Rimocostatus bugojnicus gen.et. sp. nov. (Coscinodiscophyceae, Bacillariophyta) – a new fossil diatom genus from Gračanica, Bugojno palaeolake in Bosnia and Herzegovina

Ermin Mašić, Dušica Zaova, Senka Barudanović, Nadja Ognjanova-Rumenova & Zlatko Levkov

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Rimocostatus bugojnicus gen.et. sp. nov. (Coscinodiscophyceae, Bacillariophyta) – a new fossil diatom genus from Gračanica, Bugojno palaeolake in Bosnia and Herzegovina

ERMIN MAŠIĆ[1], DUŠICA ZAOVA[2]*, SENKA BARUDANOVIĆ[1], NADJA OGNJANOVA-RUMENOVA[3] & ZLATKO LEVKOV[2]

[1]*Faculty of Science, University of Sarajevo, Sarajevo, Bosnia and Herzegovina*
[2]*Faculty of Natural Sciences, Institute of Biology, Ss. Cyril and Methodius University in Skopje, Skopje, R. North Macedonia*
[3]*Department of Palaeontology, Stratigraphy and Sedimentology, Geological Institute, Bulgarian Academy of Sciences, Sofia, Bulgaria*

A new freshwater fossil diatom taxon *Rimocostatus bugojnicus* gen. et sp. nov, from Miocene sediments at the Gračanica site, Bugojno palaeolake in Bosnia and Herzegovina, is described. The main morphological feature of the genus is the presence of marginal rimoportulae with simple, round external openings positioned on interfascicles on the valve mantle and stalked, large, wide internal slits, positioned on every costa. Marginal and valve face fultoportulae are absent. With these characteristics, the species shows similarities to both marine and freshwater species, known from recent, and primarily from fossil, diatom deposits. Thus, *Rimocostatus bugojnicus* gen. et sp. nov. is an important find for understanding the evolution of centric diatom species.

Keywords: *Balkan Peninsula, Bugojno palaeolake, Middle Miocene,* Rimocostatus, *taxonomy*

Introduction

The Balkan Peninsula accommodated a large number of freshwater lakes during the Early-Middle Miocene (Zagorcev 1992, Krstić et al. 2003, 2012, Harzhauser & Mandić 2008), the fossil diatoms of which were investigated within the IGCP Project 329, UNESCO (1992–1997). From this project, accompanying field trips and several other smaller projects and collaborations, the first biochronological schemes for this area were created and published (Ognjanova-Rumenova 2000, Ognjanova-Rumenova & Krstić 2007, Ognjanova-Rumenova & Dumurdzhanov 2008). It was possible to study the sediments of more than 45 Neogene lakes at outcrops and shallow bore holes, but only in some buried structures, such as the Sofia and Pelagonia Basins, were diatomaceous rocks thick enough and/or had adequate dating control to allow precise biostratigraphic studies (Ognjanova-Rumenova 2005, Ognjanova-Rumenova et al. 2008). Despite various systematic papers dealing with local diatom floras, changes in these lake systems over space and time are still unexplored. Another problem is that these Neogene freshwater systems are often characterized by outstanding endemism, possibly due to the origin and paleogeographic development of these paleolakes (Harzhauser & Mandić 2008). The extremely high level of endemism in the Balkan fossil diatom flora compared to other parts of Eurasia, North America and Africa, is the most striking element of their comparative diatom biostratigraphy (Fourtanier & Gasse 1988, Khursevich 1994, Serieyssol & Gasse 1991, Krebs & Bradbury 1995, Hayashi et al. 2018). Thus it was very important to investigate the relic lakes in the Balkan Peninsula – Ohrid and Prespa in Macedonia (Levkov et al. 2007, Levkov & Williams 2012), including applying new techniques, e.g., molecular methods, which have thrown new light on evolutionary events in ancient lakes (Stelbrink et al. 2018).

