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Dedicated to the late Professor Jadwiga Siemińska

Morphology and molecular phylogeny of *Gomphonemopsis sieminskae* sp. nov. isolated from brackish waters of the East China Sea coast

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Abstract. We describe the new species *Gomphonemopsis sieminskae* from brackish waters of the East China Sea littoral near Ningbo, China. Two diatom strains isolated from *Ulva* sp. were successfully grown, then analyzed by light (LM) and scanning electron (SEM) microscopy. The new species is compared to known *Gomphonemopsis* species, and similarities to *G. pseudoexigua* and *G. obscurum* are emphasized. Although the size metric data overlap and the external views are fairly similar, the two taxa differ in their valve interior. Molecular barcoding strongly discriminated *G. sieminskae* from *G.* cf. *exigua*, while *rbc*L gene-based phylogeny showed *G. sieminskae* to be sister to *Phaeodactylum tricornutum*. In this respect our results confirm the recent discovery, based on molecular data, that *Gomphonemopsis* is a close-relative taxon to *Phaeodactylum*, and place it in *Phaeodactylaceae* instead of *Rhoicospheniaceae* as inferred solely from morphology.

Key words: East China Sea, littoral zone, *Bacillariophyta*, new species, *Gomphonemopsis*, morphology, phylogeny

Introduction

Gomphonemopsis was split off as a genus from *Gomphonema* s.l. based on morphology and the marine to brackish-water habitat of the new genus versus the terrestrial freshwater of the latter (Medlin & Round 1986). *Gomphonemopsis* is a small genus, with three species transferred by Medlin and Round (1986). Medlin and Round (1986) made new combinations for *G. exigua*, *G. littoralis* and *G. pseudexigua*. Later, Guslakov et al. (1992) transferred *Gomphonema domniciae* to the genus *Gomphonemopsis*; simirarly Lange-Betalot et al. (1996) transferred *G. obscura*, and Snoeijs and Balashova (1998) transferred *G. exigua* var. *platypus*. In addition two new species including *G. lindae* (Metzeltin & Witkowski 1996) and *G. ligowskii* (Al-Handal et al. 2018) have been described in this genus. A recent paper by Sabir et al. (2018) provides a microscopic characterization of a *Gomphonemopsis* strain identified as *G.* cf. *exigua*, which together with our results suggests that many new species may await discovery in this genus.

Round et al. (1990) classified *Gomphonemopsis* in *Cymbellales* and in the family *Rhoicospheniaceae*. This classification is followed by researchers including Kociolek et al. (2019). *Gomphonemopsis* was placed in *Rhoicospheniaceae* (Chen & Zhu 1983) along with several other genera such as *Rhoicosphenia*, *Campylopyxis*, *Cuneolus* and *Gomphoseptatum*. The only character that is shared in the whole family is perforated girdle bands, indeed quite weakly developed in some *Gomphonemopsis* species. Frustules of *Rhoicosphenia*, *Cuneolus*, *Campylopyxis* and *Gomphoseptatum* are septate, while *Gomphonemopsis* do not possess this character (Medlin 1985, Medlin & Round 1986). The morphology of *Gomphonemopsis* also differs from the rest of the *Rhoicospheniaceae* in terms of raphe sternum ultrastructure. *Rhoicosphenia* and *Campylopyxis* are heterovalvar genera. In *Cuneolus* the raphe has strongly bent apical ends, as also in *Gomphoseptatum*, though the striation in the latter genus resembles that observed in *Gomphonemopsis*. Unlike the remaining genera, the frustules in *Gomphonemopsis* are isovalvar and the raphe branches terminate somewhat below the apices in simple, slightly expanded ends.

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The differences outlined above between *Gomphonemopsis* and *Rhoicospeniaceae* have recently been confirmed in research on *Gomphonemopsis* using molecular markers (Sabir et al. 2018; this study), placing this genus in the family *Phaeodactylaceae* (Silva 1962). As revealed by molecular phylogeny, *Gomphonemopsis* is the closest known relative of *Phaeodactylum tricornutum*, one of the model diatoms (Vartanian et al. 2009) on which complete genome sequencing has been performed (Bowler et al. 2008). However, in light of molecular phylogeny, it is even more difficult to point out the characters shared between *Gomphonemopsis* and *Phaeodactylum*.

