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Two new *Tertiarius* (Bacillariophyta, Coscinodiscophyceae) species from Mariovo Neogene Basin, Macedonia

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Abstract: The morphology, biostratigraphy and ecology of two new species, *Tertiarius jurijlii* and *Tertiarius mariovensis* are presented. The valve morphological variation and ultrastructure are described by light and scanning electron microscopy. *Tertiarius juriljii* is distinguished by the presence of hyaline area on the valve face; central area comprised of areolae arranged in single short or long radial rows; presence of small to large granules on the interstriae; and valve face fultoportulae arranged in radial rows around the valve center. Comparatively, in *Tertiarius mariovensis* the hyaline area around the central area is missing, and the taxon is characterized by tri– to biseriate striae in the central part of the valve face becoming uniseriate towards the valve centre and stalked rimoportula positioned between two costae inside the alveolar opening. Both species are found in nonmarine sediments of Pliocene age from Mariovo Basin, Macedonia.

Key words: Biostratigraphy, Macedonia, morphology, systematic relationships, *Tertiarius juriljii*, *Tertiarius mariovensis*

Introduction

The fossil records of the genera within the family Stephanodiscaceae (Bacillariophyta, Coscinodiscophyceae) have presented the most important biochronology of the freshwater diatoms during the Miocene and Pliocene periods (Krebs et al. 1987; Fourtanier & Gasse 1988; Khursevich 1994, 2006; Ognjanova–Rumenova 2000). Over the last two decades a number of diatom studies revealed several extinct genera within the family Stephanodiscaceae: *Concentrodiscus* Khursevich, Moisseeva et Sukhova; *Mesodictyon* Theriot et Bradbury; *Mesodictyopsis* Khursevich, Iwashita, Kociolek et Fedenya; *Tertiarius* Håkansson et Khursevich; *Tertiariopsis* Khursevich et Kociolek; *Stephanopsis* Khursevich et Fedenya and *Cyclostephanopsis* Loginova (Khursevich & Kociolek 2012). The genus *Tertiarius* was proposed for some *Cyclotella* (Kützing) Brébisson species described by Pantocsek (1892) from the Neogene fossil deposits in Romania. It is characterized by a combination of the following morphological features: a) more or less flat valves; b) hyaline area on the valve face that creates a break between the ornamentation of the central and marginal area; c) alveolae with internal domed cribra; d) simple or complex internal alveolate structure of the submarginal zone; e) marginal fultoportulae with two or three satellite pores situated on the costae (ribs); f) variable position of the rimportula(e), either in the submarginal zone of the valve face at the base of an internal costa(e) or within the alveolar chamber(s); g) valve face fultoportulae with two or three satellite pores; rarely they are absent (Håkansson & Khursevich 1997; Khursevich & Kociolek 2012).

Until now, 12 species belonging to the genus *Tertiarius* have been described from the Miocene and Pliocene sediments across the northern hemisphere (Europe, Asia and North America). Only two of them are known from the brackish–marine sediments of the Black sea, while all others have been described from freshwater environments (Håkansson & Khursevich 1997; Khursevich & Kociolek 2002; Caballero et al. 2009; Houk et al. 2010; Khursevich & Kociolek 2012).

Interesting cyclotelloid species from Mariovo Neogene Basin (Macedonia) were reported for the first time in Jenko & Gjuzelkovski (1958), but their taxonomic identifications and illustrations were provided by Jurilj. In the same publication Jurilj introduced one new species – *Cyclotella macedonica* Jurilj nom.nud. with two varieties: *C. macedonica* var. *media* Jurilj nom.nud. and *C. macedonica* var. *stictodiscus* Jurilj nom.nud. These taxa share the morphological features of the genus *Tertiarius* and thus a new combination *Tertiarius macedonica* (Jurilj) Ognjanova–Rumenova was proposed by Ognjanova–Rumenova (2001) and Ognjanova–Rumenova & Dumurdjanov (2008). Nonetheless, all these names are invalid, since they were introduced with only few illustrations, without providing formal descriptions and designation of types. This study provides a valid description of two new species *T. juriljii* sp. nov. and *T. mariovensis* sp. nov. from the Mariovo Basin, Macedonia.

Material and Methods

The Mio–Pliocene basin of Mariovo (Macedonia) is a neotectonic depression formed during the Late Miocene (Dumurdžanov et al. 2003). Based on the coal research from the outcrop and the boreholes, the thickness of the Neogene sediments in Mariovo is estimated between ca. 450–500 m. The sediments of the basin have been divided into four lithostratigraphic units, from the bottom to the top (Dumurdžanov et al. 2004):

1) The Nerezi Formation, divided into three informal units. The basal unit is ca. 120 m thick and essentially consists of gravel and sandstones of unknown age. The middle unit consists of ca. 70 m thick silty stones and claystones that grade upward into coal and claystones and finally into a 6–15 m thick layer of coal overlaid by marls and claystones. The latter one contains fossil diatom flora, and is proved to be of Late Miocene age (Ognjanova–Rumenova & Dumurdjanov 2008). The upper unit consists of ca. 60 m thick siltstones and sandstones, followed by a break of sedimentation.

2) The second lithostratigraphic unit is the Solnje Formation, a ca. 60–80 m thick sediment, dominated by gravel and sandstones of Pliocene age.

3) The Vitacevo Formation, which is of Pliocene age (Dumuržanov et al. 2004) starts with stratified tuffs overlaid by sandstones and gravel, interbedded with diatomite, tuffs and sandy claystones. Travertine layers are deposited over them and followed by a ca. 80 m thick layer of tuff and sandstones.

4) The Mariovo Formation is ca. 60–70 m thick and contains pyroclastic rocks with nine travertine layers.

The sediments used for this study were collected from a ca. 4 m thick outcrop, located in the old diatomite mine, north of the village Manastir (41°10'03"N, 21°43'42"E). Eleven samples were diatom–bearing. The diatom rich rocks belong to the Vitacevo Formation, and are mostly silty, porous and bright with whitish or yellowish color. Ten diatomite samples were collected additionally near the village Manastir, and near the road from the village Manastir to the village Zovich.

