

Transfer of the *Gomphoneis tetrastigmata* Species Complex and Related Taxa to the Genus *Gomphonella* (Bacillariophyceae)

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Abstract Since the *Gomphoneis tetrastigmata* species complex, which has four stigmata, including *G. okunoi* and *G. pseudookunoi* described by Tuji (2005), both from Japan, shares morphological characteristics with *Gomphonella olivacea* except in having four stigmata, and belong to the same clade in the phylogenetic trees based on *rbcL* and 18S rRNA. Thus, these taxa and related taxa are transferred to the genus *Gomphonella*.

Key words: *Gomphoneis*, *Gomphonella*, *Gomphonema*, new combination.

Introduction

Jahn *et al.* (2019) presented the phylogenetic position of a well-known taxon, *Gomphoneis olivacea* (Hornem.) P.A.Dawson ex R.Ross et P.A.Sims, and transferred it and related taxa to the genus *Gomphonella* Rabenh. 1853, because the clade with this taxon and related taxa is separated from the clades of the genera *Gomphonema* and *Gomphoneis*. The *Gomphoneis tetrastigmata* (Horik. et Okuno) Ohtsuka species complex (Tuji, 2005), especially *G. okunoi* Tuji, is common in Japanese rivers and lakes (Tuji, 1995; Watanabe *et al.*, 2005). The morphological characteristics of this species complex are very close to those of *Gomphonella olivacea* (Hornem.) Rabenh. except for having four stigmata. In this paper, I present the phylogenetic trees for *Gomphoneis okunoi* with *Gomphonella olivacea*, *Gomphoneis* taxa, and *Gomphonema* taxa, and discuss the taxonomical treatment of the *Gomphoneis tetrastigmata* species complex.

Materials and Methods

A field sample, TNS-AL-56464 housed in TNS (Department of Botany, National Museum of Nature and Science), was collected from a rope in the lake water at Chikubu Island, Lake Biwa, on 19 March 2008 by A. Tuji. The strain Ak513 was isolated from this field sample using agar plates [1% agar with d medium: modified WC medium described by Tuji (2000)]. This strain died. DNA was extracted using this strain by the cetyl trimethylammonium bromide (CTAB) method with glass beads, followed by polymerase chain reaction amplification (PCR) and phylogenetic reconstruction (Tuji *et al.*, 2014). The obtained sequences were deposited to the genebank (LC530727 for rRNA and LC530728 for *rbcL*).

For morphological examinations, the following field samples were examined.

1. Material numbered TNS-AL-54241c in TNS, collected from the Arakawa River by A. Tuji on 25 November, 2004. This is an isotype for *Gomphoneis okunoi* described in Tuji (2005).

2. Material numbered TNS-AL-TW-1331c from T. Watanabe collection in TNS collected

from Tikubu-shima, Lake Biwa (Sample No: C2), by T. Watanabe on 4 July, 1988. This is an isotype for *Gomphoneis pseudookunoi* Tuji described in Tuji (2005).

3. Material numbered TNS-AL-TW-6274c from T. Watanabe collection in TNS collected from the northern part of Minae, Lake Shikotsu, by T. Watanabe on 24 June, 1990. This sample was examined for *Gomphoneis tetrastigmata* in Tuji (2005).

4. Material numbered TNS-AL-TW-11527c T. Watanabe collection in TNS collected from the Seine River, France, by T. Watanabe on 20 June, 1992. *Gomphonella olivaceum* is common in this sample.

Results and Discussion

Phylogeny

The phylogenetic trees of *Gomphoneis okunoi* and related taxa determined using *rbcL* and 18S rRNA are presented in Figs. 1 and 2. *G. okunoi* (strain Ak513) is positioned at the anchor of the genus *Gomphonella* clade and separated from the clades of other genera including *Gomphonema* and *Gomphoneis*. *Gomphoneis okunoi* and the *Gomphonella* clade are monophyly, and no other genus is positioned. The supporting values between *Gomphoneis okunoi* and genus *Gomphonella* were 93 for *rbcL* and under 70 for 18S rRNA using maximum likelihood analysis. The low supporting values might be caused by the relatively short sequences of genus *Gomphonella* deposited by Jahn *et al.* (2019). The molecular information in Jahn *et al.* (2019) clarified that the *Gomphonella* taxa belong to the Cymbellales but not to the Gomphonemataceae. *Gomphoneis okunoi* should also belong to the Cymbellales, and should not belong to the genus *Gomphonema* or *Gomphoneis*, both of which are members of Gomphonemataceae.