The studies of planktonic centric diatoms within ancient Lake Ohrid yielded both a new genus (Jovanovska *et al.* 2016) and new species (Cvetkoska et al. 2012, Tofilovska et al. 2016, Zaova et al. 2020, Levkov et al. 2021). Several centric genera were found and diversified during the middle Miocene in the Balkans, such as *Cyclotella* (Kützing) Brébisson sensu lato and *Stephanodiscus* Ehrenberg. A time-calibrated phylogeny of the Thalassiosirales showed that the divergence time of *Cyclotella* sensu lato was ca. 40 My (Jovanovska et al. 2022). Other genera separated from *Cyclotella* sensu lato, e.g., *Discostella* Houk & Klee, *Lindavia* (Schutt) De Toni & Forti and *Pantocsekiella* Kiss &Ács are younger (between 6 and 10 My).

The aim of this investigation is to describe a new fossil diatom genus, *Rimocostatus*, from the Miocene

*Corresponding author. Email: zaovad@pmf.ukim.mk
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sediments of Gračanica, from the Bugojno palaeolake, Bosnia and Herzegovina, using light (LM) and scanning electron microscopy (SEM), to reveal details of its morphology and allow comparisons with other fossil centric genera.

Material and methods

Site description

The Bugojno palaeolake located in central Bosnia and Hercegovina is part of the past, tectonically formed Dinarides Lake System (DLS), which persisted during the Neogene period (Pisera et al., 2020). The Gračanica site in the southeastern part of Bugojno (43.997662° N, 17.518516° E) is a freshwater basin with lacustrine sediments containing fossil deposits (sponge spicules, chrysophyte cysts and diatoms) dated back to 15.2–14.0 Ma ago (Vasilyan 2019, Pisera et al., 2020). As a result of an active coal mine in Gračanica, Lake Zanesovići (44.011119° N and 17.507044° E) was formed (Fig 1). The lake is ~718 m long and ~260 m wide, at an altitude of 616 m a.s.l. The total water inflow originated mainly from direct precipitation and the Zaneski stream, with outflow through the River Vrbas. Lake Zanesovići is characterized by a stable hydrological regime throughout the year, with insignificant lake level oscillation of around 20–30 cm during dry periods (Mašić, 2021).

Laboratory procedure

Sediment samples were collected from the Lake Zanesovići coal mine with a grab 10 cm deep, where material from fossil deposits are washed into the lake. Diatom samples were prepared for LM and SEM using material fixed with 4% formalin in the field. For acid cleaning, the samples were treated with a few drops of cold 37% HCl and $KMnO_4$ then left overnight to remove carbonates. To oxidize the organic matter, 37% HCl was added and the samples were boiled for 30 min at 80°C. Using distilled water, The samples were rinsed several times using distilled water and then centrifuged. Diatom slides were prepared using Naphrax as mounting medium. LM observations were performed under oil immersion at 1500 × magnification using a Nikon Eclipse 80i microscope. Diatom images were taken using a Nikon Coolpix P6000 camera. For scanning electron microscopy (SEM), the material was prepared by drying clean diatom suspensions onto cover slips that were attached to the SEM stubs with carbon tape and coated with gold-palladium (Polaron SC7640 sputter coater, Quorum Technologies, Ashford, UK). An ultra high-resolution analytical field emission (FE) scanning electron microscope Hitachi SU-70 (Hitachi High Technologies, Europe, GmbH) operated at 5 kV and 10 mm distance was used for the analysis. SEM images were taken using the lower (SE-L) detector signal. Slides are deposited at the Macedonian National Diatom Collection (MKNDC) at the Institute of Biology, Faculty of Natural Sciences, Skopje.

Results

Taxonomic diagnoses

Rimocostatus Mašić, Zaova, Ognjanova-Rumenova & Levkov gen. nov. (Figs 2–42)
Frustules and valves circular in valve view, rectangular in girdle view. Valve face slightly convex or concave with radial pattern of short and strongly thickened ribs, interrupted close to the central area where irregularly arranged. Central area concentrically undulate to almost flat in smaller specimens. Marginal area with radiating striae becoming concentrically and/or irregularly organized in central area. Striae organized in fascicles. Fascicles uniseriate at valve centre, becoming biseriate to triseriate near valve mantle and multiseriate near valve mantle junction. Valve mantle with large number of fine areolae organized in 6–8 rows. Areolae with external cribrate occlusions, internally with domed cribra. Alveolar openings small round to elongated present at valve face mantle junction separated by strongly thickened costae. Valve face and marginal fultoportulae absent. Externally rimoportulae with simple, round openings positioned at interfascicles on valve mantle, internally stalked, with large and wide slit, centripetally oriented and situated on every costa. Spines small and conical, situated on valve face/mantle junction, continuing from ribs.
Typus generis: *Rimocostatus bugojnicus* Mašić, Zaova, Ognjanova-Rumenova & Levkov sp. nov.

