



Research article

Tertiarius minutulus sp. nov. (Stephanodiscaceae, Bacillariophyta) – a new fossil diatom species from Lake Ohrid

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Abstract. A new fossil diatom species, *Tertiarius minutulus* sp. nov., is described from a sediment sequence DEEP-5045-1 of Lake Ohrid. The species is characterized by small valves (3.0–8.0 µm) with a round shape, a marginal area with radially arranged costae and a central uneven area with scattered areolae. Externally, the alveoli are occluded by cribra perforated by irregularly arranged pores. Internally, the alveoli are simple and areolae are occluded with domed cribra. The marginal fulcra are situated on costae close to the valve margin, located on every 5th or 7th thick internal costa. One to three fulcra are present on the valve face, each surrounded by two to three satellite pores. One rimoportula is present, positioned on a costa at the valve face/mantle junction. The species is compared with morphologically similar taxa and a detailed differential diagnosis is provided. *Tertiarius minutulus* sp. nov. is known only as a fossil taxon from Lake Ohrid, with a first occurrence during the early stages of lake basin development.

Keywords. Ancient lake, diatom fossil record, new species, taxonomy.

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Introduction

Lake Ohrid, in the Balkan Peninsula, was formed between 1.9 and 1.3 Ma ago (Wagner *et al.* 2017). It is the oldest existing European lake and one of the few long-lived lakes around the world (Stanković 1960; Radoman 1985; Dumurdžanov *et al.* 2004; Albrecht & Wilke 2008). The lake has accumulated a wealth of sediments throughout its limnological history, which are well constrained in age and hold well-preserved fossils, of which diatoms (Bacillariophyta Karsten) are one of the most diverse and abundant groups of organisms (e.g., Cvetkoska *et al.* 2016). In order to investigate in greater details the age and origin of Lake Ohrid, and link its geological and biological evolution, a deep drilling project was performed in 2013 within the frames of the ICDP project “Scientific Collaboration on Past Speciation Conditions in Lake Ohrid (SCOPSCO)” (Wagner *et al.* 2014). A total of ~2100 m of sediment was recovered from four different sites – DEEP, Cerava, Gradište and Peštani. The drilling in the central part of the lake resulted in a recovery of a 569 m long sediment sequence (DEEP-5045-1). Observations of the diatom communities along the entire length of the core revealed the dominance of planktonic diatoms, mostly belonging to genera of the family Stephanodiscaceae Gleser & Markarova (Wagner *et al.* 2014; Cvetkoska *et al.* 2016; Jovanovska *et al.* 2016a). The majority of them belongs to *Cyclotella* (Kützing) Brébisson s. l., while high abundances of species belonging to *Stephanodiscus* Ehrenberg and *Cribrionella* Jovanovska, Cvetkoska, Tofilovska, Ognjanova-Rumenova & Levkov were also observed in the older parts of the sediment sequence (Wagner *et al.* 2014, Cvetkoska *et al.* 2016). In previous studies on this core, several new diatom species such as *Cribrionella ohridana* Jovanovska, Cvetkoska, Tofilovska, Ognjanova-Rumenova & Levkov (Jovanovska *et al.* 2016b), *Cyclotella cavitata* Tofilovska, Cvetkoska, Jovanovska, Ognjanova-Rumenova & Levkov and *Cyclotella sollevata* Tofilovska, Cvetkoska, Jovanovska, Ognjanova-Rumenova & Levkov (Tofilovska *et al.* 2016) were described. Further studies on this core resulted in a record of a new species bearing unique morphological features that is described here as *Tertiarius minutulus* sp. nov. Additional analyses of core samples from Lake Ohrid are of great importance for improving the understanding of the palaeoecology and biochronology of freshwater diatoms, their evolution and the main environmental factors that shaped the diatom community structure over geological time.

Material and methods

Lake Ohrid (North Macedonia/Albania) is located in a tectonically active graben system in western Macedonia. It is situated in a karstic graben within the Southern Balkan extensional regime (Lindhorst *et al.* 2010). Earlier studies hypothesized that the age of the lake is between 2 and 10 Ma, thus considering it the oldest European lake (Stanković 1960; Radoman 1985). Most recent results from the analyses of the DEEP site sediment sequence suggested that the lake established between 1.9 and 1.3 Ma (Wagner *et al.* 2017). The lake is ~30 km long and 15 km wide, located at an altitude of 693 m above sea level. The maximum water depth is 289 m, and the total volume of the lake is 55.4 km³ (Lindhorst *et al.* 2014). The total water inflow can be estimated to 37.9 m³s⁻¹, with ca 25% originating from direct precipitation and 25% from riverine inflow (Wagner *et al.* 2010). About 50% of the total inflow derives from karst aquifers, of which ca 8 m³s⁻¹ are supposed to come from Lake Prespa (Wagner *et al.* 2010). Within the SCOPSCO project in 2013, a total of 2100 m of sediments was recovered from four different drilling locations (DEEP, Cerava, Gradište and Peštani). In this study, only core samples from the DEEP site were used. This site is located in the central part of the lake at a water depth of 245 m (Franche *et al.* 2016), where drilling resulted in a maximum penetration of 569 m below the lake floor (Wagner *et al.* 2014). The new species, *Tertiarius minutulus* sp. nov., was observed at 452 m of the composite sequence that corresponds to an age older than 1.3 Ma (Wagner *et al.* 2019). The sediment in this part is a mixture of sand, silt and clay (Wagner *et al.* 2014).