Morphological analysis

The *Gomphoneis tetrastigmata* species complex, including *G. okunoi* and *G. pseudookunoi*, has an outline very close to that of *Gomphonella*

olivacea. This species complex and *Gomphonella olivacea* share the following features. The valves are wedge-shaped in valve view and symmetric to the apical axis. Pseudoseptae are present (Fig. 3). A longitudinal line is not present. The striae at the central area are reduced in length and make a butterfly-like central area (Figs. 4, 5, 7). The striae are biseriate (Figs. 3–7). Striae in the headpole are always biseriate (Figs. 3, 6). The areolae are small and rounded and not occluded by siliceous flaps (Figs. 3–7). An apical pore field (composed of porelli) exists at the footpole and is bisected by a terminal fissure (Figs. 3, 6). The porelli make a single row at the footpole (Fig. 6). The important difference between this species complex and *Gomphonella olivacea*, is the existence of stigmata (Figs. 4, 7). These *Gomphoneis* taxa have four stigmata (Figs. 7 B–D), but *Gomphonella olivacea* does not have any stigmata (Fig. 7A). The lack of stigmata is an important characteristic in the emended description of genus *Gomphonella* by Jahn *et al.* (2019). The external openings of the stigmata are round and are positioned in the central area (Fig. 7). The internal openings of the stigmata of this species complex are complex like in the stigmata of genus *Cymbella* or *Didymosphenia* (Fig. 4); however, the internal openings of *G. tetrastigmatum* are sometimes not observed (Fig. 5), and/or they are sometimes observed only for some of the four stigmata (Fig. 4D). The genus *Gomphosinica*, as described in Kociolek *et al.* (2015), also shares many characteristics with *Gomphonella olivacea* and the *Gomphoneis* taxa with four stigmata, except that it has only one stigma.

Gomphonella or *Gomphoneis*?

The phylogenetic trees in this study show the monophyly of *Gomphoneis okunoi* and the genus *Gomphonella*, though the emended description of the genus *Gomphonella* by Jahn *et al.* (2019) clearly excludes the taxa with stigmata. The type species of genus *Gomphoneis* is *Gomphoneis elegans* Cleve. This species has many (over four) stigmata. However, no molecular information has