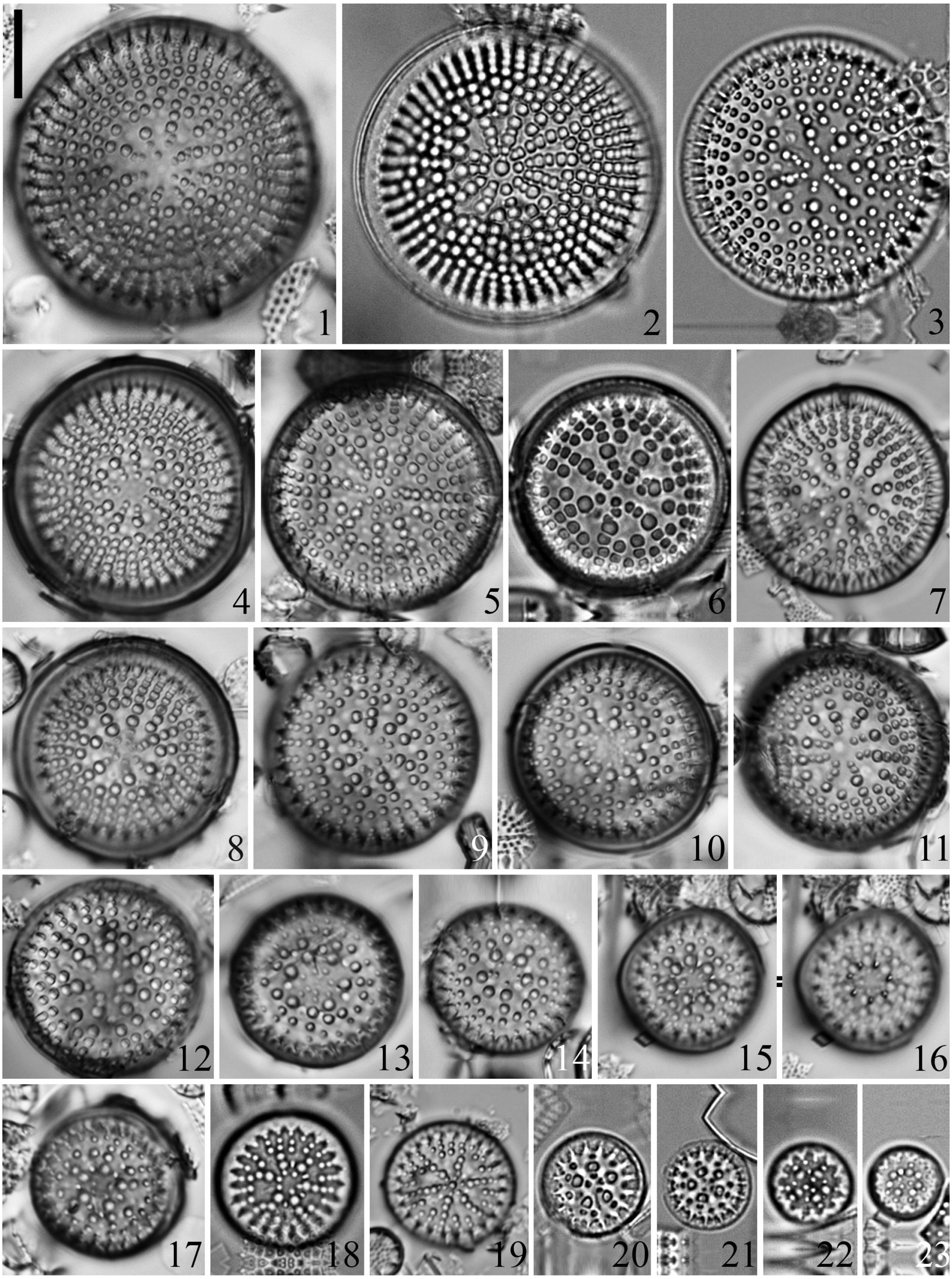
The sample cleaning procedure for the diatom analysis adopts a combination of the methods of Schrader (1973) and Hasle & Fryxell (1970) and is described in more detail in Ognjanova–Rumenova (1991). Permanent microscope diatom slides were mounted using Naphrax® and analyzed under oil immersion at ×1500 magnification with Nikon Eclipse 80*i* (Nikon Corp., Tokyo, Japan) light microscope (LM). Light microphotographs were made with a Nikon Coolpix 6000 digital camera. A total of 100 valves from different samples in the sediment sequence were measured for determining the morphological variability. Scanning electron microscopy (SEM) was performed using Jeol JSM 5510 (Jeol Ltd, Tokyo, Japan) at the Bulgarian Academy of Science and Cambridge S4 Stereoscan (Cambridge Instruments Ltd, Cambridge, UK) at the Friedrich Hustedt Study Centre for Diatoms (BRM) in Bremerhaven. SEM stubs were prepared using cleaned diatom material coated with gold–palladium (Polaron SC7640 sputter coater, Quorum Technologies, Ashford, UK). Descriptive terminology follows Anonymous (1975), Håkansson & Khursevich (1997), Håkansson (2002) and Houk et al. (2010).

