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# Three New Species of *Placoneis* Mereschkowsky (Bacillariophyceae: Cymbellales) with Comments on Cryptic Diversity in the *P. elginensis*—Group

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**Abstract:** Using genetic markers 18S V4 rDNA and *rbc*L and morphological investigation of the diatom genus *Placoneis*, we described three new species. The new species, *Placoneis baikaloelginensis* sp. nov., *Placoneis subundulata* sp. nov., *Placoneis neohambergii* sp. nov. were isolated from Russia (Lake Baikal) and Vietnam (waterbodies of Cát Tiên National Park (Đồng Nai Province) and Khánh Hòa Province). We examine relationships within the Cymbellales and show that the genera *Placoneis*, *Paraplaconeis* and *Geissleria* are phylogenetically independent. We discuss the importance of careful identification of strains used for phylogenetic analysis and we show the history of identification of several different *Placoneis elginensis* strains. After careful identification of *Placoneis elginensis* vouchers, we found that we have a few independent species. The question of cryptic or pseudocryptic species in this context is discussed.

**Keywords:** algae; voucher; cryptic diversity; Cymbellales; molecular data; morphology; *Placoneis*; Baikal; Vietnam; Cát Tiên National Park



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## 1. Introduction

The systematic position and richness of the genus *Placoneis* Mereschkowsky 1903 have been repeatedly revised and studied. Initially, on the basis of the symmetrical structure of the valve, the representatives of the genus were attributed to *Navicula* Bory 1822 [1]. In 1903, on the basis of studying the structure of the chloroplast (which is one organelle consisting of two X-shaped plates connected by an isthmus), Mereschkowsky [2] described the genus *Placoneis* and suggested, due to the nature of the chloroplast, this genus was more aligned with the "Monoplacatae" a group of taxa, including the Cymbellales, with a similarly structured chloroplast. Following this proposal, members of the genus were again consistently placed within *Navicula* (e.g., [3–5]). Eileen Cox resurrected the genus and included seven species within it and designated a generitype, *P. gastrum* (Ehrenberg) Mereschkowsky [6]. At the end of the 20th century, a targeted study of representatives of this genus using scanning electron microscopy (SEM) made it possible to identify and determine the special structure of the pore apparatus—the tectulum [7]. Over time, the richness of the genus has increased significantly due to combinations, recombinations and descriptions of new species. Kulikovskiy et al. [8] transferred species with a two-row arrangement of areolae from the genus *Placoneis* to the new genus *Paraplaconeis* Kulikovskiy, Lange-Bertalot & Metzeltin.

Representatives of *Placoneis* are widespread, mainly confined to freshwater bodies, less often with brackish ones, and they are also found in soils and mosses [6,9,10].

The use of molecular analysis in the study of the taxonomy of algae is currently a popular and convenient tool that helps to determine the boundaries of species that are closely related in morphology and to clarify the systematic position of taxa. Recently, these tools have helped identify situations where cryptic species have been identified (for example: *Sellaphora pupula* (Kützing) Mereschkovsky [11,12], *Nitzschia palea* (Kützing) W. Smith [13], *Navicula cryptocephala* Kützing [14], *Gomphonema parvulum* (Kützing) Kützing [15], *Pinnularia borealis* Ehrenberg [16], *Pinnularia subgibba* group [17,18] and *Hantzschia amphioxys* (Ehrenberg) Grunow [19]). In GenBank, gene sequences for 15 strains of this genus have been deposited, belonging to 11 taxa. In the present report, we analyze the 18S rDNA and *rbc*L genes and, accordingly, for phylogenetic analysis, we selected 10 strains with the required sequences, of which, as it turned out, four belong to *Placoneis elginensis* (Gregory) Cox. Since each *P. elginensis* strain occupies a separate position and is defined as an independent species on the phylogenetic tree built according to these markers, it became necessary to study the vouchers of these strains in order to understand whether these strains represent cryptic species. And to understand the morphological variability and boundaries of the species we studied the literature.

The purpose of the present report is to describe three new *Placoneis* species isolated into monoclonal cultures from water bodies located in Russia (Baikal Lake) and Vietnam (Cát Tiên National Park (Đồng Nai Province) and Khánh Hòa Province) based on the study of morphology and analysis of molecular data on genetic markers 18S V4 rDNA and *rbc* L. We also examine relationships within the Cymbellales and discuss the possibility of cryptic species within the *P. elginensis* species complex based on molecular data.

## 2. Materials and Methods

**Sampling**. The samples used in this manuscript were collected in Russia and Vietnam on three different expeditions at different times.

Strains B703 and B708 were isolated from sample no. 195, collected by E.S. Gusev and M.S. Kulikovskiy from moss in the swamp along the coast of Lake Baikal (Russia) in the Ayaya Bay region (55°27.279′ N; 109°54.104′ E) 24 July 2014.

Strain VN364 was isolated from a benthic sample (sample KHV 2) collected from small waterbodies near Cái River, Khánh Hòa Province, Khánh Vĩnh District, Vietnam (12°16.006′ N; 108°51.128′ E) by E.S. Gusev 20 October 2010.

Strain VN1199 was isolated from benthic sample no. Kt142, collected from temporary waterbodies situated in Cát Tiên National Park, South Vietnam (11°26.319′ N; 107°25.363′ E), 03 June 2019 by E.S. Gusev and E.M. Kezlya.

Water mineralization (specific conductance), pH and temperature measurements were made with a Hanna HI 98129 device, Hanna Instruments, Inc., Woonsocket, RI, USA. Samples from Vietnam were collected during expeditions organized and permitted by the Joint Russian-Vietnam Tropical Centre, Ecolan 1.2 and 3.2 projects.

**Culturing**. A subsample of each collection was added to WC liquid medium (Guillard and Lorenzen 1972). Monoclonal strains were established by micropipetting single cells under an inverted microscope. Non-axenic unialgal cultures were maintained in WC liquid medium at 22–25 °C in a growth chamber with a 12:12 h light:dark photoperiod.

**Preparation of slides and microscope investigation**. The culture was treated with 10% hydrochloric acid to remove carbonates and washed several times with deionized water for 12 h. Afterwards, the sample was boiled in concentrated hydrogen peroxide (≈37%) to remove organic matter. It was washed again with deionized water four times at 12 h 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 preparations 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 cam-

era (Zeiss). Valve ultrastructure was examined by means of scanning electron microscopes JSM-6510LV (IBIW, Institute for Biology of Inland Waters RAS, Borok, Russia). For scanning electron microscopy (SEM), part of the suspensions was fixed on aluminum stubs after air-drying. The stubs were sputter-coated with 50 nm of Au by means of a Eiko IB 3.

Sample and slides are deposited in the collection of Maxim Kulikovskiy at the Herbarium of the Institute of Plant Physiology Russian Academy of Sciences, Moscow, Russia

*Molecular Methods*

Total DNA of monoclonal cultures was extracted using ChelexTM 100 Molecular Biology Grade Resin (Bio-Rad, California, USA) according to the manufacturer's protocol 2.2. Fragments of 18S rDNA (359–433 bp, including V4 domain), and partial *rbc*L plastid genes (759–1101 bp) were amplified using primers D512 for and D978rev from [20] for 18S rDNA fragments and *rbc*L40+ from [21] and *rbc*L1255—from [22] for *rbc*L fragments.

Amplifications of the 18S rDNA fragments and partial *rbc*L gene fragments were carried out using the premade mix ScreenMix (Evrogen, Moscow, Russia) for the polymerase chain reaction (PCR). The conditions of amplification for 18S rDNA fragments were: an initial denaturation of 5 min at 95 °C, followed by 35 cycles at 94 °C for denaturation (30 s), 52 °C for annealing (30 s) and 72 °C for extension (50 s), and a final extension of 10 min at 72 °C. The conditions of amplification for partial *rbc*L were: an initial denaturation of 5 min at 95 °C, followed by 45 cycles at 94 °C for denaturation (30 s), 59 °C for annealing (30 s) and 72 °C for extension (80 s), and a final extension of 10 min at 72 °C.

The resulting amplicons were visualized by horizontal agarose gel electrophoresis (1.5 %), colored with SYBR Safe (Life Technologies, Carlsbad, CA, USA). Purification of DNA fragments was performed with the ExoSAP-IT kit (Affimetrix, Santa Clara, CA, USA) according to the manufacturer's protocol. 18S rDNA fragments and partial *rbc*L gene were decoded from two sides using forward and reverse PCR primers and the Big Dye system (Applied Biosystems, Waltham, MA, USA), 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 [23]. Newly determined sequences and DNA fragments from 61 other diatoms, which were downloaded from GenBank (taxa and Accession Numbers are given in the tree), were included in the alignments. Five diatom species from Rhopalodiaceae were chosen as the outgroups.

The nucleotide sequences of the 18S rDNA and *rbc*L genes were aligned separately using the Mafft v7 software and the E-INS-i model [24]. For the protein-coding sequences of the *rbc*L gene, we checked that the beginning of the aligned matrix corresponded to the first position of the codon (triplet). The resulting alignments had lengths of 439 (18S rDNA) and 1101 (*rbc*L) characters.

The dataset was analyzed using Bayesian inference (BI) method implemented in Beast ver. 1.10.1. [25] to construct phylogeny. For each of the alignment partitions, the most appropriate substitution model was estimated using the Bayesian information criterion (BIC) as implemented in jModelTest 2.1.10 [26]. This BIC-based model selection procedure selected the following models, shape parameter $\alpha$ and a proportion of invariable sites (pinvar): TIM3 + I + G, $\alpha = 0.5220$ and pinvar = 0.4850 for 18S rDNA gene; TPM1uf + I + G, $\alpha = 0.5440$ and pinvar = 0.7750 for the first codon position of the *rbc*L gene; JC + I, pinvar = 0.8630 for the second codon position of the *rbc*L gene; TVM + G, $\alpha = 0.5120$ for the third codon position of the *rbc*L gene. We used the HKY model of nucleotide substitution instead of TPM1uf and TIM3, the F81 model instead of JC, the GTR model instead of TVM given that they were the best matching models available for Bayesian inference. A Yule process tree prior was used as a speciation model. The analysis ran for 15 million generations with chain sampling every 1000 generations. The parameters-estimated convergence, effective sample size (ESS) and burn-in were checked using the software Tracer ver. 1.7.1. [25]. 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 [27]. 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).