For diatom analyses, ca 0.1 g of wet sediment was sampled and stored in Sterilin tubes at 4°C. Samples were prepared using a modification of Renberg’s technique (1990). Diatom samples were acid cleaned

by adding a few drops of cold 10% HCl and 35% H₂O₂, and left overnight to remove carbonates. The samples were then boiled ca 30 min in 37% HCl to oxidize the organic matter. Samples were rinsed several times with distilled water and subsequently centrifuged. Diatom slides were prepared using Naphrax[®]. Slides were observed under oil immersion at 1500 × magnification with a Nikon Eclipse 80i microscope, and diatom images were produced using a Nikon Coolpix P6000 camera. For scanning electron microscopy (SEM), the material was prepared by drying clean diatom suspension onto cover slips that were carbon tape attached to the SEM stubs and coated with gold-palladium (Polaron SC7640 sputter coater, Quorum Technologies, Ashford, UK). SEM observations were performed using a Cambridge S4 Stereoscan at 10 kV (Cambridge Instruments Ltd, Cambridge, UK) at the Friedrich Hustedt Study Centre for Diatoms (BM), Bremerhaven, Germany.

The holotype slide is designated and deposited at the Macedonian National Diatom Collection (MKNDK), Faculty of Natural Sciences, Skopje, Republic of North Macedonia. An isotype slide is hosted at the Friedrich Hustedt Study Centre for Diatoms (BM), Bremerhaven, Germany. Terminology follows Håkansson & Khursevich (1997), Houk *et al.* (2010) and Ognjanova-Rumenova *et al.* (2015).

Results

Phylum Bacillariophyta Karsten
Class Mediophyceae Medlin & Kaczmarska
Order Stephanodiscales Nikolaev & Harwood
Family Stephanodiscaceae Gleser & Markarova
Genus *Tertiarius* Håkansson & Khursevich

Tertiarius minutulus sp. nov.

Figs 1–3

Etymology

The specific epithet '*minutulus*' refers to its small size.

Type material

Holotype

REPUBLIC OF NORTH MACEDONIA • Lake Ohrid, core DEEP-5045-1; 41.049167° N, 20.715000° E; sediment sample from 451.92 m depth of the composite core; Mar.–Jun. 2013; MKNDK 010093.

Isotype

REPUBLIC OF NORTH MACEDONIA • 1 spec.; same collection data as for holotype; BM 81918 (slide).

Type locality

Lake Ohrid core 5045-1, site DEEP, at a depth of 451.92 m of the composite sequence.

Description

Light microscope (LM)

Valves circular, 3.5–8.0 µm in diameter, central area 3.0–6.5 µm in diameter (Fig. 1). Valve face uneven with two parts of distinctly different morphology. Marginal area with short, radiating striae exceeding 1/8–1/4 of valve diameter, with ca 24–30 striae in 10 µm. Central area uneven and colliculate with scattered central areolae that are not radially arranged. Frustules rectangular in connective view.

SEM external view (Fig. 2)

Valve face uneven and colliculate (Fig. 2A–C), with small granules in central area (Fig. 2D–E). Areolae present in central area with simple round openings. Marginal striae short, occluded by cribra, perforated with pores of variable size. Line of bigger pores bordering each costa (Fig. 2D–E). Marginal fuloportulae situated on costae, close to margin edge (Fig. 2A–B), situated on every 6th–7th costa. Externally marginal fuloportulae with simple, slightly elevated and round opening (Fig. 2C, E). One to three valve face fuloportulae present, with external small openings, round (Fig. 2A–B). Single solid ligulate girdle band present (Fig. 2A).

SEM internal view (Fig. 3)

Internal valve face flat with several central areolae occluded with domed cribra (arrow in Fig. 3B). Marginal areolae loculate, crossed with inwardly raised circumferential silica trabeculae (arrowhead in Fig. 3B), while central areolae occluded with domed cribra (Fig. 3B–C). Costae strongly thickened, ca 30 in 10 μm (Fig. 3A, E–F). Marginal fuloportulae with short tube located on short and narrow costae (Fig. 3A). Each marginal fuloportula with two satellite pores in lateral position (arrow in Fig. 3A). One to three valve face fuloportulae present. Valve face fuloportulae with short central tube surrounded with 2–3 satellite pores (arrow in Fig. 3E). One rimoportula present, located at valve face/mantle junction in the middle of costa (arrow in Fig. 3F) or on its side (arrow in Fig. 3D). Rimoportula consists of short and narrow labium with oblique slit (Fig. 3D–F).

Distribution

Freshwater fossil species observed only at its type locality, Lake Ohrid.

