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REGULAR PAPER

New and interesting *Eunotia* (Bacillariophya) taxa from the Democratic Republic of the Congo, tropical central Africa

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Background and aims – Large-celled *Eunotia* species from tropical central Africa are documented in order to define morphological variation within species and to describe new or poorly documented taxa.
Methods – Samples were collected from the Congo River between Kisangani and Bumba in the Democratic Republic of the Congo. These samples were prepared for diatom analysis and examined according to standard methods and documented using both light and scanning electron microscopy.
Key results – A single population of *Eunotia zygodon* is used to demonstrate the high degree of change in gross cell morphology during its life cycle, changes previously observed in cultures of this genus. Cells of this taxon from each extremity of the reproductive cycle could be, and probably often were, placed in discrete taxa during sample analysis, especially as this taxon occurs rather infrequently. We also describe *Eunotia leonardii* as new to science. It has an undulate valve margin and is closely related to *Eunotia serra* and *Eunotia georgii* but differs in respect to overall cell size and the structure of the terminal raphe endings. The bi-undulate *Eunotia papilio* var. *africana* is raised to the rank of species and named *Eunotia fuseyi* as it is clearly a discrete taxon from *E. papilio* s. str.

Key words – Diatoms, D.R. Congo, *Eunotia fuseyi*, *Eunotia leonardii*, *Eunotia zygodon*, new species, taxonomy, tropical Africa.

INTRODUCTION

Reports on benthic diatoms from the Democratic Republic of the Congo (D.R. Congo) have been rather limited in the past with the notable exception of Zanon (1938), Kufferath (1948, 1956a, 1956b), Hustedt (1949), Cholnoky (1970), Compère et al. (1989), Compère (1995) and Golama (1996) and with several recent papers as discussed in Cocquyt & Taylor (2015). Recently, intensive studies, including diatom collection, were initiated in the Congo Basin downstream Kisangani, thanks to the Boyekoli Ebale Congo 2010 expedition, organised by the Congo 2010 Consortium composed of the University of Kisangani, the Royal Museum for Central Africa, the Royal Belgian Institute of Natural Sciences and the National Botanic Garden of Belgium – now Botanic Garden Meise (e.g. Cocquyt et al. 2013, Cocquyt & Taylor 2015, Karthick et al. 2016, Taylor et al. 2016). After this initial large scale effort, several smaller field expeditions, during which diatom samples were also collected, were undertaken. The material used for the present studied was sampled during such a campaign in the frame of the COBAFISH project (Congo Basin: From carbon to fishes) financed by the Belgian Science Policy.

Members of the genus *Eunotia* Ehrenb. form a large percentage of the diatom populations from tropical African waters of lower pH as observed during our investigations. *Eunotia* taxa have been historically subject to 'force-fitting' in order to ascribe extant names to taxa encountered in the tropics. Often, much confusion arises with taxa that are either rare or that have seemingly obvious characteristics such as multiple dorsal undulations (e.g., *Eunotia serra* Ehrenb.). The genus *Eunotia* is also problematic as its valves may vary quite widely in structure over the cell cycle with the gametangial mother cell and the first vegetative cell having very little similarity in terms of valve outline (e.g. *E. tropica* Hust., Mayama 1995). Although this phenomenon has been demonstrated on a number of occasions in cells grown in culture, the comparative rarity of large-celled *Eunotia* in environmental samples has made this difficult to demonstrate. In this paper we describe and discuss three relatively large-celled *Eunotia*

species from tropical (moderately to very) acidic waters of rivers of D.R. Congo.

MATERIAL AND METHODS

Several samples collected in tributaries of the Congo River, downstream of Kisangani (D.R. Congo), between 2010 and 2014 were studied. Although the newly described *Eunotia* taxa are observed in many of the samples, the present paper especially deals with one sample in particular, CCA 2066 obtained on 24 Nov. 2012 from the submerged parts of *Nymphaea lotus* L. in the Lobaye River (0.48970°S 24.17728°E), a tributary of the Lomami River, Tshopo Province (formerly part of the Oriental Province), D.R. Congo by François Darchambeau and Ernest Tambwe. The Lomami River is a major tributary of the Congo River discharging in it downstream of Kisangani. The Lobaye as well as the Lomami River are large rivers, the latter a major tributary of the Congo River. Their considerable catchment area contains many villages and mining areas. As wastewater flows directly into the rivers, some impact and nutrient enrichment of the river water is to be expected. The studied sample CCA 2066 contains *Eunotia zygodon*, *Eunotia leonardii* and *Eunotia fuseyi*. The sample was fixed in a 20% v.v. final concentration ethanol solution. Subsamples for LM and SEM observations were prepared by oxidizing the material with H_2O_2 (37%) and heating at 100°C for 1 to 2 hours. Following digestion the material was rinsed several times with distilled water. A subsample from the organic–free material was mounted in Naphrax® (refractive index 1.71) for diatom community studies.

Physical parameters (temperature, conductivity and pH), collected concurrently with the diatom sample from the Lobaye River, were measured in situ with an YSI Pro-Plus multiprobe field meter equipped with a pH sensor 1001 ProSeries. Samples for nutrient analyses (NH_4, NO_3, NO_2 and SRP) were filtered and acidified in situ, and kept frozen until spectrophotometric analysis could be carried out in the laboratory of the Unité d'Océanographie Chimique, Département d'Astrophysique, Géophysique et Océanographie, University of Liège, Belgium. NO_3^- and NH_4^+ concentrations were estimated by spectrophotometry, using the dichloroisocyanurate-salicylate-nitroprussiate colorimetric method for NH_4^+ (Westwood 1981), the sulfanilamide colorimetric after vanadium reduction method for NO_3^- (APHA 1998). SRP was determined by spectrophotometry using the ammonium molybdate-potassium antimonyl tartrate method (APHA 1998). The detection limits were 0.30, 0.15, 0.03 and 8.0 µmol L^{-1} for NO_3^-, NH_4^+ and SRP respectively. Light microscope (LM) images were collected using an Olympus BX51 microscope equipped with Differential Interference Contrast optics and a 100x 1.4 N.A. oil immersion objective. An Olympus UC30 digital camera was used for photomicrographic imaging. For scanning electron microscopy (SEM), oxidized materials were rinsed with additional deionized water and isolated with micropipette on 6 mm × 6 mm cover slip. Cover slips were mounted on aluminium stubs and coated with osmium by an OPC 40A osmium plasma coater (Filgen, Nagoya, Japan). A field emission scanning electron microscope S-8020 (Hitachi, Tokyo, Japan) operated at 5 kV and 8 or 12 mm working distance was used for the analysis. The terminology used is that outlined in Anonymous (1975) and Ross et al. (1979). Cells were traced for illustration by selecting a number of cells over the size range illustrated and then tracing the exact outline of each cell, during this process the cells photographs were all maintained at a magnification of 1500 x in order to allow accurate comparison, aspect ratio of the original drawings was also maintained at 1:1 during the tracing process.

RESULTS

Eunotia zygodon Ehrenb.

Figs 1–5

Synonyms – *Eunotia tropica* Hust. sensu Hustedt (1927: 159, pl. 5, fig. 1) **synon. nov.**; *Eunotia monodon* var. *tropica* (Hust.) Hust. sensu Hustedt (in Schmidt 1933: pl. 371), **synon. nov**. – Type: Cayenne, French Guyana, sample of Ehrenberg's material nr 1108; marked valve on slide Nr R-7, Ehrenberg Collection in Museum für Naturkunde, Berlin, Germany (lecto-: STU).

Morphologic characteristics – Type, valve 53.3 µm long, 10.0 µm broad, 10–11 striae in 10 µm, marked lectotype (Reichardt 1995: 18, plate 1, fig. 16). Congo River specimens, 37.0–153.3 µm long, 11.0–14.3 µm broad, 11–15(–21) striae in 10 µm.

Taxonomic remarks – *Eunotia zygodon*, originally described from the island of Cayenne by Ehrenberg (1843), occurs rather commonly in tropical African regions (Foged 1966, Carter & Denny 1982, Cholnoky 1970). The original illustration (Ehrenberg 1843) was of a cell with two dorsal undulations, a concept that has remained rather stable. Jahn in Reichardt (1995) examined the type material of *E. zygodon* and illustrated a single cell conforming well to the original diagnosis by Ehrenberg. Metzeltin & Lange-Bertalot (1998) illustrated *E. zygodon* from tropical South America and commented that it conforms well to the type. Later, Ferrari et al. (2007) reported this taxon from Brazil, illustrating it by ten figures and providing a discussion (in Portuguese). Thus the established concept of this species seems to be rather stable, it is comparatively large, has two pronounced dorsal undulations and is therefore rather simple to identify. When we examined a sample from tropical Africa we found cells of a diatom conforming well to the description of *E. zygodon* as well as to the LM and SEM illustrations provided by Metzeltin & Lange-Bertalot (1998), but we also noted several cells of a *Eunoti*a taxon larger (longer) than *E. zygodon*, and with many more dorsal undulations. The angle of the raphe as it passed from the mantle onto the valve face was also somewhat different. In addition, the number of areolae occurring between the raphe ending and the dorsal margin of the valve face were consistently different. The shape of the apices was also different being more bluntly rounded than the established concept of *E. zygodon*. Thus we initially considered these two seemingly separate morphological entities as two separate species i.e. *E. zygodon* sensu stricto and a taxon previously identified by workers on African material (e.g. Gasse 1986, Zanon 1938) as *E. monodon* var. *tropica*. The latter taxon was a synonym of the earlier described *E. tropica* (Hustedt 1927) collected from Lake Aokiko in Japan. We considered that this morphological form deserved