## 3. Results

*Placoneis baikaloelginensis* Kezlya, Glushchenko, Kulikovskiy & Kociolek sp. nov. (Figures 1–4).

**Holotype**. Collection of Maxim Kulikovskiy at the Herbarium of the Institute of Plant Physiology Russian Academy of Science, Moscow, Russia, holotype here designated, slide No. 01493 (Figure 1H).

**Type strain**. B703, isolated in from the sample 195, deposited at collection of Maxim Kulikovskiy at the Herbarium of the Institute of Plant Physiology Russian Academy of Science, Moscow, Russia.

**Isotype**. Slide no. 01493a, strain B703, isolated in from the sample 195, deposited in herbarium of MHA, Main Botanical Garden Russian Academy of Science, Moscow, Russia.

**Type locality**. Russia, coast of Lake Baikal, Ayaya Bay, swamp, between mosses, 55°27.279′ N; 109°54.104′ E. Collected by E.S. Gusev and M.S. Kulikovskiy, 24 July 2014, pH = 7.5, conductivity = 18 μS $cm^{-1}$, t = 6 °C.

**Representative DNA sequences for VP703 strain**. Nuclear-encoded SSU rDNA partial sequence (GenBank accession MW422266 V4), plastid gene *rbc*L partial sequence (GenBank accession MW423734).

**Etymology**. The specific epithet refers to the name of the similar species *Placoneis elginensis*.

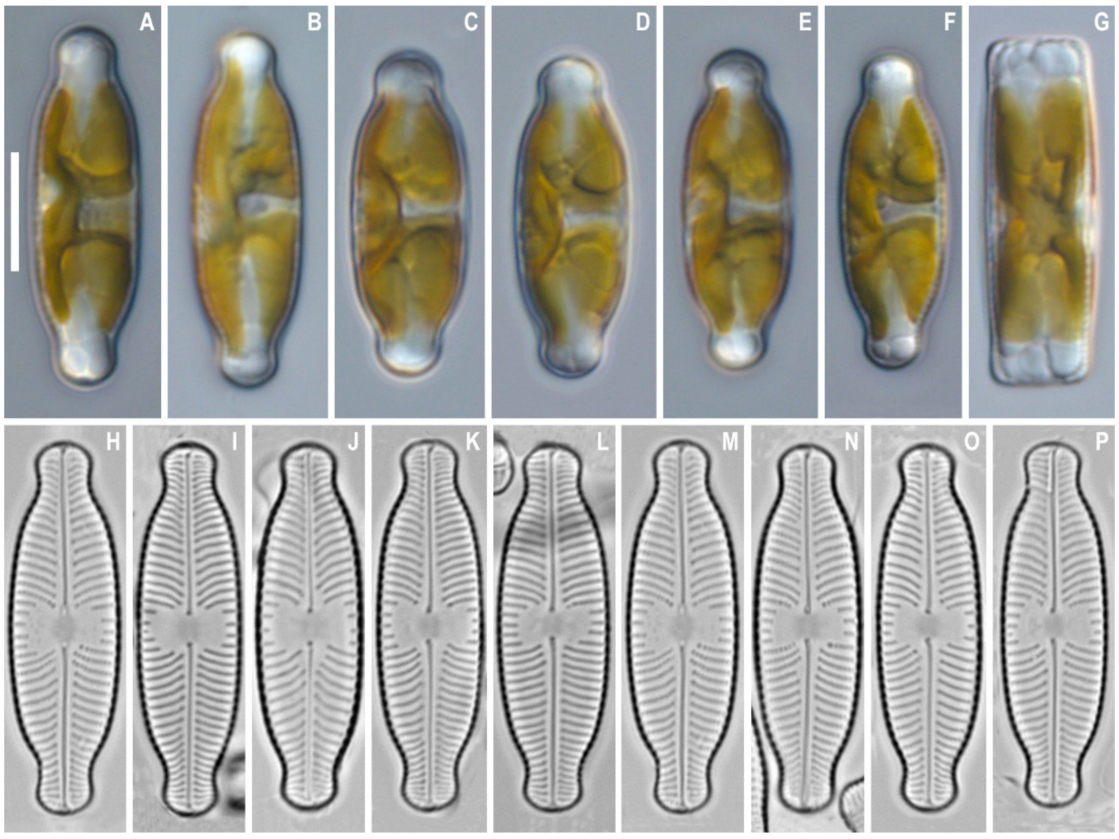


**Figure 1.** *Placoneis baikaloelginensis* Kezlya, Glushchenko, Kulikovskiy & Kociolek sp. nov. Strain B 703, slide No. 01493. Light microscopy, differential interference contrast, size diminution series. (**A**–**F**,**H**–**P**). Valves face. (**G**). Cell in girdle view. (**A**–**G**). Live cells with chloroplast structure. (**H**). Holotype. Scale bar = 10 μm.

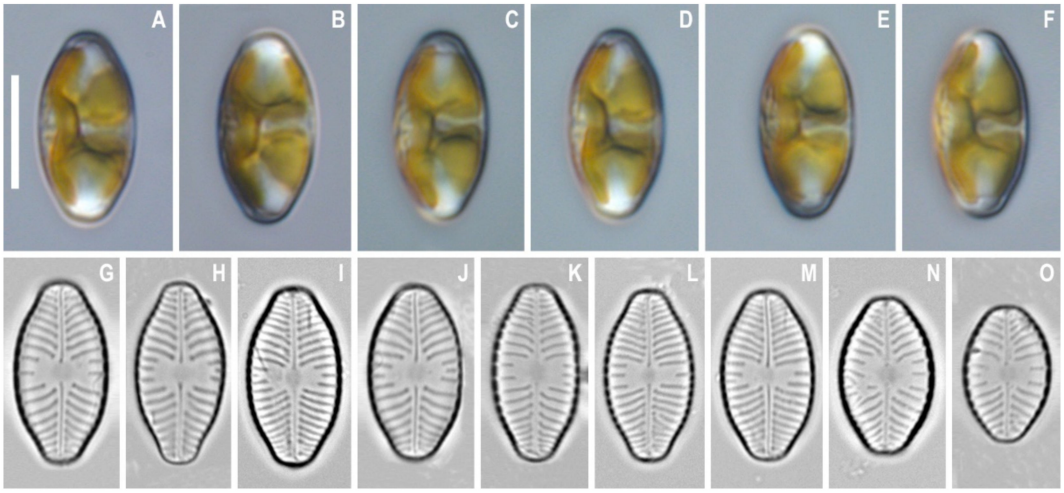


**Figure 2.** *Placoneis baikaloelginensis* Kezlya, Glushchenko, Kulikovskiy & Kociolek sp. nov. Strain B708, slide no. 01498. (**A**–**O**). Light microscopy, differential interference contrast, size diminution series. (**A**–**F**). Live cells with chloroplast structure. Scale bar = 10 μm.