Observations

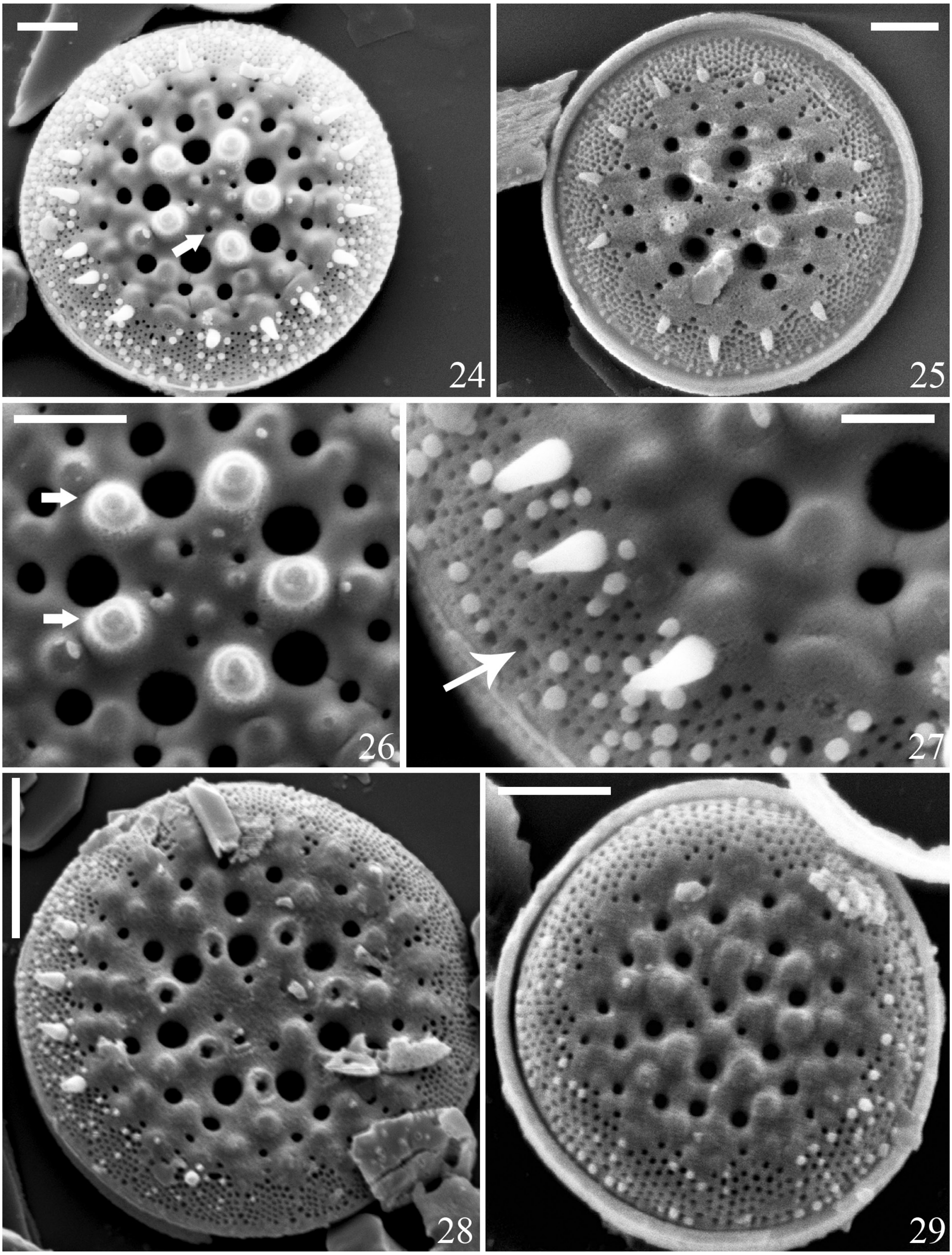
***Tertiarius juriljii* sp. nov. (Figs 1–35)**

Description: Valves circular, valve face more or less flat to slightly convex. Valve diameter 5.0–38.0 µm, central area diameter 2.0–24.0 µm. Valve face with two parts of distinctly different morphology (Figs 24, 28). Distinct hyaline area around valve face central ornamentation (Figs 25, 28). Ring of spines at central to marginal part boundary (Figs 24, 27). Small granules can be present on marginal area and valve mantle (Figs 24, 27, 29). Marginal area with radiating striae, exceeding ¼ of valve diameter. Striae ca. 50 in 10 µm. Striae in marginal area alveolate multiseriate with 5–8 rows of small perforations (Figs 24, 27). Striae in central part uniseriate with variable length, composed of variable sized areolae. Areolae loculate, larger within central part of valve 5–8 in 10 µm. Internally, valve face areolae with domed cribra (Figs 32, 33). Variable number of valve face fultoportulae (5–12) arranged in radial rows around valve center (Figs 24, 26, 28). External openings of valve face fultoportulae small, round, positioned near valve centre (arrow in Fig. 24). Internally fultoportulae with short central tube surrounded by three satellite pores (Figs 31, 32). External openings of marginal fultoportulae situated on costae (ribs) close to marginal edge (arrow in Fig. 27). Internally, openings of marginal fultoportulae on short, broad costae (Figs 32, 35). Each marginal fultoportula with two satellite pores in lateral position (arrow in Fig. 35). Internally, one rimoportula situated at the base between two costae in front of alveolar opening (Figs 30, 33).