This paper presents morphological and molecular data on new species of *Gomphonemopsis*, described based on monoclonal cultures isolated from unidentified *Ulva* sp. from brackish waters of the East China Sea near Ningbo. The new species is compared to published sources in terms of morphology and molecular phylogeny, and its similarities to known species are discussed. With this new species we increase the number of taxa in *Gomphonemopsis*, but it still holds less than ten taxa.

Materials and methods

Samples were collected in the deepwater harbor of Xiangshan (29°30′12″N, 121°35′25″E), located on the northern coast of Zhejiang Province (Figure 1). Xiangshan Harbor is surrounded by four administrative districts: Yinzhou, Fenghua, Ninghai and Xiangshan counties. The port is next to the Zhoushan Sea; these areas are separated by the Fodu Channel. Xiangshan Harbor is a semi-closed bay with a strongly developed aquaculture industry (Zhang & Xie 2014).

Sampling

Sampling was carried out in December 2016. Samples were collected from various substrates: sand, macroalgae, small pieces of rocks, and the water column (with a plankton net). The strains on which the description of the new species is based were isolated from a sample containing an unidentified seaweed (*Ulva* sp.) collected from ropes in an aquaculture pond. The samples were collected in plastic tubes with added seawater and some space left for air, then the containers were sealed with Parafilm® for protection and labeled. The samples were stored under natural light at room temperature (~25°C) and then transported to Poland.

Isolation, culturing and microscopy

At the Szczecin Diatom Culture Collection (SZCZ, University of Szczecin) the samples were transferred to Petri dishes and enriched with artificial culture medium f/2 amounting to 15 PSU (Guillard 1975). The salinity of the medium was estimated based on published environmental

