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(Bacillariophyceae: Cymbellales)
from wet soils in southern Vietnam

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A new species of *Placoneis* Mereschkowsky (Bacillariophyceae: Cymbellales) from wet soils in southern Vietnam

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ABSTRACT

A new diatom species of the genus *Placoneis* Mereschkowsky is described on the basis of molecular and morphological data. The new species, *Placoneis asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov., is morphologically characterized by slightly asymmetrical valves along the apical axis based on light and scanning electron microscopy of the exterior and interior of valves. Molecular data, which are derived from live cells, are used for phylogenetic reconstruction based on 18S V4 rDNA and *rbcL* gene markers of taxa from the genus *Placoneis* and other diatoms in the order Cymbellales D.G.Mann. Comparisons with other morphologically and molecularly closely-related species are given.

KEY WORDS

Southeast Asia,
Vietnam,
Cát Tiên National Park,
diatoms,
Bacillariophyceae,
molecular data,
morphology,
new species.

RÉSUMÉ

Une espèce nouvelle de *Placoneis* Mereschkowsky (*Bacillariophyceae*: *Cymbellales*) des sols humides du sud du Vietnam.

MOTS CLÉS
Asie du Sud-Est,
Vietnam,
Parc national de Cát Tiên,
diatomées,
Bacillariophyceae,
données moléculaires,
morphologie,
espèce nouvelle.

Une espèce nouvelle de diatomée du genre *Placoneis* Mereschkowsky est décrite sur la base de données moléculaires et morphologiques. La nouvelle espèce, *Placoneis asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov., se caractérise morphologiquement par des valves légèrement asymétriques le long de l'axe apical selon nos observations au microscope optique et électronique à balayage de l'extérieur et de l'intérieur des valves. Les données moléculaires, extraites de cellules vivantes, sont utilisées pour la reconstruction phylogénétique basée sur l'ADNr 18S V4 et les marqueurs génétiques *rbcL* des taxons du genre *Placoneis* et d'autres diatomées de l'ordre des *Cymbellales* D.G.Mann. Des comparaisons avec d'autres espèces morphologiquement et moléculairement proches sont données.

INTRODUCTION

The genus *Placoneis* Mereschkowsky was proposed by Mereschkowsky (1903: 3). He placed in this genus the representatives from the genus *Navicula* Bory (Bory 1822: 128) with a specific type of chloroplast. Since then, its taxonomic position has been revised many times with respect to its inclusion within or separation from *Navicula*. Later, a more detailed investigation using scanning electron microscopy (SEM) provided compelling evidence to separate *Placoneis* species from *Navicula sensu stricto* and restore the genus, based not only on the specific structure of the chloroplast but also on the basis of a specific type of pore apparatus (Cox 1987, 2003, 2004; Kulikovskiy & Kuznetsova 2016). Thus, in modern taxonomy, *Placoneis* includes representatives with uniseriate striae with a unique type of pore occlusion, termed the tectulum (Cox 2004; Kulikovskiy *et al.* 2014, 2016; Kulikovskiy & Kuznetsova 2016; Kociolek *et al.* 2017), and a chloroplast, which is a single organelle consisting of two X-shaped plates connected by a wide isthmus (Mereschkowsky 1903; Cox 1987, 2003).

At the time of Cox's (2003) revision, *Placoneis* included 31 species and infraspecific taxa. Currently, the genus has increased significantly not only due to the transfer of some species from the genera *Navicula* and *Pinnularia* Ehrenberg (e.g. Miho & Lange-Bertalot 2006; Levkov *et al.* 2007; Lange-Bertalot & Wojtal 2014), but largely due to the description of new taxa from all over the world (Metzeltin *et al.* 2005; Miho & Lange-Bertalot 2006; Levkov *et al.* 2007; Metzeltin *et al.* 2009; Kociolek & Thomas 2010; Torgan *et al.* 2010; Levkov & Williams 2011; Kulikovskiy *et al.* 2012; Gong *et al.* 2013; Straube *et al.* 2013; Lange-Bertalot & Wojtal 2014; Fofana *et al.* 2014; Pomazkina *et al.* 2019). Currently the genus includes 136 species, 17 varieties and three forms that are taxonomically accepted (Guiry & Guiry 2016; Kociolek *et al.* 2022).

The first results of molecular studies of the phylogenetic position of the genus *Placoneis* indicated that the genus *Cymbella* C.Agardh *sensu lato* (Bruder & Medlin 2007, 2008) was a close ally. Subsequent work, with greater taxon sampling, suggested that *Placoneis* was more closely related to *Gomphonema* Ehrenberg (Kermarrec *et al.* 2011). In one

of the most ambitious studies of the phylogenetic relationships of the order *Cymbellales* Nakov *et al.* (2014) showed that *Placoneis* is not monophyletic, and that the *Cymbellales* overall requires revision.

According to the description of *Placoneis* by Mereschkowsky "all species live free in fresh or brackish water, rarely in saline waters" (cited in Cox 1987: 145). Although most of the taxa are typically freshwater, there are many records of certain species from aerophilic habitats, including soils. For example, *P. elginensis* (W.Gregory) E.J.Cox and *P. hambergii* (Hustedt) Bruder were shown to inhabit soil and mosses of northern Germany (Bruder & Medlin 2007), while *P. gastrum* (Ehrenberg) Mereschkowsky was recorded in soil from the territory of the Podkarpacie Province, Poland (Noga *et al.* 2014). *Placoneis elliptica* (Hustedt) Ohtsuka, *Placoneis* sp. cf. *pseudanglica* (Lange-Bertalot) E.J.Cox, and *P. undulata* (Østrup) Lange-Bertalot, Ohtsuka were recorded in soils of paddy fields of northern Laos (Fujita & Ohtsuka 2005), and *P. porifera* (Hustedt) E.J.Cox, *P. undulata* (Østrup) Ohtsuka & Fujita, *P. gastrum* (Ehrenberg) Mereschkowsky f. *nipponica* (Skvortzow) Ohtsuka & Fujita, and *P. elginensis* were noted in soil of paddy fields in Central Japan. It should be noted that the above species are considered typical and widespread for fresh water bodies (Cox 2003; Levkov & Williams 2012; Kulikovskiy *et al.* 2016). Findings in soils may indicate their adaptability to aerophytic conditions. However, for an accurate understanding of the distribution of species and reliable identification of taxa, molecular data are needed.