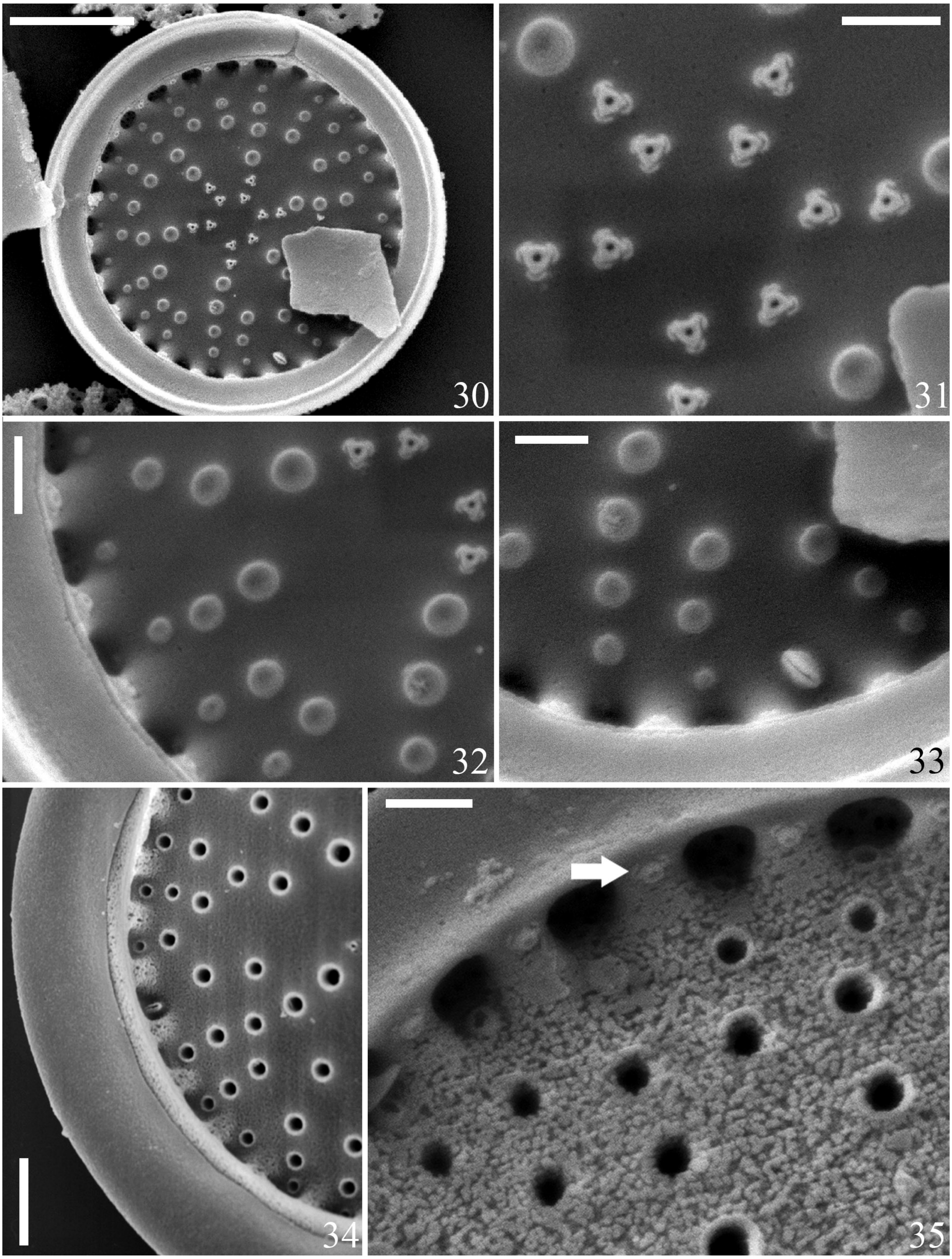
Etymology: The specific epithet (*juriljii*) is dedicated to Dr. Anto Jurilj, who first observed and illustrated



Figs 1–23. *Tertiarius juriljii*, LM valve views, Mariovo, Macedonia: (1–23): diminution series, (3): Holotype specimen. Scale bar 10 µm.



Figs 24–29. *Tertiarius juriljii*, SEM external valve views, type material: (24) valve view, showing the external openings of the valve face fultoportula (arrow), (25) view of the whole valve, (26) detailed view of the valve centre, showing the distinct valve large granules (white arrows), (27) girdle view showing the spines and the external opening of the fultoportula (white arrow), (28, 29) valve views. Scale bars 5 µm (28), 2 µm (24–26, 29), 1 µm (27).



Figs 30–35. *Tertiarius juriljii*, SEM internal valve views, type material: (30) valve view, (31) detailed view of the mid–valve showing the valve face fultoportulae, (32) detailed view of the valve margin showing the marginal fultoportulae and the areolae covered by domed cribra, (33) close view of the valve margin showing the internal opening of the rimoportula, (34) view of a corroded specimen, (35) close view of the marginal fultoportulae surrounded with two satellite pores (white arrow). Scale bars 5 µm (30), 3 µm (34), 1 µm (31–33, 35).

this cyclotelloid form.

Type locality: Outcrop near village Manastir, Mariovo, Macedonia. Lat. 41°10'03"N, Long. 21°43'42"E.

Holotype (designated here): Slide: MaB–I/02, outcrop near village Manastir, Mariovo Neogene Basin – in Coll. Ognjanova–Rumenova, Institute of Geology, Bulgarian Academy of Sciences, Sofia.

Isotype (designated here): Slide MKNDC NOR01/A, Macedonian National Diatom Collection (MKNDC), Skopje.

Type material: Diatomite, outcrop near village Manastir, Vitacevo Formation in Mariovo Neogene Basin, Macedonia. Coll. Date: 14 June 1995, Leg: N. Ognjanova–Rumenova, Accession no. Manastir I/002.

Age range: Pliocene.

Differential diagnosis: *Tertiarius juriljii* is clearly distinguished from similar *Tertiarius* species by the shape of the hyaline area on the valve face; the central part of the valve comprised of areolae arranged in a single short or long radial rows; presence of variable sized granules and depressions and the valve face fultoportulae arranged in radial rows around the valve center.

Tertiarius porosus Khursevich et Kociolek (2002, figs 48–58) has a comparable valve size and similar ornamentation of the interestriae (with granules) to *T. juriljii*. However, it can be easily differentiated from *T. juriljii* by several morphological features. In *T. porosus* the valve face fultoportulae are with two satellite pores and scattered in the valve central part. The rimoportulae are located at the base of the elongated alveolae and usually are in higher number (up to 4). The spines at the central to marginal part boundary are absent in *T. porosus.*

Smaller valves of *T. juriljii* resemble *T. distinctus* Khursevich et Kociolek (2002, figs 1–5, 12–22) with respect to the valve's central part ornamentation. The latter can be distinguished from *T. juriljii* by the number, structure and position of the valve face fultoportulae (1–5 with two satellite pores and located in the valve centre in *T. distinctus*); absence of spines at the central to marginal part boundary; and the internal rimoportula opening located at the base of a costae or within the alveolar chamber in *T. distinctus* (Khursevich & Kociolek 2002).