Differential diagnosis

The main differential features of *T. minutulus* sp. nov. that can be observed in LM are its small valve size (diameter = 3.5–8.0 μm) and the presence of central scattered areolae that are not radially arranged. *Tertiarius pygmaeus* (Pantocsek) Håkansson & Khursevich (1997: 22) is characterized by a valve diameter ranging from 8.0–16.0 μm , and occasionally the areolae are not arranged in radial striae (e.g., Pantocsek 1892: figs 2: 22, 4: 59; Houk *et al.* 2010: fig. 296: 1, 4). Important differences between *T. minutulus* sp. nov. and *T. pygmaeus* can be observed with SEM. The marginal striae in *T. pygmaeus* do not have a complex alveolar structure and are composed of regular rows of fine pori. Externally, distinct

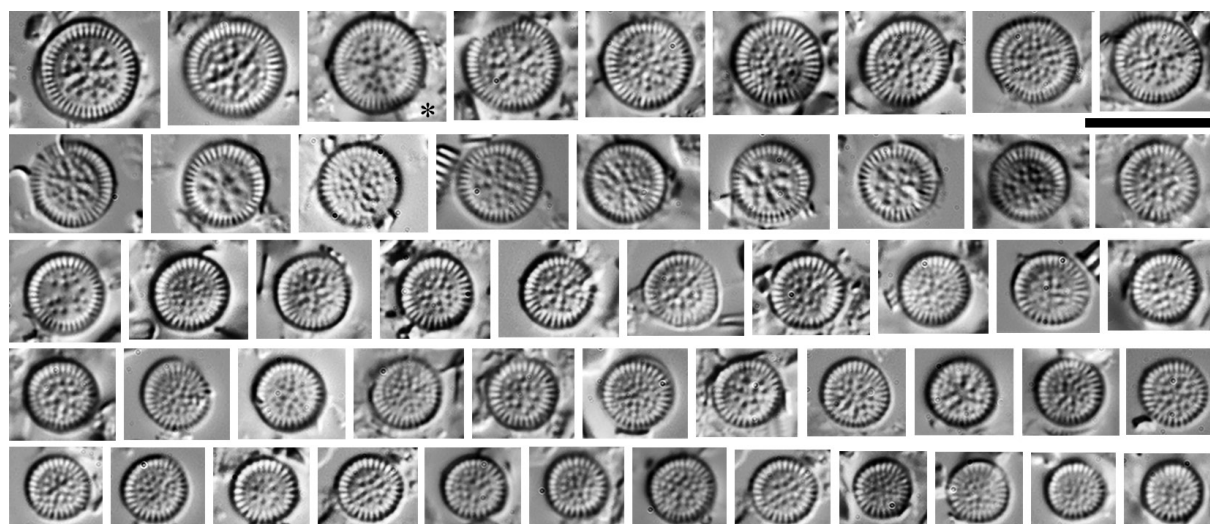


Fig. 1. *Tertiarius minutulus* sp. nov., LM valve views, Lake Ohrid, North Macedonia. The asterisk indicates the holotype. Scale bar = 10 μm .