The study of algae in the territory of the Cát Tiên National Park was started by the authors in 2014. A high species diversity was found and new species from genera *Aulacoseira* Thwaites, *Eunotia* Ehrenberg, *Gomphonema* and *Luticola* D.G.Mann were described from this park (Glushchenko *et al.* 2016, 2017a, b, 2018). The new taxon was isolated from soil, but the sample was taken on the banks of the Đông Nai River, about 10-15 m from the channel. During the wet season, the river overflows and floods this area. The study of the composition of the river diatom flora was not carried out. This work provides a morphological description and molecular data on the 18S V4 rDNA and *rbcL* gene markers for a new species from the genus *Placoneis*.

MATERIAL AND METHODS

STUDY AREA

Cát Tiên National Park is located in southern Vietnam, 150 km northeast of Ho Chi Minh City (Fig. 1). The park was established in 1978 and consists of three parts with a total area of 73 878 ha (Blanc *et al.* 2001). The region belongs to the bioclimatic type of monsoon tropical climate with summer rains (Nguyen Khanh Van *et al.* 2000). The main part of the territory is occupied by forests, which are of the monsoon, semi-deciduous type. These forests are characterized by high biological diversity and high productivity, second only to moist tropical forests in this respect (Blanc *et al.* 2001). The second largest river, the Đồng Nai, flows through the park.

SAMPLING

The material containing the new taxon was collected from Vietnam, Cát Tiên National Park, Đồng Nai Province ($11^{\circ}26'05.6''\text{N}$, $107^{\circ}25'44.8''\text{E}$) by E.S. Gusev and E.M. Kezlya on 6 June 2019 during an expedition of the Joint Russian-Vietnamese Tropical Research and Technological Centre (the “Ecolan 1.2” project), and designated under slide no. 06728. It was collected from wet soil, which had a pH of 6.3. Samples in general were taken as follows: first, the surface of the test site was examined in order to detect macrogrowth of algae, then a combined sample was taken from an area of 10–30 m². The composite sample consisted of 5–10 individual samples. For an individual sample, the topsoil was removed from an area of 5 to 20 cm². After selection, the instruments were cleaned and sterilized with alcohol. Samples were placed in plastic zip bags and labeled. Immediately after selection, the absolute humidity (20.8%) was determined in the laboratory room by the “hot drying” method (Vadjunina & Korchagina 1986), then brought to the air-dry state and packaged.

MEASUREMENT OF THE pH

To measure pH, we weighed 30 g of soil to which 150 ml of distilled water was added (Arinushkina 1970). The suspension was poured into a clean glass beaker and measurements were performed using the Hanna Combo (HI 98129) device (Hanna Instruments Inc., United States).

CULTURING

Gathered materials were processed in the laboratory of molecular systematics of aquatic plants of Institute of Plant Physiology of the Russian Academy of Science (IPP RAS). In order to prepare cultures, the soil sample was thoroughly mixed and a small amount (15–20 g) was placed into a Petri dish (diameter 60 mm), then saturated with distilled water up to 60–80% of full moisture capacity. Then, the Petri dish was placed into an illuminated climate chamber. After being in the chamber for ten days, for algae detection, distilled water (3–5 ml) was added to the soil sample, shaken slightly, then the liquid was transferred to another Petri dish and observed with an inverted microscope Zeiss Axio Vert A1. Such observations were carried out every 10–14 days. Algal cells were extracted with a micropipette, washed in 3–5 drops of sterile



FIG. 1. — Geographical position of the studied area (in red). Grey area is the Đồng Nai Province.

distilled water and placed into a 300 µl well on a plate for enzyme-linked immunoassay with WC liquid medium (Guillard & Lorenzen 1972). Non-axenic unialgal cultures were maintained at 22–25°C in a growth chamber with a 12/12 hours light/dark photoperiod.

PREPARATION OF SLIDES AND MICROSCOPIC ANALYSES

The culture was treated with 10% hydrochloric acid to remove carbonates and washed several times with deionized water for 12 hours. Afterwards, the sample was boiled in concentrated hydrogen peroxide (*c.* 37%) to remove organic matter. It was washed again with deionized water four times at 12 hours intervals. After decanting and filling with deionized water up to 100 ml, the suspension was pipetted onto coverslips and left to dry at room temperature. Permanent diatom slides were mounted in Naphrax[®]. Light microscopic (LM) observations were performed with a Zeiss Axio Scope A1 microscope equipped with an oil immersion objective (× 100, n.a. 1.4, differential interference contrast [DIC]) and AxioCam ERc 5s camera (Zeiss). Valve ultrastructure was examined using a scanning electron microscope (JSM-6510LV housed at IBIW,

Institute for Biology of Inland Waters RAS, Borok, Russia). For scanning electron microscopy, part of the cleaned material was fixed on aluminum stubs after air-drying. The stubs were sputter-coated with 50 nm of Au with a Eiko IB 3.

Sample and slides are deposited in the collection of Maxim Kulikovskiy at the Herbarium of the IPP RAS, Moscow, Russia.

EXTRACTION OF DNA AND AMPLIFICATION

Total DNA of monoclonal cultures was extracted using Chelex™ 100 Molecular Biology Grade Resin according to the manufacturer's protocol 2.2. Fragments of 18S rDNA (384 bp, including V4 domain), and partial *rbcL* plastid genes (978 bp) were amplified using primers D512for and D978rev from Zimmermann *et al.* (2011) for 18S rDNA fragments and *rbcL*40+ from Ruck & Theriot (2011) and *rbcL*1255- from Alverson *et al.* (2007) for *rbcL* fragments.

Amplifications of the 18S rDNA fragments and partial *rbcL* gene fragments were carried out using the premade mix ScreenMix (Evrogen, Russia) for the polymerase chain reaction (PCR). The conditions of amplification for 18S rDNA fragments were: an initial denaturation of five minutes at 95°C, followed by 35 cycles at 94°C for denaturation (30 seconds), 52°C for annealing (30 seconds) and 72°C for extension (50 seconds), and a final extension of ten minutes at 72°C. The conditions of amplification for partial *rbcL* were: an initial denaturation of five minutes at 95°C, followed by 45 cycles at 94°C for denaturation (30 seconds), 59°C for annealing (30 seconds) and 72°C for extension (80 seconds), and a final extension of ten minutes at 72°C.