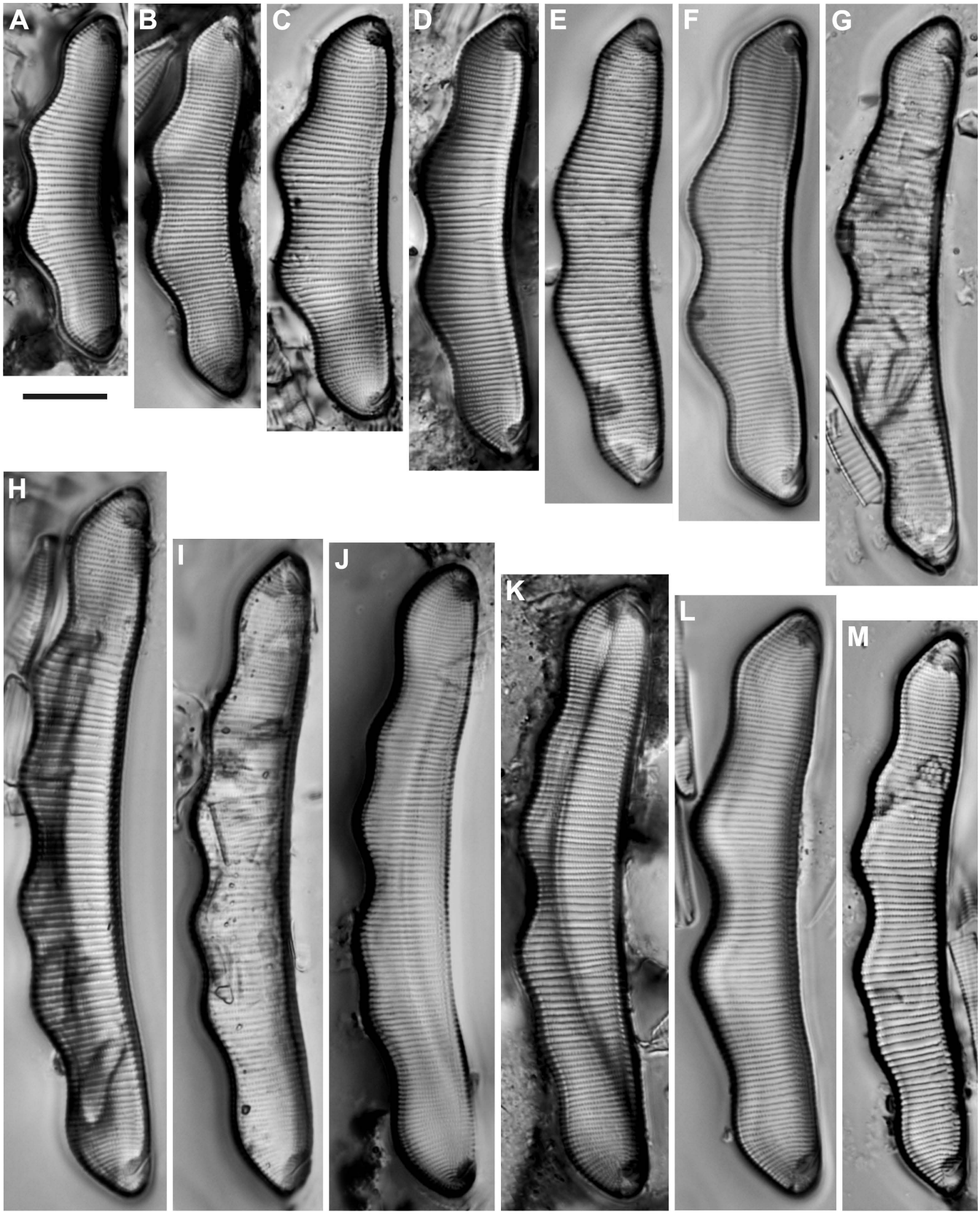

Figure 1 – *Eunotia zygodon*, LM. Size diminution series from a single population. Note especially G and M which are transitional forms between the bi-undulate and multi-undulate cells. Scale bar = 10 µm.

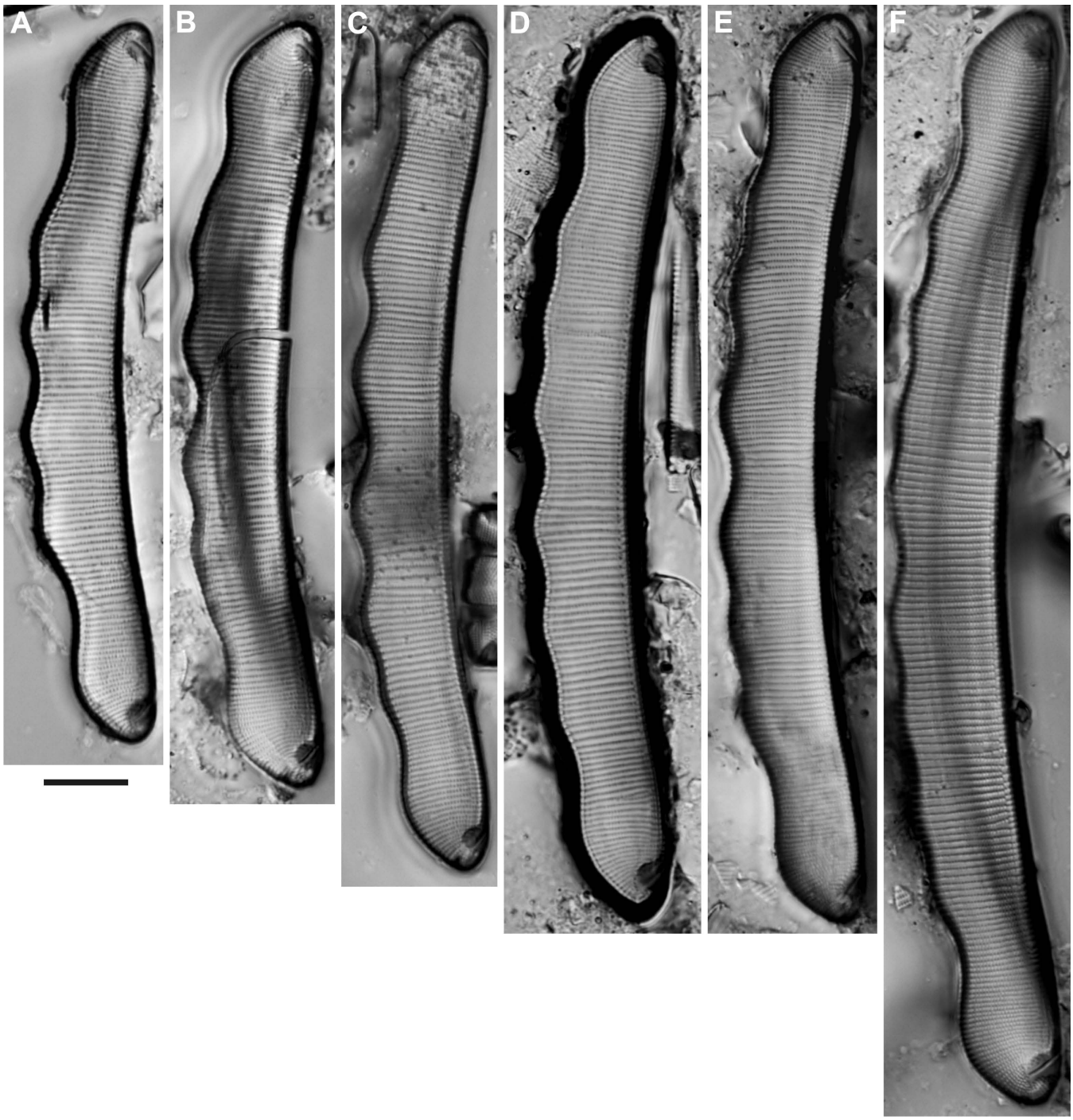

Figure 2 – *Eunotia zygodon*, LM. Size diminution series from a single population continued from Figure 1. Multi-undulate cells. Scale bar = 10 µm.

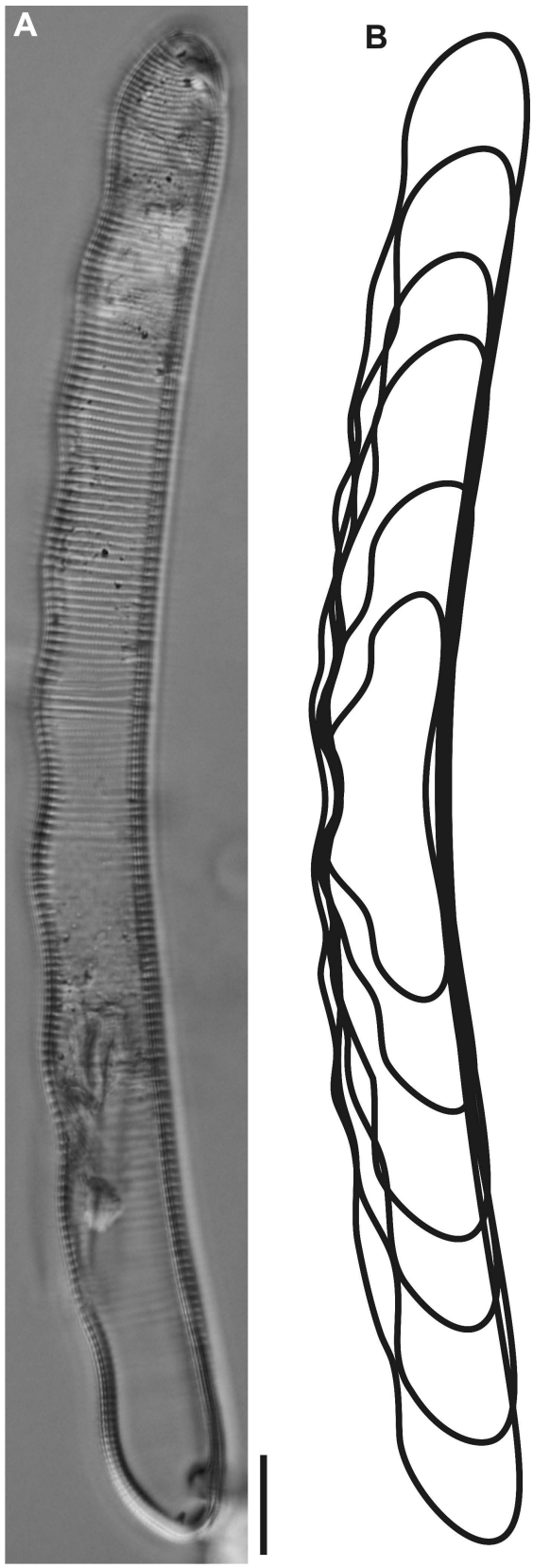

Figure 3 – *Eunotia zygodon*, LM: A, multi-undulate auxospore initial cell; B, traced outlines of selected cells from figures 1–3 showing geometric size reduction and reduction in number of dorsal undulations over the cell cycle. Scale bar = 10 µm.