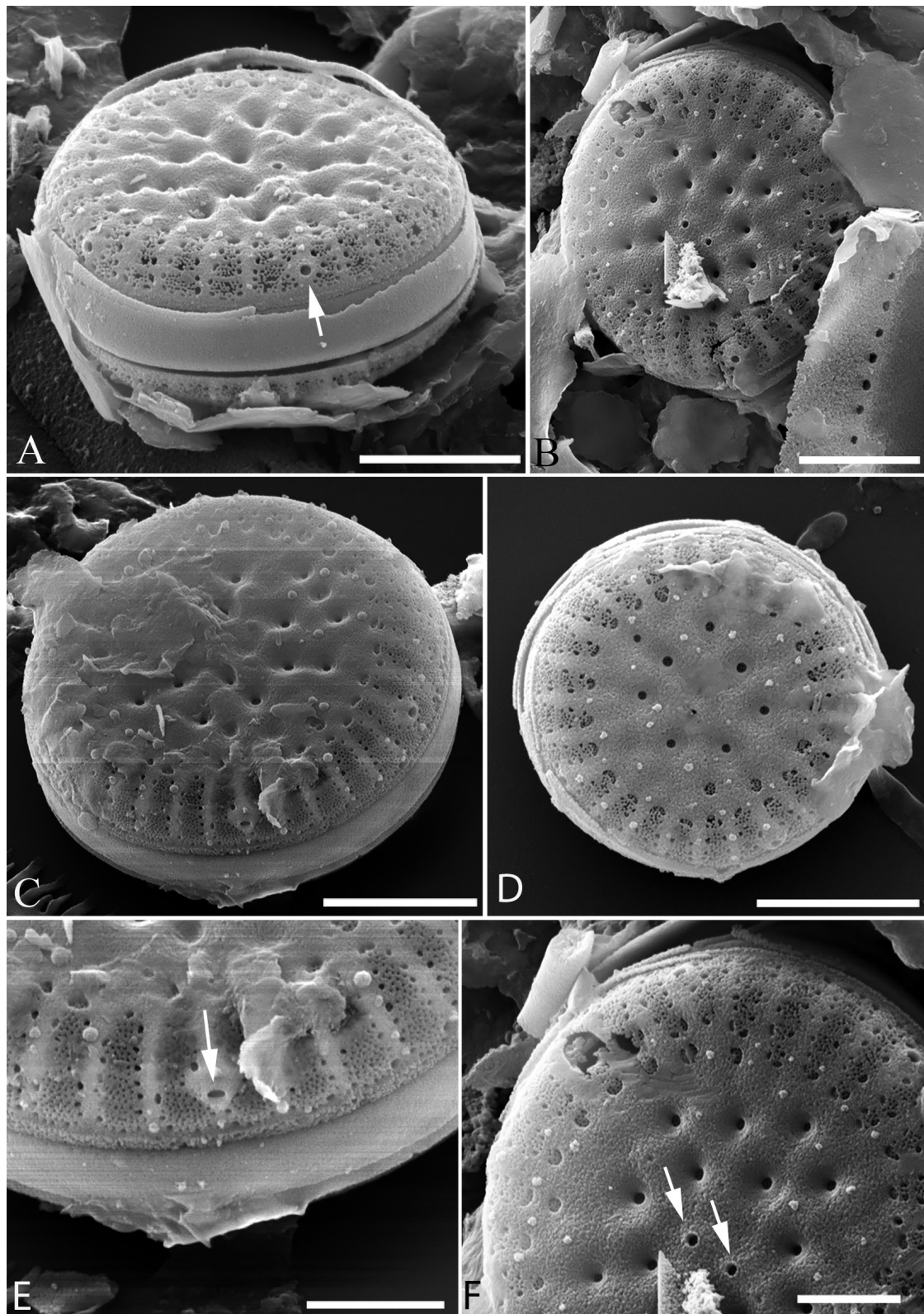


Fig. 2. *Tertiarius minutulus* sp. nov., SEM external valve views. **A.** Valve view showing the marginal openings of the fultoportulae (see white arrow). **B–D.** View of the whole frustule. **E.** Close view of marginal openings of the fultoportula (see white arrow). **F.** Close view of the external openings of the valve face fultoportulae (see white arrows). Scale bars: A–D = 2.0 μm ; E–F = 1.0 μm .