The resulting amplicons were visualized by horizontal agarose gel electrophoresis (1.5%), colored with SYBR Safe (Life Technologies, United States). Purification of DNA fragments was performed with the ExoSAP-IT kit (Affimetrix, United States) according to the manufacturer's protocol. 18S rDNA fragments and partial *rbcL* gene were sequenced from two sides using forward and reverse PCR primers and the Big Dye system (Applied Biosystems, United States), followed by electrophoresis using a Genetic Analyzer 3500 sequencer (Applied Biosystems).

Editing and assembling of the consensus sequences were carried out by comparing the direct and reverse chromatograms using the Ridom TraceEdit program (ver. 1.1.0) and Mega7 (Kumar *et al.* 2016). Newly determined sequences and DNA fragments from 59 other diatoms, which were downloaded from GenBank (taxa and accession numbers are given in the tree, Fig. 5), were included in the alignments. Five diatom species from the Rhopalodiaceae (Karsten) Topachev'skyj & Oksiyuk were chosen as the outgroup.

The nucleotide sequences of the 18S rDNA and *rbcL* genes were aligned separately using the Mafft v7 software and the E-INS-i model (Katoh & Toh 2010). For the protein-coding sequences of the *rbcL* gene, we checked that the beginning of the aligned matrix corresponded to the first position of the codon (triplet). Final alignments were then carried out: unpaired sites were visually determined and removed from the beginning and the end of the resulting matrices. After removal of the unpaired regions, the aligned 18S rDNA gene

sequences (438 bp) were combined with the *rbcL* gene sequences (1017 bp) into a single matrix using Mega v7 software.

The data set was analyzed using Bayesian inference (BI) method implemented in Beast ver. 1.10.1. (Drummond & Rambaut 2007) to construct phylogeny. For each of the alignment partition, the most appropriate substitution model was estimated using the Bayesian information criterion (BIC) as implemented in jModelTest 2.1.10 (Darriba *et al.* 2012). This BIC-based model selection procedure selected the following models, shape parameter α and a proportion of invariable sites (pinvar): TIM3 + I + G, $\alpha = 0.5320$ and pinvar = 0.4810 for 18S rDNA gene; TPM1uf + I + G, $\alpha = 0.4530$ and pinvar = 0.7360 for the first codon position of the *rbcL* gene; JC + I + G, $\alpha = 0.0270$ and pinvar = 0.5180 for the second codon position of the *rbcL* gene; GTR + I + G, $\alpha = 1.5550$ and pinvar = 0.2950 for the third codon position of the *rbcL* gene. We used the HKY model of nucleotide substitution instead of JC and TIM3, the GTR model instead of TPM1uf, given that they were the best matching model available for Bayesian inference. A Yule process tree prior was used as a speciation model. The analysis ran for 10 million generations with chain sampling every 1000 generations. The parameters-estimated convergence, effective sample size (ESS) and burn-in period were checked using the software Tracer ver. 1.7.1. (Drummond & Rambaut 2007). The initial 25% of the trees were removed, the rest retained to reconstruct a final phylogeny. The phylogenetic tree and posterior probabilities of its branching were obtained on the basis of the remaining trees, having stable estimates of the parameter models of nucleotide substitutions and likelihood. Maximum Likelihood (ML) analysis was performed using the program RAxML (Stamatakis *et al.* 2008). The nonparametric bootstrap analysis with 1000 replicas was used. The statistical support values were visualized in FigTree ver. 1.4.4 and Adobe Photoshop CC (19.0). Using Mega7 software, the *p*-distances were determined to calculate the sequence similarity with the formula $(1 - p) \times 100$.

RESULTS

Family CYMBELLACEAE Kützing
Genus *Placoneis* Mereschkowsky

Placoneis asymmetricus

Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov.
(Figs 2-4)

HOLOTYPE. — Vietnam. Đồng Nai Province, Cát Tiên National Park, from soil sampled at Đồng Nai riverside, 11°26'05.6"N, 107°25'44.8"E, 03.VI.2019, E.S. Gusev & E.M. Kezlya, slide no. 06728 (oxidized material of cultured strain VP57). Deposited in the collection of Maxim Kulikovskiy at the Herbarium of the Institute of Plant Physiology, Russian Academy of Science, Moscow, Russia (Fig. 2P).

ISOTYPE. — Same data as holotype, slide no. 06728a. Deposited in the collection of the Main Botanical Garden of the Russian Academy of Science, Moscow, Russia (MHA).