Tertiarius pygmaeus can be differentiated from *T. jurilljii* by the presence of a distinct hyaline area around the valve face central ornamentation; the arrangement of the areolae on the valve face central part (areolae scattered on the central part of the valve); the structure and position of the valve face fultorportulae (with two satellite pores, scattered in the central part); and the position of the rimoportula (on a side of the costa and inside of the alveolar chamber).

Tertiarius pantocseki (Fricke) Khursevich et Kociolek (= *Stephanodiscus pantocseki* Fricke in Schmidt et al. 1874 –1959) has several similar morphological features as *T. juriljii.* In *T. pantocseki* the valve face fultoportulae are arranged in ring and have 3 satellite pores internally; the conic spines on the valve face/mantle junction; and granules on the costae are present (Khursevich & Kociolek 2012). However, both species can be differentiated by the valve size (18–55 µm in *T. pantocseki*); ornamentation of the valve face central part (with one to several isolated areolae in *T. pantocseki*); number of satellite pores surrounding marginal fultoportulae (three in *T. pantocseki*); and costae density (2–4 in 10 µm in *T. pantocseki*).

***Tertiarius mariovensis* sp. nov. (Figs 36–105)**

Description: Valves circular, valve face more or less flat (Figs 36–92). Valve diameter 4.0–19.0 µm, central area diameter 1.5–13.5 µm. Valve face with two parts of distinctly different morphology (Figs 93, 94, 97). Small granules present on marginal area and valve mantle (Fig. 95, 96). Marginal area with radiating striae, exceeding ¼ of valve diameter, ca. 50 in 10 µm. Striae on valve mantle and marginal area alveolate multiseriate with 8–10 rows of small perforations (Figs 93, 94). Striae biseriate in valve central part and uniseriate with variable length on valve centre, 6–12 in 10 µm. Areolae loculatae, 12–16 in 10 µm, internally with domed cribra (Figs 99, 101, 103, 104). Interstriae distinct (Fig. 102), coarser towards valve centre. Variable number of valve face fultoportulae (2–9) arranged in a ring around valve center (Figs 94, 97, 98). External openings of valve face fultoportulae small, round, easily discernable from areolae openings (arrow in Fig. 97). Internally, valve face fultoportulae with short central tube surrounded by three satellite pores (Figs 99, 100, 103, 104). Alveolus complex, composed of folds or narrow ridges of silica (arrow in Figs 104, 105). Internally, openings of marginal fultoportulae on basis of thick costae (Figs 101, 102, 104, 105). Each marginal fultoportula with two satellite pores in lateral position (arrow in Fig. 102). Internally, one rimoportula situated between two costae inside alveolar opening (Figs 101, 104).

Etymology: The specific epithet (*mariovensis*) refers to the type locality of this species – Mariovo Neogene Basin.

Type locality: Outcrop near village Zovich, Mariovo, Macedonia. Lat. 41°05'19" N, Long. 21°41'58" E.

Holotype (designated here): Slide MKNDC 008531/A, Diatomite near the village Manastir; Mariovo Neogene Basin.

Isotype (designated here): Slide MaB–IV/20, Institute of Geology, Bulgarian Academy of Sciences, Sofia.

Type material: Diatomite, near village Zovich, Vitacevo Formation in Mariovo Neogene Basin, Macedonia. Coll. Date: 15 July 2012, Leg. E. Jovanovska, Accession no. 008531 (MKNDC).

Age range: Pliocene.

Differential diagnosis: *Tertiarius mariovensis* is characterized by the following distinct morphological features that clearly separate it from similar taxa: tri– to biseriate striae in the central part of the valve face becoming uniseriate towards the valve centre; distinct, coarse costae; absence of large granules in the central part of the valve; rimoportula stalked, positioned between two costae inside alveolar opening. In addition, a surrounding hyaline area around the valve face central ornamentation is missing (Figs 94, 97). *Tertiarius mariovensis* seems to be quite variable in the arrangement of areolae in the central part of the valve. In some specimens the areolae are arranged in radial rows throughout, while in others the radial rows become scattered towards the valve centre (compare Figs 36–67 with Figs 68–92). Since additional morphological differences were not documented with the LM and SEM observations, these two morphologies are considered as one species. Phenotypic plasticity could be the reason for such variation within the species, and as such *Tertiarius mariovensis* should be subject of future morphometrical analyses.