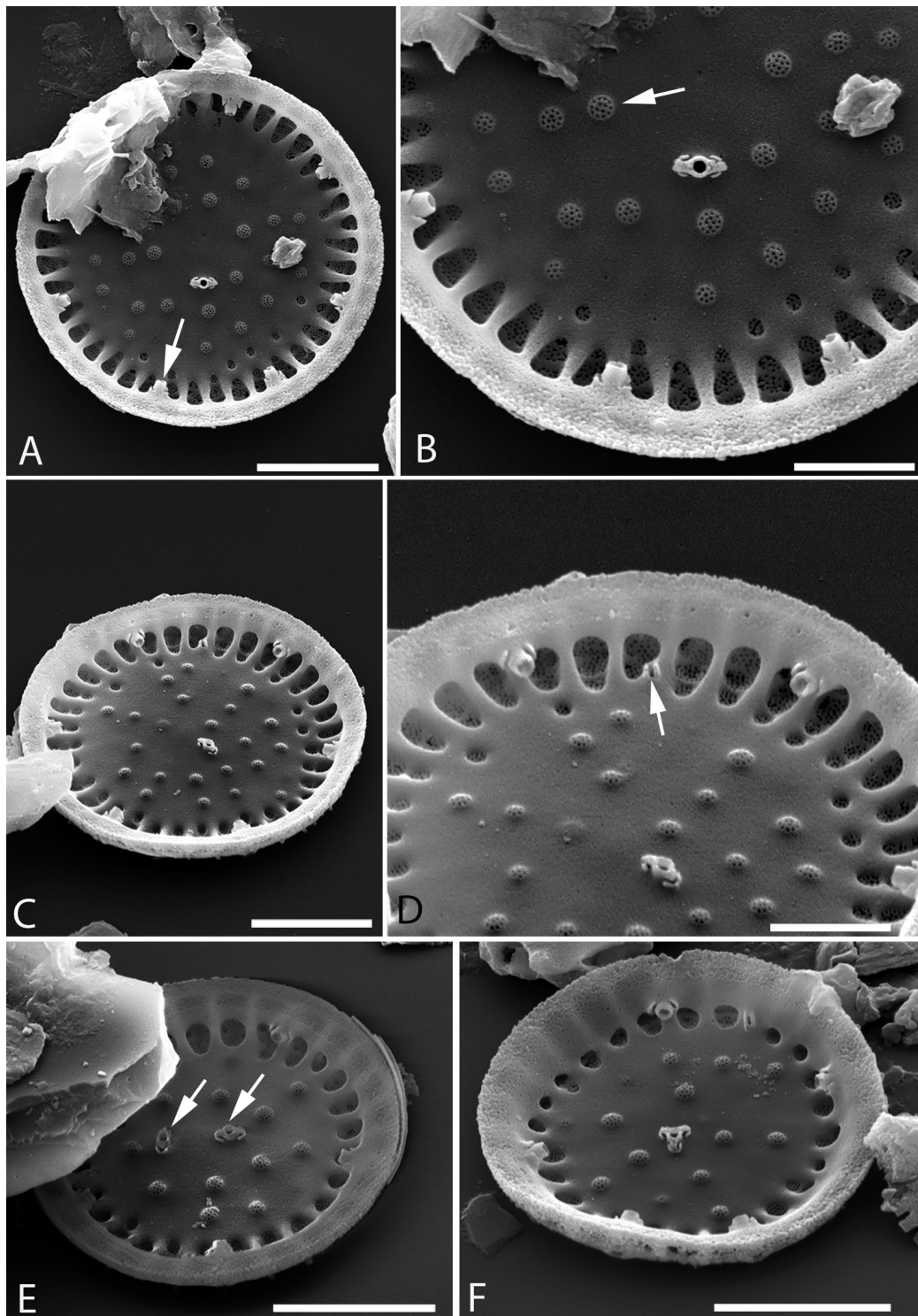


Fig. 3. *Tertiarius minutulus* sp. nov., SEM internal valve views. **A.** View of the whole frustule and the marginal fultoportulae surrounded by two satellite pores, cowlings absent (see white arrow). **B.** Close view of marginal areolae with circumferential silica trabeculae and central areolae occluded by domed cribra (see white arrow). **C.** View of the whole frustule. **D.** Close view of rimoportula (see white arrow) located on costa at junction between valve face/mantle. **E.** Internal valve view showing the central fultoportulae with short central tube surrounded with 2–3 satellite pores (see white arrows). **F.** Valve view. Scale bars: A, C, E–F = 2.0 μm ; B, D = 1.0 μm .

spines are present on every costa. Internally, the alveolus is round and small in *T. pygmaeus* whereas it is larger and more elongated in *T. minutulus* sp. nov. In *T. pygmaeus*, the marginal fuloportulae are present on each costa, the number of valve face fuloportulae is one to seven scattered in the central area, and the rimoportula is situated inside of the alveolar opening on a side of costa. Additionally, the costae are much thicker and with a lower density (8–10 in 10 μm) in *T. pygmaeus* than in *T. minutulus* sp. nov.

Another species with small-sized valves is *T. mariovensis* Ognjanova-Rumenova, Jovanovska, Cvetkoska & Levkov (diameter 4.0–19.0 μm), recently described from a diatomite near the village Manastir, Mariovo Neogene Basin, Republic of North Macedonia (Ognjanova-Rumenova *et al.* 2015: 56, figs 36–105). This species was also observed in several sediment samples from Lake Ohrid DEEP-5045-1 core. However, both species can easily be differentiated by the structure of the central area. In *T. mariovensis*, the areolae in the central area form radially arranged striae. The number of valve face fuloportulae in *T. mariovensis* is higher (2–9) and the tube of the central fuloportula is always surrounded by three satellite pores. Additionally, the marginal fuloportulae are positioned on every costa, while the rimoportula is located inside and in the middle of the alveolus, but not connected to a costa as in *T. minutulus* sp. nov. Another small-sized species, *Tertiarius distinctus* Khursevich & Kociolek (2002: 333, figs 1–5, 12–22), has a comparable valve size as *T. minutulus* sp. nov. (diameter 4.5–14.0 μm), but coarser areolae arranged in short or long radial striae composed of large areolae, while the marginal striae are composed of 4–6 rows of small pori. Marginal fuloportulae are present on every costa, while in the central area there are 1–5 valve face fuloportulae. The rimoportula is located near the base of a costa within the alveolus. Differences between *T. distinctus* and *T. minutulus* sp. nov. can be noticed in the structure of the marginal and central striae, the number of valve face and marginal fuloportulae, and the position of the rimoportula. Smaller specimens of *T. indigenus* Khursevich & Kociolek (2002: 336, figs 6–11, 23–32) have a valve size comparable to *T. minutulus* sp. nov. (diameter 5.0–27.5 μm). However, differences between these two species can be noticed in the striae structure (longer marginal striae composed of 4–5 regular rows or pori), central area (colliculate central area with 3–5 large areolae and several ‘bumps’), presence of fuloportulae on each costa and one to several rimoportulae located on thinner costae within the alveolar chamber.

Tertiarius baicalensis Khursevich & Fedenya (in Khursevich *et al.* 2003: 306, figs 1: 1, 2, 4, 5, 14) is characterized by circular valves with flat face and diameter of 6.4–16.6 μm , three to 17 valve face fuloportulae arranged in radial rows and one to four sessile rimoportulae located in the submarginal zone of the valve face. Differences between *T. minutulus* sp. nov. and *T. baicalensis* can be noticed in the ornamentation of the central area (areolae organized in radial striae in *T. baicalensis*), number and position of valve face fuloportulae, number of satellite pores on marginal fuloportulae (three satellite pores in *T. baicalensis*), and position of rimoportula(e) that are located in the submarginal zone of the valve face (not connected with costa or within the alveolar chamber).