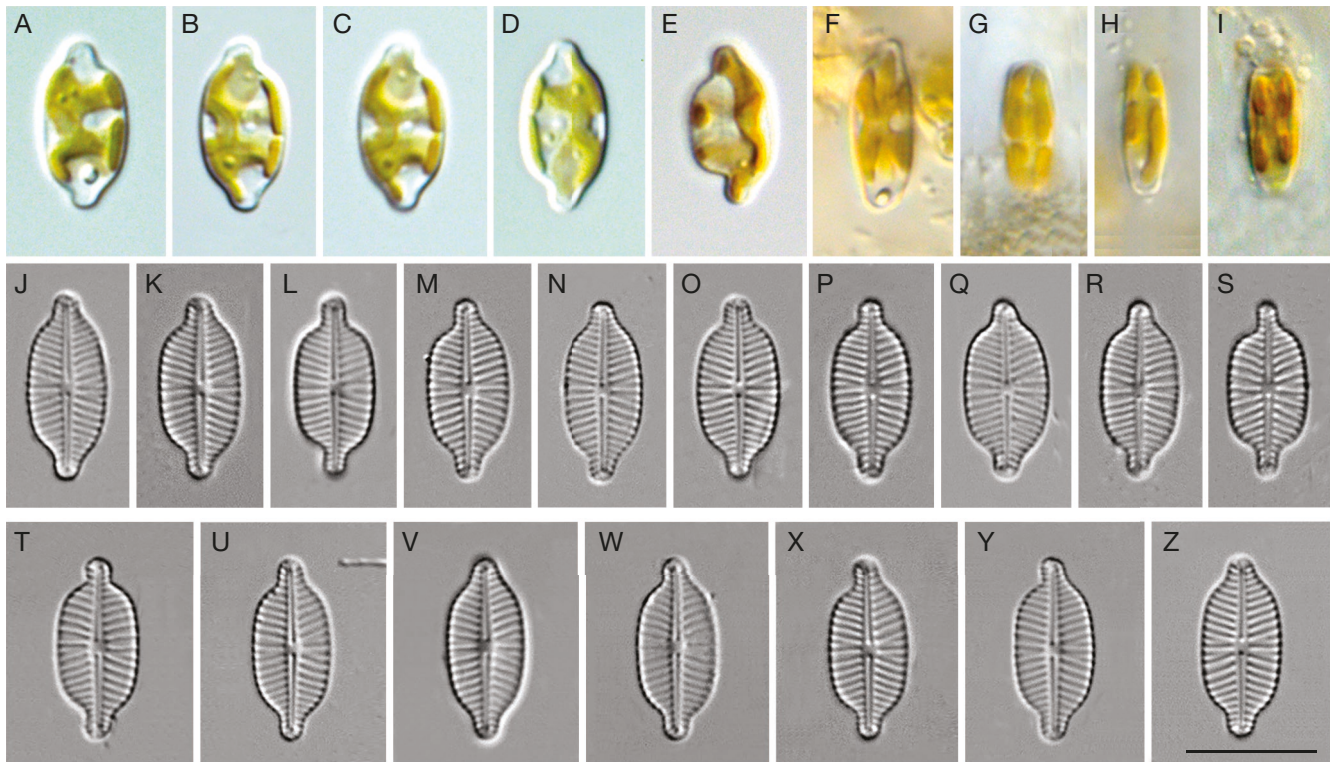


Fig. 2. — *Placoneis asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov.: A-I, live cells with chloroplast structure; J-S, cleaned cells from strain VP57, slide no. 06728; T-Z, valves from a natural sample, slide no. 07219; A-E, J-Z, valve face; F-I, girdle views. P represents the holotype. Scale bar: 10 µm.

TYPE LOCALITY. — Vietnam. Đồng Nai Province, Cát Tiên National Park, Đồng Nai riverside, 11°26'05.6"N, 107°25'44.8"E.

ETYMOLOGY. — The specific epithet refers to the typical asymmetry of the valves of this species.

SEQUENCE DATA. — Nuclear-encoded SSU rDNA partial sequence (GenBank accession: MW979509), plastid gene *rbcL* partial sequence (GenBank accession: MW987572).

DISTRIBUTION. — So far only known from the type locality.

DESCRIPTION

LM (Fig. 2)

Cells solitary, rectangular in girdle view, with a single chloroplast with medially positioned centre and lobes beneath both valves (Fig. 2A-I). Chloroplasts share the characteristic organization typical for the genus (Mereschkowsky 1903; Cox 1987, 2003). The chloroplast is single, large, and extends along the inner surface of the cell. The central column (isthmus) is offset to one side from the central axis and forms a slight bend. Libroplast is missing or represented by only one granule (Fig. 2A, F). The chloroplast has four irregular outgrowths (similar to petals) extending from the isthmus. Valve are linear-elliptical, slightly asymmetric along the longitudinal axis (Fig. 2J-Z). Apices are bluntly rostrate, often asymmetric, breadth 1.3-2.0 µm. Valve length 10.3-15.4 µm, valve width 6.0-6.8 µm. Axial area narrow, almost linear, very slightly widened towards the valve middle. Central area is absent. Raphe filiform, straight, central pores are small. The structure of distal raphe ends not discernible in the

LM. Striae radiate, 17-21 in 10 µm, in the valve middle 2-3 irregularly shortened striae are present on each side. Areolae not resolvable in LM.

SEM external views (Fig. 3)

Valve face is flat. Raphe narrow, linear (Fig. 3A). Proximal raphe ends straight, small, slightly expanded (Fig. 3B). Distal raphe ends hook-shaped, extending to the valve mantle and unilaterally bent (Fig. 3A, C). Striae composed of 3-11 rounded or weakly elongated areolae, continuing onto the valve mantle. Areolae 45-50 in 10 µm. Stigma is absent.

SEM internal views (Fig. 4)

The raphe is straight (Fig. 4A). Proximal raphe ends are hooked-shaped and deflected to one side (Fig. 4B). Distal ends terminating on small helictoglossae and may be slightly deflected to opposite sides (Fig. 4A, C). The striae are bordered by broad interstriae (virgae) (Fig. 4C). Areolae small, square or round. Two isolated areolae are present at the apices (Fig. 4C). Volate occlusions were not preserved during the material treatment.

MOLECULAR INVESTIGATION

To evaluate the phylogenetic position of the strain VP57, we sequenced the V4 region of the ribosomal 18S rDNA and chloroplast *rbcL* genes. The evolutionary distance matrix based on the V4 region showed that *P. asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. shared 91.4%-96.4% similarities with other *Placoneis* spe-

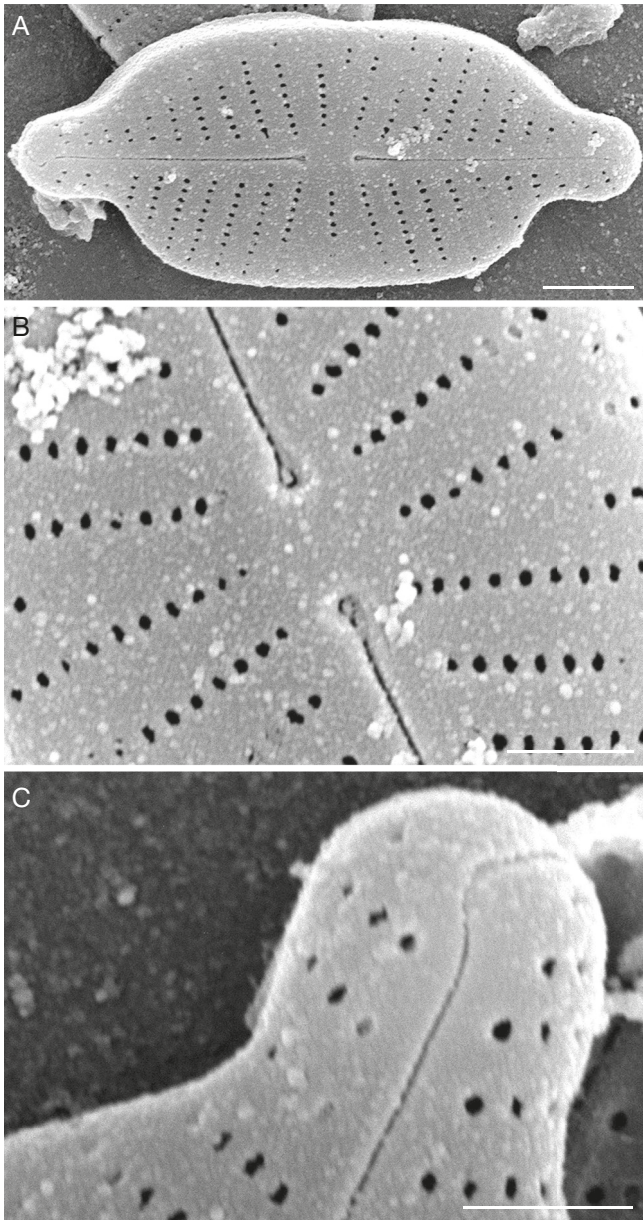


FIG. 3. — SEM external views of *Placoneis asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. (strain VP57): **A**, entire valve; **B**, detail, central area; **C**, valve apex. Scale bars: A, 2 μ m; B, C, 1 μ m.