the rank of species as it differed from *E. tropica,* as illustrated by Mayama (1995), and thus began an intensive investigation of the sample in question to determine the size range and other key characteristics. The more valves we examined though it became clear that we in fact were observing a single taxon rather than two discrete entities; we found a very clear diminution series showing the transition between the typical *E. zygodon* like cells to the *E. monodon* var. *tropica* like cells (figs 1–3).

What also helped to elucidate this problem was the digital tracing of the valve outline, with the inclusion of the raphe. By drawing a large cell and proportionally decreasing the size including shape of the valve apices and the angle of the raphe, the cell changed to conform to that of the smaller cells. No other digital manipulation than the proportional shrinking of the superimposed outline of the cell was used, which may be taken to replicate, to some extent at least, the geometrical progression in size reduction during asexual reproduction. We also then traced a number of cells of different sizes (fig. 3); after they were superimposed on each other that the cell width at the centre remains more or less the same and the way in which the undulations of the dorsal margin decrease in number over the cell cycle is also illustrated. The cells illustrated here (figs 1–3) therefore all belong to the same taxon i.e. *E. zygodon*. Manguin (1949) also illustrated *E. zygodon* cells with a broad ranging morphology from Madagascar, including a rather large cell that he identified as *E. zygodon* var. *elongata* Hust. In a later study from Madagascar Metzeltin & Lange-Bertalot (2002) lumped these different length cells with differing numbers of dorsal undulations under the epithet *E. tropica*. We also examined characteristics other than the valve length and outline under SEM. In cells of approximately 40, 80 and 130 µm in length, we determined the number of areolae between the valve margin and the mantle margin at the centre of the raphe. Mayama (2001) demonstrated that the characteristics of this region remain stable during the cell cycle. We consistently found 3–5 areolae above the raphe and 6–8 areolae below the raphe even though the smallest and largest cells measured differed in length by almost 90 µm.

Other characteristics were examined under the SEM and found to be consistent over the cells; these included the shape of the raphe and axial area on the valve mantle, the structure of the raphe and the raphe ending on the valve face, the position of the apical spine (fig. 5D) and the position of the rimoportula (fig. 5F). We also noted small foramina lips, after corrosion of the occluding membranes (cf. *E. pseudopectinalis* Hust.) (fig. 5C).

Eunotia leonardii J.C.Taylor & Cocquyt, **sp. nov.**

Figs 6–10

Type: D.R. Congo, Lobaye River, sample CCA 2066 (holo-: slide BR 4400, BR; the valve representing the type is illustrated here in fig. 6N; iso-: slide PUC–13-567, PUC; iso-: slide Zu 10/63 Friedrich Hustedt Diatom Collection, BRM). – Type locality: D.R. Congo, Tshopo Province (part of the formerly Oriental Province), Lobaye River, 0.48970°S, 24.17728°E, tributary of the Lomami River which is a major tributary of the Congo River.

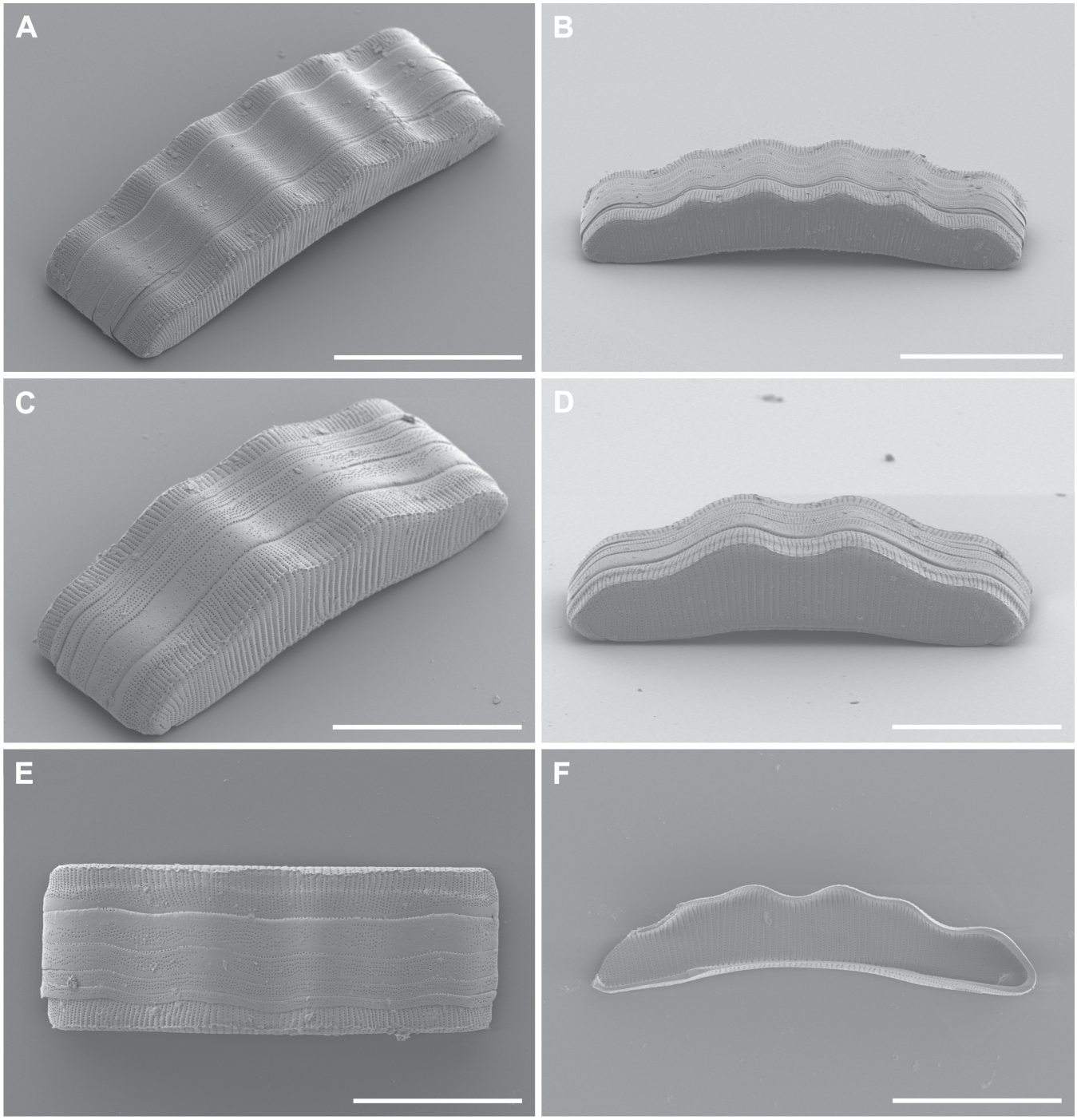

Figure 4 – *Eunotia zygodon*, SEM: A & B, external view of multi-undulate valve; C & D, external view of bi-undulate vale of *E. zygodon*; E, external view of the dorsal copulae of *E. zygodon*; F, internal view of valve. Scale bars: A & B = 30 µm; C–F = 20 µm.