Another small-celled species, *Cribrionella ohridana*, was recently described from the same core from Lake Ohrid (Jovanovska *et al.* 2016b). Both species share several characters such as small size (2.0–7.5 μm in diameter), the presence of areolae in the central area and a single rimoportula situated on a costa, marginal fuloportulae located on each 4th–5th costa surrounded by two satellite pores and the presence of inwardly raised circumferential silica trabeculae. However, differences between these two species can be noticed in the absence of valve face fuloportula in *C. ohridana* and areolae internally not occluded with domed cribra as in *T. minutulus* sp. nov.

One of the dominant and very frequent species in fossil diatom assemblages of Lake Ohrid is *Cyclotella minuscula* (Jurilj) Cvetkoska. *Cyclotella minuscula* was recently transferred to *Lindavia* (Schütt) De Toni & Forti as *L. minuscula* (Jurilj) Nakov, Guillory, Julius, Theriot & Alverson (Nakov *et al.* 2015) and later to the genus *Pantocsekiella* Kiss & Ács as *P. minuscula* (Jurilj) Kiss & Ács (Ács *et al.* 2016).

Table 1 (continued on the next page). Morphological and ultrastructural characteristics of known species of *Tertitarius* (Guiry & Guiry 2019). NAD = no available data.

| Species | Valve size (µm) | Central area size (µm) | Number of striae in 10 µm | Structure and position of mantle fultoportulae | Structure and position of valve face fultoportulae (FP) | Structure and position of rimoportulae (RP) | Source |
|--|-----------------|------------------------|---------------------------|---|--|---|---------------------------------------|
| <i>Tertitarius minutulus</i> sp. nov. | 3.5–8.0 | 3.0–6.5 | 25–30 | on every 6 th –7 th costa, with two satellite pores | 1–3 FP with 2–3 satellite pores | one RP at the valve face/mantle junction on a costa | this study |
| <i>Tertitarius baicalensis</i> Khursevich & Fedenya | 4–16 | 1.5–8 | 10–20 | on every costa, near the valve edge with three satellite pores | 3–17 FP with two satellite pores | 1–4 RP in the sub-marginal zone of the valve face | Khursevich <i>et al.</i> 2003 |
| <i>Tertitarius chernomoricus</i> Khursevich & Kociolek | 10–18 | NAD | 6–8 | on every costa with two satellite pores | NAD | one RP at the end of the submarginal zone of the valve face | Khursevich & Kociolek 2012 |
| <i>Tertitarius distinctus</i> Khursevich & Kociolek | 4.5–14 | 4–8.5 | 10–20 | on every costa with two satellite pores | 1–5 FP with two satellite pores; | one RP near the base of costae or within a chamber of the alveolus | Khursevich & Kociolek 2002 |
| <i>Tertitarius elgeri</i> (Hustedt) V.Houk, R.Klee & H.Tanaka | 20–110 | 10.5–24.5 | 13–15 | on every costa with two satellite pores | absent | one RP between striae, connected with a short rib at the valve margin and one of the adjacent costa | Houk <i>et al.</i> 2010 |
| <i>Tertitarius indigenus</i> Khursevich & Kociolek | 5–27.5 | 2.5–11.5 | 6–8 | on every costa with two satellite pores | absent | 1–4 RP on the thinner costae within the alveolar chambers | Khursevich & Kociolek 2002 |
| <i>Tertitarius hidalgensis</i> Caballero, Khursevich & Velasco de León | 8–32 | 11–20 | 10–15 | on every costa with two satellite pores | 4–10 FP, forming a ring near the valve centre with 2–3 satellite pores | one RP between two costae inside alveolar opening | Caballero <i>et al.</i> 2009 |
| <i>Tertitarius jurjii</i> Ognjanova-Rumenova, Jovanovska, Cvetkoska & Z.Levkov | 5.0–38.0 | 2.0–24.0 | 5–8 | on every costa with two satellite pores | 5–12 FP, with three satellite pores | one RP at the base between two costae in front of alveolar opening | Ognjanova-Rumenova <i>et al.</i> 2015 |

Table 1 (continued). Morphological and ultrastructural characteristics of known species of *Tertiarius* (Guiry & Guiry 2019). NAD = no available data.