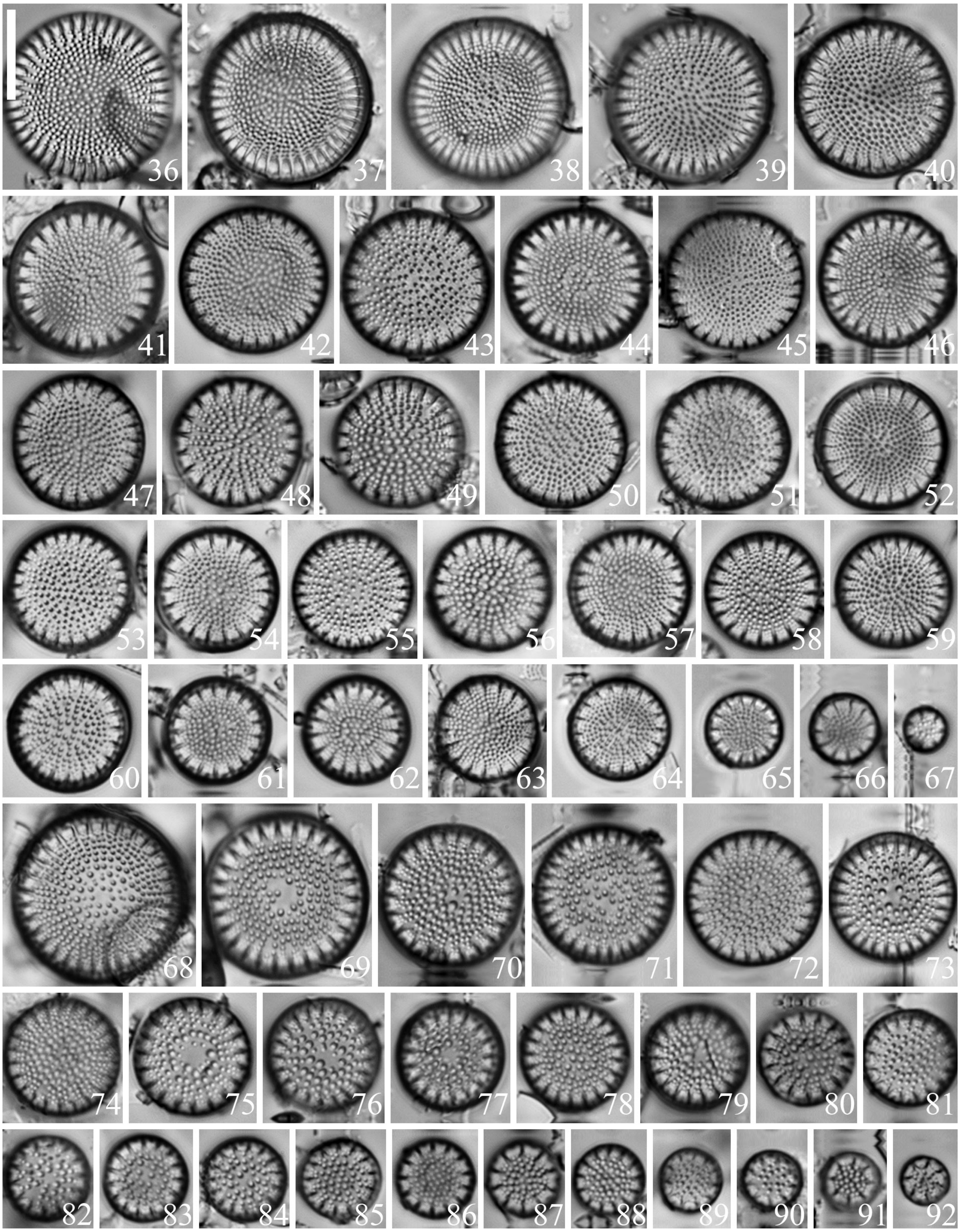
Tertiarius mariovensis can be easily associated with *T. transilvanicus* (Pantocsek) Håkansson et Khursevich (Håkansson & Khursevich 1997, figs 16–27; Houk et al. 2010, figs 297: 1–6; 298: 1–6). However, both species can be distinguished by several morphological features, and in *Tertiarius transilvanicus* these characters are: presence of distinct hyaline area around the central part of the valve face; striae uniseriate at the entire central part of the valve; valve face fultoportulae arranged in a ring near the marginal part with two satellite pores; internal opening of the rimoportula located on a recessed costa; and small, almost round internal openings of the alveolae. *Tertiarius transilvanicus* var. *disseminatepunctatus* (Pantocsek) Håkansson et Khursevich (Håkansson & Khursevich 1997, figs, 28–34; Houk et al. 2010, figs 299: 1–8) can be separated from *T. mariovensis* by: the presence of distinct hyaline area around the central part of the valve face; the scattered areolae on the central part of the valve; areolae arranged in a ring at the hyaline area; valve face fultoportulae arranged in a ring near the marginal part and with two satellite pores; and the internal rimoportula located on a side of a costa. *Tertiarius elgeri* (Hustedt) Houk, Klee et Tanaka is much larger than T. *mariovensis* (20–110 μm vs. 4–19 μm), with larger marginal area and striae composed of 3–4 rows of small areolae. Striae are uniseriate at the central part of the valve, composed of small round areolae (in comparison to the much larger areolae in *T. mariovensis*). In addition *T. elgeri* is missing valve face fultoportulae, but one to several rimoportulae are present, which are internally located between the striae, stalked and connected with a short rib to one of the adjacent costae.