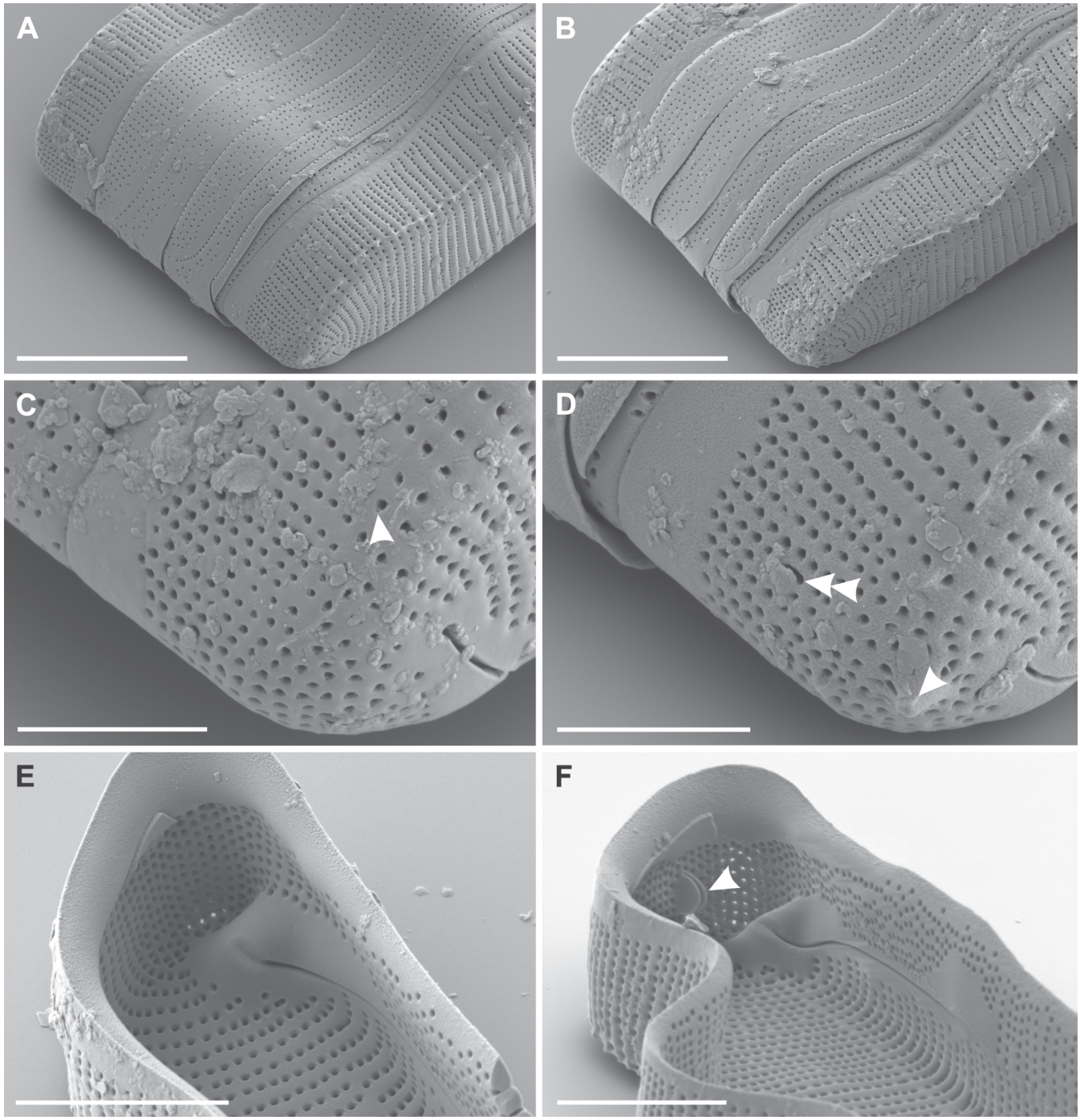

Figure 5 – *Eunotia zygodon*, SEM: A & B, external view of valve apices; C & D, detailed view of cell apices, note foramina lips (arrow C) apical spine (arrow D) and external opening of the rimoportula (double arrow D); E & F, internal view of the valve apices. Note the thickened terminal nodule and the position of the internal opening of the rimoportula (arrow F). Scale bars: A & B = 10 µm; C & D = 3 µm; E & F = 5 µm.

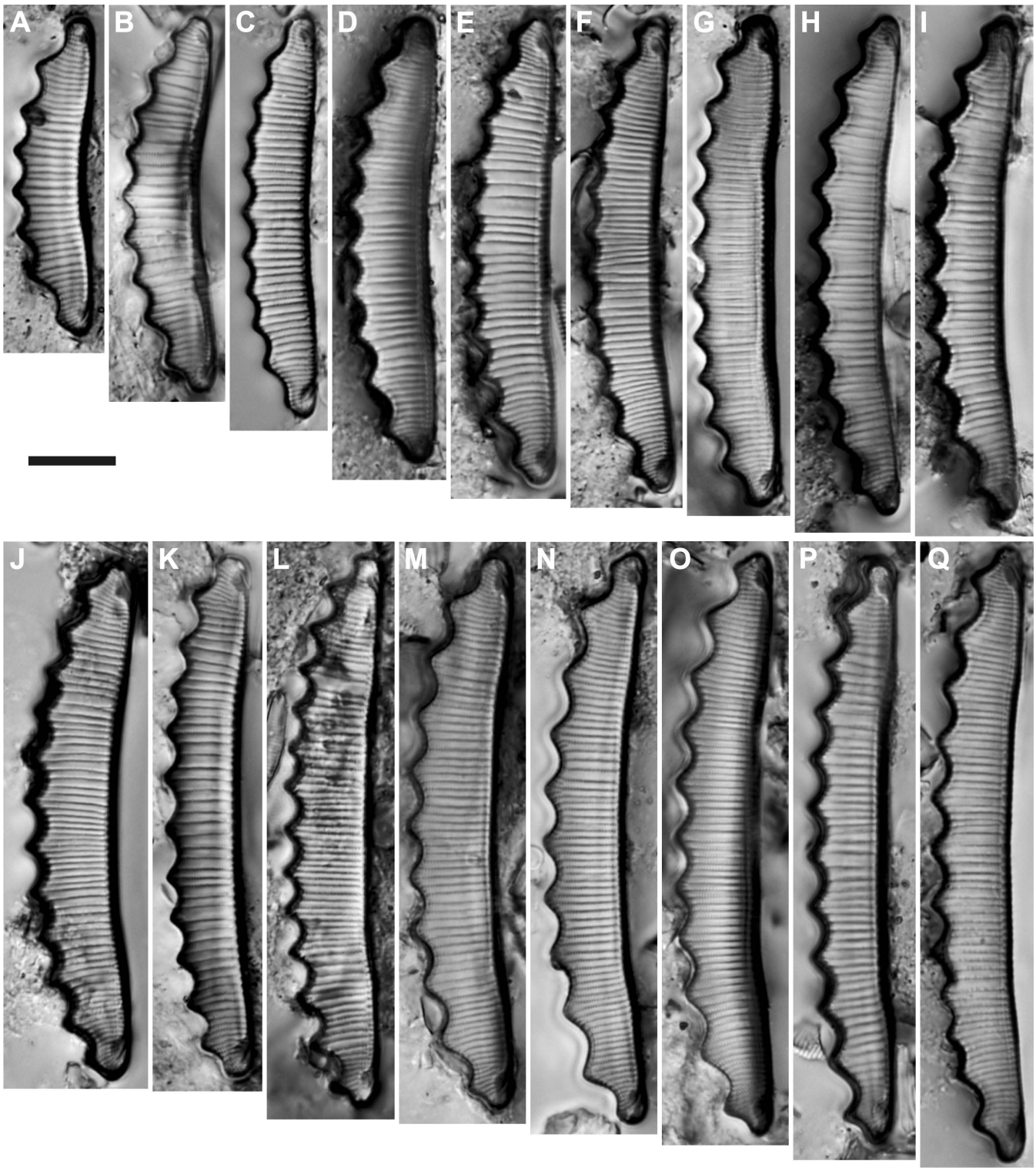

Figure 6 – *Eunotia leonardii* sp. nov., LM from sample CCA 2066, slide BR 4400. Size diminution series. Scale bar = 10 µm.

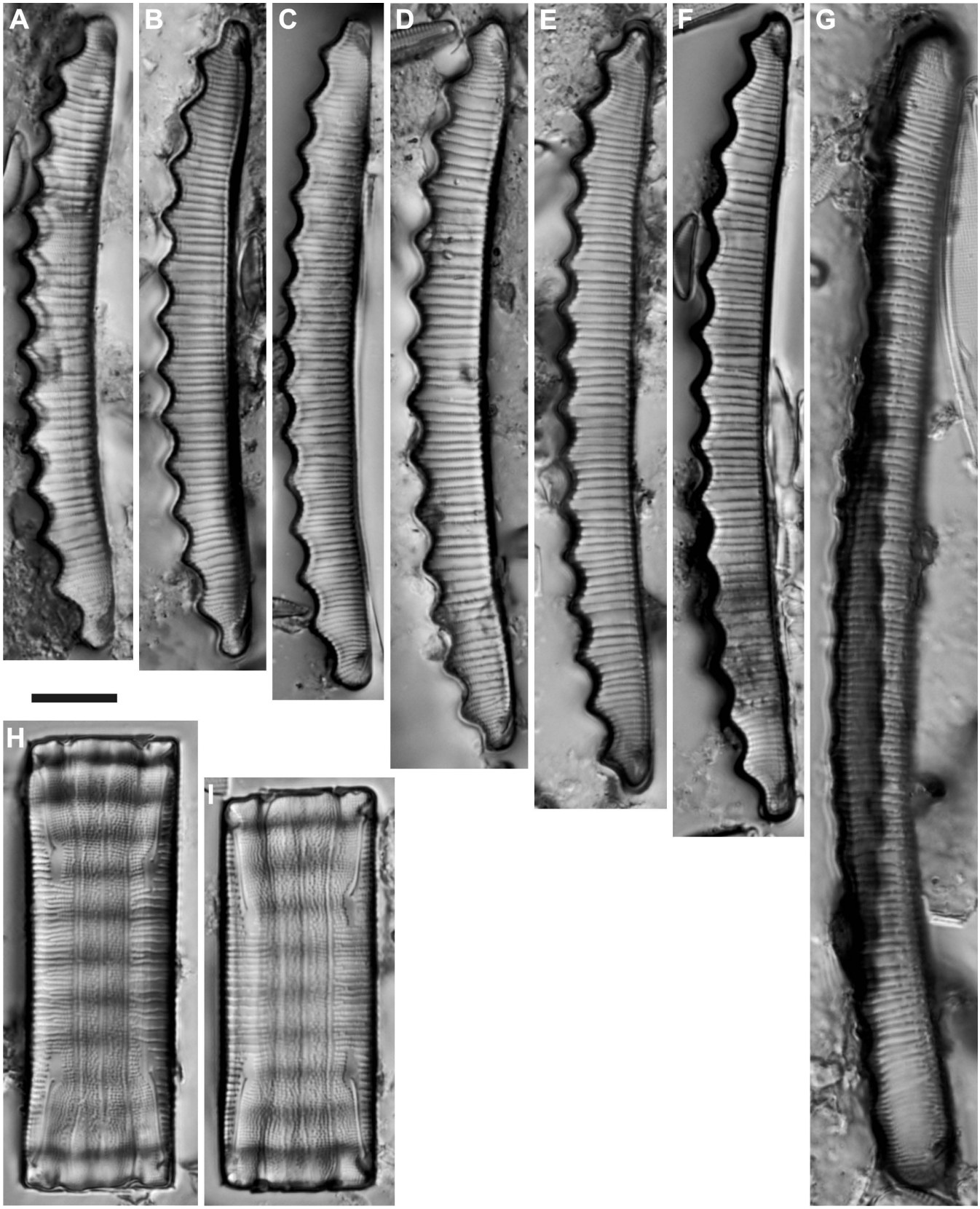

Figure 7 – *Eunotia leonardii* sp. nov., LM from sample CCA 2066, slide BR 4400. Size diminution series continued from figure 6. G, post auxospore initial cell; H–I, girdle view of ventral side of cell. Scale bar = 10 µm.

