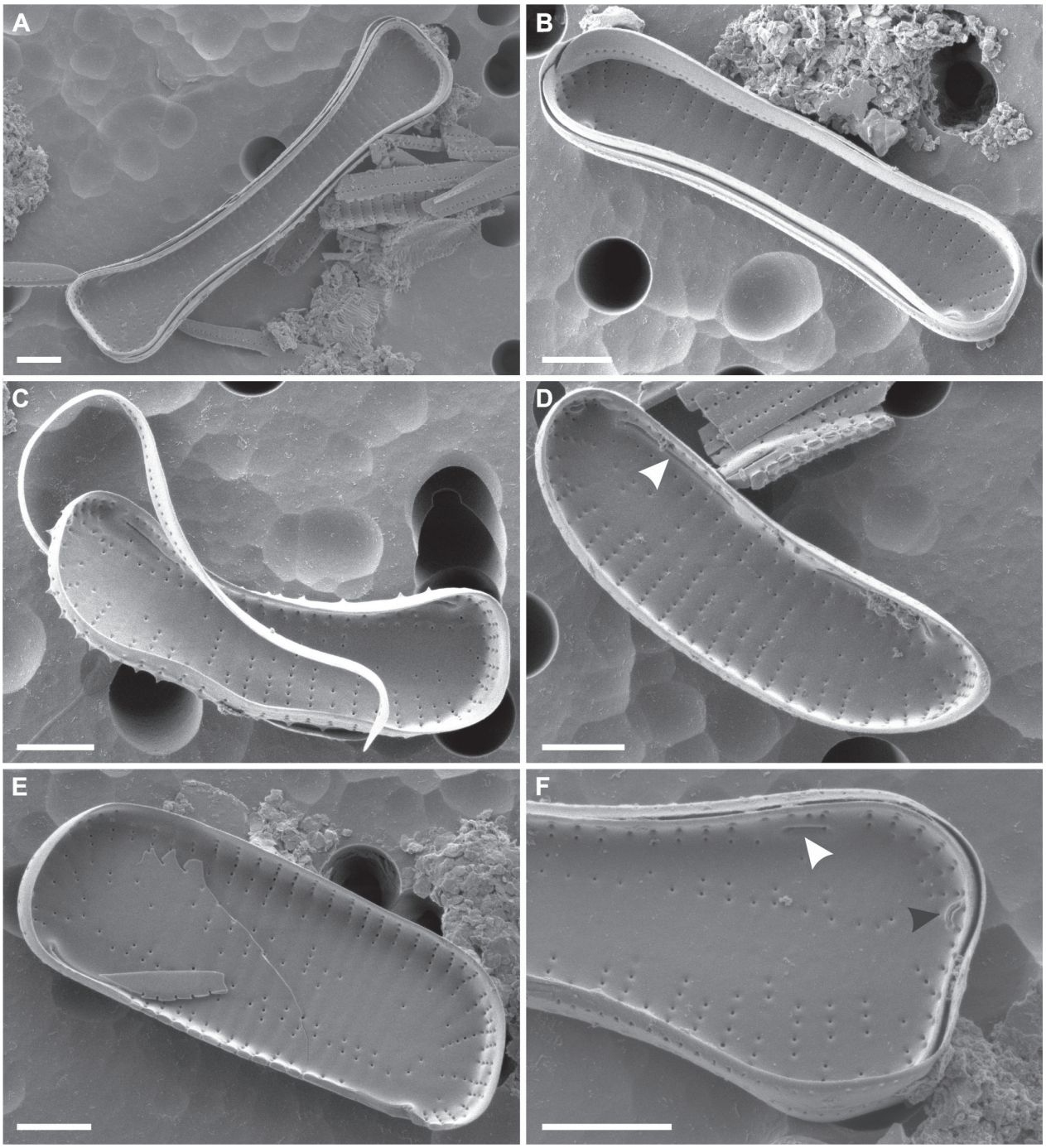


Figure 4. *Eunotia magnaparva* sp. nov., SEM from type material, sample CCA 4521. **A**–**F**. Internal view of valve showing the internally unoccluded areolae, striae discontinuous over the valve face. **D**. Structure of the raphe (arrowhead), terminating in a somewhat reduced helictoglossa. **F**. Highly reduced raphe (white arrowhead), rimoportula present near the ventral side (black arrowhead). Scale bars = 2 μm.