Discussion

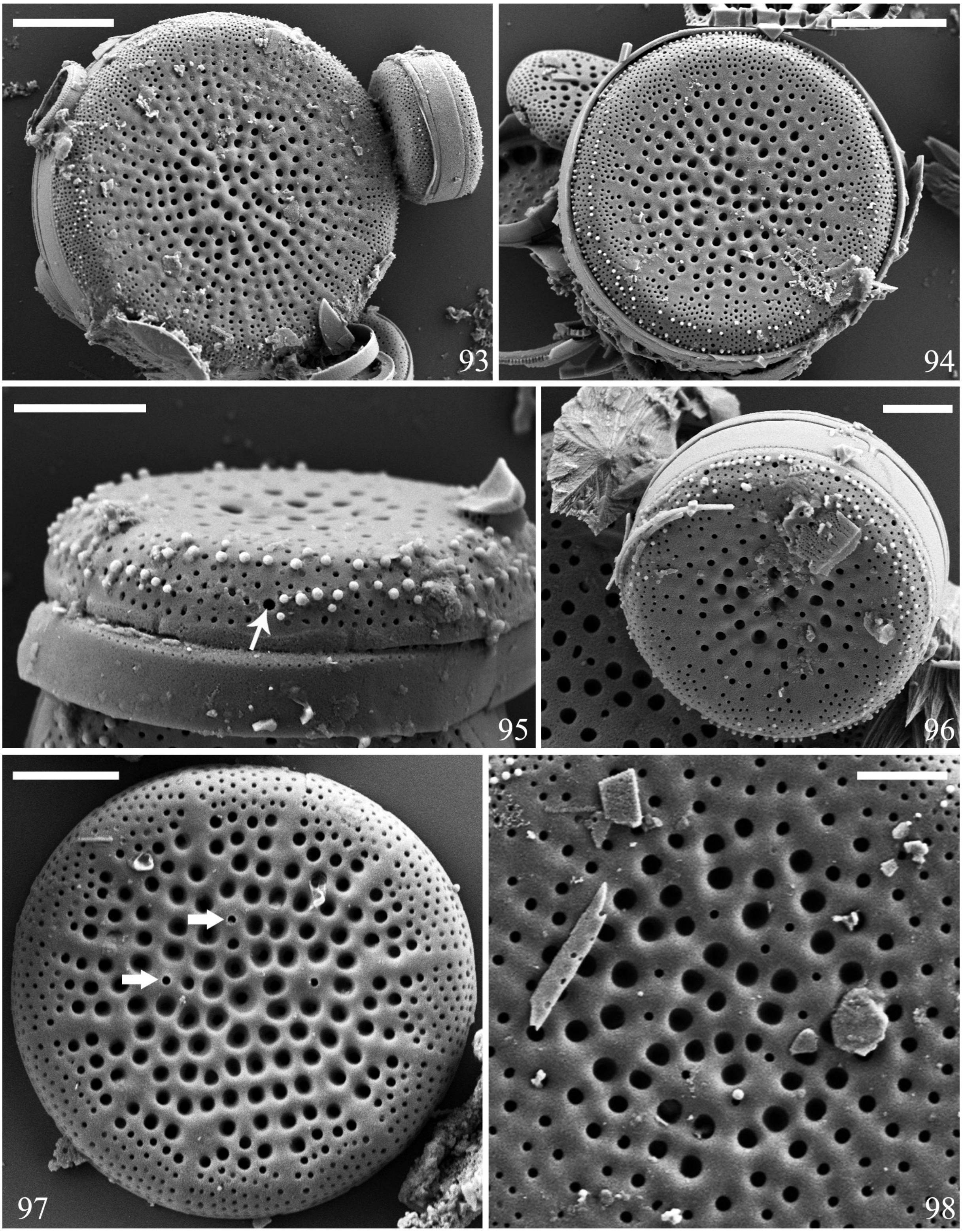
The Neogene represents a very important period for phylogenetic development of the genera within the family Stephanodiscaceae (Khursevich 2006). The Early Pliocene is followed by gradual extinctions of species from the genus *Actinocyclus* in the Central Balkan Peninsula (Ognjanova–Rumenova 2000). During this period, *Actinocyclus* taxa [*A. makarovae* (Temniskova–Topalova et Ognjanova–Rumenova) Temniskova–Topalova et Ognjanova–Rumenova, *A. fungiformis* Temniskova–Topalova, Khursevich et Valeva, *A. krasskei* (Krasske) Bradbury et Krebs, etc.] have been replaced with different planktonic genera like *Pliocaenicus* F.E.Round et Håkansson, *Cyclostephanos* F.E.Round ex Theriot, Håkansson, Kociolek, Round et Stoermer, and various *Stephanodiscus* Ehrenberg and *Cyclotella* (Kützing) Brébisson representatives [some of them known only as extinct forms (Ognjanova–Rumenova 2000)]. Unlike the other genera, the genus *Tertiarius* was reported only from the Pliocene sediments of the Mariovo Basin. However, recent observations suggest presence of *Tertiarius* species in the sediment record of Lake Prespa of older age (ca. 90 cal ka BP, Cvetkoska pers. obs.). A few years ago Levkov et al. (2007) reported a taxon, *Stephanodiscus* sp. (2007, figs 10: 8–14) from the recent flora of Lake Prespa, which most probable belongs to the genus *Tertiarius*.

It is very difficult to trace the phylogeny and evolution of the family Stepahanodiscaceae in the Neogene Lake system of the Balkans, because all investigated sediment profiles represent only a brief period of the geological timescale (Ognjanova–Rumenova 2000, 2006). Therefore it is preferable to include continuous cores from the regional ancient lakes in order to observe a longer geological timescale (e.g. lakes Ohrid and Prespa). Analyses on long continuous cores (Upper Miocene–Holocene) could be used as an important archive for following the temporal changes in the morphological characters of the family, like in Lake Baikal (Khursevich 2006; Kuzmin et al. 2009; Khursevich & Kociolek 2012). Detailed analyses on Lake Baikal core samples show that the extinct genera from the family Stephanodiscaceae replaced each other from the base to the top of the studied sections of two cores that demonstrates the pattern of the morphological evolution as follows: i) change of the cribrum position in areola (from internal flat cribrum to internal domed cribrum); ii) change in the stria morphology; iii) change of the structure of the marginal fultoportulae on the valve mantle; and iv) change of the number of rimoportulae (Khursevich 2006).