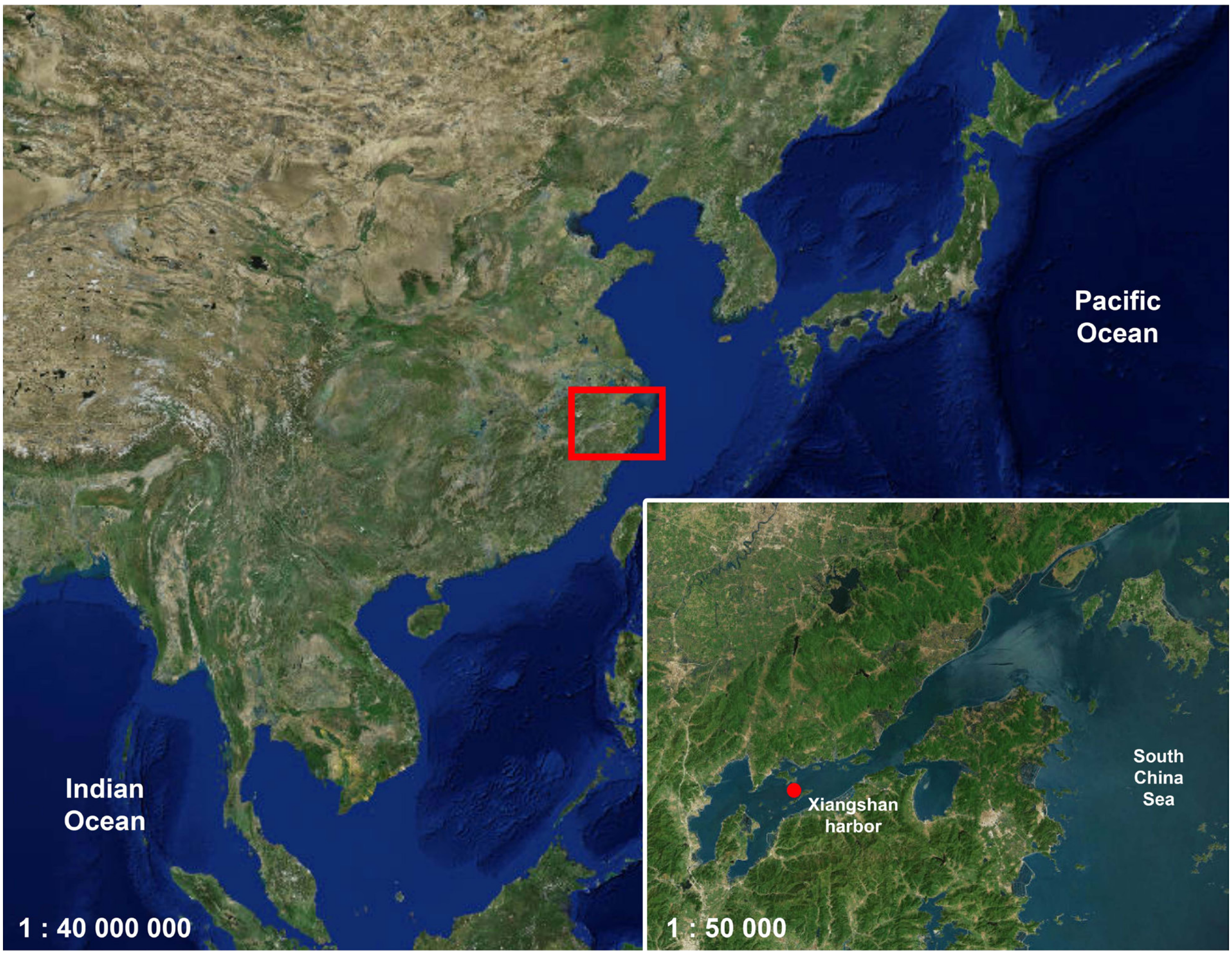

Figure 1. Map showing the location of the study area.

data (Zhang & Xie 2014). After two weeks, single diatom cells were isolated using a Pasteur glass micropipette. Isolation was done using capillary tube technique (Andersen & Kawachi 2005) under an inverted Nikon Eclipse light microscope. Living cultures were stored in a growth chamber at 20°C under a 12 h photoperiod (white light, ~100 μmol photons m^{-2} s^{-1}). After about four weeks the monoclonal cultures were transferred to Erlenmeyer flasks (100 ml) for biomass upscaling. To prepare LM slides and for SEM observation, small amounts of biomass were transferred to 50 ml beakers and 10 ml 30% H_2O_2 was added, then the samples were boiled until the organic matter was mineralized. Cleaned samples were rinsed with deionized water to reach neutral pH. The samples were pipetted onto coverslips and mounted on slides with Naphrax. Slides with the new cleaned frustules and valves are stored at SZCZ.

DNA extraction, molecular barcoding and phylogeny

Total DNA was extracted following Doyle and Doyle (1990). The biomass of cultures in exponential growth phase was separated from the supernatant by gentle centrifugation. The complete *rbc*L gene was retrieved from an ongoing work on the organellar genomes of *G. sieminskae*. A total of 60 million paired-end reads was obtained from a BGISEQ-500 platform by the Beijing Genomics Institute (Shenzhen, China). Data were assembled using SPAdes 3.12.0 (Bankevich et al. 2012). The contigs corresponding to the plastidic genome were retrieved by customized stand-alone BLAST searches (McGinnis & Madden 2004). The complete *rbc*L gene was extracted with the help of ORFfinder software from NCBI (Rombel et al. 2002) and deposited in GenBank under accession number MK689241. This sequence was aligned using Clustal Omega (Sievers et al. 2011) with *rbc*L from various *Cymbellales* species, and also from *Luticola ventricosa* and *Phaeodactylum tricornutum*. Sequences were then trimmed to ~1350 bp final length. The alignment was used for maximum likelihood phylogeny, which was conducted with MEGA X (Kumar et al. 2018), using the GTR+I+G model and 1000 bootstrap replications.