Distribution. The herbarium sheets sampled for diatom investigation (CCA 4510, 4511, 4521) on which *Eunotia magnaparva* sp. nov. was observed all came from the region around Eala, in the Province of Équateur, D.R. Congo. So far, this taxon was not observed in other materials from D.R. Congo.

Ecology. This species was found on *Utricularia* and *Nymphaea lotus*. No further data was collected at the time of sampling.

Etymology. The epithet *magnaparva* refers to the variability of this species in terms of size over the cell cycle, magna being large and parva small, reflecting that the cells range from particularly small to comparatively large.

Registration. http://phycobank.org/104056

Notes. The diminution series as presented in Fig. 1 shows a notable degree of difference in the cell morphology over the cell cycle. These differences are apparent in the shape of the apices, the cell length to breadth ratio, as well as in the structure of the raphe. The width of the cells dramatically decreases with an increase in cell length. In larger cells the raphe may be highly reduced and only present at the junction of the valve face and mantle (Fig. 3A, B), while in smaller cells the raphe is longer and extends onto the valve face (Fig. 2D).

Eunotia globicephala J.C.Taylor, Cocquyt & G.Walsh, **sp. nov.**

Figs 5–6

Type. REPUBLIC OF THE CONGO • Confluence of outflow from Lake Nanga and the Kouilou River; 4°19'37.9"S, 11°52'01.0"E; sample 13-335; holotype: slide SANDC–13-335 (SANDC), the valve representing the type is illustrated in Fig. 5F; isotype: slide BR 4756 (BR).

LM description. Valves linear, arcuate, morphologically variable over cell cycle. Ventral margins convex, dorsal margins concave. Apices distinctly capitate in large cells, subcapitate in smaller cells. Apices rostrate to weakly cordiform, strongly asymmetrical in larger cells, expanded ventrally, slightly indented in larger cells forming a shallow cleft. Cells planar to highly flexed round the apical axis, flexing may reach 90° (Fig. 5G). Terminal nodule barely visible. Cells weakly heteropolar in girdle view, most often exhibiting flexing around the apical axis. Cells constricted mid-valve. Valve length 25–40 µm, width 1.0–2.5 µm in the middle, and 3.0–4.5 µm at the poles. Striae visible under LM, 15–22 in 10 µm.

SEM description. Striae uniseriate externally and internally, composed of round areolae, occluded externally by hymenes. Striae continue onto valve mantle near the apices, becoming discontinuous close to the apices (Fig. 6F). Striae interrupted externally at the