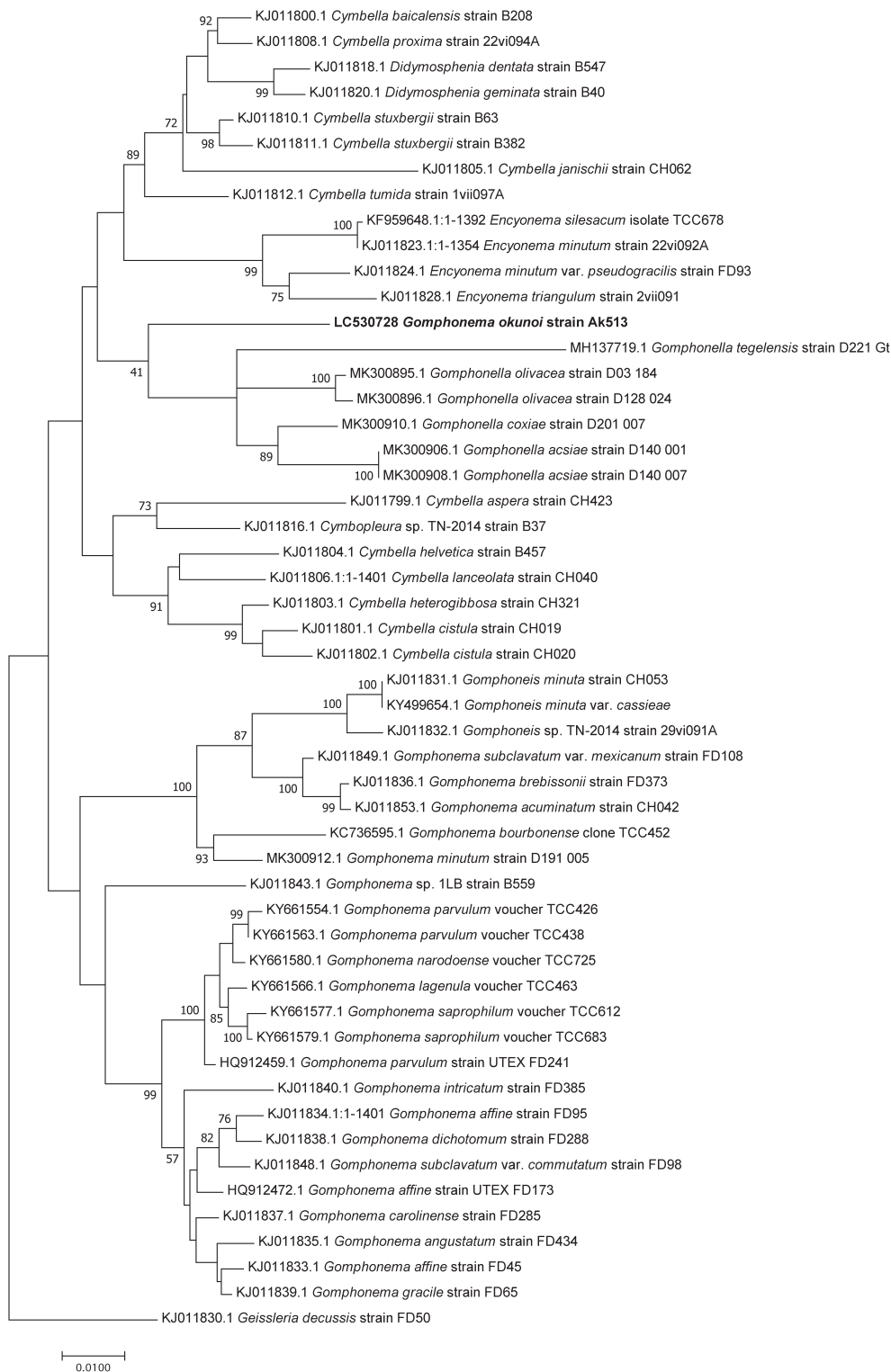


Fig. 1. Phylogenetic position of *Gomphoneis okunoi* and related taxa determined by maximum likelihood (ML) method from *rbcL*. Taxonomic names are followed by GenBank accession numbers. Numbers at branches indicate neighbor joining/ML bootstrap support values (only values higher than 70 are shown).