Rimocostatus bugojnicus Mašić, Zaova, Ognjanova-Rumenova & Levkov sp. nov. (Figs 2–42)

LM description (Figs 2–24): Valves circular 8.5–24.0 µm in diameter. Valve face slightly convex or concave. Central area concentrically undulate, becoming almost flat in smaller specimens. Marginal area with radiating striae becoming concentrically and/or irregularly organized in central area. Striae 7–15 in 10 µm. Striae organized in fascicles. Fascicles uniseriate at the valve centre, becoming biseriate to triseriate towards the valve mantle and multiseriate near the valve mantle junction.

SEM external view (Figs 25–33): Valve face uneven, slightly convex or concave with radial arrangement of short strongly thickened ribs, interrupted close to the central area where irregularly arranged (Figs 25, 28). Areolae rounded to slightly irregularly shaped, organized in fascicles (Figs 29, 32). Fascicles radial and multiseriate at the valve face/mantle junction, often with 4–5 rows of areolae, biseriate and becoming concentrically uniseriate and/or irregularly organized towards the valve centre (Figs 32, 33). Valve mantle with large number of fine areolae organized in 6–8 rows. Areolae with external cribrate occlusions (black arrowhead in Fig. 31). Spines small and

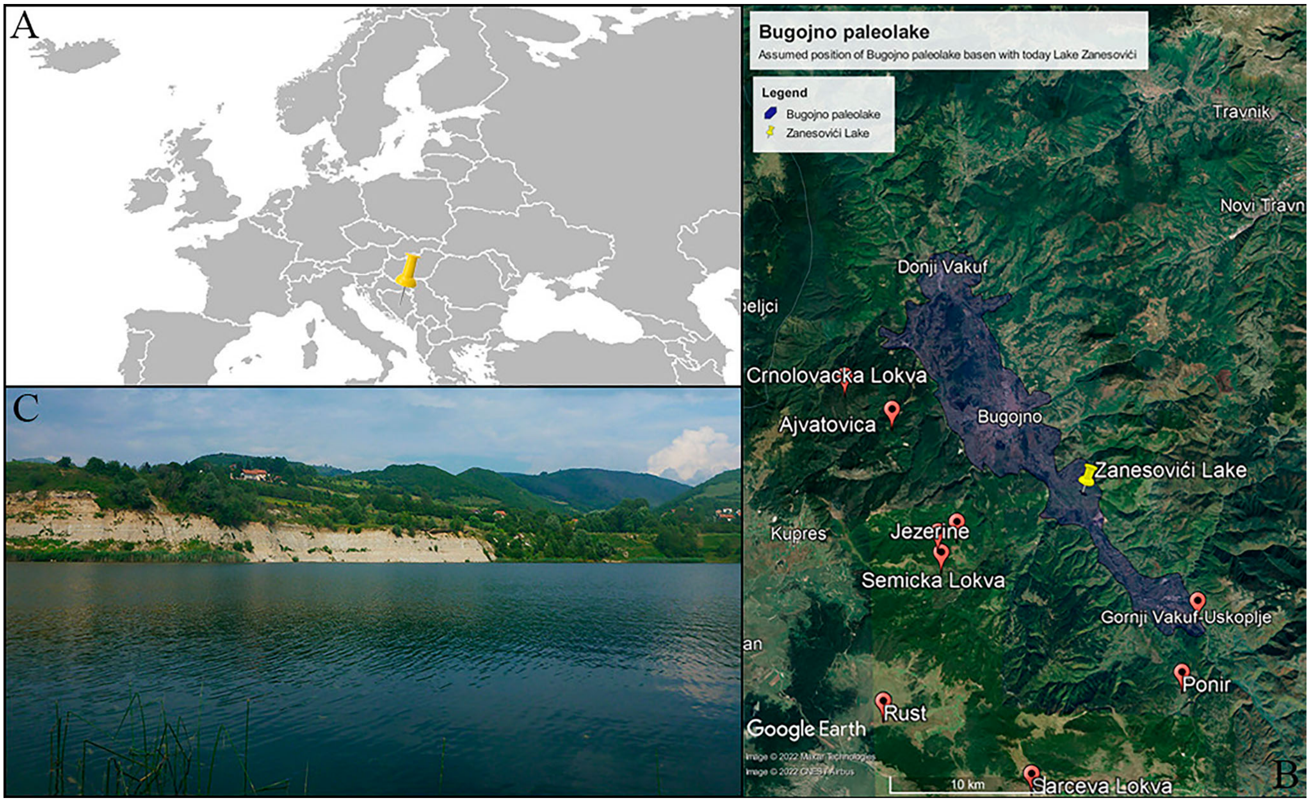


Fig. 1. Map showing the location of Lake Zanesovići and Bugojno palaeolake. A) Location of Bosnia and Herzegovinaon the Balkan Peninsula. B) Topographic map of Lake Zanesovići with assumed position of Bugojno palaeolake. C) Photo of Lake Zanesovići formed on active coal mine on the ground of Gračanica site.

conical, situated at valve face/mantle junction, continuing from ribs (black arrow in Fig. 31). Valve face and marginal fultoportulae absent. Rimoportulae with simple, round external openings on interfascicles on the valve mantle (white arrow in Fig. 31).

SEM internal view (Figs 34–42): Valve face concentrically undulate to flat (in small specimens) with convex to concave central area. Areolae with round openings, organized in fascicles, probably occluded with domed cribra, but corroded in all specimens. Remnants of occlusions visible in Fig. 41 (black arrow). Fascicles radial and multiseriate at the valve face mantle junction, biseriate at the valve margin, becoming uniseriate and/or concentrically uniseriate or irregularly organized at the valve centre. Alveolar openings small round to elongated present at the valve face mantle junction separated by strongly thickened costae (Figs 40–42). Valve face and marginal fultoportulae absent. Rimoportulae, stalked, with large and wide slit, centripetally oriented and situated on every costa (Figs 41, 42; white arrow).

Holotype: Slide 11080 in Macedonian National Diatom collection.

Isotype: BM 100309 in Natural History Museum, London.

Type locality: Bugojno palaeolake basin, Lake Zanesovići on the Gračanica site in central Bosnia and Herzegovina.