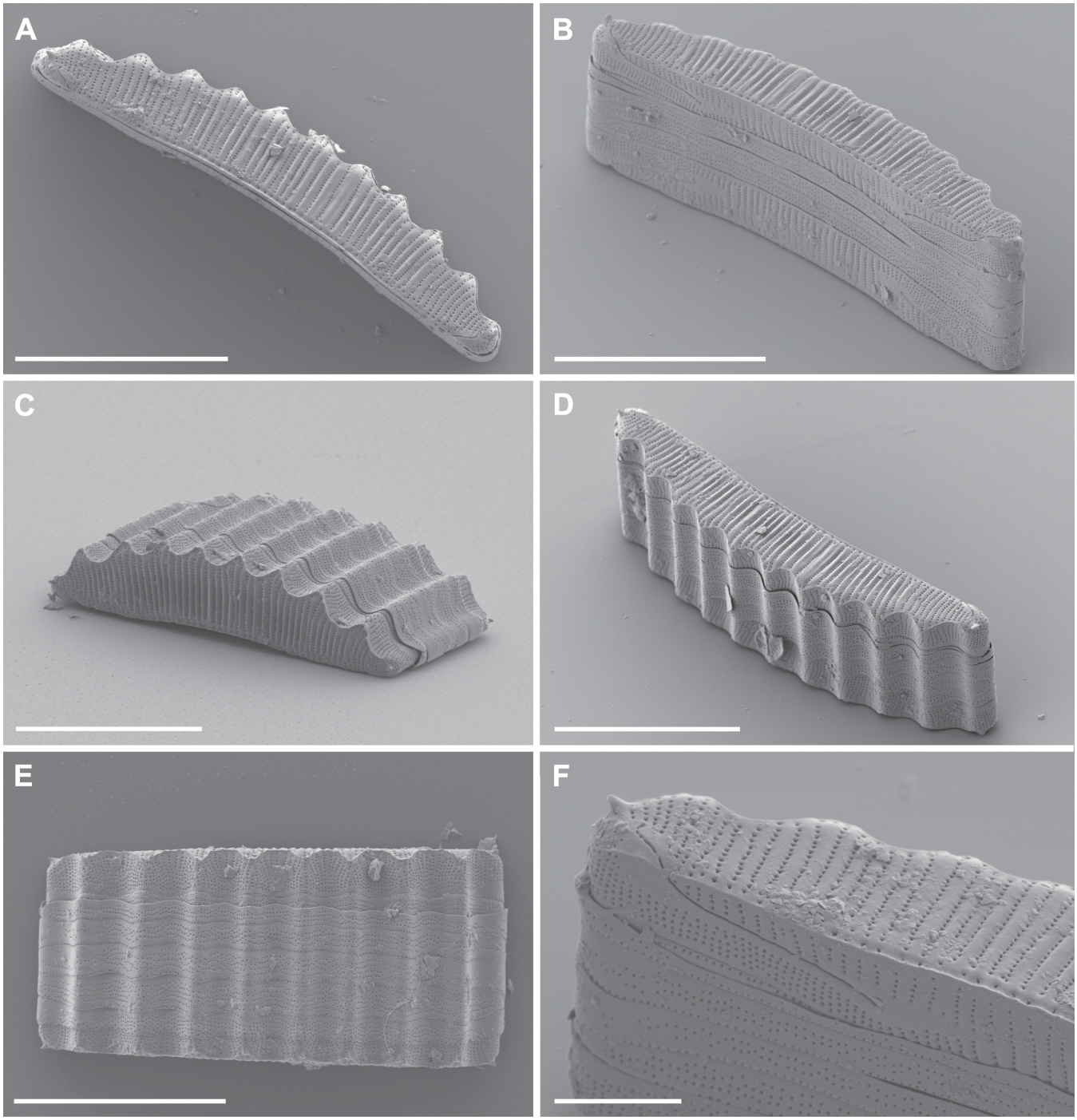

Figure 8 – *Eunotia leonardii* sp. nov., SEM from sample CCA 2066: A–E, external view of complete frustules; F, external detail of apex, note structure of raphe and the apical spine. Scale bars: A–E = 20 µm; F = 5 µm.

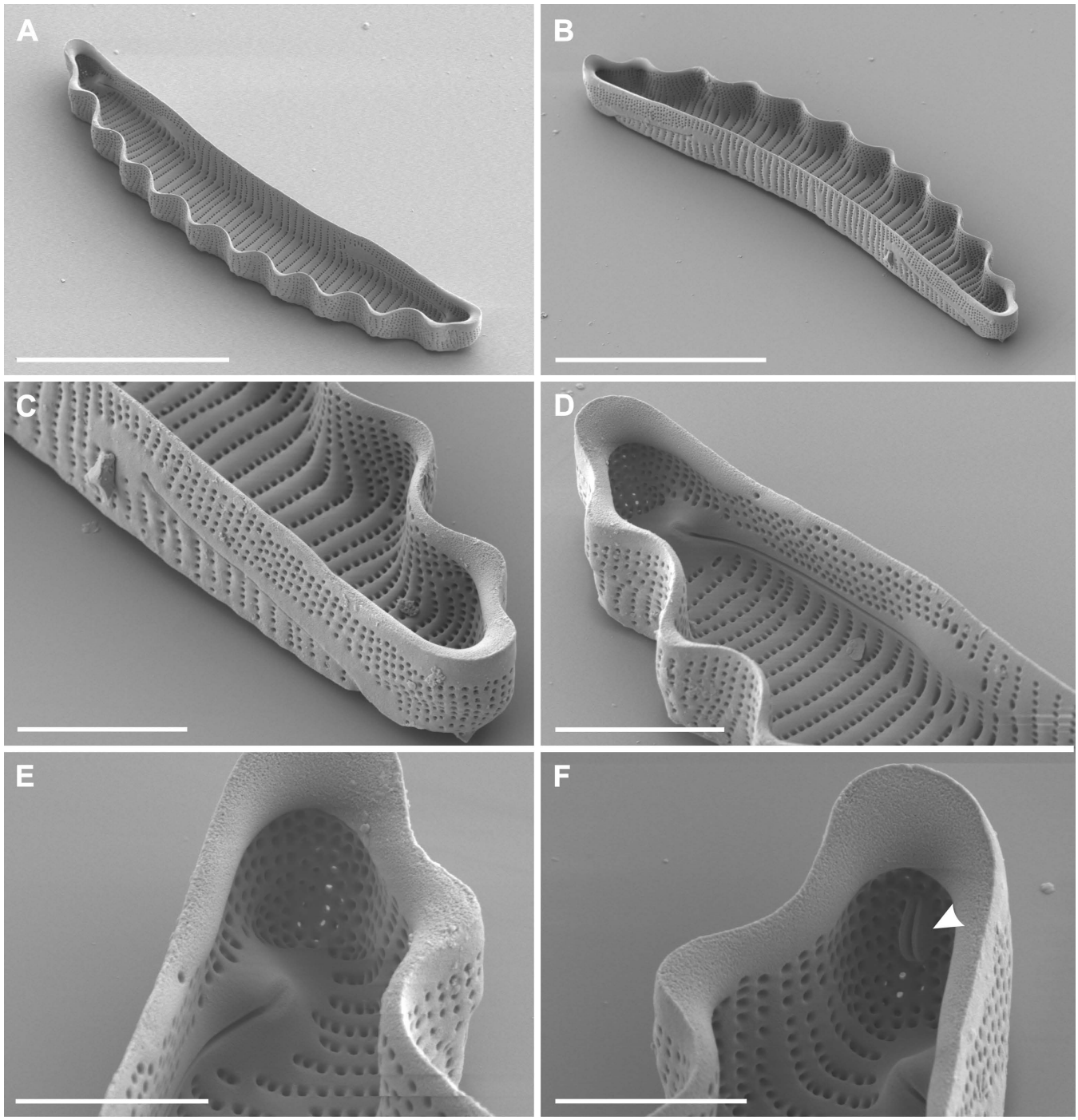

Figure 9 – *Eunotia leonardii* sp. nov., SEM from sample CCA 2066: A & B, internal oblique views of valve; C & D, internal view of detail of apices; E, internal view of the helictoglossa; F, internal view of the rimoportula (arrow). Scale bars: A & B = 20 µm; C & D = 5 µm; E & F = 3 µm.