cies (Table 1) and 91.9%–94.4% similarities with the closest *Geissleria* Lange-Bertalot & Metzeltin and *Paraplaconeis* Kulikovskiy, Lange-Bertalot & Metzeltin strains in the phylogenetic tree. However, some species are more similar to each other. *Placoneis elginensis* showed sequence similarity in the range of 97.8%–100% to *P. abiskoensis* (Hustedt) Lange-Bertalot & Metzeltin, while *P. cattiensis* Glushchenko, Kezlya, Kulikovskiy & Kociolek showed 96.9%–99.2% to *P. elginensis* (Table 1). Results of the *rbcL* gene showed that the *p*-distance of the VP57 to strains of *Placoneis* ranged from 95.2% to 96.2% (Table 2). The range of differences from 0.5% to 5.0% has been established for formally reported *Placoneis* species. At the same time, the difference between *Geissleria* species was 4.5%.

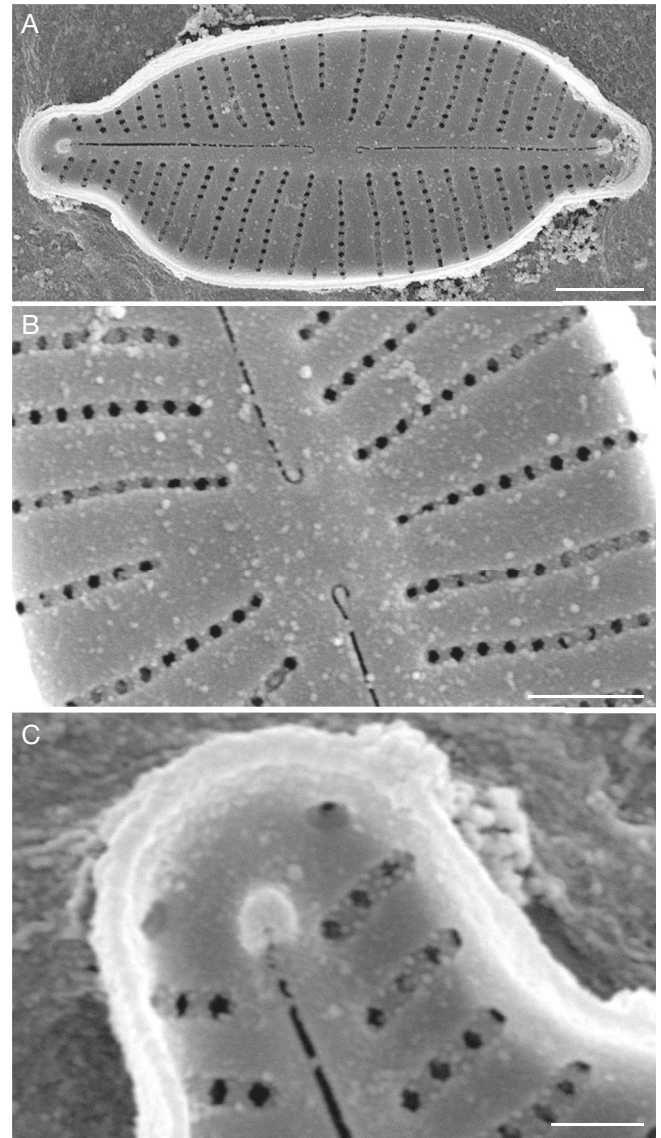


FIG. 4. — SEM internal views of *Placoneis asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. (strain VP57): **A**, entire valve; **B**, detail, central area; **C**, valve apex. Scale bars: A, 2 μ m; B, 1 μ m; C, 0.5 μ m.

Overall, the phylogenetic analysis by the genetic markers 18S V4 and *rbcL* shows the Cymbellales to be a monophyletic group. All specimens of the genus *Encyonema* Kützing grouped together, albeit with low support values, suggesting that the the genus *Encyonema* is monophyletic. This clade of *Encyonema* species is sister to a clade that includes species of *Gomphonema* C.G.Ehrenberg, *Didymosphenia* M.Schmidt, *Cymbella* C.Agardh, *Cymbopleura* (Krammer) Krammer, *Placoneis*, *Geissleria* and *Paraplaconeis*. The monophyletic lineage includes *Placoneis*, *Geissleria* and *Paraplaconeis* and, of these, *Placoneis* is shown to be monophyletic. Of the eight distinct *Placoneis* taxa, *Placoneis asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. (strain VP57) appeared most closely related to strains of *Placoneis hambergii* AT-160Gel09, to which it is sister, and *Placoneis clementis* FD419 (Fig. 5). Together these three species form a monophyletic group (with moderate to strong statistical support; ML 90; BI 100).