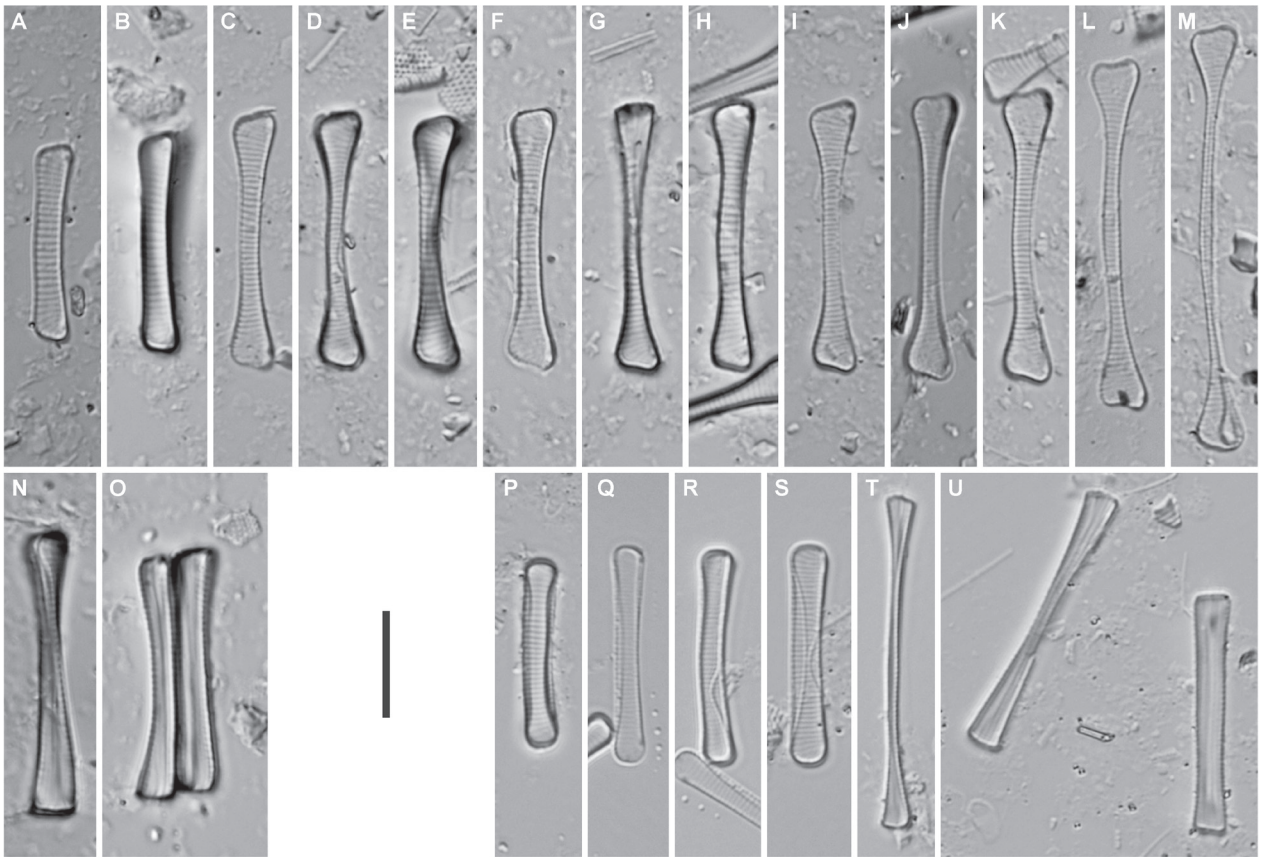


Figure 5. *Eunotia globicephala* sp. nov., LM from type material, sample 13-335. Type represented by Fig. 5F. **A**–**M**. Size diminution series, valve view. **N**–**O**. Girdle view. *Eunotia tukanorum*, LM from sample CCA 1847. **P**–**S**. Diminution series, valve view. **T**–**U**. Girdle view. Scale bar = 10 µm.

margin by a slightly thickened silica ridge. Striae evenly spaced, parallel at the centre of the valve becoming radial at the apices (Fig. 6A, B). Raphe slits clearly visible externally and internally, extending onto the valve face terminating very close to the valve apices (Fig. 6D). Valve mantle shallow, at 90° to the valve face (Fig. 6E). Conical spines present at valve face/mantle junction in larger cells (Fig. 6C, E), absent in smaller cells (Fig. 6A). Internally,