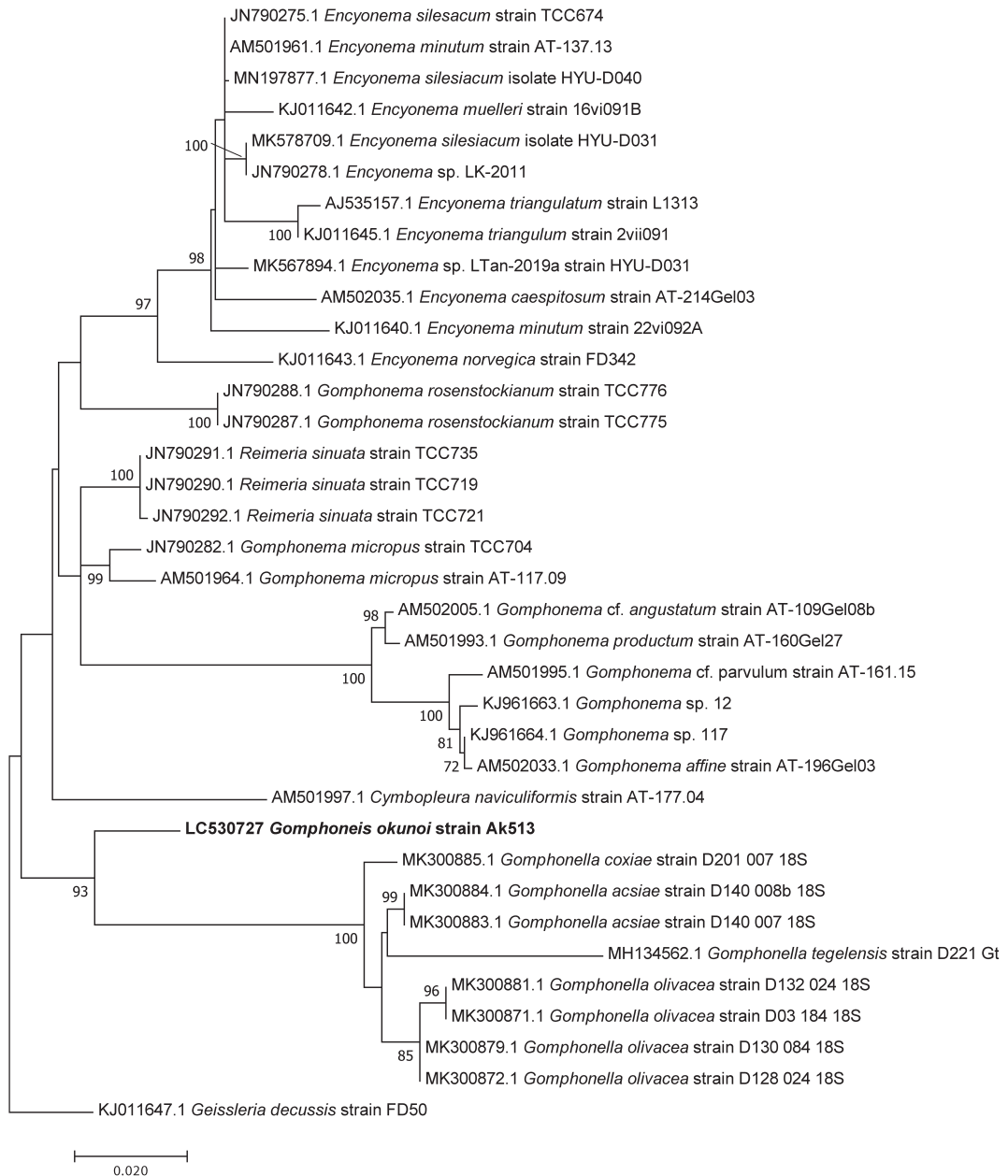


Fig. 2. Phylogenetic position of *Gomphoneis okunoii* and related taxa determined by maximum likelihood (ML) method from 18S rRNA. Taxonomic names are followed by GenBank accession numbers. Numbers at branches indicate neighbor joining/ML bootstrap support values (only values higher than 70 are shown).

been reported for this taxon. If *G. elegans* and the genus *Gomphonella* are monophyly, and should be regarded as the same genus, *Gomphonella* Rabenh. 1853 has priority over *Gomphoneis* Cleve 1894. Under the circumstances, it

is better to expand the definition of the genus *Gomphonella* to include the *Gomphoneis* taxa with four stigmata. The similarity of the ultrastructure between the *Gomphoneis tetrastigmatum* species complex and *Gomphonella olivacea*