LM: description – Valves with weakly arched dorsal margin, with many undulations (16–24 in 100 µm), parallel to slightly convex ventral margin. Ends often heteropolar, bluntly to acutely rounded. Length 36.0–137.0 µm, breadth 7.5–11.0 µm. Terminal raphe ends extend far onto the valve face and are more or less straight. Striae 9–16 in 10 µm. Puncta of transapical striae indistinct. Chain-forming frustules.

SEM: description – Striae evenly or unevenly spaced 9–16 in 10 µm becoming more densely spaced near the ends in all specimens, 14–20 in 10 µm. Few or no shortened striae intercalated at the dorsal margin. Striae composed of rounded areolae, 40–44 in 10 µm. Areolae occluded externally by hymenes anchored at 3 points giving the areolae a slightly triangular appearance (fig. 10D). Terminal nodules distinctly distant from the ends lying at the junction between valve mantle and valve face (fig. 9E). Terminal raphe fissures curved and extending with an angle of mostly c. 90–110° onto the valve face, more or less halfway towards the dorsal margin (fig. 8F). Internally the raphe terminates apically in a small helictoglossa with the cell wall rather thickened at this point (fig. 9E). A single rimoportula is found at one of the apices (fig. 9F) and a single terminal spine is found at both apices (fig. 8A). Girdle composed of multiple copulae, with a maximun of five observed (fig. 8E).

Etymology – The epithet *leonardii* is in honour of Jean Léonard, a Belgian botanist who worked at the Institut National pour l'Etude Agronomique du Congo (INEAC) and who collected algae material in the vicinity of Kisangani which is deposited at the herbarium of the National Botanic Garden of Belgium (BR) housed at the Botanic Garden Meise, Belgium.

Ecology – Temperature of 24.2°C, pH of 5.01, conductivity of 17.4 $\mu S.cm^{-1}$, 0.054 $mg.l^{-1}$ NH_4^+, 0.0009 $mg.l^{-1}$ NO_2^-, 0.16 $mg.l^{-1}$ NO_3^-, and 0.038 $mg.l^{-1}$ SRP.

Distribution – Lobaye and Lomami River basins in the Democratic Republic of the Congo, tropical Africa.

Taxonomic remarks – The chief differences between *Eunotia leonardii* and *E. georgii* Metzeltin & Lange-Bert. lie in the shape of the apices (bluntly to acutely rounded as opposed to acutely), the curvature of the ventral margin, which is never strongly concave in *E. leonardii*, the shape and angle of the raphe ending on the valve face, and the smaller range of striae in *E. georgii* (12–14 in 10 µm as opposed to 9–14 in 10 µm for *E. leonardii*). The largest difference lies perhaps in

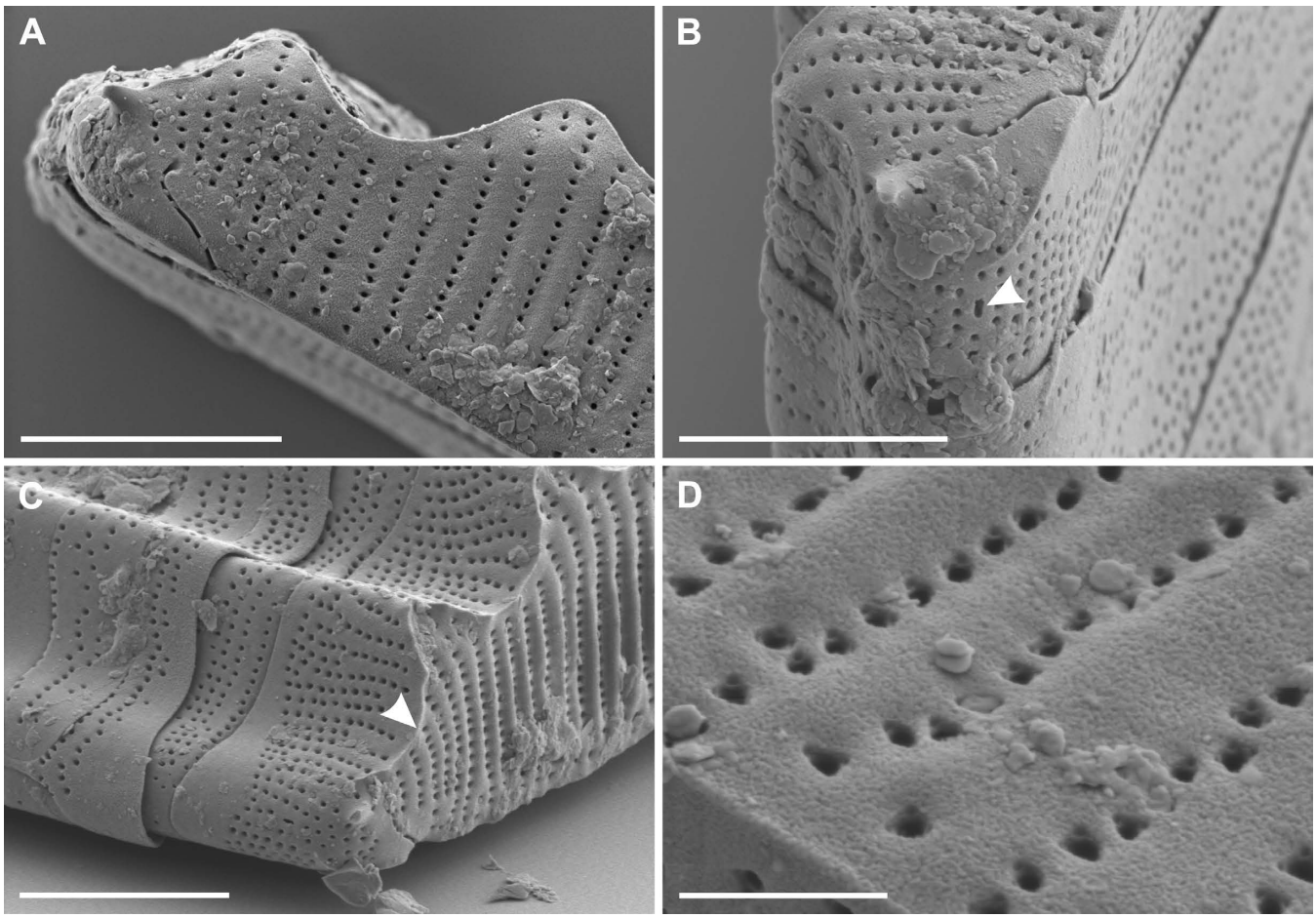

Figure 10 – *Eunotia leonardii* sp. nov., SEM from sample CCA 2066: A, external view of apex showing structure of the terminal raphe ending; B, external view of apex, note position of rimoportula (arrow); C, external view of apex and copulae, note marginal ridge of silica (arrow); D, external view of areolae, occlusion has been corroded away during cell treatment. Scale bars: A, C & D = 5 µm; B = 3 µm.

the number of areolae: *E. georgii* has 27–30 in 10 µm while *E. leonardii* has 40–44 in 10 µm.

Eunotia leonardii differs from *E. serra* in the shape of the valves, which have a weakly arched dorsal margin with many undulations in *E. leonardii* opposed to the rather strongly arcuate valves with more or less concave ventral margins and strongly convex dorsal margins with even undulations in *E. serra*. In *E. serra* the valve ends are rounded like the wave crest or obliquely truncated in the case where it fuses with a distal undulation. The number of undulations varies in *E. serra* from 6 to 18; the valves are 40–150 µm long and 12–16 µm broad. Striae and areolae of *Eunotia leonardii* are distinctly more densely spaced than in *E. diadema* Ehrenb. and *E. tetraodon* Ehrenb., areolae 24–27 in 10 µm.

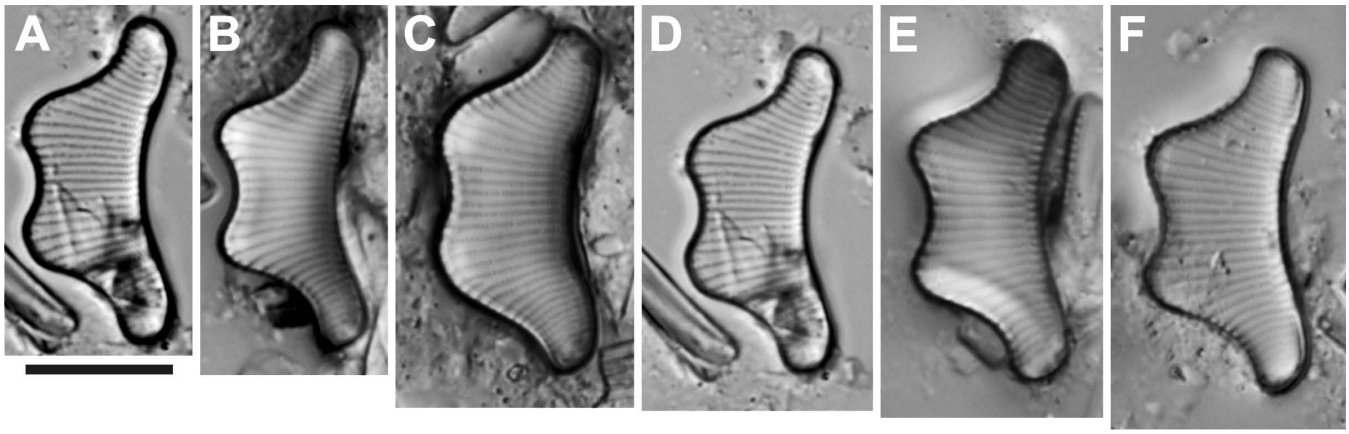

Figure 11 – *Eunotia fuseyi* sp. nov., LM from sample CCA 2066, slide BR 4401. Size diminution series. Scale bar = 10 µm.