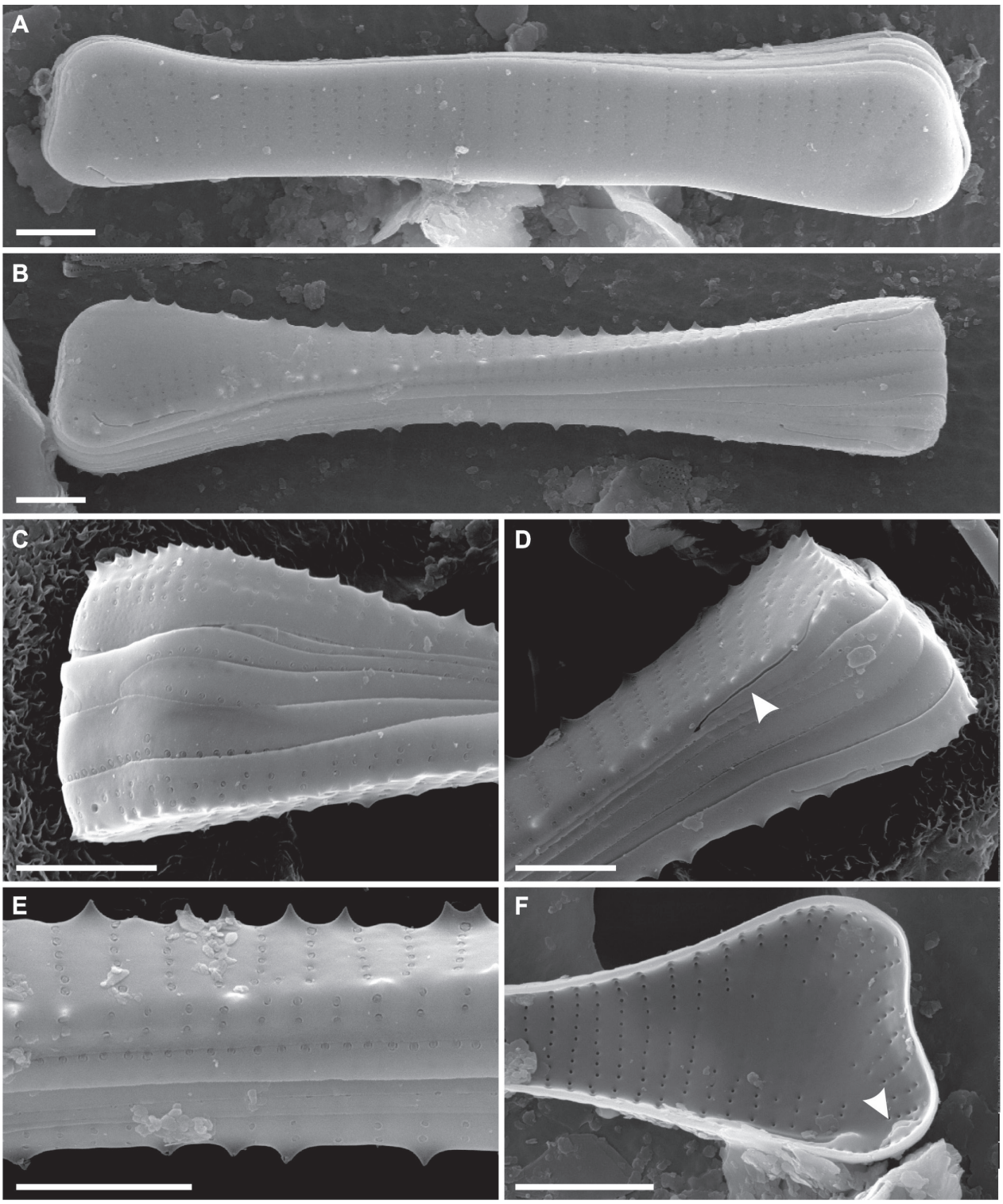


Figure 6. *Eunotia globicephala* sp. nov., SEM from type material, sample 13-335. **A**. External view of valve of smaller cell, no marginal spines present. **B**. External view of valve of large cell, flexed 90° around the apical axis. **C**. External view of dorsal margin and apices, note the marginal spines and open copulae. **D**. External view of the ventral mantle showing the position of the raphe (arrowhead). **E**. External view of mid-valve showing the conical marginal spines and areolae occluded externally. **F**. Internal view of the valve apex showing helictoglossa and rimoportula (arrowhead) located very close to the ventral margin. Scale bars = 2 μm.