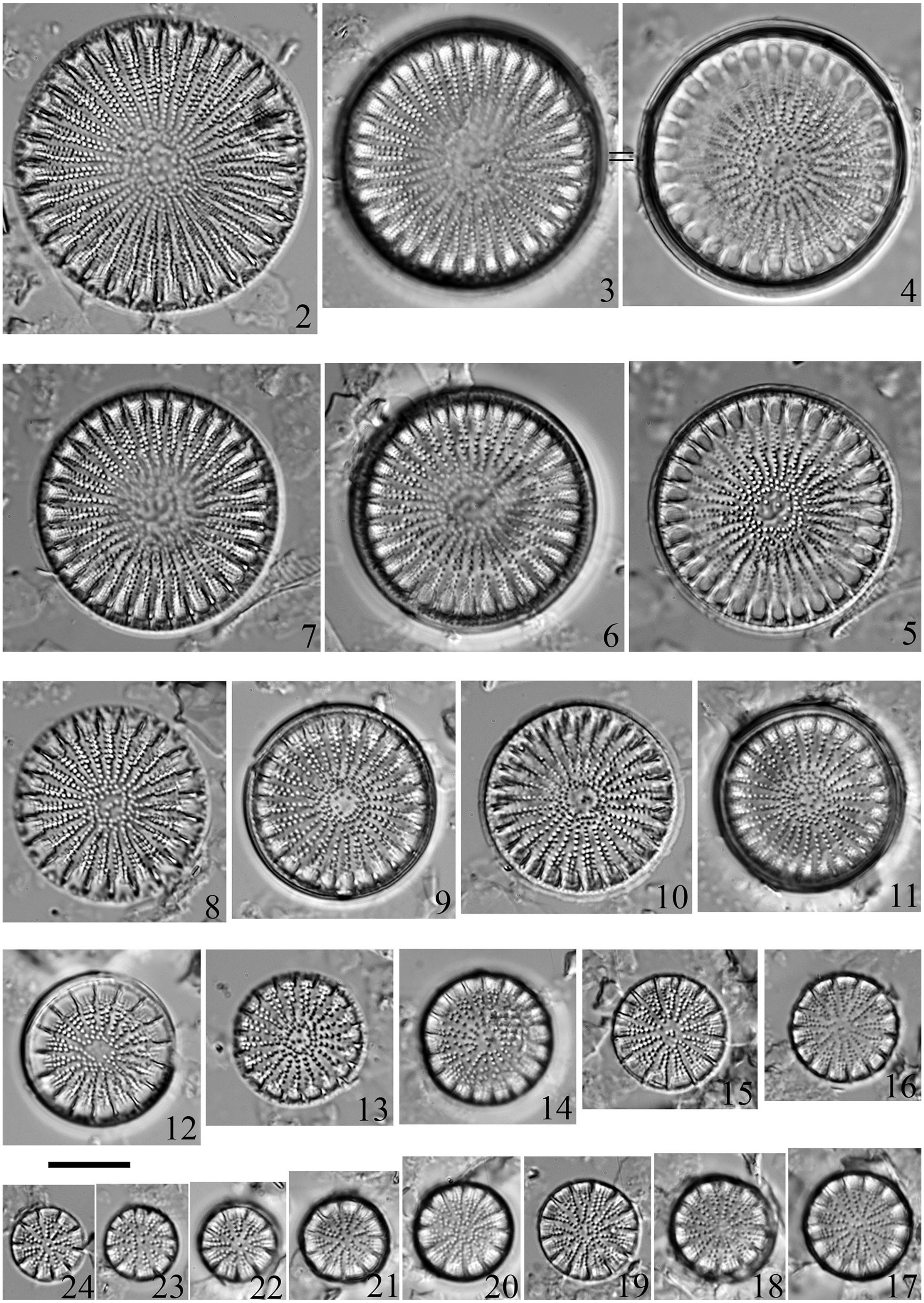
Type material: Lake Zanesovići 10 cm sediment depth, GPS: 44° 0′39.52″N; 17°30′34.46″E. coll. date: 07.2014., leg. E. Mašić.

Ecology: Freshwater fossil species.