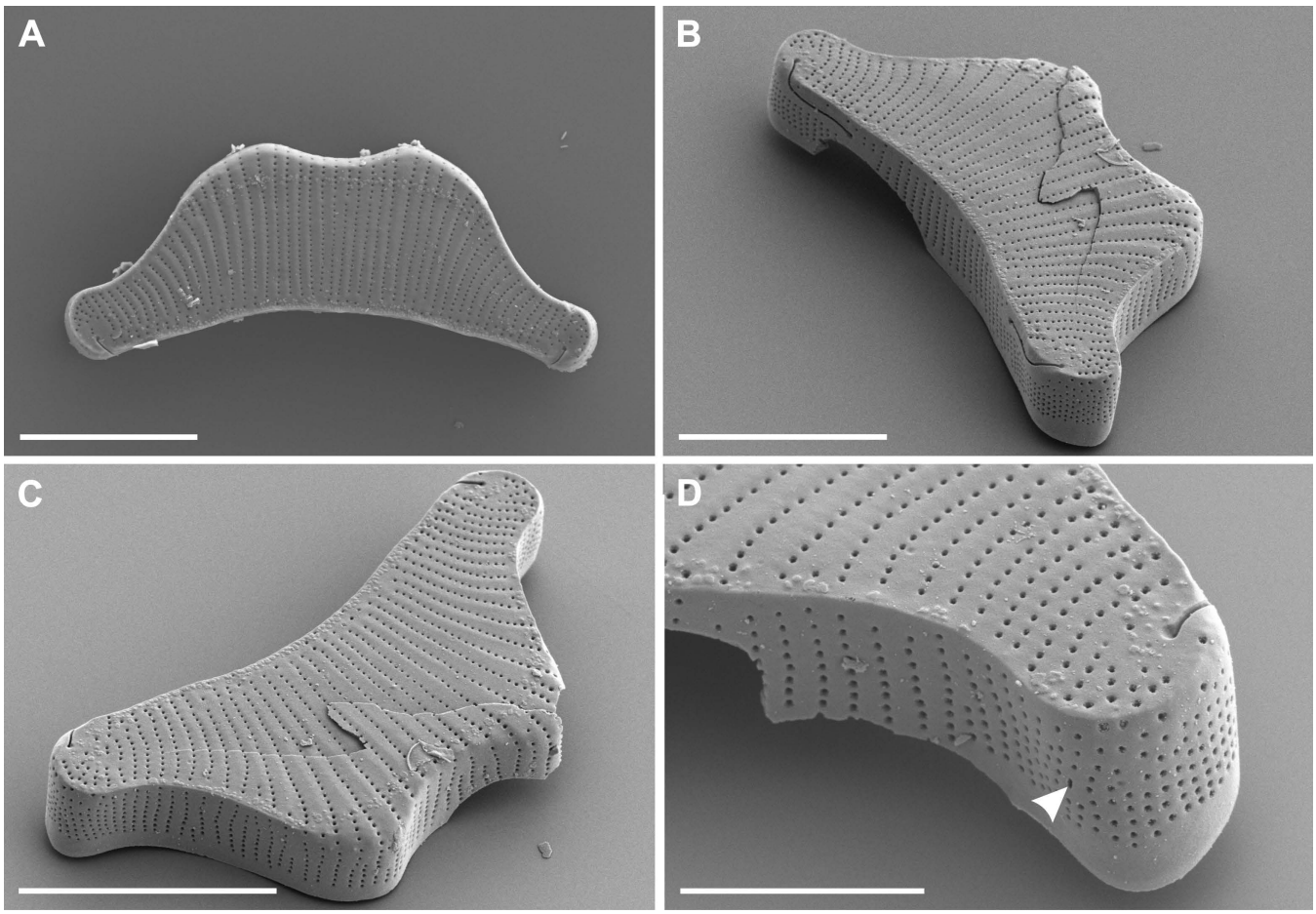

Figure 12 – *Eunotia fuseyi* sp. nov., SEM from sample CCA 2066: A–C, external view of complete valves; D, external detail of apex, note external opening of rimoportula (arrow). Scale bars: A–C = 10 µm; D = 4 µm.

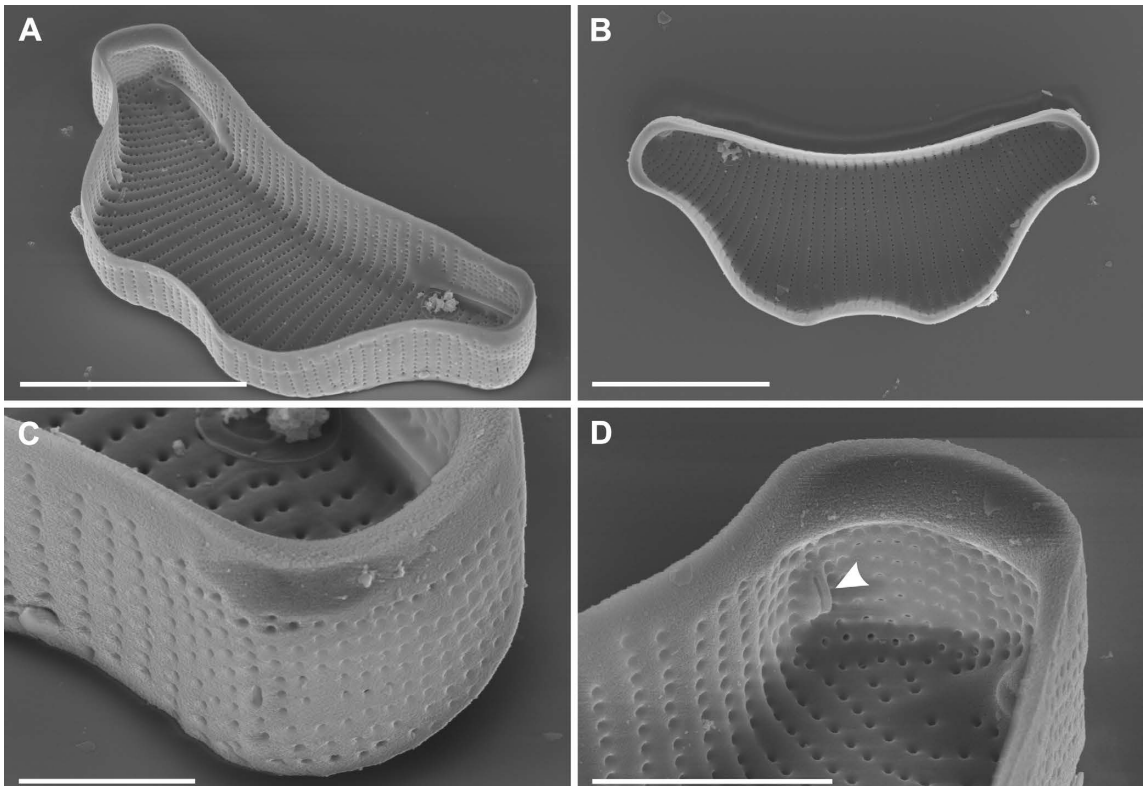

Figure 13 – *Eunotia fuseyi* sp. nov., SEM from holotype sample CCA 2066: A & B, internal view of complete valve; C, external view of apex showing position of external opening of rimoportula; D, internal view of the apex showing the rimoportula (arrow). Scale bars: A–B = 10 µm; C = 2 µm; D = 3 µm.

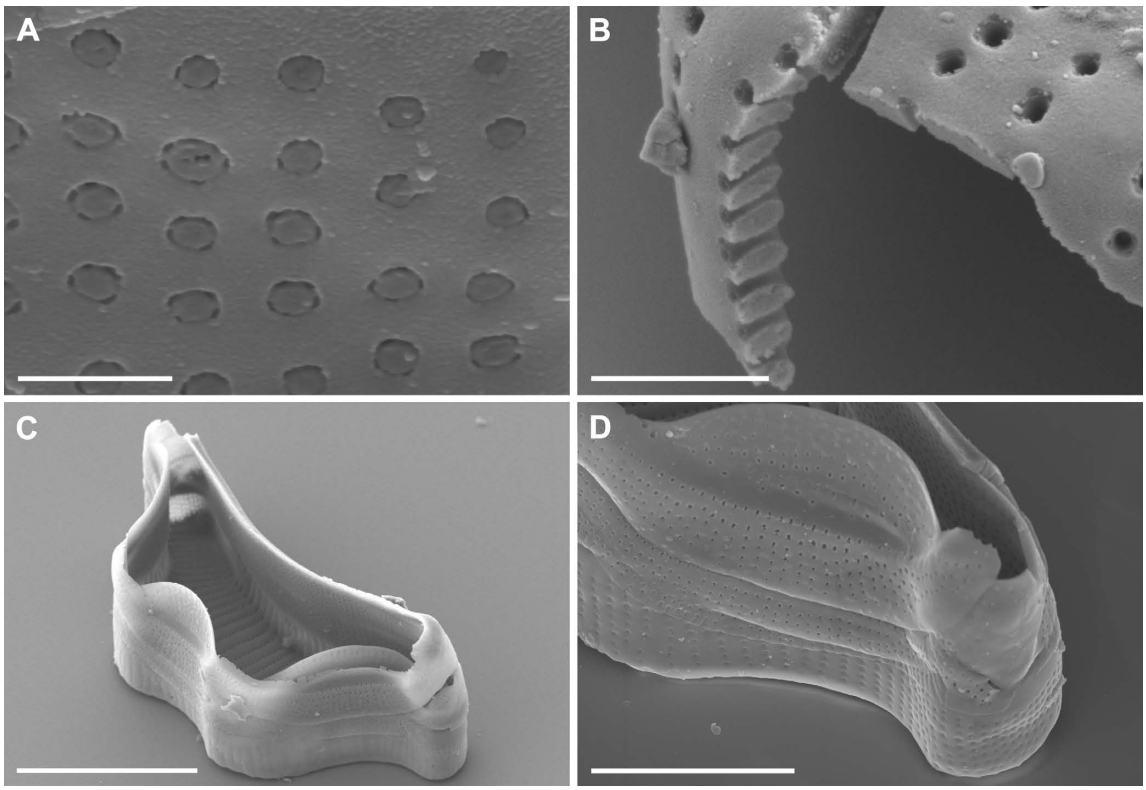

Figure 14 – *Eunotia fuseyi* sp. nov., SEM from sample CCA 2066: A, external view of areolae, note external occlusions; B, broken cell showing structure of areolae; C, valve with copulae; D, detail of copulae. Scale bars: A = 0.5 µm; B = 1 µm; C = 10 µm; D = 5 µm.