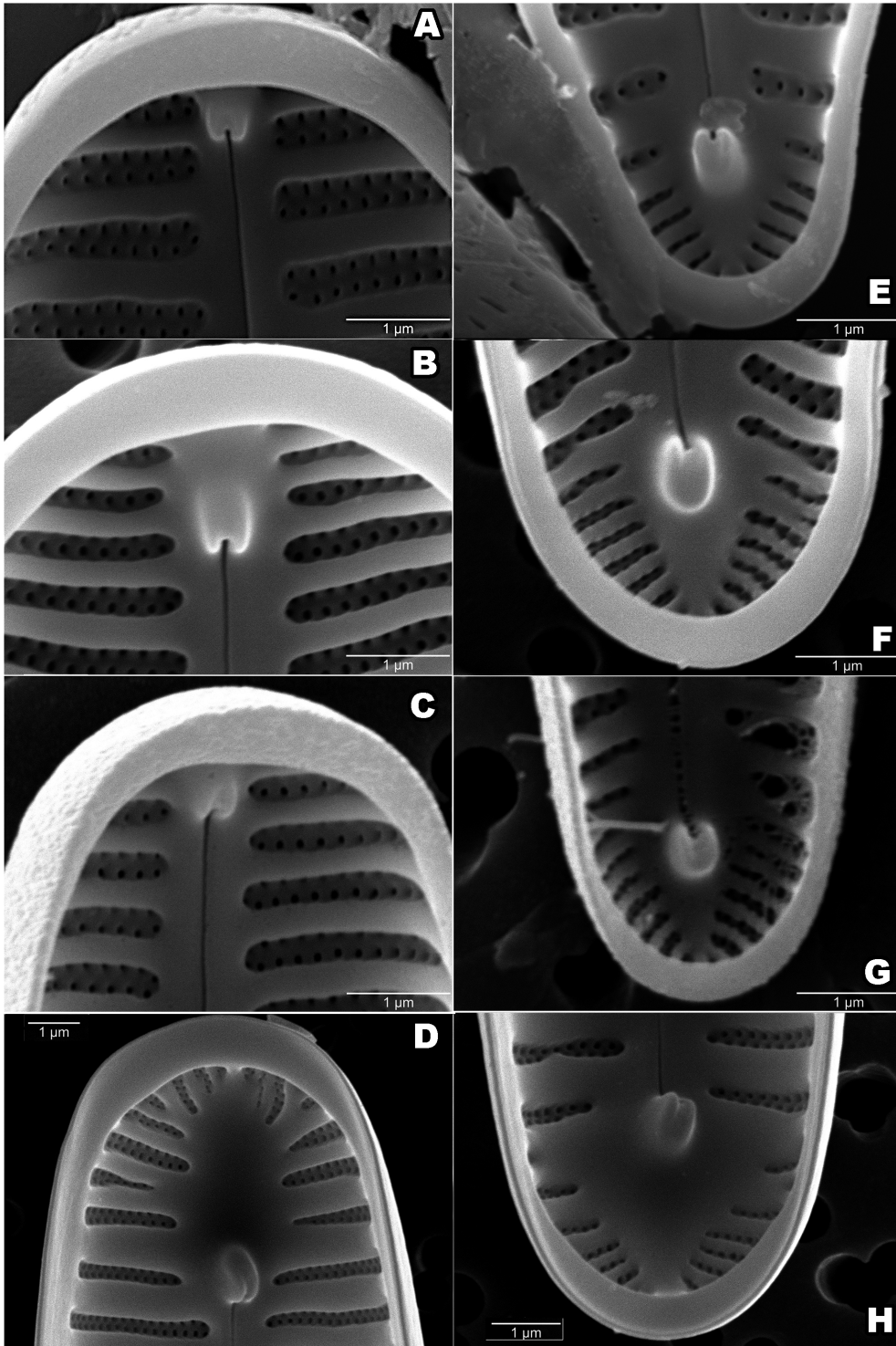


Fig. 3. Internal valve view. A–D. Headpoles. E–H. Footpoles. A, E. *Gomphonella olivacea*. B, F. *Gomphoneis okunoi*. C, G. *Gomphoneis pseudookunoi*. D, H. *Gomphoneis tetrastigmatum*. Scales: 1 µm.