| Species | Valve size (µm) | Central area size (µm) | Number of striae in 10 µm | Structure and position of mantle fultoportulae | Structure and position of valve face fultoportulae (FP) | Structure and position of rimoportulae (RP) | Source |
|---|-----------------|------------------------|---------------------------|--|---|---|---------------------------------------|
| <i>Tertiarius mariovens</i> Ognjanova-Rumenova, Jovanovska, Cvetkoska & Levkov | 4.0–19.0 | 1.5–13.5 | 6–12 | on every costa with three satellite pores | 2–9 FP, arranged in a ring around valve centre with three satellite pores | one RP at the end of the submarginal zone of the valve face | Ognjanova-Rumenova <i>et al.</i> 2015 |
| <i>Tertiarius pantocsekii</i> (Fricke) Khursevich & Kociolek | 18–55 | 2.5–3.2 | 3–5 | on every costa with three satellite pores | NAD | one RP at the end of the submarginal zone of the valve face | Khursevich & Kociolek 2012 |
| <i>Tertiarius porosus</i> Khursevich & Kociolek | 10–60 | 6.5–3.2 | 5–7 | on every costa with two satellite pores | 1–4 FP, with two satellite pores | 1–4 RP at the base of an elongated chamber | Khursevich & Kociolek 2002 |
| <i>Tertiarius pygmaeus</i> (Pantocsek) Håkansson & Khursevich | 8–14(16) | 6.5–1.1 | 6 | on every costa with two satellite pores | 6–8 FP, with two satellite pores | one small RP positioned on a side of a costa | Houk <i>et al.</i> 2010 |
| <i>Tertiarius rodadae</i> Kociolek & Khursevich | 6–45 | 2–2.7 | 3–8 | on every costa with two satellite pores | 3–6 FP, with two satellite pores | one RP at the submarginal zone of the valve face | Khursevich & Kociolek 2002 |
| <i>Tertiarius satsumaensis</i> (H. Tanaka & V. Houk) Nakov <i>et al.</i> | 4.5–9.5 | 3.5–7 | 16–24 | on every 3 rd –9 th costa on the lower part of the mantle with two satellite pores | 2–3 with two satellite pores associated with the depressions | one RP on thickened costa | Houk <i>et al.</i> 2010 |
| <i>Tertiarius tempereiformicus</i> (Khursevich) Nakov <i>et al.</i> | 14.3–60 | 21.5–51.5 | 7–10 | on every costa with two satellite pores | absent | up to four RP on thickened costa | Khursevich <i>et al.</i> 2001 |
| <i>Tertiarius transylvanicus</i> (Pantocsek) Håkansson & Khursevich | 22–36 | 17–29 | 6–8 | on every costa with two satellite pores | in variable number with two satellite pores | one RP located on a side of a costa | Houk <i>et al.</i> 2010 |

Both species, *Tertiarius minutulus* sp. nov. and *Cyclotella minuscula*, have a similar valve size (diameter 3–7 µm in *C. minuscula*), a colliculate central area, a small marginal zone, a single valve face central fuloportula, a marginal fuloportula with two satellite pores located on each 4th–10th costa and a single rimoportula positioned at a rib, beneath the ring of marginal fuloportulae. Difference between these two species can be observed only in a presence/absence of areolae in the central area: in *C. minuscula* areolae are absent, while in *T. minutulus* sp. nov. they are present.

Discussion

Earlier studies have described 17 taxa (16 species and one variety) which belong to the genus *Tertiarius* (Guiry & Guiry 2019), Table 1. The initial description of the genus *Tertiarius* is based on sediment samples of Miocene and Pliocene age from Köpez (Transylvania, Romania). Furthermore, new species of *Tertiarius* have been described from deposits in the western USA (Khursevich & Kociolek 2002), Pliocene sediments from Mexico (Caballero *et al.* 2009) and Middle Pliocene sediments from Lake Baikal in Asia (Khursevich *et al.* 2003). In addition, two species from this genus, viz., *T. jurilii* Ognjanova-Rumenova, Jovanovska, Cvetkoska & Z.Levkov and *T. mariovensis*, are described from sediments of Pliocene age from Mariovo Basin, North Macedonia (Ognjanova-Rumenova *et al.* 2015). The presence of *Tertiarius mariovensis* is also recorded from sediments of Lake Ohrid, at ca 630 ka (Francke *et al.* 2016). According to Khursevich & Kociolek (2012), the biostratigraphic range of species from the genus *Tertiarius* in the Northern Hemisphere extends into the Miocene to Pliocene periods. Interestingly, both *T. mariovensis* and *T. minutulus* sp. nov. have been observed in Middle Pleistocene up to Quaternary sediments. These two recent records of *Tertiarius* probably represent the latest occurrence of the genus in freshwater habitats, again supporting the notion of Lake Ohrid being refugium for many species throughout its existence.