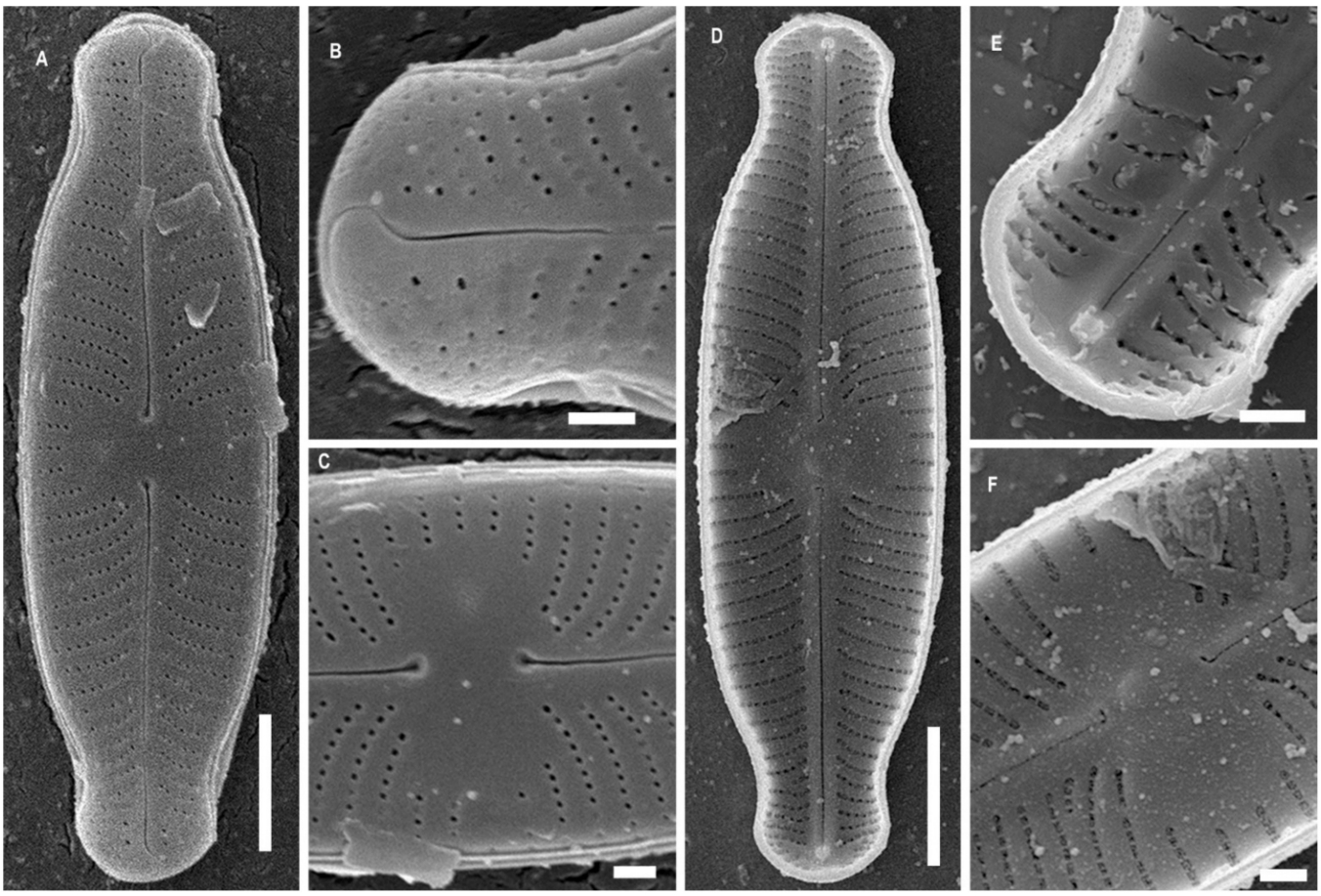


**Figure 3.** *Placoneis baikaloelginensis* Kezlya, Glushchenko, Kulikovskiy & Kociolek sp. nov. Strain B 703, sample No. 01493. Scanning electron microscopy: (**A**–**C**) External views. (**D**–**F**) Internal views. (**A**,**D**) The whole valve. (**C**,**F**) Central area details. (**B**,**E**) Valves ends. Scale bars (**A**,**D**) = 5 μm; (**B**,**C**,**E**,**F**) = 1 μm.

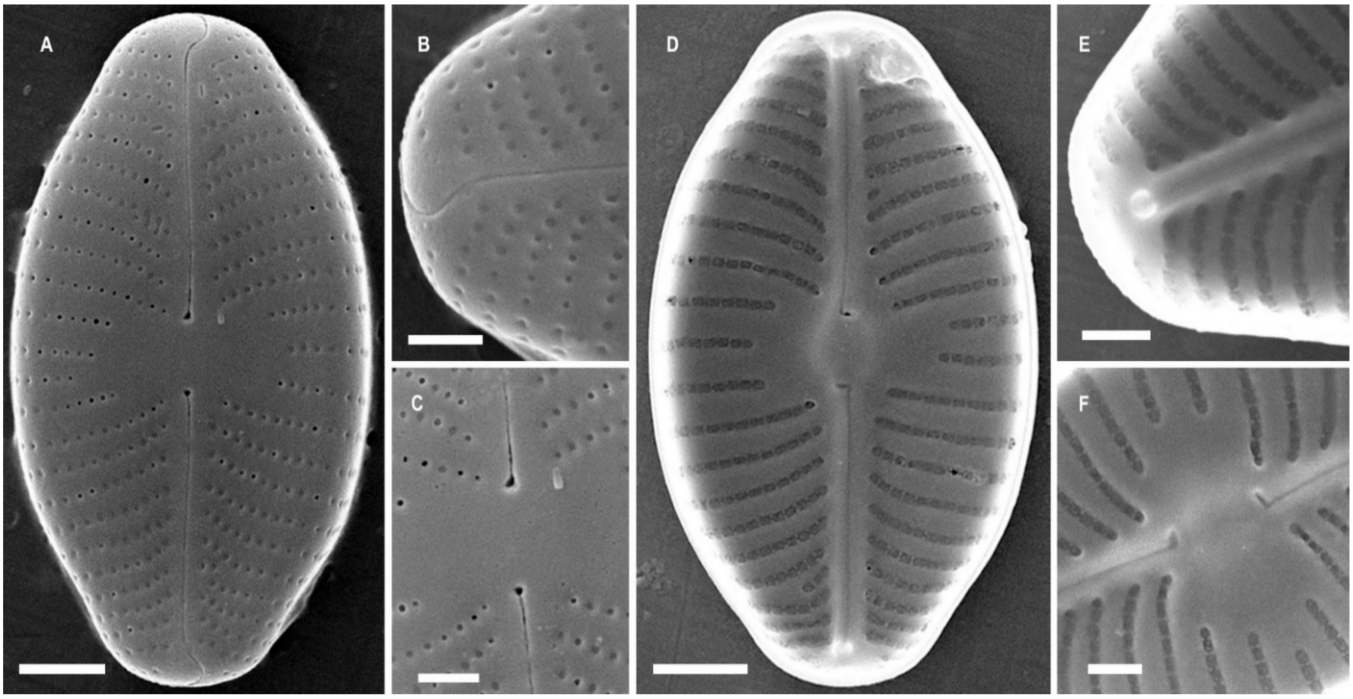


**Figure 4.** *Placoneis baikaloelginensis* Kezlya, Glushchenko, Kulikovskiy & Kociolek sp. nov. Strain B708, sample no. 01498. Scanning electron microscopy: (**A**–**C**) External views. (**D**–**F**) Internal views. (**A**,**D**) The whole valve. (**C**,**F**). Central area details. (**B**,**E**) Valves ends. Scale bars (**A**,**D**) = 5 µm; (**B**,**C**,**E**,**F**) = 1 µm.

**Distribution**. As yet, known only from the type locality.

**Description**. LM (Figures 1 and 2). Cells solitary, rectangular in girdle view (Figure 1G). Valves linear-elliptical to elliptic-lanceolate with broadly rounded, subcapitate ends. Length 12.2–31.6 µm, breadth 7.8–9.3 µm, apex width 4.5–5.0 µm. Central area large, transversely-expanded, rounded or bow-tie-shaped from 1/2 to 3/4 width of valve. Axial area narrow, linear, sometimes slightly widening to the middle of the valve. Raphe filiform. Proximal raphe ends drop-shaped, straight or slightly deflected to one side, distal raphe ends curved to one side. Striae radiate, becoming parallel to convergent at the valve ends, 13–15 (17) in 10 µm. Areolae difficult to resolve in the LM. Chloroplast has a typical organization inherent in representatives of the genus, being a single H-shaped plastid, with one arm lying against each side of the girdle, connected by a narrow central isthmus.

SEM (Figure 3). In external views (Figure 3A–C), the raphe is narrow, linear (Figure 3A). Proximal raphe ends are straight or slightly deflected to one side, drop-shaped (Figure 4C). Distal raphe ends hook-shaped, extending to the valve margin (Figure 3A,C). Striae are composed of 5–11 rounded areolae, extending to valve margin (Figure 3A,B). Areolae 30 in 10 µm. Internally (Figure 3D–F), the raphe is straight, lying in a raised raphe-sternum (Figure 3D). Proximal valve ends deflected to one side (Figure 3F). Distal raphe ends terminate as small helictoglossae (Figure 3E). Areolae are small, rounded, and covered by vola-like occlusions (Figure 3E,F).

Another strain *Placoneis baikaloelginensis* sp. nov. B708 was isolated from the sample 195 as well (slide No. 01498). It shows smaller cells in the size diminution series of type strain (Figure 2A–O). Valves elliptic-lanceolate with broadly rounded ends. Length 12.2–16.7 µm, breadth 7.8–8.5 µm. Morphology of central and axial areas, raphe, areolae same with type strain B703. The strains B708 and B703 are identical in the SEM (Figure 4A–F) and form one branch with maximum statistical support on the phylogenetic tree (Figure 5). Representative DNA sequences for VP708 strain: nuclear-encoded SSU rDNA partial sequence (GenBank accession MW422267 V4), plastid gene *rbc*L partial sequence (GenBank accession MW423735).