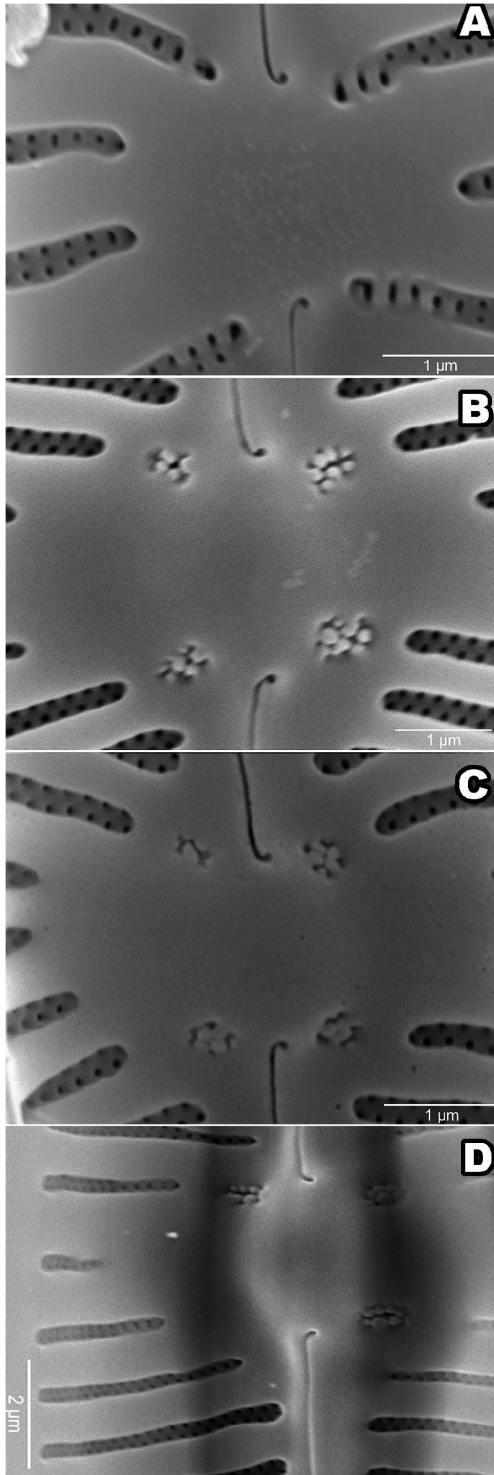


Fig. 4. Internal valve view. A–D. Central area. A. *Gomphonella olivacea*. B. *Gomphoneis okunoi*. C. *Gomphoneis pseudookunoi*. D. *Gomphoneis tetrastigmatum*. Only three stigmata are observed. Scales: 1 µm.

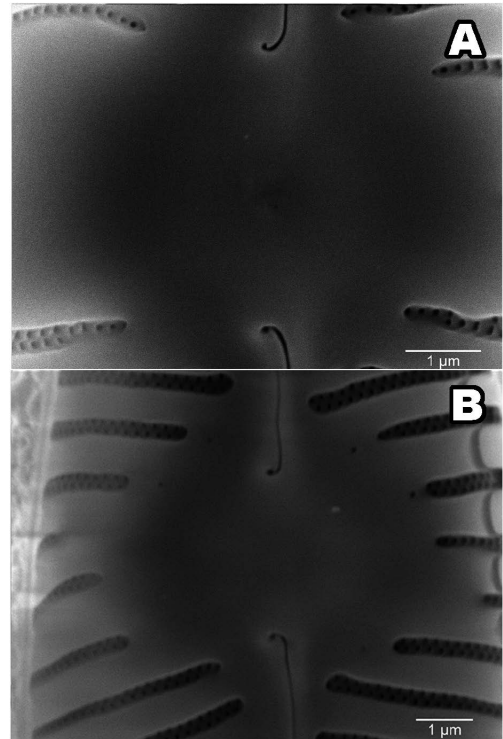


Fig. 5. Internal valve view. A–B. Central area. *Gomphoneis tetrastigmatum*. No stigma is observed. Scales: 1 µm.

also supports the expansion of the definition of the genus *Gomphonella*. *Gomphosinica* may be a synonym of the genus *Gomphonella*, because their morphological characteristics are identical except for the presence of only one stigma. However, no molecular information has been published, and this issue requires further study.

Transfer of Gomphoneis taxa with four stigmata to genus Gomphonella

***Gomphonella quadripunctata* (Østrup) Tuji, comb. et stat. nov.**

Basionym: *Gomphonema olivaceum* var. *quadripunctatum* Østrup, Nov. Hedw. 48: 85. f. 11. 1908.

Synonym: *Gomphonema quadripunctatum* (Østrup) Wislouch, Ber. dt. Bot. Ges. 42: 166. 1924.

Gomphoneis quadripunctatum (Østrup) P.A.Dawson ex R.Ross et P.A.Sims, Bacill. 1: 162. 1978.