terminal raphe branches terminating onto small but well-defined helictoglossae (Fig. 6D). Girdle composed of several open, perforated copulae, bearing one row of occluded areolae (Fig. 6C). One rimoportula present, always located close to the terminal raphe ending at the ventral part of one of the apices (Fig. 6F).

Distribution. At present only known from the type locality.

Ecology. This species was collected at the confluence of Lake Nanga and the Kouilou River. The site is situated in a seasonally flooded, forest-grassland-wetland mosaic in the Kouilou plain. Water at the time of sampling was slightly turbid with circumneutral pH (6.94), low conductivity (74.8 $\mu S.cm^{-1}$), and moderate oxygen levels (53.8% saturation).

Etymology. The epithet *globicephala* refers to the genus of the long-finned pilot whale [*Globicephala melas* (Traill, 1809)] as the apices of *Eunotia globicephala* bear a strong resemblance to the head and rostrum of this whale when seen in profile.

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Notes. In the *Eunotia globicephala* population observed from the Republic of the Congo cells can be planar to highly flexed around the apical axis, flexing may reach 90° (Figs 5G, N, 6B). Flexing was only observed in larger cells. This twisting may have an advantage for colony formation in that twisted cells will allow for the formation

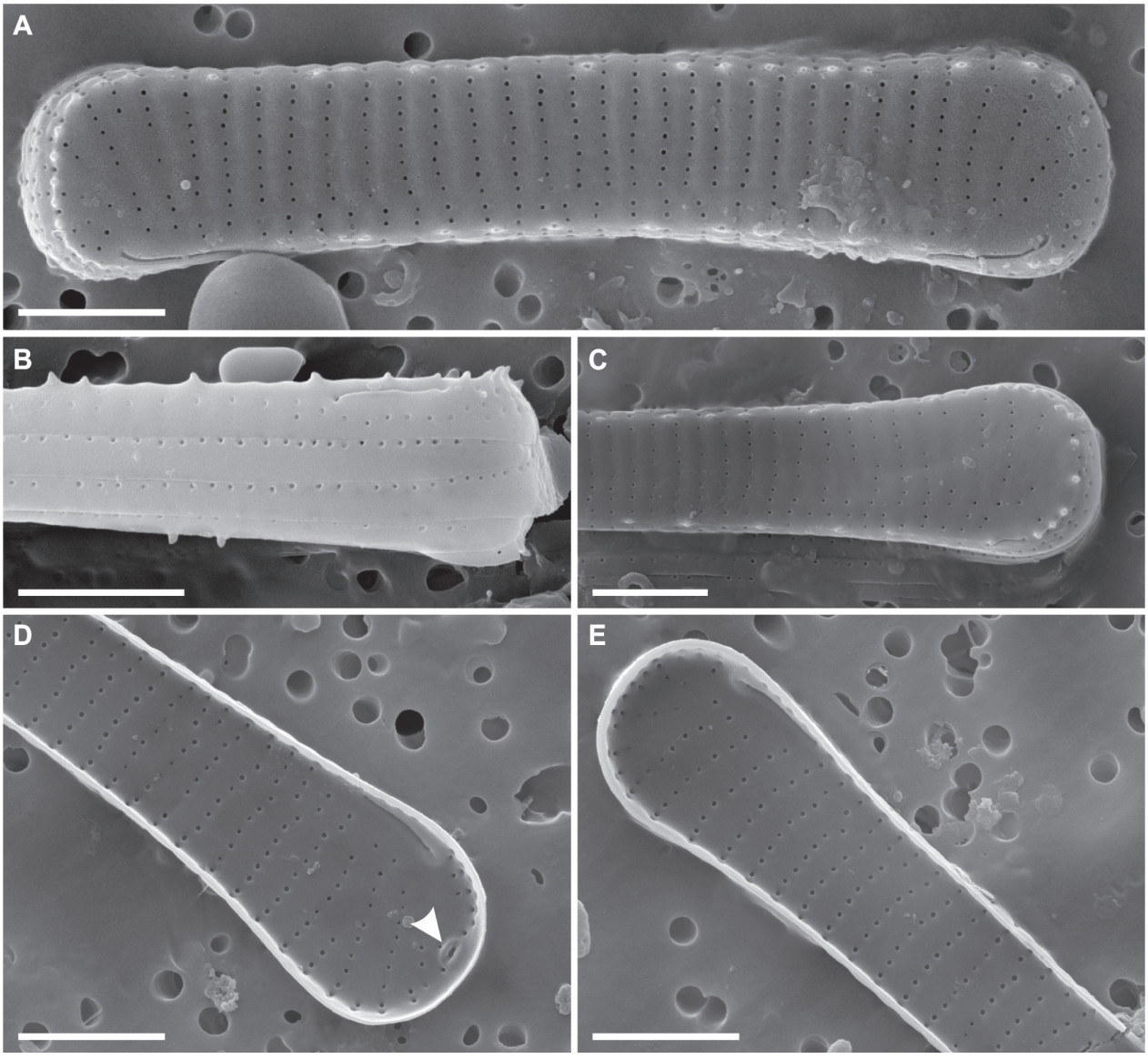


Figure 7. *Eunotia tukanorum*, SEM from sample CCA 1847. **A**. External view of valve of smaller cell, marginal spines present, raphe extending onto the valve face. **B**. External view of valve mantle and copulae (perforate), note the reduced raphe restricted to the mantle and the conical marginal spines. **C**. External view of apex, note the highly reduced raphe on the ventral margin between the valve face and mantle. **D**. Internal view of apex showing the rimoportula located close to the centre of the apex (arrowhead). **E**. Internal view of apex showing small helictoglossa and raphe restricted almost entirely to the margin. Scale bars = 2 µm.