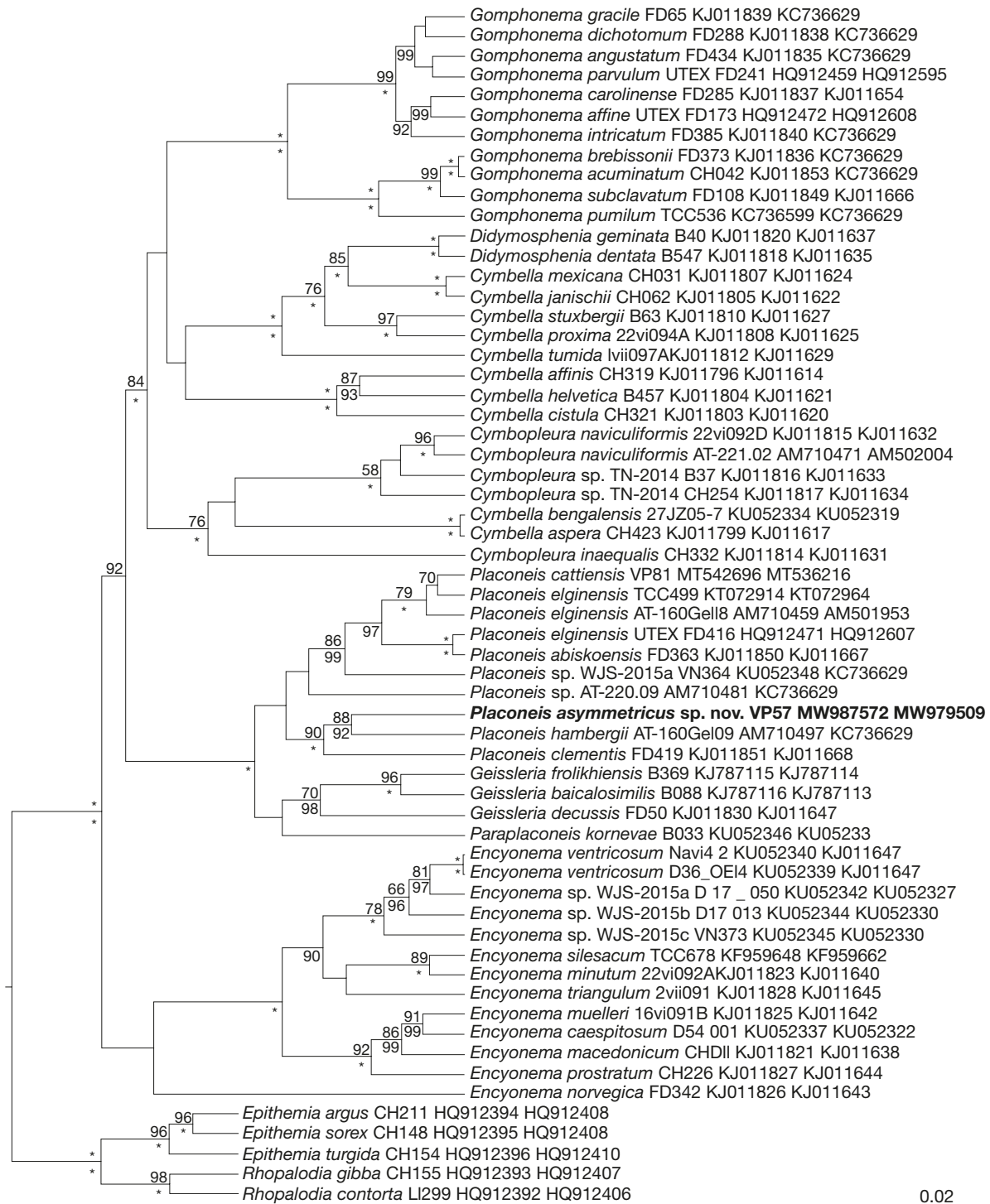


FIG. 5. — Phylogenetic position of *Placoneis asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. (indicated in **bold**) within the Bacillariophyceae Haeckel based on Bayesian analysis of 60 partial *rbcL* and partial 18S rDNA genes sequences. Values above the horizontal lines are bootstrap support from ML analyses (< 50 are not shown); values below the horizontal lines are Bayesian posterior probabilities (< 90 are not shown). All sequences have strain numbers (if available) and GenBank numbers. Species from Rhopalodiaceae (Karsten) Topachevs'kyj & Oksiyuk were used as an outgroup. * is 100% statistical support.

DISCUSSION

Overall, our analysis of the phylogenetic relationships of the Cymbellales agrees with past studies (Kermarrec *et al.* 2011; Nakov *et al.* 2014). In the analysis of those genera with

asymmetry about the apical axis (“cymbelloid diatoms”), we see that *Encyonema* is monophyletic (Fig. 5), while *Cymbella* and *Cymbopleura* are non-monophyletic. While this group of cymbelloid diatoms contains over 2000 taxa (Kociolek *et al.* 2022), and the number of taxa included in molecular studies

TABLE 1. — Percent similarity (*p*-distance) matrix based on the V4 region of the 18S rDNA gene sequences (363 bp) for 14 strains.

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 <i>Placoneis asymmetricus</i> sp. nov. VP57	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2 <i>Placoneis hambergii</i> AT-160Gel09	96.4	–	–	–	–	–	–	–	–	–	–	–	–	–
3 <i>Placoneis clementis</i> FD419	93.0	95.3	–	–	–	–	–	–	–	–	–	–	–	–
4 <i>Placoneis</i> sp. AT-220.09	91.4	92.2	91.1	–	–	–	–	–	–	–	–	–	–	–
5 <i>Placoneis</i> sp. WJS-2015a VN364	92.5	93.3	92.2	93.6	–	–	–	–	–	–	–	–	–	–
6 <i>Placoneis cattiensis</i> VP81	93.3	94.4	93.3	95.3	95.3	–	–	–	–	–	–	–	–	–
7 <i>Placoneis elginensis</i> UTEX FD416	94.7	96.1	94.4	94.2	95	96.9	–	–	–	–	–	–	–	–
8 <i>Placoneis elginensis</i> TCC499	94.2	95.3	94.1	95.5	96.1	98.6	97.8	–	–	–	–	–	–	–
9 <i>Placoneis elginensis</i> AT-160Gel18	93.9	95.3	94.1	95.8	95.8	99.2	97.8	99.4	–	–	–	–	–	–
10 <i>Placoneis abiskoensis</i> FD363	95.0	96.1	94.4	94.4	95.3	97.2	100	97.8	98.1	–	–	–	–	–
11 <i>Geissleria decussis</i> FD50	93.0	95.2	94.1	93.6	93.3	95.8	96.4	96.7	96.7	96.4	–	–	–	–
12 <i>Geissleria baicalosimilis</i> B088	94.4	95.5	94.1	94.4	94.2	96.7	96.7	97.2	97.5	96.9	97.8	–	–	–
13 <i>Geissleria frolikhiensis</i> B369	93.9	94.4	94.1	94.2	94.4	96.7	96.1	97.2	97.5	96.4	96.7	98.6	–	–
14 <i>Paraplaconeis kornevae</i> B033	91.9	93.0	92.2	94.2	93.6	95.5	94.4	95.3	95.8	94.7	94.4	95.0	95.0	–

TABLE 2. — Percent similarity (*p*-distance) matrix based on the *rbcl* gene sequences (963 bp) for 7 strains.