Results

Taxonomy

Gomphonemopsis sieminskae Krzywda, Gastineau, Ch. Zhou & Witkowski, sp. nov. (Fig. 2)

Diagnosis: Frustules wedge-shaped in girdle view, with rounded corners, with single chloroplast. Valves heteropolar with footpole slightly narrower and acutely rounded, and head pole broader and obtusely rounded, 9–18 μm in length and 2.0–2.5 μm in width (n = 30). Axial area narrow, linear, expanding in the middle into the rectangular central area. Raphe filiform, external proximal endings distinct, somewhat expanded, apical endings in LM barely resolvable. Transapical striae composed of single areola, in the middle slightly radiate, becoming parallel and finally slightly convergent at apices, 18–22 in 10 μm.

Holotype: slide no. SZCZ1724 deposited in A. Witkowski Diatom Culture Collection, Faculty of Geosciences, University of Szczecin.

Iconotype: Fig. 2C'.

Type locality: on specimens of unidentified *Ulva* sp. from the coast of the East China Sea in the Xiangshan deepwater harbor, 29°30′12″N, 121°35′25″E, leg. M. Krzywda & Ch. Zhou, 9 Dec. 2016.

SEM description. External view. Valve face flat, abruptly bent towards mantle. Transition from valve face to mantle consists of hyaline silica strip. Axial area distinct, slightly widening from apices towards central area. Central area butterfly-shaped, originating through the absence of the middle stria pair. Central area expanded up to valve margin. Raphe filiform, composed of two slightly undulating branches. In broken specimen, raphe slit shaped like "less than" symbol (<). Both proximal and distal external raphe ends slightly expanded into tear-like shape. Proximal raphe ends distant from each other, slightly bent towards valve primary side, apical ends straight, terminating well below valve apex, surrounded by well-developed terminal nodule (Fig. 2C', D'). Valve mantle relatively deep and steep, perforated with single row of elongate to circular areola. Valve mantle areola size decreases from apex towards foot pole (Fig. 2E'). Transapical striae composed of solitary, relatively large areola changing the orientation along the raphe branches from slightly radiate close to central area, to parallel, and finally to convergent close to apices. At footpole, two small isolated pores observed between regular striae forming areolae. In some specimens, small solitary pores observed. Girdle composed of a few copulae. Copulae usually ligulate but plain; however, in both strains some copulae with a single row of indistinct pores were observed; and in a few cases in strain 1724, girdle bands with a double row of pores (43 in 10 μm) were observed (Fig. 2H').

Internal view. Valve interior flat, with raphe positioned within somewhat elevated sternum. Internal proximal raphe ends closer than external ones, but likewise slightly expanded in tear-like shape. Internal distal raphe ends terminate in small and simple helictoglossae. Striae forming areolae depressed between viminae. Ultrastructure of areolae occlusions not observed in our study. Solid silica transition from valve face to mantle appears internally flat. At footpole, 1–2 isolated pores present at either one or both sides of helictoglossa, whereas at headpole such pores were not observed (Fig. 2F', G').

Etymology. This species is dedicated to the memory of the late Professor Jadwiga Siemińska, our teacher and friend.

Taxonomic comments

Gomphonemopsis sieminskae shows superficial similarity to *G. pseudexigua*, as the size ranges of the two species partly overlap (Table 1). In both species, stria density is the same, 18–22 in 10 μm. The two species have a single row of areolae on the valve mantle. However, the middle areola in *G. pseudexigua* is smaller than the remaining valve mantle areolae, whereas in the newly described species all valve mantle areolae are of the same size. The major difference between the species is the presence of the internal