***Gomphonella olivaceoides* (Hust.) Tuji, comb. nov.**

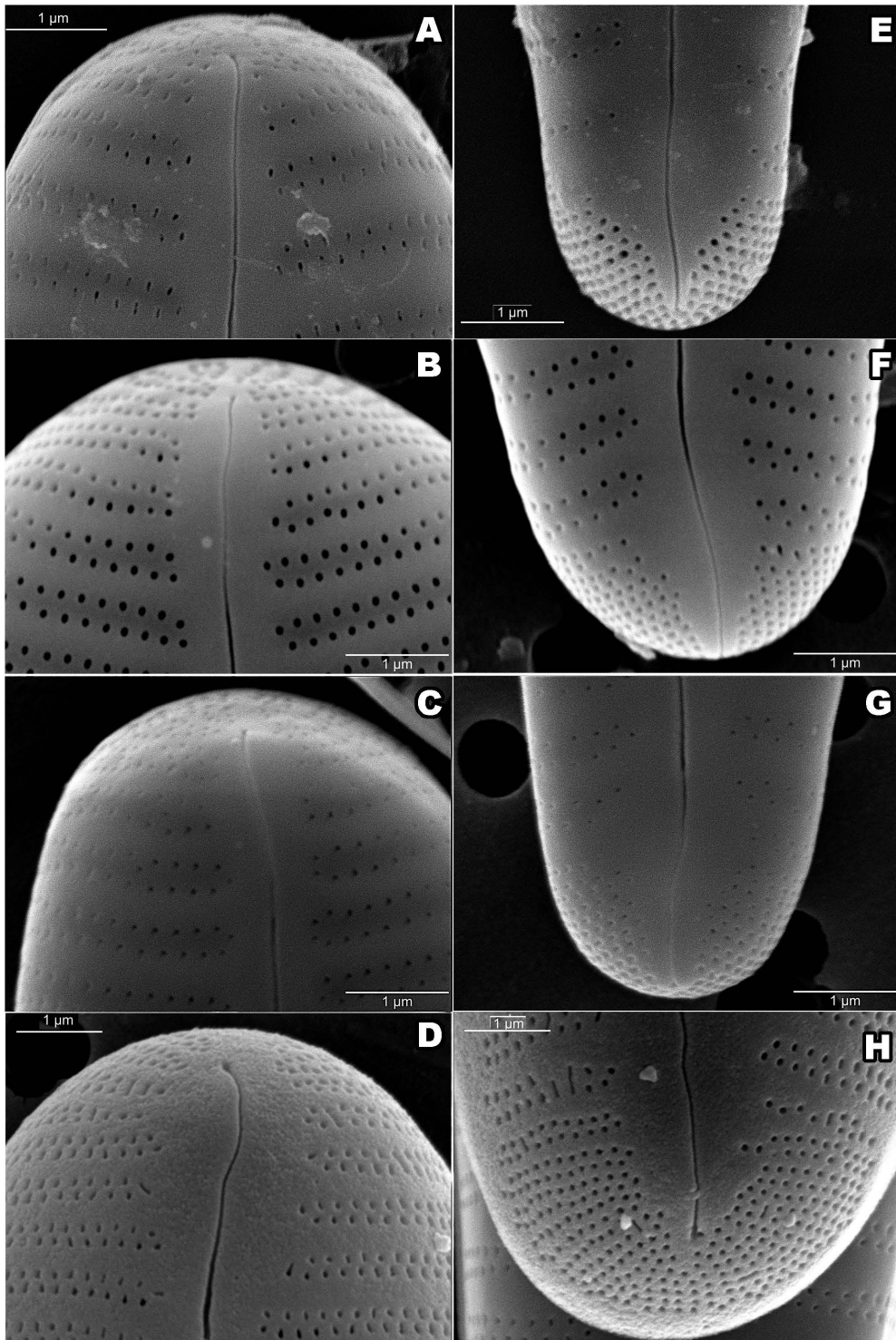


Fig. 6. External valve view. A–D. Headpoles. E–H. Footpoles. A, E. *Gomphonella olivacea*. B, F. *Gomphoneis okunoi*. C, G. *Gomphoneis pseudookunoi*. D, H. *Gomphoneis tetrastigmatum*. Scales: 1 µm.