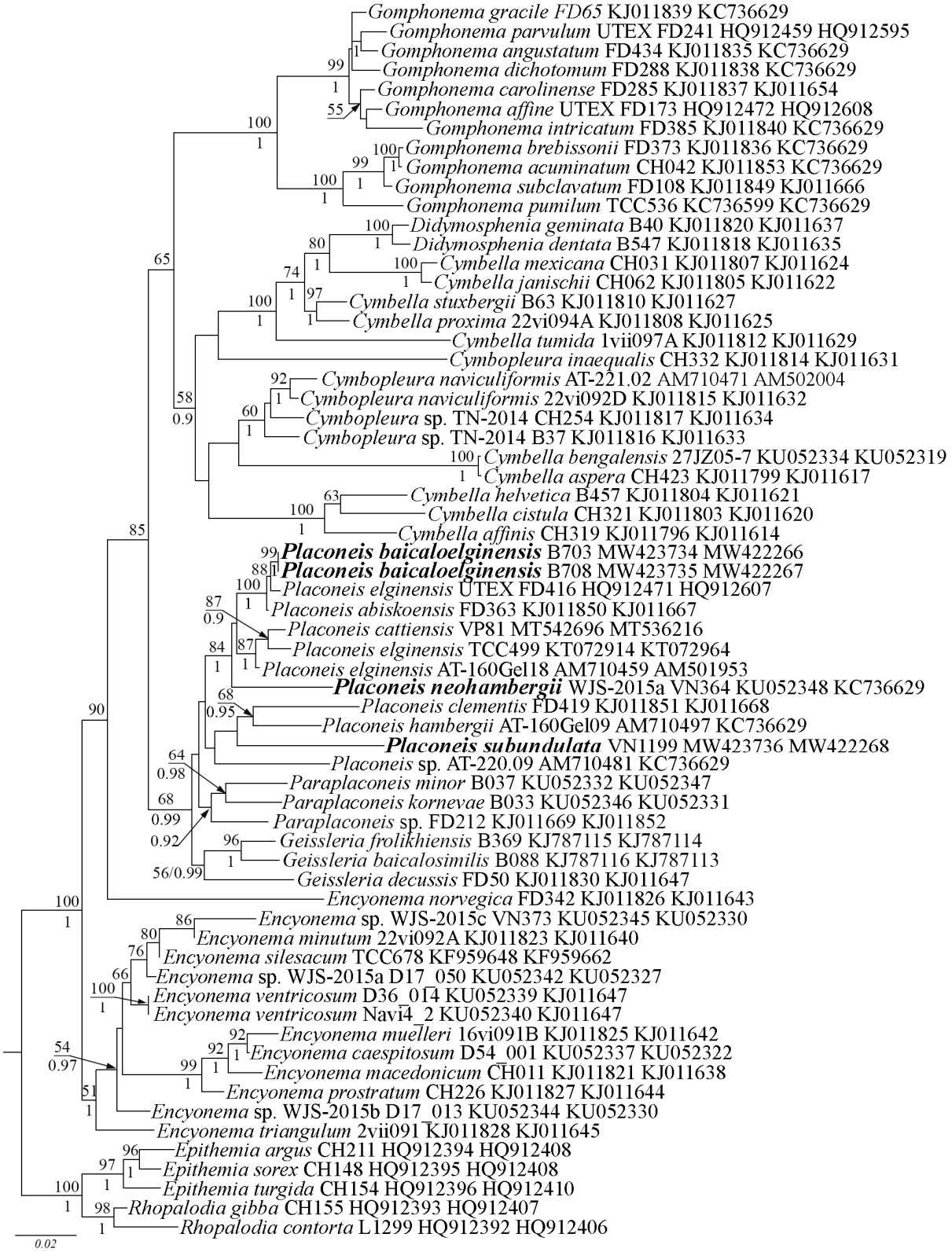


**Figure 5.** Maximum likelihood tree for *Placoneis baicaloelginensis* sp. nov., *Placoneis neohambergii* sp. nov. and *Placoneis subundulata* sp. nov. (indicated in bold) constructed from a concatenated alignment of 64 partial *rbc*L and partial 18S rDNA sequences of 1540 characters. Values above the horizontal lines (on the left of slash) are bootstrap support from ML analyses (<50 are not shown); values below the horizontal lines (to the right of slash) are Bayesian posterior probabilities (<0.9 are not shown). All sequences have strain numbers (if available) and GenBank numbers. Species from Rhopalodiaceae were used as an outgroup.

*Placoneis subundulata* Kezlya, Glushchenko, Kulikovskiy & Kociolek sp. nov. (Figures 6 and 7).

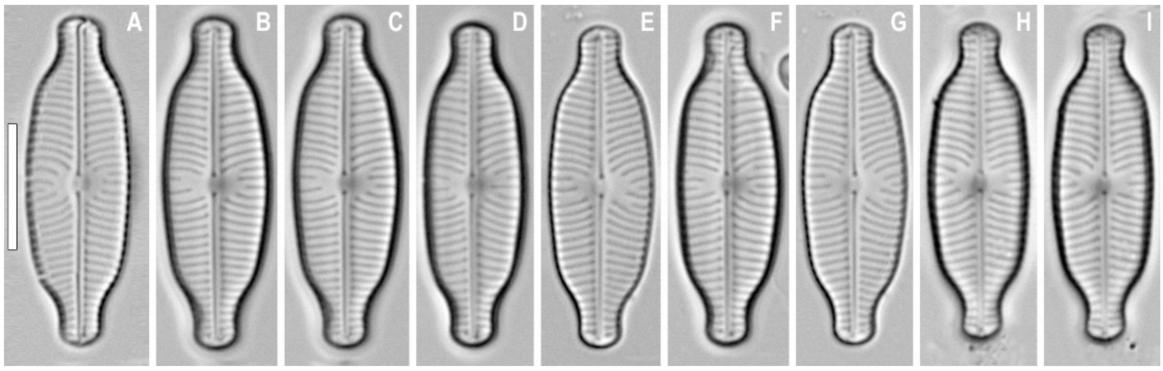


**Figure 6.** *Placoneis subundulata* Kezlya, Glushchenko, Kulikovskiy & Kociolek sp. nov. Strain VN1199, slide no. 01682. (**A**–**I**) Light microscopy, differential interference contrast, size diminution series. (**G**) Holotype. Scale bar = 10 µm.

**Holotype**. Collection of Maxim Kulikovskiy at the Herbarium of the Institute of Plant Physiology Russian Academy of Science, Moscow, Russia, holotype here designated, slide No. 01682 (Figure 6G).

**Type strain**. Strain VN1199, isolated in from the sample Kt142, deposited at collection of Maxim Kulikovskiy at the Herbarium of the Institute of Plant Physiology Russian Academy of Science, Moscow, Russia.

**Isotype**. Slide no 01682a, deposited in collection of MHA, Main Botanical Garden Russian Academy of Science, Moscow, Russia.

**Type locality**. Vietnam, Cát Tiên National Park, temporary reservoir (11°26.319′ N, 107°25.363′ E), benthos. Collected by E.S. Gusev and E.M. Kezlya, 03 June 2019, pH = 7.63, t = 28 °C.

**Representative DNA sequences for strain VN1199**. Nuclear-encoded SSU rDNA partial sequence (GenBank accession MW422268 V4), plastid gene *rbc*L partial sequence (GenBank accession MW 423736).

**Etymology**: The specific epithet refers to the name of similar species *Placoneis undulata*.

**Distribution**. As yet known only from the type locality.

**Description**. LM (Figure 6A–I). Valves linear-elliptic, margins of valve slightly triundulate (sometimes only one side). Ends rostrate. Length 25.5–27.0 µm, width 7.5–8.5 µm, apex width 3.0–3.5 µm. Central area irregular in shape (transverse, rounded, rhombic, bow-tie-shaped, asymmetrical) because of alternating shorter and longer striae to 1/2 width of valve. Axial area narrow, linear. Raphe filiform. Proximal raphe ends drop-shaped; distal raphe ends are curved in one side. Striae radiate, becoming parallel in the ends, 14.0–15.0 in 10 µm. Areolae not discernable in the LM.

SEM (Figure 7A–F). In external views, valve face is flat (Figure 7A). Raphe is narrow, linear (Figure 7A–C). Proximal raphe ends are straight, drop-shaped (Figure 7B). Distal raphe ends are hook-shaped, extend to valve margin (Figure 7C). Striae are composed in 5–12 elongated or slit-shaped areolae, extending to valve margin (Figure 7A–C). Areolae number 35 in 10 µm. In internal views (Figure 7D–F). The raphe endings are straight and lie in a prominent and raised raphe-sternum (Figure 7D). Proximal valve ends are deflected to one side, almost on 90° (Figure 7E). Distal raphe ends terminating on small helictoglossae (Figure 7D,F). Areolae are rounded covered by volate occlusions (Figure 7D,F).

*Placoneis neohambergii* Kezlya, Glushchenko, Kulikovskiy & Kociolek sp. nov. (Figures 8 and 9).

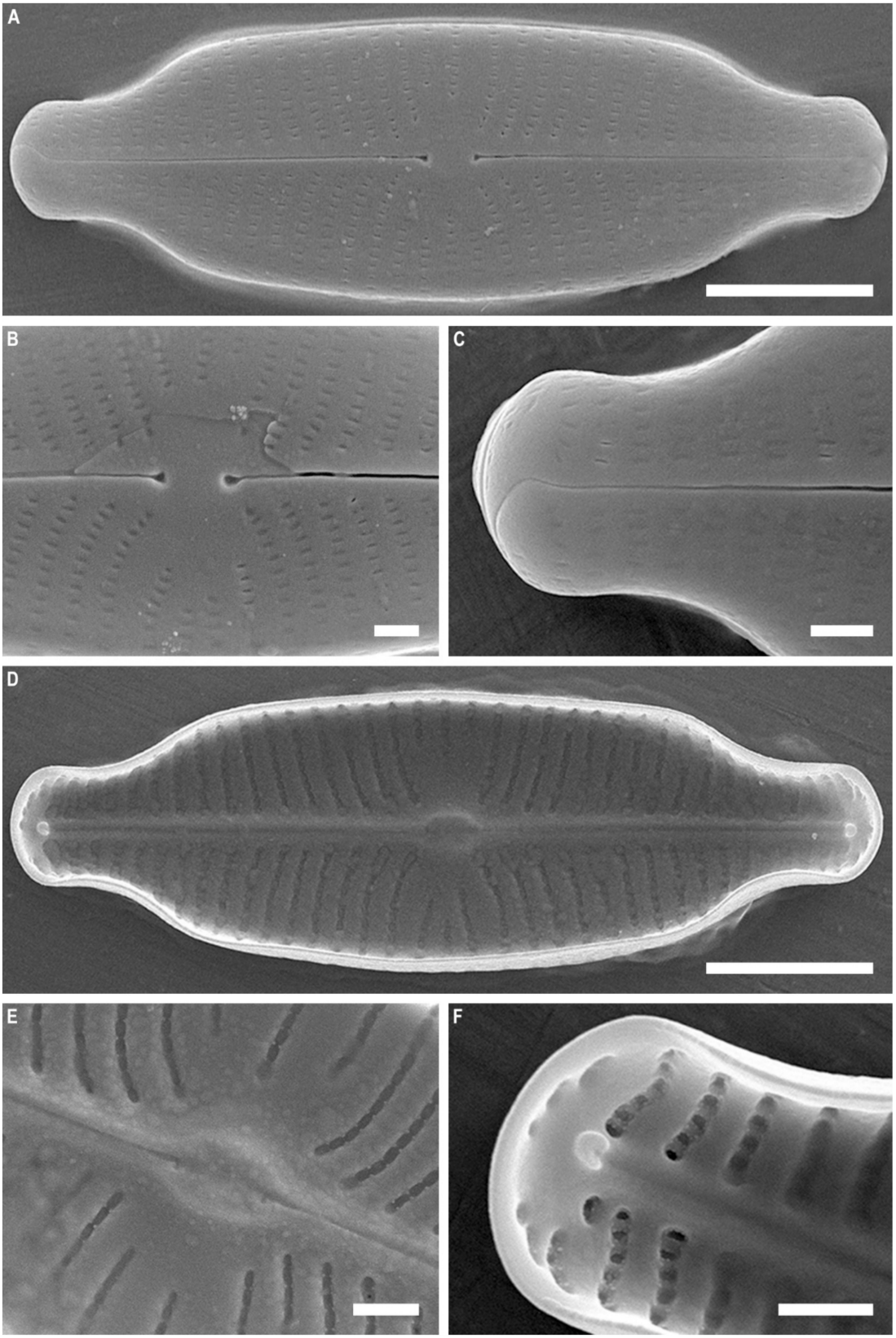


**Figure 7.** *Placoneis subundulata* Kezlya, Kulikovskiy & Kociolek sp. nov. Strain VN1199, sample no. 01682. Scanning electron microscopy: (**A**–**C**). External views. (**D**–**F**). Internal views. (**A**,**D**) The whole valve. (**B**,**E**) Central area details. (**C**,**F**) Valves ends. Scale bars (**A**,**D**) = 5 μm; (**B**,**C**,**E**,**F**) = 1 μm.