Strain	1	2	3	4	5	6	7
1 <i>Placoneis asymmetricus</i> sp. nov. VP57	–	–	–	–	–	–	–
2 <i>Placoneis clementis</i> FD419	95.2	–	–	–	–	–	–
3 <i>Placoneis abiskoensis</i> FD363	95.2	95.0	–	–	–	–	–
4 <i>Placoneis cattiensis</i> VP81	96.2	95.5	97.7	–	–	–	–
5 <i>Placoneis elginensis</i> UTEX FD416	95.4	95.4	99.5	98.0	–	–	–
6 <i>Geissleria decussis</i> FD50	94.7	94.4	94.9	94.9	95.4	–	–
7 <i>Geissleria frolikhiensis</i> B369	95.2	94.9	95.7	95.4	96.2	95.5	–

is comparatively low, further work is required to resolve and diagnose the commonly used and accepted genera within this lineage.

The cells of *Placoneis asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. have a single chloroplast with lobes beneath both valves connected by an isthmus, uniseriate striae, and areolae with porous occlusion tectulum. Based on these features it belongs to the genus *Placoneis*, as defined by Cox (2004). The main morphological features of this species are characterized by an asymmetric valve contour, small size, high density of striae and areolae, and absence of a central area.

Phylogenetic analysis based on 18S V4 and *rbcl* gene regions showed that *Placoneis asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. (strain VP 57) forms a clade with *Placoneis hambergii* (AT160_Gel09_AM710497) and *Placoneis clementis* (Grunow) E.J.Cox (FD419_KJ011851) (Fig. 5). Another large clade is formed by *P. cattiensis*, *P. elg-*

inensis, *P. abiskoensis* and *Placoneis* spp. *Placoneis asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. is easily separated from the above-mentioned species by its morphological characteristics (Table 3). Percent dissimilarity 1.3%-1.6% of aligned V4 regions has been effectively used in discriminating *Nupela indonesica* Kulikovskiy, Maltsev, Glushchenko & Kociolek and *Nupela lesothensis* (Schoeman) Lange-Bertalot (Kulikovskiy et al. 2020). In this study, the highest V4 region sequence similarity (100%) was detected between the strains *P. abiskoensis* FD363 and *P. elginensis* UTEX FD416. The highest 18S rDNA gene sequence similarity of *P. asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. with other *Placoneis* strains is 96.4%, which provided the molecular evidence to support the recognition of the new species we have described.

Placoneis asymmetricus Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. and *P. hambergii* (Bruder & Medlin 2007; Kulikovskiy et al. 2016) differ in valve outline and apices.

TABLE 3. — Comparison of species from the genus *Placoneis* Mereschkowsky on the basis of own and literature data. *, counted by us from published data.

	<i>P. asymmetricus</i> sp. nov.	<i>P. hambergii</i>	<i>P. clementis</i>	<i>P. anglophila</i>	<i>P. neoexigua</i>	<i>P. nanoclementis</i>	<i>P. vadosa</i>
Valve outline	Linear-elliptical	Elliptic-lanceolate	Lanceolate	Elliptical to linear-elliptical	Broadly elliptical to linear-elliptical	Broadly elliptical-lanceolate to elliptical	Elliptic-lanceolate
Valve apices	Abruptly rostrate	Slightly rostrate	Moderately protracted rostrate	Rostrate to subcapitate	Shortly rostrate to slightly subcapitate	Abruptly protracted rather broadly subrostrate to rostrate	Protracted capitate
Valve apices width (µm)	1.3-2.0	2.8-3.3*	2.8-4.0*	2-3	2.0-2.5*	1.9-2.5*	2.8-3.0*
Axial area	Narrow, linear	Narrow, lanceolate	Narrow, linear	Narrow, linear	Narrow, linear	Narrow, very slightly expanded proximally	Narrow, linear
Central area	Absent	Absent	Rounded, 1/3 of the valve width	Rounded, < 1/4 of the valve width	Moderately expanded transapically, c. 1/3 of the valve width, variable in shape*	Extended transapically, c. 1/2 of the valve width, variable in shape	Small, weakly transversely extended, c. 1/4 of the valve width
Stigmata	Absent	Absent	2	Absent	Absent	2	1
Valve length (µm)	10.3-15.4	16-25	25*-60	18-32	14-40	14-23	15.6-17.8
Valve width (µm)	6.0-6.8	6-8	10.3*-16	7-12	6.6-11.0	8.0-8.6	7.2-8.0
Striae	Uniseriate, slightly radiate throughout, 17-21 in 10 µm	Uniseriate, radiate throughout, 15-18 in 10 µm	Uniseriate, radiate, density 8-10 in 10 µm proximally, becoming 14-15 close to the ends	Uniseriate, gently radiate throughout, 10-13 in 10 µm	Uniseriate, radiate throughout up to the ends, c. 10-12/10 µm in proximal parts of the valve becoming 18/10 µm close to the apices	Uniseriate, radiate throughout, 12-13 in 10 µm, close to the ends becoming more strongly radiate, 14-16 in 10 µm	Uniseriate, radiate throughout, 14-16 in 10 µm
Areolae	Not discernible in the LM, 45-50 in 10 µm	Not discernible in the LM, 40 in 10 µm*	Density is 28 in 10 µm	Not discernible in the LM, 30-34 in 10 µm	Distinct in the LM, 26-30 in 10 µm	Not discernible in the LM, 40-44 in 10 µm	Not discernible in the LM, 30 in 10 µm in the center and 40-45 at the ends
Distribution	South-East Asia (Vietnam)	Holarctic	Holarctic	Presumably cosmopolitan	Central Europe	Germany (Altmark, Sachsen-Anhalt)	Russia (Lake Baikal)
References	This study	Bruder & Medlin 2007; Kulikovskiy <i>et al.</i> 2016	Cox 1987; Lange-Bertalot & Wojtal 2014	Krammer & Lange-Bertalot 1985; Cox 1987, 2003; Metzeltin <i>et al.</i> 2005; Miho & Lange-Bertalot 2006; Kulikovskiy <i>et al.</i> 2010, 2016	Miho & Lange-Bertalot 2006	Lange-Bertalot & Wojtal 2014	Pomazkina <i>et al.</i> 2019

The valves of the new species are linear-elliptic, slightly asymmetrical, whereas in *P. hambergii* they are elliptic-lanceolate. Valve apices in *P. asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. are clearly rostrate, their width is 1.3-2.0 µm, while in *P. hambergii* they are wider (2.8-3.3 µm) and only slightly rostrate. These two species also differ in the

density of areolae: 45-50 in 10 µm in *P. asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. versus 40 in 10 µm in *P. hambergii*.