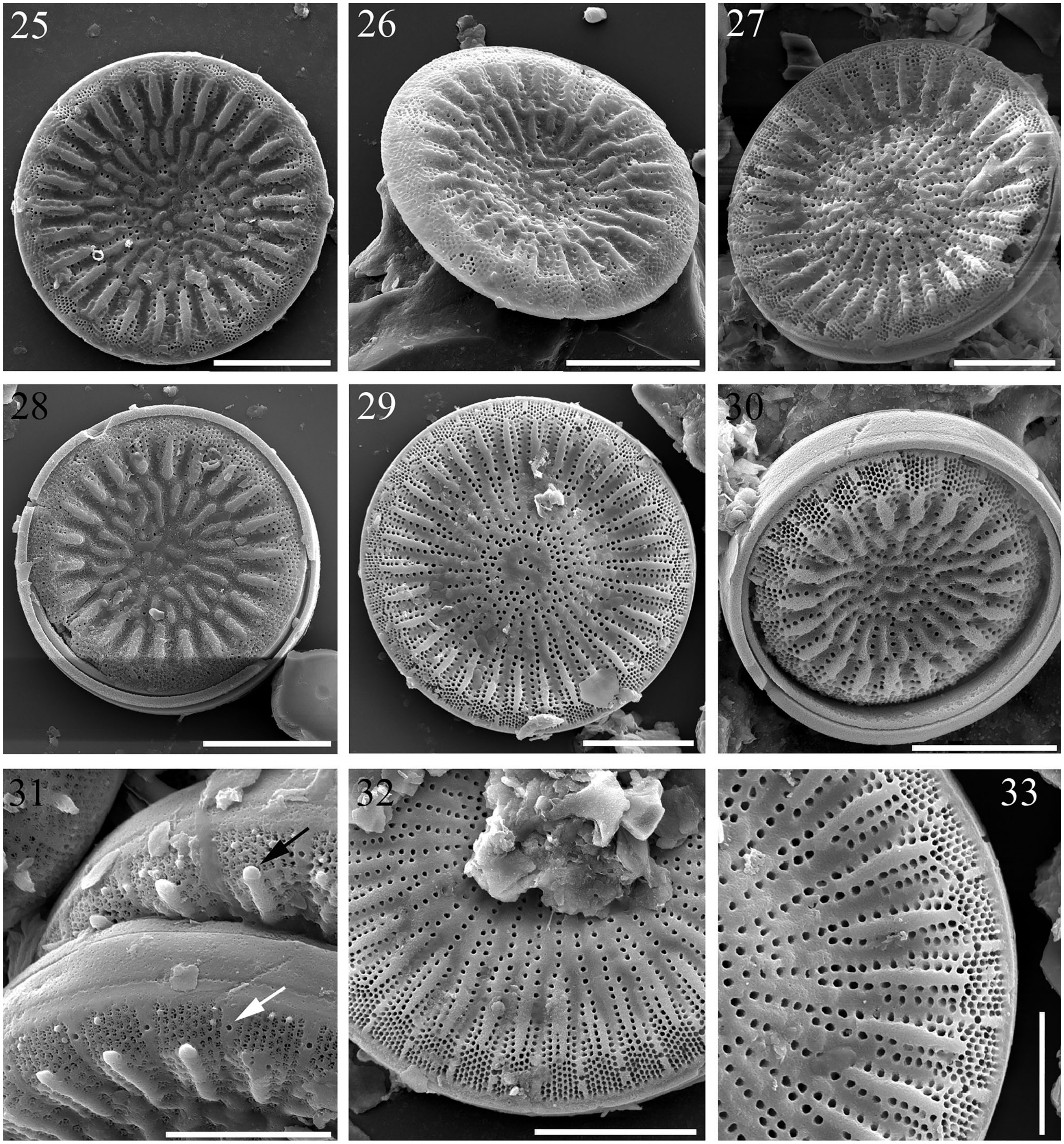
Etymology: The genus name '*Rimocostatus'* refers to the presence of a rimoportula on every costa and the species name '*bugojnicus*' to the type locality, Bugojno palaeolake.

Discussion

The most similar taxa to *R. bugojnicus* were from the family *Heliopeltaceae* H.L. Smith. *Lepidodiscus* Witt (1886, p. 163), described from marine sediments dating back to the Eocene (Round et al. 1990), shares several morphological features: both taxa have circular valves with concentric undulation; the areolae are organized in radiate fascicles, becoming randomly scattered in the central area; alveolar openings separated by strongly thickened costae; the presence of shallow areolate valve mantle; and the most important shared character is the absence of central and marginal fultoportulae but the presence of evenly arranged