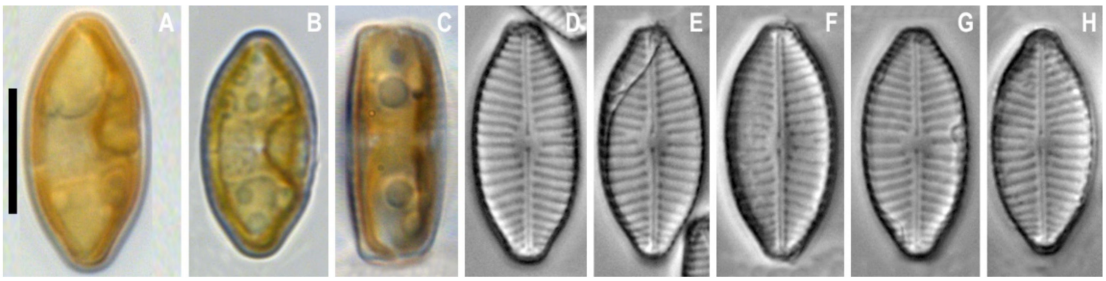


**Figure 8.** *Placoneis neohambergii* Kezlya, Glushchenko, Kulikovskiy & Kociolek sp. nov. Strain VN364, slide no. 00134. (**A**–**H**) Light microscopy, differential interference contrast, size diminution series. (**A**–**C**) Live cells with chloroplast structure. (**D**) Holotype. Scale bar = 10 μm.

**Holotype**. Collection of Maxim Kulikovskiy at the Herbarium of the Institute of Plant Physiology Russian Academy of Science, Moscow, Russia, holotype here designated, slide No. 00134 (Figure 8D).

**Type strain**. Strain VN364, isolated in from the sample KHV 2, deposited at collection of Maxim Kulikovskiy at the Herbarium of the Institute of Plant Physiology Russian Academy of Science, Moscow, Russia.

**Isotype**. Slide no. 00134a, deposited in collection of MHA, Main Botanical Garden Russian Academy of Science, Moscow, Russia.

**Representative DNA sequences for strain VN364**. Nuclear-encoded SSU rDNA partial sequence (GenBank accession KC736629 V4), plastid gene *rbc*L partial sequence (GenBank accession KU052348).

**Type locality**. Vietnam, Khánh Hòa Province, Khánh Vĩnh District, unnamed reservoir near Cái River (12°16.006′ N, 108°51.128′ E), benthos. Collected by E.S. Gusev, 20 October 2010, pH = 6.8, conductivity = 88 μS $cm^{-1}$, t = 28 °C.

**Etymology**: The specific epithet refers to the name of similar species *Placoneis hambergii*.

**Distribution**. As yet known only from the type locality.

**Description**. LM (Figure 8A–H). Cells solitary, rectangular in girdle view (Figure 8C). Valves elliptical- lanceolate with barely protracted broadly rounded ends. Length 17.0–19.0 μm, breadth 7.5–8.0 μm, apex width 2.0–3.0 μm. Central area small, rounded or not distinct, confined one or two shorter striae. Axial area narrow, linear. Raphe filiform. Proximal raphe ends drop-shaped, straight. Striae weakly radiate, subparallel, 13–14 (17) in 10 μm. Areolae not discernible in the LM (Figure 8D–H). Chloroplast has a typical organization inherent in representatives of the genus, being a single, H-shaped plastid, with one arm lying against each side of the girdle, connected by a narrow central isthmus (Figure 8A–C).

SEM (Figure 9A–F). In external views the raphe is narrow, linear (Figure 9A–C). Proximal raphe ends are straight, and the distal raphe ends are hook-shaped and extending to the valve margin (Figure 9A–C). Striae are composed of 5–11 rounded areolae, extending to valve margin (Figure 9A,C). Areolae number 35 in 10 μm.

In internal views (Figure 9D–F), the raphe is straight, lying in a raised raphe-sternum (Figure 9D). Proximal valve ends are deflected to one side (Figure 9E). Distal raphe ends terminate on small helictoglossae (Figure 9D,F). Areolae are small, rounded, and covered with vola-like occlusions (Figure 9D,F).

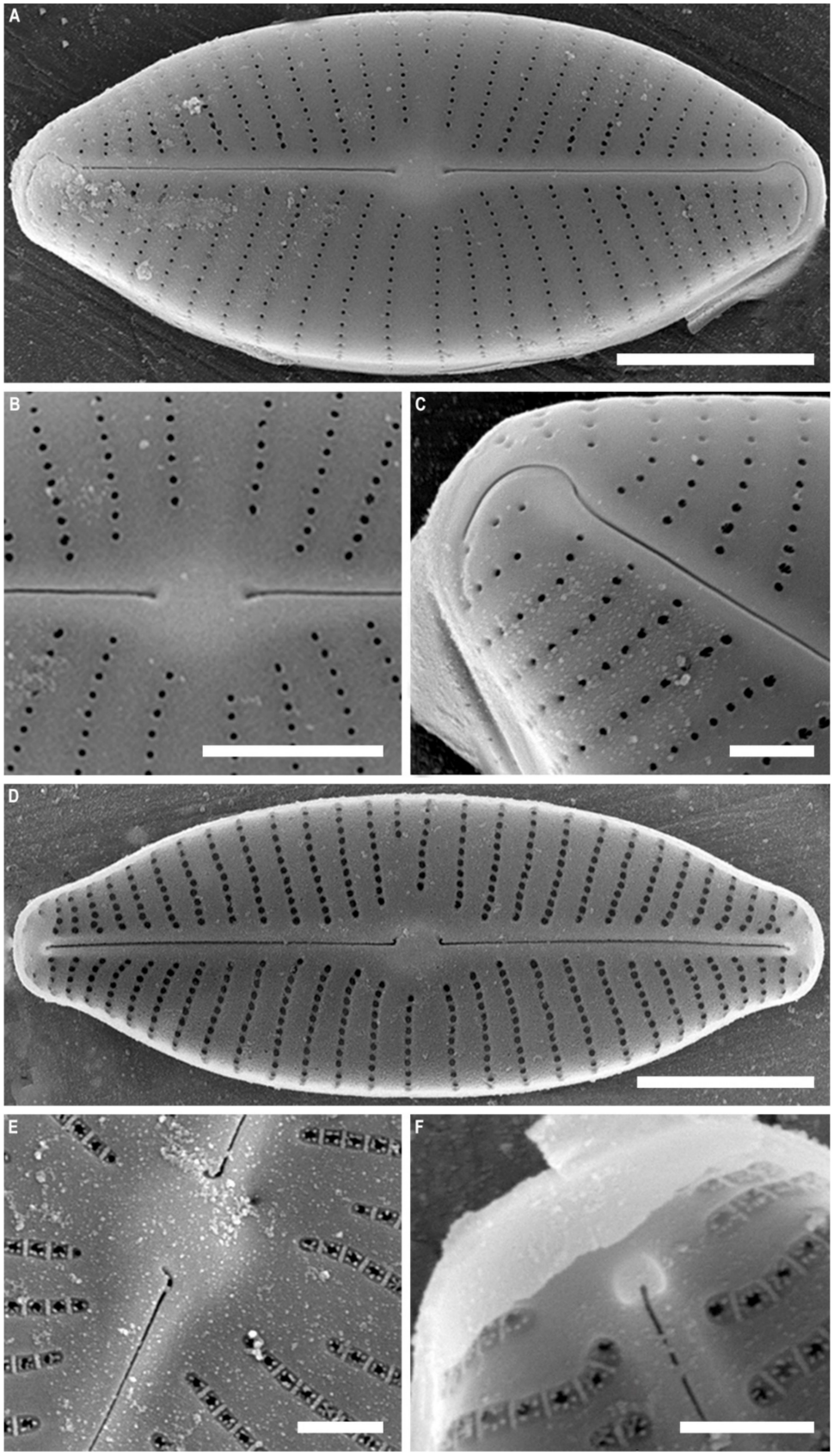


**Figure 9.** *Placoneis neohambergii* Glushchenko, Kezlya, Kulikovskiy & Kociolek sp. nov. Strain VN364, sample no. 00134. Scanning electron microscopy: (**A**,**D**) The whole valve. (**B**,**E**) Central area details. (**C**,**F**) Valves ends. Scale bar (**A**,**D**) = 5 μm; (**B**) = 2.5 μm; (**C**,**E**,**F**) = 1 μm.