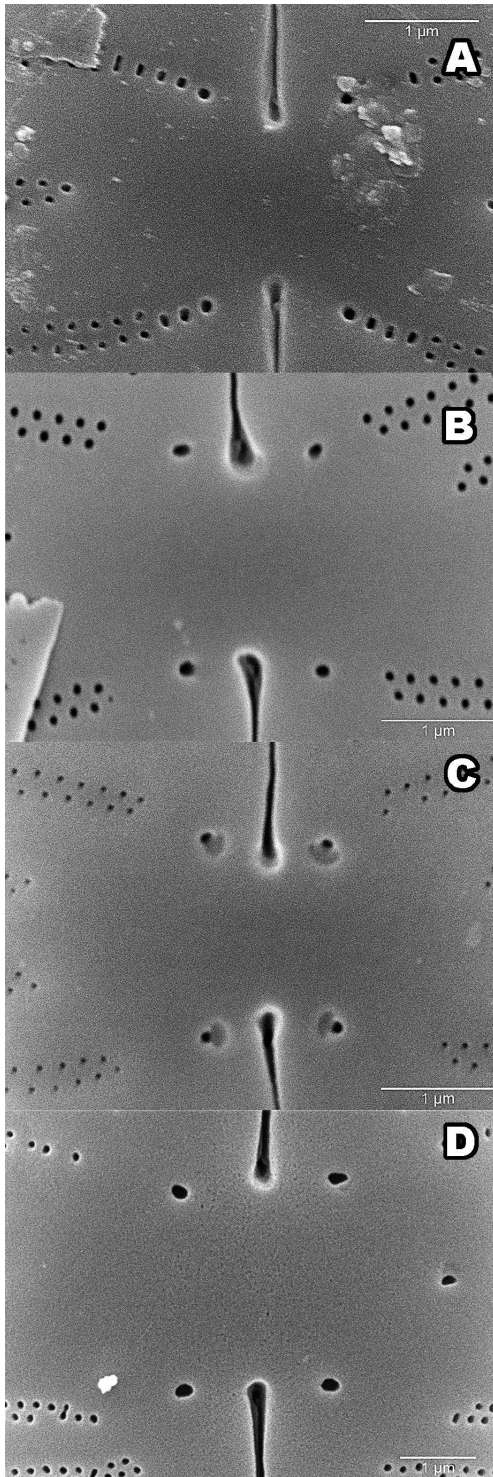


Fig. 7. External valve view of central area. A. *Gomphonella olivacea*. B. *Gomphoneis okunoi*. C. *Gomphoneis pseudookunoi*. D. *Gomphoneis tetrastigmatum*. Scales: 1 μ m.

Basionym: *Gomphonema olivaceoides* Hust., Arch. Hydrobiol. 3: 397. pl. 37. f. 9–12. 1950.

Synonym: *Gomphoneis olivaceoides* (Hust.) J.R.Carter and Bailey-Watts, Nov. Hedw. 33: 566. pl. 9. f. 49–51. 1981. excl. figs et desc.

Gomphoneis olivaceoides (Hust.) J.R.Carter and Bailey-Watts ex. Tuji, Bull. Natl. Sci. Mus., Tokyo, Ser. B 31: 90–91. 2005.

Gomphonella calcifuga (Lange-Bert. et E. Reichardt) Tuji, comb. nov.

Basionym: *Gomphonema calcifugum* Lange-Bert. & E. Reichardt in Lange-Bert., Iconogr. Diatomol. 6: 53. 1999, nom. nov.

Synonym: *Gomphonema olivaceum* var. *minutissimum* Hust. in Pascher, Süsw.-Fl., ed. 2, 10: 378. f. 720. 1930.

Gomphonella tetrastigmata (Horik. et Okuno) Tuji, comb. nov.

Basionym: *Gomphonema tetrastigmatum* Horik. et Okuno in Okuno, Bot. Mag. Tokyo. 58: 10. f. 3e. 1944.

Synonym: *Gomphoneis tetrastigmata* (Horik. et Okuno) Ohtsuka, Diat. 18: 32. 2002.

Gomphonella okunoi (Tuji) Tuji, comb. nov.

Basionym: *Gomphoneis okunoi* Tuji, Bull. Natl. Sci. Mus., Tokyo, Ser. B 31: 92–95. pl. 12. f. 1–10. pl. 13. f. 1–4. pl. 14. f. 1–5. 2005.

Gomphonella pseudookunoi (Tuji) Tuji, comb. nov.

Basionym: *Gomphoneis pseudookunoi* Tuji, Bull. Natl. Sci. Mus., Tokyo, Ser. B 31: 97. pl. 15. f. 1–15. pl. 16. f. 1–5. pl. 17. f. 1–5. 2005.

Gomphonella hutchinsoniana (R.M.Patrick) Tuji, comb. nov.

Basionym: *Gomphonema olivaceoides* var. *hutchinsoniana* R.M.Patrick, Limnol. Oceanogr. 16: 414. f. 1. 1971.

Gomphonella perolivaceoides (Levkov) Tuji, comb. nov.

Basionym: *Gomphonema perolivaceoides* Levkov, Iconogr. Diatomol. 16: 65–66. pl. 177. f. 1–21. 2007.

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