SEM observations indicate that *T. minutulus* sp. nov. shares characteristics with the genus *Lindavia* (Schütt) De Toni & Forti (= *Handmania* M.Peragallo = *Puncticulata* Håkansson). The genus *Tertiarius* is considered as fossil with a biostratigraphic range from Miocene to Pliocene, while *Lindavia* (= *Handmannia* M.Peragallo) has a longer range from Middle Eocene to present. *Tertiarius* is characterized by laterally positioned rimoportula on a fuloportula-bearing costa, while in *Lindavia*, the rimoportula is located on the valve face (Nakov *et al.* 2015). In the latter study, the authors proposed a broad concept of the genus *Lindavia*, but Ács *et al.* (2016) narrowed the concept with a description of the newly erected genus *Pantocsekiella* Kiss & Ács (in Ács *et al.* 2016: 62). Both *Lindavia* and *Pantocsekiella* have the rimoportula situated on the valve face. However, the position of the rimoportula can be variable (Houk *et al.* 2010, Cvetkoska *et al.* 2014). Similarly, in *Tertiarius*, the position of the rimoportula is variable: in some species it is located in the alveolus (e.g., *T. mariovensis*), on the side of costa (e.g., *T. pygmaeus*), or in the marginal zone (*T. baicalensis*). In most species of *Lindavia*, the rimoportula is located on the valve face, but in *L. thienemannii* (Jurilj) Nakov, Guillory, Julius, Theriot & Alverson, it is associated with a costa and situated near the valve face/mantle junction (Levkov unpubl. data). Another important feature of *T. minutulus* sp. nov. is the arrangement of marginal fuloportulae (on every 6th–7th costa). In all species of *Tertiarius* so far observed with SEM, marginal fuloportulae are located on every costa whereas it is on every 3rd–7th costa in species of *Lindavia* (Houk *et al.* 2010).

In general, species of *Tertiarius* and *Lindavia* can be differentiated mainly by the position of the rimoportula. *Tertiarius minutulus* sp. nov. shares the features of both genera and might be placed in any of these two genera. Based on the number and position of fuloportulae, *T. minutulus* sp. nov. can be related to *Lindavia*, but based on the position of rimoportula it is closer to *Tertiarius*. Since the occurrence of this fossil species is in relatively deeper geological time, molecular analyses that would uncover its phylogenetic status are yet rather impossible (see for example ancient DNA limitation in Wilke *et al.* 2016).

Khursevich & Stachura-Suchoples (2008) consider the genus *Tertiarius* to be closely related to the genus *Pliocaenicus* F.E.Round & Håkansson (Round & Håkansson 1992: 116) based on the position of the internal domed cribra on valve face areolae, the presence of alveolae mainly on the valve mantle and the external opening of the rimoportula lacking tubuli. The main features that distinguish these two genera are the valve undulation, absence of externally domed interfascicles, different type structure of the rimoportula (i.e., fan-shaped, sessile or raised) and different location of rimoportula – on the valve surface in *Pliocaenicus* and on the valve mantle in *Tertiarius*. Based on these differential features, *T. minutulus* sp. nov. can be distinguished from *P. hercynicus* F.E.Round & Håkansson and *P. undulatus* F.E.Round & Håkansson (Round & Håkansson 1992).

Tofilovska *et al.* (2016: 230) discussed in detail the validity of the transfer of *Cyclotella minuscula* to *Lindavia*, pointing out the variability of a main synapomorphic character, the position of the rimoportula, that is quite variable in this species (see also Levkov *et al.* 2007 and Cvetkoska *et al.* 2014). Similarly, as in *Cribrionella ohridana* and *T. minutulus* sp. nov., the rimoportula in *Cyclotella minuscula* is located at the base of costa. Having in mind that the position of rimoportula should be considered as a main synapomorphic character, then these three species (*Cyclotella minuscula*, *Cribrionella ohridana* and *Tertiarius minutulus* sp. nov.) might represent a natural group and could be placed in the same genus. This raises another important question about the stability of characters considered as synapomorphic for any particular genus. As was pointed out, the position and location of rimoportula, the presence of valve face fuloportulae, the number of satellite pores on the fuloportulae, the presence of central lamina, etc. are variable characters within each genus. In such case, the only possible and obviously accepted solution in diatom systematic is to use a combination of characters that is unique for each genus. However, this approach can result in the future in separation and description of new cyclotelloid genera, based on already noted and differentiated morphological groups by Khursevich & Kocielek (2012). There is a general consensus that the number of diatom genera is underestimated, but the main task is to find appropriate and stable morphological characters that are synapomorphic for all members of the genus. There are several recent studies that resulted in synonymization of newly described genera (e.g., *Neofragilaria* D.M.Williams & F.E.Round, *Synedrella* Round & N.I.Maidana, *Lacunicula* Lange-Bertalot, Cavacini, Tagliaventi & Alfinito, etc.), since the characters used for their separation are present in other genera. However, whether this is the case with *Tertiarius* and *Lindavia* and whether both genera represent a single genus, remains an open question.

It seems that in the older species of *Tertiarius*, the rimoportula is located near the valve margin (within the alveolus or connected with the costa) while in *T. minutulus* sp. nov., it is located on the costa. In contemporary species of *Lindavia*, the rimoportula is located on the valve face distantly from the costa. It might be hypothesized that during the evolution, the rimoportula moved from the valve margin to the valve face. A similar pattern can be noticed in *Cyclotella* vs *Pantocskiella*, where in *Cyclotella* s. str. the rimoportula is located on the valve mantle while in *Pantocsekiella* it is on the valve face. In some fossil species of *Cyclotella* (e.g., *Cyclotella iris* Brun & Héribaude-Joseph) the rimoportula is located on the transition of valve the mante to the valve face (e.g., Houk *et al.* 2010: fig.s 183: 5). However, additional SEM observations especially on fossil species of *Lindavia* and *Pantocsekiella* are necessary to obtain a precise answer.

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