*Molecular Investigation*

Phylogenetic analysis yielded a monophyletic Cymbellales (Figure 5). The first branching dichotomy shows the genus *Encyonema* as monophyletic (except for one taxon, *E. norvegica* (Grunow in A.Schmidt et al.) Bukhtiyarova), and a branch that includes naviculoid, cymbelloid and gomphonemoid diatoms. In this latter branch, there is first a single branch comprised of *E. norvegica*, then a dichotomy between a monophyletic group of naviculoid taxa (represented by *Geissleria*, *Paraplaconeis* and *Placoneis*, each of which is monophyletic) and a branch of cymbelloid and gomphonemoid diatoms. The branch with cymbelloid and gomphonemoid diatoms shows a branch comprised of cymbelloid diatoms and a branch comprised of *Gomphonema* taxa; the genus *Gomphonema* is shown here to be monophyletic. Within the branch containing cymbelloid diatoms, neither *Cymbella* nor *Cymbopleura* are shown to be monophyletic, and the genus *Didymosphenia* is again confirmed to be a part of the cymbelloid diatoms and not the gomphonemoid diatoms.

## 4. Discussion

The phylogenetic analysis presented here on the Cymbellales, the most comprehensive to date in terms of taxon sampling, supports the results shown in previous analyses [28,29] that diatoms with naviculoid symmetry are part of the monophyletic Cymbellales lineage. These results also confirm the relationship of *Didymosphenia* with members of the genus *Cymbella*, as suggested with morphological [30] and molecular data (e.g., [28]). These results also support the idea that neither *Cymbella* or *Cymbopleura* as currently conceived (e.g., [31]) are monophyletic. Since together these genera are large in terms number of described taxa [32], it is likely that further taxon sampling, including the generitype of *Cymbella* (*C. cymbiformis*), will be necessary before natural groups and the associated classification system that is derived from those relationships can be determined. The separation of *E. norvegica* from the other 10 representatives of the genus *Encyonema* also requires additional research. While this work gives us the best understanding to date of the relationships within this large order of freshwater diatoms, many genera are absent from the analysis, including all of the endemics from Asia [33,34]. Further work is required to more fully understand the relationships of this group and evaluate character evolution and biogeography of its members.

Within *Placoneis*, two branches can be recognized. One includes *P. clementis*, *P. hambergi* and *P. subundulata* sp. nov, the latter taxon being one of the new species described herein. The other branch contains *P. elginensis*, *P. cattiensis*, *P. abiskoenis*, and two new species described here, namely *P. baicaloelginensis* sp. nov. and *P. neohambergii* sp. nov. There are several strains represented in this analysis that were originally given the taxonomic designation "*P. elginensis*". In this analysis, these strains are within two separate branches within the genus, and do not form a monophyletic group.

*P. elginensis* is an example of a widespread species whose morphological variability and boundaries have been revised more than once. This species was described in 1856 by W. Gregory as *Pinnularia elginensis*, then transferred into *Navicula* by Ralfs (Ralfs in [35]). A sample of the type material is at the collection of the Natural History Museum, London, slide 11751 [36]. In 1986, in connection with the revision of the *Navicula*, Krammer & Lange-Bertalot illustrated the lectotype, but added to their review the material from slide BM23510 (lectotype for *Navicula tumida* syn. *N. anglica* Ralfs (*N. tumida* W. Smith) of the same collection. They also reviewed lectotypes for *Navicula anglica* f. *minor* (VH Type de synopsis 59), *N. dicephala* var. *neglecta* = var. *undulata* (Østrup in coll. Hustedt), and *N. neglecta* in coll. Krasske, and considered them all conspecific. Therefore, in the view of Krammer and Lange-Bertalot [5] the range of morphological variation within *P. elginensis* was considered to be rather wide. The authors did not notice clear differences in the material studied, considering the variety of valve shapes to be morphological variation of this species. Thus, at that time, *Placoneis* (as *Navicula elginensis* sensu lato) included valves of both elliptical and linear shapes with a rounded or transversely widened central area.

On the basis of comparative studies of many populations of *Placoneis*, Lange-Bertalot revised his previous opinion [20,37] and removed from *P. elginensis* sensu lato new species of smaller size with parallel outlines of the valves. These included *P. paraelginensis* Lange-Bertalot, which confirms the differentiation of *P. elginensis* sensu sricto. However, according to opinion of Cox [36], the illustrations provided by Rumrich et al. ([38], p. 361, taf. 60, Figures 17–20) for *P. paraelginensis* most likely include three taxa and suggested that a more detailed study of this species was required. Later, when revising the genus *Placoneis*, the type material was revised by Cox [36]. She characterized the lectotype based on slide BM 11751, and slide BM23510 was identified as the lectotype for *P. anglica*. However, in describing the distribution of *P. elginensis* Cox notes: "Because a number of different taxa have been included under this name, its distribution requires closer investigation". Thus, a group of species assigned to *P. elginensis* sensu lato was repeatedly revised [36–38], and as a result, a new species *P. paraelginensis* was described on the basis of morphometric characters [38], and new combinations and new status of taxa have been proposed (*P. pseudanglica* (Lange-Bertalot) E.J. Cox [6], *P. ignorata* (Schimanski) Lange-Bertalot, *P. undulata* (Østrup) Lange-Bertalot [38], *P. rostrata* (A. Mayer) E.J. Cox, *P. anglica* (Ralfs) E.J. Cox [36].

Molecular data are currently available for only 4 strains of *P. elginensis*. Two of these strains (UTEX FD416 and FD212) belong to UTEX Culture Collection of algae (https://utex.org, accessed on 28 August 2021), and the specimens were isolated from Minnesota, USA. Strain AT160Gel18 has vouchers listed on the Protist Central site (http://protistcentral.org, accessed on 28 August 2021), and was isolated from northern Germany (52°057.65′ N 08°020.67′ E. Poggenpohls Moor, puddle, soil). The culture is maintained by the Botanic Garden and Botanical Museum Berlin-Dahlem, FU Berlin, Germany [39]. The fourth strain, TCC499 strain is at Thonon Culture Collection of freshwater microalgae and was sampled from Mayotte Island Kwale River upstream site, France [40,41]. Analysis of the morphometric characteristics of *P. elginensis* vouchers (Table 1) shows that three of the four strains are consistent with the description of the type species in terms of the valve shape, the arrangement of striae, and the structure of the central and axial area.

**Table 1.** Comparison of morphological features of vouchers of *Placoneis elginensis*.

| | ***P. elginensis* TCC499** | ***P. elginensis* AT160Gel18** | ***P. elginensis* UTEX FD416** | ***P. elginensis* FD212** | ***P. elginensis* Type [36]** |
|---|---|---|---|---|---|
| **Outline** | Linear | Linear | Elliptic-lanceolate to elliptic | Linear | Linear |
| **Apex width (µm)** | 2.8–3.2 * | 3.1 * | no | 2.8–3 * | 4–4.5 |
| **Valve length (µm)** | 27.6–28.4 * | 25–26 * | 8.5–10 * | 14–18 * | 30–36 |
| **Valve breadth (µm)** | 8–8.8 * | 6.6 * | 5.44–6.46 * | 5.6–6 * | 9–10 |
| **Striae** | 12–14 * | 15 * | 16–17 * | 13–15 * | 11 |
| **Sampling origin** | France, Ile de Mayotte Kwale River upstream site Pierre Rivière | Europe, Germany 52°57.65′ N; 08°20.67′ E AlgaTerra Culture | Minnesota, USA | Minnesota, USA | Elgin, Scotland |
| **Locality** | Thonon Culture Collection | AlgaTerra Culture Collection | UTEX Culture Collection | UTEX Culture Collection | Natural History Museum Collection, London. Slide BM 11751 |

* counted from published data.

Differences are found in the cell size, number of striae, and width of the valve ends, which are most likely attributed by researchers to the morphological variability of this taxon (Table 1). The voucher of the TCC499 strain has slightly smaller valves relative to the type (27.6–28.4 µm vs. 30–36 µm), narrower valve ends (2.8–3.2 µm vs. 4.0–4.5 µm), and

a higher striae density (12–14 in 10 μm vs. 11 in 10 μm). Illustrations of the AT160Gel18 voucher are presented in the articles of Bruder [10] and in the AlgaTerra collection [39] and include a LM photo of one valve, four SEM images and a photo of two living cells. However, figures referenced in the text for *P. elginensis* are labeled as *P. paraelginensis*.

The cells in the illustrations are more similar to *P. paraelginensis* in outline (valves are almost parallel) and morphometric parameters (Table 1). Thus, according to the presented voucher, the AT160Gel18 strain does not belong to *P. elginensis*, but belongs to *P. paraelginensis*. Thus, morphological and molecular evidence both help to explain the independent position of isolates originally designated as "*P. elginensis*" on the tree (Figure 5).

The voucher of strain FD212 (labelled herein as "*Paraplaconeis* sp.") has much smaller valves relative to the type of *P. elginensis*, (length 14–18 μm vs. 30–36 μm, width 5.6–6.0 μm vs. 9–10 μm), higher striae density (13–15 in 10 μm vs. 11 in 10 μm). It should be noted that in our study, relative to its position on the phylogenetic tree, strain FDD212 occupies a position on the branch with other species of the genus *Paraplaconeis*. Originally the phylogenetic position of this strain was determined by Nakov et al. [28] in a study of the molecular phylogeny of Cymbellales based on nuclear encoded small ribosomal subunit rDNA (SSU) and large ribosomal subunit rDNA (LSU), the chloroplast encoded *rbc*L gene and chloroplast encoded photosystem I and II genes, *psa*B and *psb*A. The phylogenetic position of "*P. elginensis* FD212" in the tree prepared by Nakov et al. [28] was approximately the same as the tree presented here, combined with strain of *Geissleria decussis* (Østrup) Lange-Bertalot and Metzeltin. The data available at that time allowed the authors to make the assumption that " ... the placement of *Geissleria* Lange-Bertalot & Metzeltin renders *Placoneis* paraphyletic" [28]. In our study, species of the genus *Geissleria* form a separate branch near with *Paraplaconeis* group of species. The position of strain FD212 on the branch with *Paraplaconeis* suggests that *Placoneis. elginensis* FD212 is erroneously identified and most likely belongs to *Paraplaconeis*. We need more information for this strain using SEM to investigate the congruence of molecular data and morphology.