of rather more complex three-dimensional net-like structures rather than flattened colonies as are found in *Asterionella formosa* Hassall. As cell attachment usually is in the form of a mucus pad forming on the cell apex near to the ventral margin a cell twisted through 90° would provide an attachment site that would be perpendicular to that cell. A three-dimensional net-like structure could provide greater resistance to sinking and help maintain the position of the colony within the euphotic zone. Interestingly, the raphe never seems to be reduced in this taxon as is the case in *E. zasuminensis* and *E. magnaparva*.

Eunotia tukanorum C.E.Wetzel & D.C.Bicudo

Fig. 7

Samples. The cells observed from CCA 1847 and CCA 1849 have morphological characteristics that we consider to be consistent with the description of *E. tukanorum* as presented in Wetzel et al. (2010). We include a description of the population from D.R. Congo.

LM description. Valves linear, arcuate with parallel margins. Ventral margins concave, dorsal margins convex. Apices slightly protracted and slightly expanded, rounded to bluntly rounded to slightly rectangular. Helictoglossae on the terminal nodules visible under LM as a small dot on the ventral margin. In girdle view, cells thin, isopolar, constricted in the middle portion. Striae parallel and equidistant, becoming radial towards the apices and always visible in LM. Valves measured in the present study (n = 18) with the following dimensions: length 19.5–31.0 µm, width 2.0–3.0 µm in the centre of the valve, and 2.5–3.0 µm at the apices. Stria density 20–22 striae per 10 µm in the centre of the valve. Valves measured from the type population (Wetzel et al. 2010) had the following dimensions: length 9–30.5 µm, width 2.5–3.5 µm in the centre of the valve, and 3.0–3.5 µm at the apices. Stria density 23–27 striae per 10 µm in the centre of the valve. Observed cells with characteristics consistent in general with the type population with the exception of the stria densities. Costa et al. (2017), however, documented a population from Brazil with lower stria density and consistent with the striae counts obtained in the present study (Table 1).