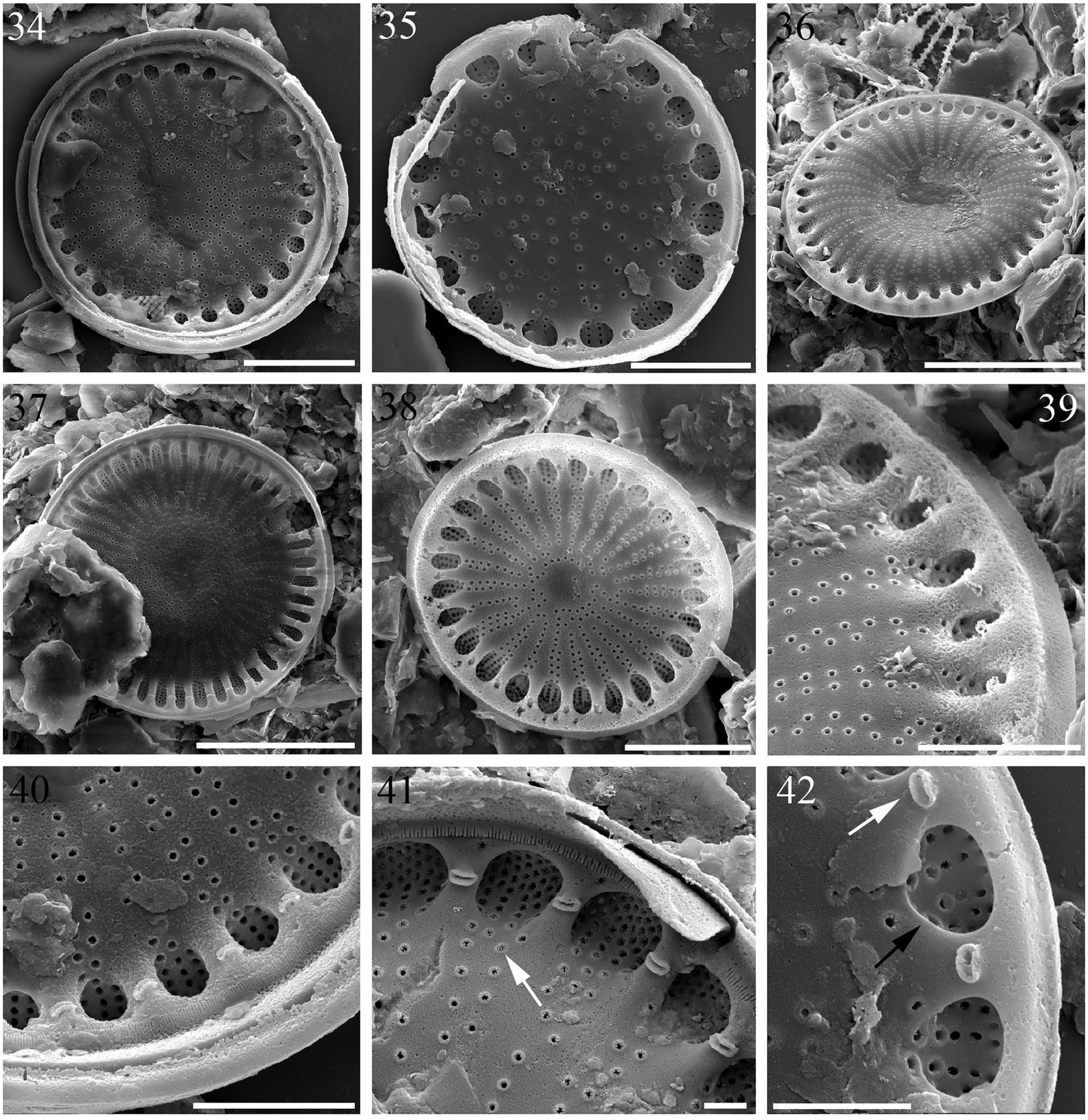
Figs 2–24. *Rimocostatus bugojnicus* gen.et. sp. nov, LM valve views. Scale bar = 10 µm.



Figs 25–33. *Rimocostatus bugojnicus* gen.et. sp. nov. SEM external valve views. Figs 25–30. View of the whole frustule. Fig. 31. Close view of the external openings of marginal rimoportulae (see white arrow) and small conical spines situated on valve face/mantle junction, continuing from ribs (see black arrow). Figs. 32–33. Close view of the external openings of areolae organized in fascicles. Scale bars: 25–30, 32 = 10 μm; 31, 33 = 5 μm.

rimoportulae situated on interfascicles. However, the rimportulae in *R. bugojnicus* are centripetally orientated and situated on a costa closely related to the interfasicles, unlike *Lepidodiscus elegans* Witt in which they are radially orientated, situated on interfascicles related to alveolar openings (Round et al. 1990).

Another similar taxon to *R. bugojnicus* in the *Heliopeltaceae* is *Actinoptychus* Ehrenberg, described from predominantly fossil deposits (Kozyrenko et al. 2008). There are similarities in the presence of marginal rimoportulae in both taxa, but they are easily differentiated by the sectored valve face, with elevated and depressed



Figs 34–42. *Rimocostatus bugojnicus* gen.et. sp. nov. SEM external valve views. Figs. 34–38. View of the whole frustule. Figs. 39–40. Close view of the internal openings of areolae organized in fascicles. Fig. 41. Areolae with small round openings, covered with domed cribra (see white arrow). Fig. 42. Rimoportulae, stalked, with large and wide slit, centripetally oriented and situated on every costa (see white arrow). Detail view of alveolar chambers (see black arrow). Scale bars: 34, 38 = 10 µm; 35, 39, 40 = 5 µm; 36 = 20 µm; 41 = 1 µm; 42 = 2 µm. Alveolar openings small round to elongated present at the valve face mantle junction separated by strongly thickened costae.

parts, in *Actinoptychus*. Moreover, the *Actinoptychus* valve face is often ornamented with additional wart-like outgrows, siliceous ridges or other distinctively patterned features that were not seen in *R. bugojnicus* (Round et al. 1990).