Another voucher of *P. elginensis*, UTEX FD416, differs significantly from the type in the shape of the cells and in size (Table 1). The photographs show small cells (8.5–10.0 μm in length) with a lanceolate shape, which are apparently attributed to the *P. elginensis*, which has shrunk during its life cycle according to the illustration of Cox ([6] (p. 148, Figure 24). Nevertheless, it occupies a position within *Placoneis* in the phylogenetic tree (Figure 5).

Despite the similarity in the shape of the valves, as well as the structure of the axial and central areas, each of the vouchers has unique morphometric features (in the size of valves, width of ends, density of striae) that differentiates them from each other and from type. Phylogenetic analysis based on the regions of the *rbc*L and 18S rDNA genes indicates that each strain occupies a distinct position in phylogenetic tree (Figure 5) and, therefore, is an independent species. As a result, the *P. elginensis* AT160Gel18 strain should be renamed as *P. paraelginensis*. The *P. elginensis* TCC499 strain can be attributed to a pseudo-cryptic species with respect to the type. The *P. elginensis* FD212 strain was probably erroneously identified because molecular data indicate that it belongs to *Paraplaconeis*. The voucher of the *P. elginensis* UTEX FD416 strain does not have clear morphometric characteristics by which this strain could be identified as *P. elginensis*.

Two strains of *Placoneis baikaloelginensis* sp. nov. form a group with high statistical support (ML99, BI 100) with strains *P. abiskoensis* FD363 and *P. elginensis* UTEX FD416 (ML100, BI100). Valves of the *P. baikaloelginensis* B703 strain have morphometric parameters corresponding to the typical characteristics for the described species (Figure 2A–P), and in the *P. baikaloelginensis* B708 strain, valves are small, almost elliptical, without characteristic subcapitate ends, because they represent smaller valves during valve diminution series (Figure 3A–I). Despite differences in valve shape during valve diminution series these strains form one branch with maximum statistical support on the phylogenetic tree (Figure 5).

Micrographs of valves of the *P. abiskoensis* FD363 voucher (http://protistcentral.org/Photo/get/photo_id/3676, accessed on 28 August 2021) show that valves are characterized by much smaller sizes and the width of the ends in comparison with the type (Table 2). The valve length in the micrographs does not exceed 27.5 µm in length and the width is 6.6 µm, while type species is characterized by being 38–47 µm long and 9–11 µm wide [36]. Valve ends in the illustrated voucher specimens are narrower (3.1 µm vs 5–6 µm) than the type. The similarity with the description of the type species is observed in the shape of valves (linear with parallel valves), the number and arrangement of striae, and the structure of the central area. This strain was probably wrongly identified. Our species *P. baikaloelginensis* sp. nov. is similar to the *P. abiskoensis* FD363 voucher in valve length (25.0–27.5 µm in FD363 vs 12.2–31.6 µm in *P. baikaloelginensis* sp. nov.), arrangement of striae, and structure of axial and central areas, but differs in having a linear-elliptical shape of the valve (with convex, rather than parallel margins). In addition, *P. baikaloelginensis* sp. nov. has wider valve ends (4.5–5.0 µm vs. 3.1 µm) and higher striae density (13–15 in 10 µm vs. 11–13 in 10 µm).

Comparison of *P. baikaloelginensis* sp. nov. morphology to the voucher of *P. elginensis* UTEX FD416 (http://protistcentral.org/Photo/get/photo_id/1210, accessed on 28 August 2021) showed significant differences (Table 2). Valves of the voucher of *P. elginensis* UTEX FD416 strain have small (up to 10 µm length) elliptical or elliptic-lanceolate valves with narrow cuneate ends, radial striae, a narrow axial area and a small rounded central area.

There are no shared morphological features with our new species, including with the small-cell strain B708, which is easily distinguished by larger valves (length 12.2–16.7 µm, width 7.8–8.5 µm vs. 8.5–10.0 µm and 5.4–6.5 µm in *P. elginensis* UTEX FD416) with widely rounded, slightly drawn ends and a large (at least 1/2 valve width) central area (Figure 4E–I).

The type of *P. elginensis* [6] (p. 155 Figures 56–58) is morphologically most similar to *P. baikaloelginensis* sp. nov., based on features of valve size, arrangement of striae and structure of the axial and central areas.

Differences between the two species are observed primarily in the shape of the valve. Valves of our new species are linear-elliptical, while in *P. elginensis* they are linear, slightly narrower (width 7.8–9.0 µm vs. 8.2–10.0 µm) and have wider subcapitate ends (4.9–5.0 µm vs. 4.0–4.5 µm). The two taxa also differ in stria density. *P. baikaloelginensis* sp. nov. is characterized by higher density of striae (13–15 in 10 µm versus 11.0 in 10 µm in *P. elginensis*) [36]. There are no differences in the structure of the axial and central area, or in the arrangement of the striae.

Two species with similar valve outlines that can be confused with *P. baikaloelginensis* sp. nov. are *P. significans* (Hustedt) Lange-Bertalot and *P. subgastriformis* (Hustedt) E.J. Cox (Table 2). The most characteristic difference between these two species is the presence a stigma in the central area, and that valves are relatively wider. The shape of the valve ends in *P. significans* is rostrate. In our species the ends are subcapitate. Also, these species can be easily distinguished by the structure of the central area. *P. baikaloelginensis* sp. nov. has a large, transversely-expanded or butterfly-shaped central area, occupying up to 3/4 width of valve axial area, whereas *P. significans* and *P. subgastriformis* have axial areas that are small and rounded. Another difference is the density of striae: in our species striae number 13–15 in 10 µm, but in *P. significans* they number 10–11 in 10 µm, and in *P. subgastriformis* they number 9–11 in 10 µm (Table 2).

*Placoneis subundulata* sp. nov. is most closely related to *P. hambergii* AT_160Gel09 and *P. clementis* FD419. The new species can be easily distinguished from these two species by the shape of the valve. *P. subundulata* sp. nov. cells are linear-elliptical with clearly drawn, beak-shaped ends, while in *P. hambergii* AT_160Gel09 and *P. clementis* FD419 valves are an elliptical-lanceolate in shape.

The valve shape of *P. subundulata* sp. nov. is linear-elliptical, similar to species such as *P. elginensis*, *P. paraelginensis*, *P. ignorata* (some specimens with linear valves), and *P. cattiensis* (Table 3). However, the valve margin of *P. subundulata* sp. nov. is slightly wavy. This feature easily distinguishes the new species from the above taxa.

**Table 2.** Comparison of *Placoneis baikaloelginensis* sp. nov. with similar species.

| | ***P. baikaloelginensis* sp. nov.** | ***P. abiskoensis*** | ***P. elginensis*** | ***P. paraelginensis*** | ***P. significans*** | ***P. subgastriformis* sp. nov.** |
|---|---|---|---|---|---|---|
| **Outline** | Linear-elliptical | Linear | Linear | Linear | Linear-elliptical to elliptic-lanceolate | Lanceolate -elliptical |
| **Apex shape** | Subcapitate | Rostrate to subcapitate | Subcapitate | Subcapitate | Rostrate, more or less protected, obtusely rounded | Subcapitate |
| **Apex width (μm)** | 4.9–5 | 5–6 * | 4–4.5 | 3–4 * | 3.9–4 * | 3.8–4.3 |
| **Axial area** | Narrow, slightly widening to the middle of the valve | Narrow, slightly widening to the middle of the valve | Narrow, linear, barely or not broadened towards the center | Narrow, linear | Narrow, linear | Narrow, linear |
| **Central area** | Large, transversely-expanded or butterfly-shaped to 3/4 width of valve | Transversely-expanded, butterfly-shaped or rounded, confined by 3–4 shortened striae | Larger than half of the valve width, butterfly-shaped | Rounded, slightly transversely-expanded, confined by 3–4 shortened striae | Small, with one stigma | Rounded, with one stigma |
| **Valve length (μm)** | 12.2–31.6 | 38–47 | 30–36 | 20–30 | 20–30 | 26–33 |
| **Valve breadth (μm)** | 7.8–9 | 9–11 | 8.2–10 | 6.5–8 | 10–10.5 | 9–11 |
| **Striae type, number in 10 μm** | Uniseriate, radiate, becoming parallel to convergent at the valve ends, 13–15 | Uniseriate, radiate, 7.5–12 | Uniseriate, radiate (angle to the raphe: 74), with one pair perpendicular to raphe very close to the ends, 9–12.4 | Uniseriate, radiate, becoming subparallel to convergent at the valve ends, 12–18 | Uniseriate, radiate, 10–11 | Uniseriate, radiate, slightly curved 9–11 |
| **Areolae, number in 10 μm** | Not discernible in the LM, 30 | Distinct in the LM, 26–30 | Difficult to resolve in the LM, 22.4–28.9 | Difficult to resolve in the LM, 36* | Indiscernible in LM | Not discernible in the LM |
| **Distribution** | Baikal | | Holarctic | Holarctic | Lake "Schmaler Luzin" in North Germany; Ohrid and Prespa lakes, Shkodra (Albanian part) | Widespread species |
| **References** | This study | [33,36,42,43] | [36,44,45] | [20,33,36,44,46,47] | [5,46] | [33,47] |

* counted from published data.

The greatest similarity of the new species is observed with *P. undulata*, whose valve edges are also wavy. It should be noted that *P. subundulata* sp. nov and *P. undulata* are similar in shape of the valves, structure and size of the central area, and the radial arrangement of striae (Table 3). The differences are as follows: in *P. undulata* the valve margins are clearly triundulate; in *P. subundulata* sp. nov. valves are only slightly wavy. The two species also differ in the outline: in *P. undulata* the valves are elliptical, but in *P. subundulata* sp. nov. they are linear-elliptical. The most noticeable difference between these species is the density of striae: in *P. undulata* stria density is 12 in 10 μm, but in *Placoneis subundulata* sp. nov. the stria density is 14–15 in 10 μm. It should also be noted that the valves of the new species are larger (25.5–27.0 in length) than those of *P. undulata* (18.0–19.0 μm in length).