SEM description. Externally and internally, areolae composed of small round pores (Fig. 7A), extending across the valve face and onto the mantle (Fig. 7B). Valve face bordered by small spines, especially at the apices (Fig. 7C). Raphe almost entirely restricted to the valve mantle on larger cells (Fig. 7B) and often highly reduced (Fig. 7C) although extremely variable and occasionally continuing onto the valve face at the terminal ends (Fig. 7A), more often observed in smaller cells. Valve mantle shallow and perpendicular to the valve face (Fig. 7B). Internally, raphe distally terminating onto a small helictoglossa (Fig. 7C), restricted to the valve mantle. One small rimoportula found at one end of the valve close to the centre of the apex (Fig. 7E).

Ecology. The fishponds in which the species was found are used to farm *Tilapia* and *Clarius*. Water slowly enters the ponds to keep them full and the fish are fed. Concurrently with the collection of sample CCA 1847, the following parameters were measured: water temperature 30°C, electrical conductivity 16 µS.cm^{-1}, dissolved oxygen concentration 0.88 mg.L^{-1}. Parameters measured for sample CCA 1849 were: water temperature 29.9°C, electrical conductivity 16 µS.cm^{-1}, dissolved oxygen concentration 1.81 mg.L^{-1}.

Notes. Wetzel et al. (2010) cite several defining characteristics for this taxon including the valve outline, weakly silicified delicate cells, hyaline striae, and thin cells in girdle view. The taxon from the D.R. Congo identified as *Eunotia tukanorum* and illustrated in the present study has characteristics consistent with these.

DISCUSSION

Eunotia globicephala sp. nov. and *E. magnaparva* sp. nov. are morphologically most closely related to *E. zasuminensis* described originally from a Polish lake (Cabejszekówna 1937). The type material of *E. zasuminensis* has been lost. However, Wetzel et al. (2010) examined and illustrated a population of this taxon from Finland using both LM and SEM observations. Using these observations, we can differentiate both novel taxa from *E. zasuminensis* based on the following morphological characteristics (summarised in Table 2): the position of the raphe is closer to the apices than in *E. zasuminensis*, the raphe is consistently longer in *E. globicephala* than that of *E. zasuminensis*. The raphe of *E. magnaparva* is rather variable but longer in smaller cells. The shape of the apices differs from *E. zasuminensis*, cordiform with a larger swelling of the apex to the ventral side in *E. globicephala* and cordiform with a deeper cleft in *E. magnaparva*. In *E. zasuminensis*, the rimoportula is located in the cleft of one bilobate apex, whereas in *E. globicephala* and *E. magnaparva* the rimoportula is located towards the ventral margin near the helictoglossa. *Eunotia zasuminensis* has twisting around the apical axis whereas, however, in *E. globicephala* the degree of twist (up to 90° in some instances; Fig. 5G, N) seems to be higher. The helictoglossa is much reduced in *E. zasuminensis* and much more pronounced in both *E. globicephala* and *E. magnaparva*. All three taxa have a slight swelling of the valve in the centre of the cell as well as some degree of dorsiventral symmetry.

Eunotia is a genus in which considerable morphological variation can be noted over a diminution series from larger cells to smaller cells. One such example, *Eunotia zygodon,* is illustrated and discussed by Taylor et al. (2016). In the *Eunotia asterionelloides* complex, Wetzel et al. (2010) also illustrate similar distinct changes in size over the cell cycle of *E. tukanorum*. At the extremes of such a diminution series, cells may appear entirely distinct from each other in terms of apex shape, symmetry, and a variety of other characters. For this reason, we illustrate an exhaustive

Table 2. Morphological characteristics of some taxa from the *Eunotia asterionelloides* species complex. The new taxa are indicated in bold.