Mayama & Kobayasi (1990) examined materials from Degernä diatomite, the material from which *E. serra* was originally described. They found specimens with the following characteristics: valves 96–128 µm long and 13–16 µm broad with about 11 striae in 10 µm.

Eunotia fuseyi J.C.Taylor & Cocquyt, **sp. nov.**

Figs 11–14

Synonym – *Eunotia papilio* [as *papillo*] var. *africana* (Fusey) Fusey, **nom. inval.** in Fusey (1966: 61, pl. 1, figs 1– 4). – Type: D.R. Congo, Lobaye River, sample CCA 2066 (holo-: slide BR 4401, BR; the valve representing the type is illustrated here in fig. 11F; iso-: slide NWU–13-567, PUC; iso-: slide Zu 10/64 Friedrich Hustedt Diatom Collection, BRM). – Type locality: Democratic Republic of the Congo (DRC), Tshopo Province (part of the formerly Oriental Province), Lobaye River, 0.48970°S, 24.17728°E, tributary of the Lomami River which is a major tributary of the Congo River.

LM: description – Valves bigibbous. Ventral margin more or less concave. Dorsal margins slightly to strongly convex with respect to the two humps, separated by a depression of variable depth. Ends abruptly to strongly protracted, wide to narrow. Sometimes obliquely capitate and reflexed dorsally, never broadly rounded. Length 15.0–40.0 µm, breadth at widest parts 6–10 (12) µm. Terminal nodules offset from the poles. Terminal raphe fissures rather short on valve face, more or less 1/3 of the way towards the dorsal margin. Proximal raphe endings straight, running parallel and just below the junction of the valve face and mantle. Striae variable, 14–20 in 10 µm becoming only slightly more densely spaced near the ends. Areolae about 32 in 10 µm.

SEM: description – Striae evenly spaced, 14–20 in 10 µm becoming only slightly more densely spaced near the apices in all specimens. Very few (1–2) or no shortened striae intercalated at the dorsal margin. Striae composed of rounded areolae, 32 in 10 µm. Areolae occluded externally by hymenes anchored at 4 to 6 points and thus appearing more or less round (fig. 14A). Terminal nodules relatively close to the ends and not distinct (fig. 13A & B). Terminal raphe fissures short, straight and extending with an angle of mostly c. 110° onto the valve face, extending more or less ¼ to ⅓ of the distance towards the dorsal margin (fig. 12C & D). Internally the raphe terminates apically in a very small helictoglossa with the cell wall only slightly thickened at this point (fig. 13A & B). A single rimoportula is found at one of the apices located towards the dorsal side (fig. 13D). Terminal spines are absent. Girdle composed of at least two or more copulae (fig. 14C & D).

Etymology – The epithet *fuseyi* is in honour of Pierre Fusey, "chef de Travaux au Laboratoire de Cryptogamie du Muséum national d'Histoire naturelle" at Paris, France in the 1960s, and who studied algal samples from the Central African Republic. He described about 35, unfortunately invalid, new diatom taxa.

Ecology – Temperature of 24.2°C, pH of 5.01, conductivity of 17.4 $\mu S.cm^{-1}$, 0.054 $mg.l^{-1}$ NH_4^+, 0.0009 $mg.l^{-1}$ NO_2^-, 0.16 $mg.l^{-1}$ NO_3^-, and 0.038 $mg.l^{-1}$ SRP.

Distribution – Lobaye and Lomami River basins in the Democratic Republic of the Congo, tropical Africa, also more widely in tropical Africa including the Republic of the Congo.

Taxonomic remarks – Fusey described *E. papilio* var. *africana* as a variety from the Central African Republic, first in 1964 with a diagnosis in French and again in 1966, this time with a Latin diagnosis. However, under article 9.1 of the ICBN (McNeill et al. 2012) this name is invalid as two or more collections are mentioned and neither a type slide is designated nor a type is indicated in either of the descriptions. Reference is made to a number of photographs without date and locality information within the caption. We are however sure that this species is conspecific with the newly described *E. fuseyi* as it matches the measurements given by him as well as his illustrations (Fusey 1964, 1966). We believe that this variety deserves the rank of species as it is very different from the concept of *E. papilio* (Ehrenb.) Grunow sensu stricto. *Eunotia papilio* was originally described as *Himantidium papilio* Ehrenb. in 1843 and later-transferred to *Eunotia* by Grunow (1868). In his examination of the type material Reichardt (1995) recorded the following characteristics of *E. papilio* from the lectotype: 22–31 µm long, broadest region 11–16 µm, 8–12 in the narrowest part; 6 striae per 10 µm near the dorsal side to 12 near the ventral side, raphe short and curves onto the mantle; 18–26 puncta in 10 µm. *Eunotia fuseyi* has a higher number of areolae, a higher number of striae, the ventral margin is generally less convex, cells are often less constricted in the centre of the dorsal margin and the raphe runs along the junction of the valve face and valve mantle and is almost straight before curving onto the valve face.

DISCUSSION

Initially, during the examination of the *Eunotia zygodon* cells, we were convinced that there were two or even more distinct taxa present in our samples. We examined these different valves repeatedly grouping them according to length or breadth or number of undulations on the dorsal margin. We also examined the raphe endings on the valve face and could find no clear differences in these features. The more cells we photographed and examined the less clear the apparent differences became. This led us to trace the cells (fig. 3B) and then to superimpose the traced images over one another. By doing this it became obvious that we had a rather complete representation of the cell line and associated cell size reduction. Many *Eunotia* species have been initially described with reference to the undulations on the dorsal valve and this is evidenced by the names chosen for the species (e.g. *Eunotia triodon* Ehrenb. *E. diodon* Ehrenb. etc.), thus intrinsically linking the number of dorsal undulations to the species concept. This has always been the case for *Eunotia zygodon*, represented in literature as having two dorsal undulations; this is also concurrent with its initial description and illustration. As discussed in the introduction it may be possible that this species is rarely found in samples and when it is found there are only a few cells present. Also as earlier discussed this species forms long chains with the cells of more or less the same size in terms of cell length so that if only a

single chain or fragment of chain is sampled it will give the impression that cell size (length) and number of dorsal undulations are rather stable characters, however these are stable within the chain of cells but other chains may have a different valve morphology and it is only when numerous chains are sampled that this becomes obvious. This has been very well documented in cultures of the closely related (in terms of morphology) *Eunotia tropica* by Mayama (1995) but as far as we are aware has not been documented in naturally occurring *E. zygodon* populations. We do not propose that dorsal valve margin undulations will always be variable in number across all taxa of *Eunotia*, but it seems that several taxa have a stable number of undulations, e.g. *E. papilio* and *E. fuseyi* described herein. Caution is necessary when using this as a key character for identification (see discussion of *E. pectinalis* in Lange-Bertalot et al. 2011). In the case of *E. zygodon* we concur with Mayama (2001) that more stable regions of the valve face be chosen for confirmation of species identification.

The chain-forming *Eunotia leonardii* has multiple valve undulations and occurs *en-masse* in tropical African waters. At first view it shares some similarity with *Eunotia serra* but the similarities are very superficial. *Eunotia leonardii* has 8 to 18 valve undulations, compared to 6–18 undulations of *E. serra*, but the chief distinguishing character is the shape of the apices and curvature of the ventral margin.

Eunotia fuseyi was originally invalidly described as *E. papilio* var. *africana* as discussed above. As with many other taxa given the rank of variety the ascribed variety has little relation to the nominate variety, in this case there is a similarity to *E. papilio* var. *papilio*: both taxa have two dorsal undulations. However, other key characters are distinctly different, e.g., cell shape, striae density and areolae density, and this coupled with the original invalid description has led us to describe this entity as a distinct taxon requiring the rank of species. Ascribing taxa the rank of variety is problematic in modern diatom systematics as this rank is deemed unnatural. This is a problem worldwide and now many taxa, formerly at the rank of variety, are given the rank of species. Many taxa from Africa were ascribed the rank of variety, sometimes forma, in particular the rank '*africana*'. This was a convenient systematic tool for those working on African diatoms. If the taxon in question bore a superficial resemblance to one previously described from Europe or elsewhere, then the most convenient solution was to create a variety or forma. No blame is ascribed for this as species concepts changed over time and past studies were carried out without the benefit of scanning electron microscopy and other tools allowing for the detailed examination of diatom frustules. However, those interested in the study of the African diatom flora will have to systematically raise to the rank of species many of the taxa bearing epithets such as 'var. *africana*' or 'f. *africana*', but always after a thorough investigation of the concerned taxa.

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