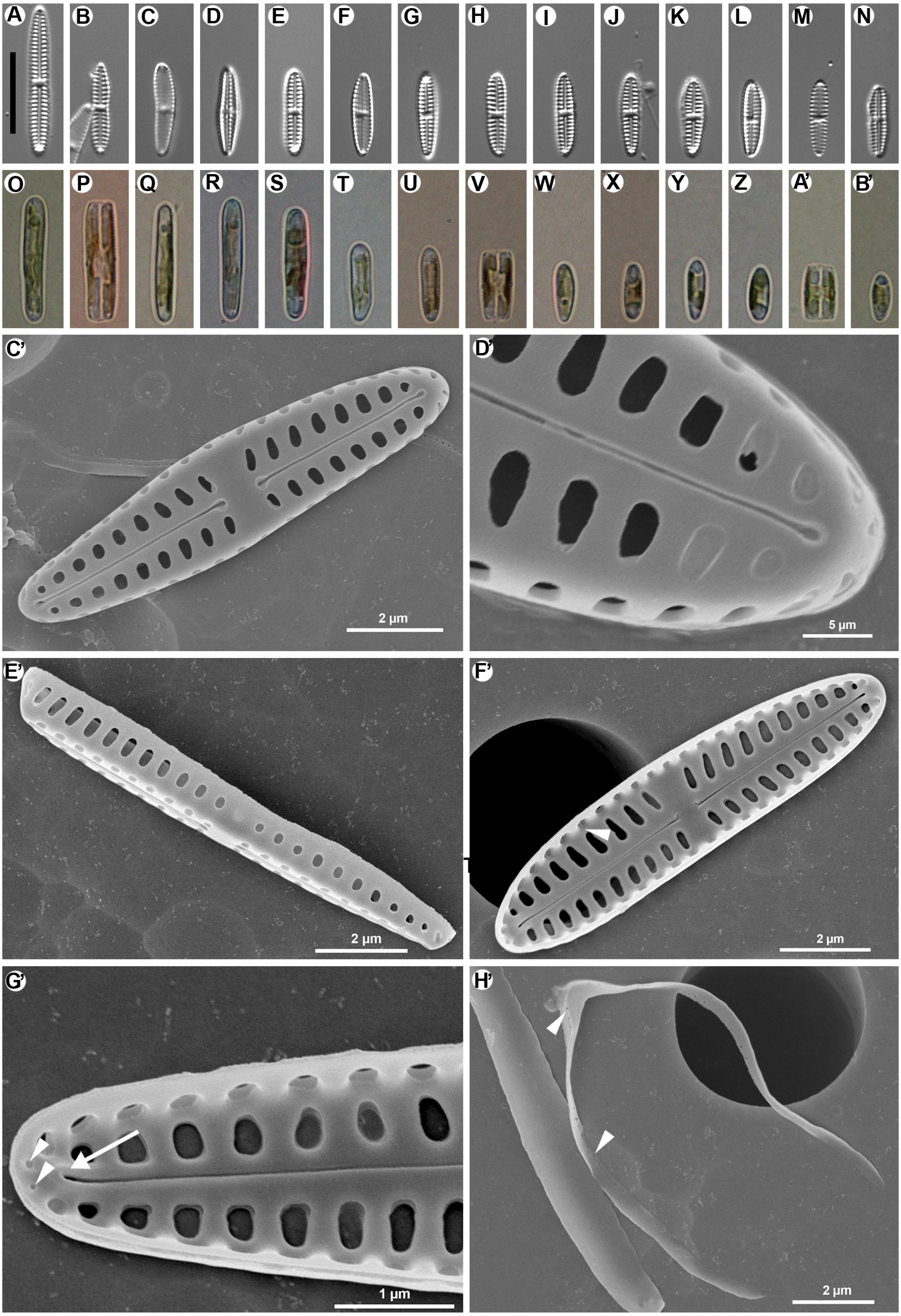

Figure 2. *Gomphonemopsis sieminskae* (clone SZCZM1724). A–N – light micrographs, showing morphology of cleaned valves from culture; O–B' – light micrographs, showing plastids in living cells from culture; C'–H' – scanning electron micrographs; C' – external view with flat valve face, arrowhead marks Voigt discordance, note the abrupt transition towards the mantle, and the proximal external raphe ends bent towards the valve primary side (arrows); D' – close-up of apical part of specimen (illustrated in Fig. C'); E' – girdle view, showing valve mantle perforated with single row of elongate to circular areolae; F' – valve internal view with raphe positioned within somewhat elevated sternum, note the siliceous strip dividing the striae into two parts (arrowhead); G' – close-up of footpole apex internal view, with apical raphe end terminating in a small, simple helictoglossa (arrow), note the presence of two isolated pores (arrowheads); H' – complete copula with single row of indistinct pores marked by arrowheads. Scales: A–B' = 10 µm.

siliceous strip dividing the transapical striae into two parts in *G. sieminskae*, and the absence of such a character in *G. pseudexigua* (Medlin & Round 1986; figs 49, 51).