Taxon	Valve length (µm)	Valve breadth (centre) (µm)	Valve breadth (apices) (µm)	Striae/10 µm	Apex shape	Raphe reduced in large cells?	Reference
Eunotia zasuminensis	25.0–38.0	1.0–2.0	2.5–4.0	23–37	Cordiform, symmetrical	Yes	Wetzel et al. (2010)
Eunotia magnaparva	**8.5–40.0**	**2.0–4.0**	**2.5–8.0**	**15–24**	**Strongly cordiform**	**Yes**	**This study**
Eunotia globicephala	**25.0–40.0**	**1.0–2.5**	**3.0–4.5**	**15–22**	**Cordiform, asymmetrical**	**No**	**This study**
Eunotia tukanorum	9.0–30.5	2.5–3.5	3.0–3.5	23–27	Rounded to slightly flattened	Yes	Wetzel et al. (2010)
Eunotia tukanorum	14.5–28.0	2.5–3.5	–	19–22	Rounded to slightly flattened	–	Costa et al. (2017)

diminution series of *Eunotia magnaparva* (Fig. 1A–AS) based on as many cells as we could observe from a single sample. In addition, other samples were observed and comparable results were obtained (not illustrated here). These additional samples demonstrated a similar size range and continuity from larger to smaller cells. This series is also supported by a selection of SEM micrographs (Figs 2–4) that show what we consider to be key defining characteristics for this taxon. We have paid attention to the striation of the apices in terms of angle, curvature, and how the striae are interrupted, all of which remain constant over the cell cycle. In addition, the number and position of the rimoportulae remains constant. The occlusion of the round areolae by hymenes is also consistent in structure between the large and smaller cells.

As to why raphe length differs over the cell cycle, it could be surmised that this phenomenon is connected to the amount of contact the cells will have with a substrate. The majority of taxa within the *Eunotia asterionelloides* complex are considered planktonic with cells linking to form branched chains that remain suspended in the water column (Wetzel et al. 2010). The raphe is a structure generally accepted to be associated with diatom motility, while suspended in the phytoplankton cells would need to have little or no motility. As the cell cycle advances and cells are reduced in size, surface area and thus buoyancy would decrease. As buoyancy decreases, cells will be more likely to settle out of the water column, come into contact with a substrate and at this point motility may be useful in order that cells could then move towards the light. Regarding the presence of a raphe in planktonic *Eunotia*, Furey (2021) discusses at length the almost complete reduction of the raphe in some taxa. Furey (2021) postulates that although movement in such environments would be minimal the presence of a raphe indicates that movement would be possible. As discussed above, this becomes particularly important for smaller (shorter) cells which may gain a competitive advantage by having a larger raphe system and being more motile. Although the type sample (CCA 4521) for *Eunotia magnaparva* was collected from standing waters, it seems that the substratum (in this case *Utricularia*) possibly provided a habitat for small motile forms of this species. This would have to be tested though by sampling true open water populations vs near shore or benthic populations. There are enough stable characters (e.g. stria density, structure and position of the rimoportulae, structure of the striae at the apices, disjunct striae, etc.) to demonstrate that all the cells in the diminution series belong to a single species. If, for example, the cell illustrated in Fig. 1A and Fig. 1AO had been observed in separate samples, it would have been easy to assume that these are distinct species not related to each other, especially with LM observations.

Collecting diatoms from herbarium samples has proved interesting in the past for reconstructing environmental conditions (Yallop et al. 2009; Okito et al. 2021) but as the present study has shown they may also be a valuable source of diatom samples for taxonomic work. Dried herbarium sheets would in most cases provide an ideal environment for diatom preservation as the silica cell walls of the diatoms on dried material can persist indefinitely in a protected environment.

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