Another fossil species with similar features is *Fideliacyclus wombatiensis* Siver, Wolfe & Edlund described from non-marine sediments from northern Canada (Siver et al. 2016). This species is also characterized by the absence of fultoportulae, but the presence of rimoportulae spaced in a ring along the valve margin. The rimoportulae in *R. bugojnicus* are stalked, large with a wide slit, unlike those in *F. wombatiensis* which lack a stalk and have a thin slit. However, the main difference between *R. bugojnicus* and *F. wombatiensis* is seen in the stria structure: in *R. bugojnicus* the striae are organized in fascicles with alveolar openings at the valve face mantle junction (Figs 31-33), while in *F. wombatiensis* striae are chambered and widely spaced, separated by a hypocaust forming a continuous enclosed space within the cell wall (Siver et al. 2016). This stria organization, as well as the presence of marginal rimoportulae, is more reminiscent of species from the family *Hemidiscaceae* Hendey ex Hasle (Round et al. 1990; Kozyrenko et al. 2008).

Rimocostatus bugojnicus also shows similarities with some other taxa from the *Hemidiscaceae,* such as *Actinocyclus* Ehrenberg and *Roperia* Grunow ex Pelletan. *Roperia* is recent genus, with a few described marine species i.e. *Roperia tesselata* (Roper) Grunow ex Pelletan, *Roperia excentrica* Cheng & Chinand and *Roperia latiovala* Chen & Qian. In general, these species have flat circular valves with pseudonodules near the valve rim. The areolae are circular, loculate, with external cribra and large round internal foramina (Round et al. 1990). The similarity of *Roperia* species to *R. bugojnicus* can be seen in the presence of stalked marginal rimoportulae spaced in a ring around the valve margin. However, internally the rimoportulae of *Roperia* are slit-like and tilted at an angle to the valve face junction, whereas in *R. bugojnicus* they are simple, round openings, positioned on interfascicles on the valve face mantle (Figs 41, 42).

Actinocyclus is an important component of the Miocene period, known from both marine and freshwater environments (Round et al. 1990; Bradbury & Krebs 1995; Temniskova-Topalova & Ognjanova-Rumenova 1997; Kozyrenko et al. 2008; Hayashi et al. 2018). The similarities between *R. bugojnicus* and *Actinocyclus* species [e.g., *A. makarovae* (Temniskova-Topalova & Ognjanova-Rumenova) Temniskova-Topalova & Ognjanova-Rumenova and *A. fungiformis* Temniskova, Chursevich, Valeva] are seen in the presence of marginal rimoportulae with labiate processes and the absence of fultoportulae (Figs 34–42). Additionally, the striae of both genera are organized in radial fascicles and areolae are occluded internally by domed cribra, although *Actinocyclus* has chambered areolae in. Additionally, *Rimocostatus* and *Actinocyclus* can be easily differentiated by the presence of thick ribs on the valve face of *R. bugojnicus* (Fig. 25), the presence of small alveolar openings separated by strongly thickened costae (Figs 40–42) at the valve face mantle junction, as well as the presence of a pseudonodulus.

Taxa similar to *R. bugojnicus* have been also found in the recent flora, represented by freshwater *Stephanodiscus* Ehrenberg and particularly *Cyclostephanos* Round, members of the family *Stephanodiscaceae* Makarova (Houk et al. 2014). Radially arranged fascicles of areolae with intervening hyaline interfascicles are observed in all three genera, as well as a concentrically undulate central area. An additional resemblance between *R. bugojnicus* and some *Cyclostephanos* species i.e. *Cyclostephanos dubius* (Hustedt) Round and *C. novaezeelandiae* (Cleve) Round, is the presence of alveolar chambers between ribs near valve margin. However, the distinctive marginal rimoportulae of *R. bugojnicus* are not present in *Stephanodiscus* or *Cyclostephanos*, which instead have fultoportulae. Thus this species can be easily differentiated.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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