Molecular phylogeny

The employed method easily produced a complete 1470 bp *rbc*L gene. Megablast analyses already suggested a close relationship between *Gomphonemopsis* and *Phaeodactylum tricornutum*, which was later clearly evident in the ML phylogeny. As illustrated in Figure 3, the two genera appeared as sister clades, with strong node values (>90), and both were rejected from the larger upper-clade encompassing *Cymbellales*. Also, the identity between the *rbc*L partial sequences of *G. sieminskae* and *Gomphonemopsis* cf. *exigua* strain SA18 (GenBank accession number MH064098.1) was only 91.27% after trimming and as calculated by online application of Clustal Omega.

Discussion

The newly described species *Gomphonemopsis sieminskae* was isolated from brackish waters of the East China Sea. The two strains maintained in culture apparently belonged to an epiphytic assemblage on seaweed identified only to generic level as *Ulva* sp. This is consistent with the general habitat characteristics for taxa in *Gomphonemopsis*, as underlined by Sabir et al. (2018). Based on morphology data acquired by LM and SEM examination, we describe it as a species new to science and name it *G. sieminskae*. Taking into consideration the unresolved name *G.* cf. *exigua* discussed by Sabir et al. (2018), our taxon is the ninth one within the genus, and the second one with molecular data and phylogeny.

Morphologically, and particularly in valve external view, *Gomphonemopsis sieminskae* resembles *G. pseudexigua*. Both species have a distinct central area which developed due to the absence of the middle stria at both sides of the central nodule. Likewise, they possess a striated valve mantle. There is a subtle difference between the two species in the development of the mantle striae: in *G. sieminskae*, all areolae are of similar size, whereas in *G. pseudexigua* the middle stria is smaller than the remaining ones positioned on the mantle. In addition, the two species show a significant overlap in size and stria density data (see species description). The major difference between the two taxa is observed in the valve interior. That of *G. sieminskae* shows a distinct apically oriented siliceous rim which separates the transapical striae into two areola, one on the valve face and one on the mantle. Such a siliceous rim is missing in *G. pseudexigua*, and the transapical striae are internally composed of a single areola. The two species also have similar salinity requirements: both occur in brackish waters, with the former species tolerant of salinities below 7 psu (Simonsen 1959; Hinz et al. 2012). The water salinity of the type habitat of *G. sieminskae* was 15 psu.

Krasske (1939) described *Gomphonema obscurum* from marine waters in Chile. This species was transferred in *Gomphonemopsis* as *G. obscura* (Lange-Bertalot et al. 1996). The Krasske species is well documented in LM, but SEM illustrations based on an examination of original material are unavailable. Perhaps this is why *G. obscura* is rarely identified in floristic and palaeoceanographic studies (e.g. Witoń & Witkowski 2006). In light of the missing SEM images of *G. obscura*, a comparison of our species with the latter one must be based on LM images and size measurement data. The major difference between the two taxa is lower stria density in *G. obscura* (16 in 10 μm) as compared to the new taxon (18–22 in 10 μm). The valves of *G. obscura* also have a somewhat larger width range (2–3 μm) than *G. sieminskae* (2.0–2.5 μm) (Table 1).

Gomphonemopsis sieminskae show morphological similarity to *G. domniciae* (Guslyakov et al. 1992, p. 82, Fig. CXX: 5). There is a size measurement data overlap between our species and *G. domniciae*. In general, *G. domniciae* has shorter and narrower valves, with more robust striae, than our species. Valve length in the former is 6–12 (6–8 μm in original description; Guslyakov 1981, p. 802), versus 9–18 in our species. The width of *G. domniciae* (2–3 μm, although given as 1.7–2.5 μm in the original diagnosis) is somewhat larger than in *G. sieminskae* (2.0–2.5 μm). Finally, the striae are somewhat coarser in *G. domniciae* and range from 10 to 16 in 10 μm (in original description given as 10–18 in 10 μm) (Table 1). Stria density is higher in *G. sieminskae* (18–22 in 10 μm). The type habitat of *Gomphonemopsis domniciae* is the Black Sea in Odessa Bay, with brackish-water conditions (16.5 psu), where it was observed in samples from seaweed and on stones, and in the Caspian Sea (Guslyakov 1981; Guslyakov et al. 1992). *Gomphonemopsis* species strongly

Table 1. Valve dimensions and stria density of *Gomphonemopsis* taxa, according to literature and authors' measurements.