*Placoneis neohambergii* sp. nov. occupies a separate, independent position in the phylogenetic tree and it is not closely related to the strains of *P. hambergii* used for construction of phylogenetic tree (Figure 5). As evident from Figure 8A–H, the cells have all the features characteristic for the genus, the main ones being the structure of the chloroplast (Figure 8A–C) and the pore occlusions (Figure 9E,F).

In terms of morphometric parameters, *P. neohambergii* sp. nov. is most similar to species with elliptical-lanceolate valves, such as *P. hambergii*, *P. opportuna*, and to species with elliptical valves such as *P. witkowskii* and *P. ovilus* (Table 3).

*P. neohambergii* is most similar morphologically to *P. hambergii*, and they can be easily confused. The ranges of the sizes are very close: in *P. neohambergii* sp. nov. valve length is 17–19 μm, breadth is 7.5–8.0 μm, and width at the apex is 2.5–3.0 μm, while in *P. hambergii* these parameters vary within close ranges in terms of length 16.0–25.0 μm, breadth 6.0–8.0 μm and apex width 2.0–2.5 μm. These species have almost the same cell shape, but in *P. hambergii* they are relatively more narrowed towards the ends and from this the cells are more elongated, resembling a boat in shape. The cell shape of *P. neohambergii* sp. nov. can be characterized as elliptical with rostrate ends. In addition, the species differ in the density of striae and areolae: the new species has 12–14 striae in 10 μm with a very small angle of inclination and 35 areolae in 10 μm. In *P. hambergii*, the density of striae and areolae is higher (15–18 in 10 μm and 45 areolae in 10 μm, respectively). The orientation of the striae also differs: in the new species they are almost parallel, while in *P. hambergii* the striae are clearly radiate. Differences in the structure of the axial and central areas should also be noted: in *P. neohambergii* sp. nov. the axial area is narrow and linear and central area is small, irregularly-shaped and confined by 1–2 alternating longer and shorter striae, whereas in *P. hambergii* there is a lanceolate axial area, and the central area is not evident.

*Placones opportuna* (Hustedt) Chudaev & Gololobova cells are elliptical at maximum size, and elliptical-lanceolate when they decrease during the life cycle. And since the axial and central areas have the same structure as *P. neohambergii* sp. nov., small valves of *P. opportuna* can easily be confused with the new species. Distinguishing *P. neohambergii* sp. nov. from *P. opportuna* is achieved primarily by the presence of slightly drawn-out cuneate ends. In *P. opportuna*, the ends are not pronounced, but rather widely rounded. Another notable difference is the orientation and the density of striae: in *P. opportuna*, striae are clearly radiate and denser (15.1–16.6 (18) in 10 μm), whereas in the new species they are almost parallel, 12–14 in 10 μm. The results of studying these species under SEM indicate that the new species also has a smaller number of areolae (35 in 10 μm vs 42.7–45.7 in 10 μm in *P. opportuna*).

*Placoneis witkowskii* Metzeltin, Lange-Bertalot & García-Rodríguez was described in 2005 from Uruguay [48]. Largest cells of this species include elliptical cells with clearly cuneate ends; they clearly differ from the new species in valve shape. Valves in the lower size range have indistinct ends and are very similar to *P. neohambergii* sp. nov. Thus, smaller cells of *P. witkowskii* can be confused with the new species because they are close in valve shape, size, striae density, structure of the central and axial area (Table 1). The difference between these species is the orientation of the striae. In the new species, the striae are located almost parallel, whereas in *P. witkowskii* they are clearly radiate, in the center area the angle of inclination is 40–45°.

**Table 3.** Comparison of *Placoneis subundulata* sp. nov. and *Placoneis neohambergii* sp. nov. with similar species.

| | ***P. subundulata* sp. nov.** | ***P. undulata*** | ***P. cattiensis*** | ***P. ignorata*** | ***P. neohambergii* sp. nov.** | ***P. hambergii*** | ***P. opportuna*** | ***P. witkowskii*** | ***P. ovilus*** |
|---|---|---|---|---|---|---|---|---|---|
| **Outline** | Linear-elliptical, with slightly triundulate valve margins | Elliptic-undulate | Linear-elliptical | Linear-elliptical to elliptic-lanceolate | elliptical- lanceolate | Elliptic-lanceolate | Elliptic-lanceolate to elliptic | Elliptic | Strictly elliptic |
| **Apex shape** | Rostrate to subcapitate | Rostrate | subcapitate | Rostrate to broadly rounded | barely protracted broadly rounded | Slightly or distinct rostrate | Broadly rounded | Broadly protracted subrostrate | Slightly cuneate |
| **Apex width (µm)** | 3–3.5 | 2.5 | 2.5–3 | 2.8–3 * | 2–3 | 2–2.5 | - | - | - |
| **Axial area** | Narrow, linear | Narrow, slightly widening to the middle of the valve | Narrow, slightly widening to the middle of the valve | Narrow, linear | Narrow, linear | Linear—lanceolate | Narrow, linear | Narrow, linear | Narrow, slightly expanded towards the center |
| **Central area** | Transversely-expanded, asymmetrical, rounded or butterfly-shaped confined by 3–4 shortened striae from 1/4 to 1/2 width of valve | Transverse elliptic, confined by 3–4 shortened striae to 1/2 width of valve | Large, transverse, rarely asymmetrical, from 1/2 to 2/3 width of valve | Transversely-expanded, rectangular, confined by 2 shortened striae | Small, rounded or not distinct, confined one or two shorter striae | Not expressed, confined by two shortened striae | Small, weakly expressed, confined by shortened striae | Small, ill-defined in outline by single longer stria in the middle and two shorter striae on either side | Small, irregularly confined by a few alternating longer and shorter striae |
| **Valve length (µm)** | 25.5–27 | 18–19 | 24–25 | 12–25 | 17–19 | 16–25 | 7.9–14.4 (20) | 14–24 | 18–23 |
| **Valve breadth (µm)** | 7.3–8 | About 7 | 6.7–7.4 | 7–8 | 7.5–8 | 6–8 | 5.4–7.3 (8) | 8–10 | 8.6–9.3 |
| **Striae type, number in 10 µm** | Uniseriate, radiate becoming subparallel in the ends, 14–15 | Uniseriate, radiate, 12 | Uniseriate, radiate becoming subparallel in the ends, 12–14 | Uniseriate, radiate, 11–14 | Uniseriate, slightly radiate or subparallel, 12–14 | Uniseriate, radiate, 15–18 | Uniseriate, radiate, 15.1–16.6 (18) | Uniseriate, radiate throughout, 12–15 | Uniseriate, radiate throughout and somewhat curved, 12–13.5 |
| **Areolae, number in 10 µm** | Indiscernible in LM, 35 | Indiscernible in LM | Not discernible in the LM, 45–50 | Not discernible in the LM | Indiscernible in LM, 35 | Indiscernible in LM, 45 * | Indiscernible in LM, 40–42.7 (45.7) | Not to discern in the LM, (mach more 30) | Discernible in the LM, 27 |
| **Distribution** | Indonesia | North Tirol, Austria | Vietnam | Widespread species | Vietnam | Holarctic | Widespread species | Laguna Blanca, Department of Maldonado, Uruguay | Arroyo Sause, Arroyo del Aiguá, Department of Maldonado, Uruguay |
| **References** | This study | [5,20,36,49] | [50] | [20,33,36,42,44] | This study | [10,33,48] | [33,45] | [48] | [48] |

* counted from published data.

*Placoneis ovilus* Metzeltin Lange-Bertalot & García-Rodríguez was also described from Uruguay [48], and it is similar to *P. neohambergii* sp. nov. in terms of valve outline [48] (p. 393, Figures 22 and 23), stria density and size range (in *P. neohambergii* sp. nov. valve length—17–19 µm, breadth 7.5–8.0 µm, in *P. ovilus* valve length—18–23 µm, breadth 8.6–9.3 µm). However, in the new species, valves have slightly extended rostrate ends (Figure 6F–J) while in *P. ovilus* the apices are not protracted, the cells are elliptical, and the ends are simply narrowed ([48] p. 393, Figures 20–24) or broadly rounded [48] (p. 393, Figures 25 and 26). The structure of the axial area is different between the two as well: in *P. neohambergii* sp. nov. the axial area is narrow and linear while in *P. ovilus* the axial area is slightly expanded towards the center. The orientation of the striae is also different: in the new species, the striae are almost parallel but in *P. ovilus* they are radiate throughout and somewhat curved. These species also differ in the density of the areolae. In *P. neohambergii* sp. nov. areolae are indiscernible in LM (35 in 10 µm), whereas in *P. ovilus* areolae are discernible in LM (27 in 10 µm).

Our molecular investigation shows that *Placoneis* as a genus is monophyletic and is not paraphyletic as was discussed previously. Strains of *Placoneis* comprise an independent branch separate from *Geissleria* strains and *Paraplaconeis*. Our results also show that *Paraplaconeis* is a genus that phylogenetically independent from closely-related taxa. However, this genus is yet uniformly recognized [51]. Another very interesting result of our molecular study and morphological comparison of *P. elginensis* vouchers is that, despite slight differences in morphometric parameters (with the exception of the UTEX FD 416 voucher), phylogenetic analysis clearly separates these strains into different taxa. This situation is probably not unique. We need to start analyzing more strains of the same species from different parts of the world in order to assess whether morphologically similar populations are mysterious or perhaps pseudo-cryptic taxa, according to Mann [52]. The taxonomic instability of *P. elginensis* described here is a good example that we need more thorough taxonomic work. This work is important not only for the taxonomy of diatoms, but also for the use of molecular markers and identification for barcoding and assessing water quality (see [40,41]).

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