Placoneis clementis FD419_KJ011851 strain was deposited into the UTEX collection (<https://utex.org/pages/search-results?q=placoneis>), and a picture serves as a voucher (available

at http://protistcentral.org/Photo/get/photo_id/3682; Nakov et al. 2014). The valves shown in the photograph are similar in valve outline and striation pattern to *P. clementis*, but there is no possibility to assess the morphometric characteristics, because there is no scale bar. In addition, the typically distinct stigma in this species is not visible on the valves shown on the voucher. Therefore, *Placoneis clementis* strain FD419_KJ011851 currently requires, in our opinion, clarification of its taxonomic identity. Lange-Bertalot & Wojtal (2014) studied “diversity in species complexes of *Placoneis clementis*” and identified several new species (*Placoneis clementispronina* Lange-Bertalot & Wojtal, *Placoneis nanoclementis* Lange-Bertalot & Wojtal and *Placoneis parvapolonica* Lange-Bertalot & Wojtal). However, if we consider specifically *Placoneis clementis* and the entire complex of these species (Cox 1987: 148, figs 28-33; Lange-Bertalot & Wojtal 2014: 406, 409, figs 1-13, 14-44) in comparison with *Placoneis asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov., a number of differences could be noted. The valve outline of *P. clementis* is lanceolate or elliptic-lanceolate, whereas in the new species it is linear-elliptical. The striae density in species of this group does not exceed 16 in 10 µm, and the number of areolae is less than 44 in 10 µm. In *P. asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. both striae and areolae are denser (17-21 in 10 µm and 45-50 in 10 µm, respectively). The central area contains two stigmata; *P. asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. lacks any stigma.

Placoneis nanoclementis Lange-Bertalot & Wojtal, as well as two other *Placoneis* taxa (*Placoneis anglophila* (Lange-Bertalot) Lange-Bertalot, *Placoneis neoexigua* Lange-Bertalot & Miho) are very similar to *P. asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. when their smaller valves are compared. Nevertheless, the new species is easy to distinguish by a number of morphological features. Widespread species *Placoneis anglophila* (Cox 1987: 147, 152, figs 11, 12, 17-19, 47, 2003: 64, 65, figs 66, 67, table 2, 2004: 40, figs 19-23; Miho & Lange-Bertalot 2006: 305, figs 12-19; Kulikovskiy et al. 2016: 687, figs 1-4) (as *P. pseudanglica*) and *P. asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. are similar in valve outline (linear-elliptical), in the shape of the valve apices (rostrate) and in the arrangement of striae (slightly radiate). However, *P. asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. differs in having smaller valves (length 13.4-15.4 µm, width 6.0-6.8 µm versus 18-32 µm and 7-12 µm in *P. anglophila*, respectively), a higher density of striae (17-21 in 10 µm in *P. asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. versus 10-13 in 10 µm in *P. anglophila*) and areolae (45-50 in 10 µm versus 30-34 in 10 µm, respectively). Finally, *P. asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. lacks a central area, whereas in *P. anglophila* a small (< ¼ of the valve width) and rounded central area is present.

Another species very close to *Placoneis anglophila* and, respectively, to our species is *Placoneis neoexigua* Lange-Bertalot & Miho (Miho & Lange-Bertalot 2006: 303, figs 1-11). Detailed analysis of the comparison between *P. anglophila*

and *P. neoexigua* is given in Miho & Lange-Bertalot (2006: 305, figs 12-26). Many of the main morphological features of these two species almost completely overlap (valve outline and dimensions, number of striae in 10 µm). The differences are only in “the characteristic small apical frets separating the areolae of *P. anglophila* are lacking in *P. neoexigua*” (Miho & Lange-Bertalot 2006), and number of areolae. Thus, *P. asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. differs from both these species in valve outline (asymmetric, linear-elliptical). Smaller valves of *P. neoexigua* are also often widely lanceolate, and such valves were never observed in *P. asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. New species is characterized by a higher number of striae and areolae in 10 µm (17-21 striae and 45-50 areolae in 10 µm versus 10-12 striae [up to 18 only at the ends of valves] and 26-30 areolae in *P. neoexigua*). The central area in *P. asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. is lacking, whereas in both *P. neoexigua* and *P. anglophila* a small and rounded central area is present.

Some similarities are observed between *Placoneis asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. and *Placoneis nanoclementis* (Lange-Bertalot & Wojtal 2014: 409, 416, figs 14-28, 71-76). The length and width of the valves of *Placoneis asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. is close to the lower dimensional boundaries of *P. nanoclementis* (length 13.4-15.4 µm and width 6.0-6.8 µm in the new taxa versus length 14-23 µm and width 8.0-8.6 µm in *P. nanoclementis*). The valve outline of *P. asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. differs by being linear in outline versus elliptical or broadly lanceolate in *P. nanoclementis*. In *P. asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. both the striae and the areolae are denser (17-21 striae and 45-50 areolae in 10 µm, respectively, versus 12-16 striae and 40-44 areolae in 10 µm in *P. nanoclementis*). In addition, a stigma is lacking in the central area in *Placoneis asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov., whereas in *P. nanoclementis* two stigmata are clearly visible (Table 3).

Another small-celled *Placoneis* species that can be confused with *P. asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. is a rare species described from Lake Baikal, *Placoneis vodosa* Pomazkina & Rodionova (Pomazkina et al. 2019). The two taxa have a similar valve dimensions (in the new species, the length and width are 13.4-15.4 µm and 6.0-6.8 µm, respectively, while in *P. vodosa* they are 15.6-17.8 µm and 7.2-8.0 µm, respectively); shortened striae are present in the central part of the valves. However, *P. asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. differs with the more linear valve outline and the typical asymmetry along the apical axis (in *P. vodosa*, the shape of valves is elliptical-lanceolate), higher density of striae and areolae (17-21 striae and 45-50 areolae in 10 µm versus 14-16 striae and 30-45 areolae in 10 µm in *P. vodosa*, respectively).

Thus, our molecular studies have shown that *P. asymmetricus* Glushchenko, Kezlya, Kulikovskiy & Kociolek, sp. nov. belongs to a monophyletic *Placoneis* and occupies a separate

phylogenetic position in relation to other species with high statistical support. The morphological features of the valve structure studied by us confirm the description of this species as new to science.

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