Taxa	Lenght (μm)	Width (μm)	Striae/10 (μm)	References
G. domniciae (Guslakov) Guslakov	6–8	1.7–2.5	10–18	Guslyakov 1981, Guslyakov et al. 1992
G. littoralis (Hendey) Medlin	14–22	2–3	16–19	Medlin & Round 1986
G. pseudoexigua (Simonsen) Medlin	3.5–15.0	1.5–2.5	18–22	Medlin & Round 1986
G. exigua (Kützing) Medlin	9–34	2–6	16–30	Medlin & Round 1986
G. lindae Witkowski, Metzeltin & Lange-Bert.	16.0–18.5	2.5–3.0	18–24	Metzeltin & Witkowski 1996
G. ligowskii Al Handal & Thomas	11–17	1.5–2.5	11–14	Al-Handal et al. 2018
G. obscura (Krasske) Lange-Bert.	10–17	2–3	16	Lange-Bertalot et al. 1996
G. exigua var. *platypus* (Østrup) Snoeijs	9.5–24.0	3.0–4.5	17.5–21.0	Snoeijs & Balashova 1998
G. sieminskae Krzywda, Gastineau, Ch. Zhou & Witkowski	9–18	2.0–2.5	18–22	This paper

Figure 3. *Rbc*L-based ML tree. New taxon *G. sieminskae* bolded for clarity.

resembling *G. domniciae* in terms of coarser stria density have also been observed in material from the Puck Bay on the southern Baltic Sea (Witkowski unpubl.).

Unfortunately, TEM images of *G. domniciae* (Guslyakov 1981) do not illustrate the girdle, although in the species description it is stated that central area expands onto the mantle. However, the specimen illustrated in Gulsyakov et al. 1992 (Fig. XCC: 5) shows the presence of valve mantle areolae, but the quality of the image in the referenced plate is not good enough to determine whether the central area continues over the valve mantle.

The newly described species shows a certain degree of similarity to *G. littoralis*. In general, the size measurements of *G. littoralis* are similar to those of our species, with a slight overlap in length (14–22 µm vs 9–18 µm, respectively) and in stria density (16–19 in 10 µm vs 18–22 in 10 µm, respectively) (Table 1). Valve width overlaps in the two species, in *G. littoralis* ranging from 2 to 3 µm, and in *G. sieminskae* ranging from 2.0 to 2.5 µm (Table 1). The two species have similar raphe sternum systems, with filiform raphe branches terminating below the apices, a narrow axial area and a large central area. In *G. sieminskae*, however, the central area is limited in its extent only to the valve face, while in *G. littoralis* it extends also on the valve mantle (Medlin & Round 1986, fig. 51–53). Unlike *G. sieminskae*, which is a brackish-water form, *G. littoralis* inhabits fully marine and oceanic coasts (Hendey, 1977; Witkowski et al. 2000).

Molecular phylogeny

The ML phylogeny clearly associated *Gomphonemopsis* spp. with *Phaeodactylum tricornutum*, confirming the results of Sabir et al. (2018). The localization of *Luticola ventricosa* as sister to the *Gomphonemopsis* + *Phaeodactylum* cluster was less obvious in our tree than in Sabir et al. (2018). While our tree is *rbc*L-based, Sabir et al. (2018) reconstructed the phylogeny of *Gomphonemopsis* and *Phaeodactylum* using a concatenated SSU-*psb*C-*rbc*L dataset; in our opinion, this explains the differences between these tree topologies. In the near future, after completion of an ongoing project on the whole organellar sequencing of *G. sieminskae*, we intend to use the gathered data to elaborate an accurate multigene phylogeny for this species.

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