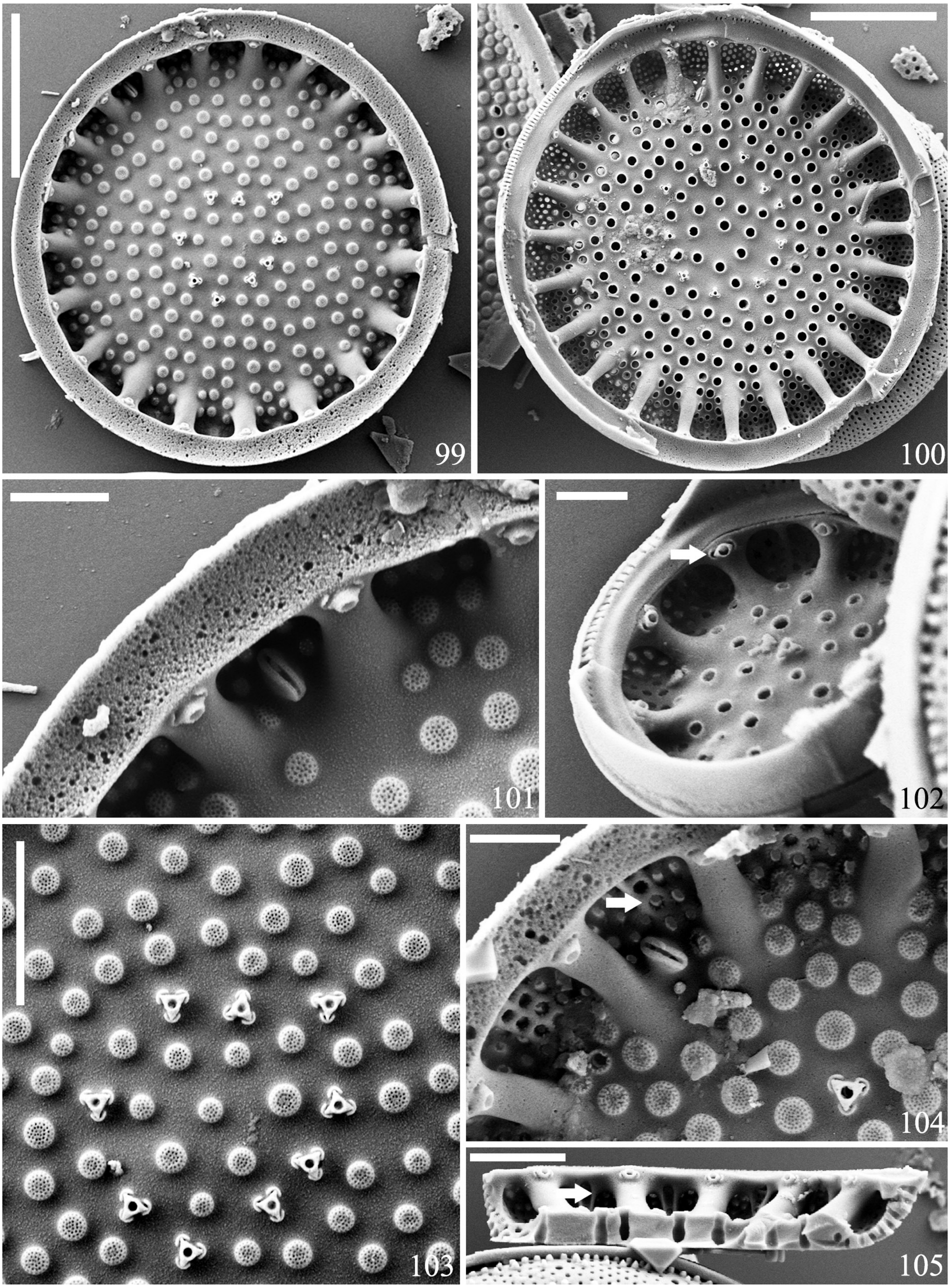
The differences in the structure of the alveolae within the genus *Tertiarius* might also be the result of evolutionary patterns and processes, which have in turn resulted with the alignment of the species into two morphological groups: i) simple structure of



Figs 36–92. *Tertiarius mariovensis*, LM valve views, Mariovo, Macedonia, diminution series. Scale bar 10 µm.



Figs 93–98. *Tertiarius mariovensis,* SEM external valve views: (93, 94) view of the whole valve, (95) girdle view showing the external opening of the fultoportula (white arrow), (96) view of the whole frustule, (97) close view of the external openings of the valve face fultoportula in corroded specimen (white arrows), (98) detailed view of the central part of the valve in corroded specimen. Scale bar 5 µm (93, 94), 2 µm (95–98).



Figs 99–105. *Tertiarius mariovensis,* SEM internal valve views: (99) valve view, (100) view of a corroded specimen, (101) close view of the valve margin showing the internal opening of the rimoportula and the marginal fultoportulae, (102) showing the marginal fultorportulae surrounded by two satellite pores (white arrow), (103) detailed view of the mid–valve showing the valve face fultoportulae, (104) close view of the valve margin showing the internal opening of the rimoportula, marginal fultoportulae, areolae covered by domed cribra and strongly thickened interstriae in the alveolar chamber (arrow), (105) Broken valve, showing the marginal fultoportulae and the alveolar chamber with recessed costae (white arrow). Scale bar 5 µm (99, 100), 2 µm (103), 1 µm (101, 102, 104).

the alveolae; ii) simple and complex structure of the alveolae (Khursevich & Kociolek 2012). However, morphological differentiation in the structure of the alveolae is not observed in the stratigraphic range (Houk et al. 2010; Khursevich & Kociolek 2012). Due to this, we may assume that the structure of the alveolae is not related to the phylogenetic development of the genus *Tertiarius*.

Distribution and ecology

The Balkan Peninsula realm was dry during most times of marine flooding of the Mediterranean area in the Early Pliocene. A great water ingression, coming from the east covered the Balkan Peninsula and formed a large lake system in the area (Krstić et al. 2008). The Mariovo Basin was part of this system. According to the palaeogeography, palaeoclimatology and biostratigraphy, the upper stratigraphic units of the Mariovo Basin are of Late Pliocene age (Krstić et al. 2008). The Pliocene sediments extend in the northern and southern parts of Mariovo (Dumurdžanov et al. 2003). The largest area of the basin is covered by pyroclastics of different kind, but in the realm among the villages Beshishte, Manastir and Zovich, limestone occurs. Diatomite is restricted to this area under the limestone cover. The macroflora from the diatomite, near the village Manastir, was determined by Pantić (1956) and later revised by Mihajlović (in Dumurdžanov et al. 2003). This fossil flora is of Pliocene age. The Pliocene age of the Vitacevo Formation was also confirmed by K/Ar radiometric ages from the volcanic rocks of the topmost formation part that range from 5.0 to 1.8 Ma (Boev & Yanev 2001).

However, the presence of the diatomite deposits indicates a deeper lake, but limestone sedimentation indicates gradual changes and lake shallowing (Dumurdžanov et al. 2003). The natural eutrophication of the lake was in progress, confirmed by the most abundant accompanying taxa in the diatom assemblages: *Stephanodiscus carconensis* Grunow, *S. carconensis* var. *pusillus* Grunow, *Aulacoseira granulata* var. *angustissima* (Müller) Simonsen, *Ellerbeckia arenaria* (Moore) Crawford, *Epithemia turgida* (Ehrenberg) Kützing and various species belonging to the genus *Fragilaria* Lyngbye sensu lato (Ognjanova–Rumenova & Dumurdjanov 2008; Krstić et al. 2008).

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