Plant Ecology and Evolution 152 (2): 248–261, 2019
https://doi.org/10.5091/plecevo.2019.1614

REGULAR PAPER

Taxonomy and diversity of a little-known diatom genus *Simonsenia* (Bacillariaceae) in the marine littoral: novel taxa from the Yellow Sea and the Gulf of Mexico

Byoung-Seok Kim[1,8,*], Andrzej Witkowski[2,8], Jong-Gyu Park[3], Chunlian Li[4,2], Rosa Trobajo[5], David G. Mann[5,6], So-Yeon Kim[1], Matt Ashworth[7], Małgorzata Bąk[2] & Romain Gastineau[2]

[1]Department of Oceanography, College of Ocean Science and Engineering, Kunsan National University, Gunsan 54150, Republic of Korea
[2]Palaeoceanology Unit, Faculty of Geosciences, Natural Sciences Education and Research Centre, University of Szczecin, Mickiewicza 16a, 70-383 Szczecin, Poland
[3]Department of Marine Life and Applied Sciences, College of Ocean Science and Engineering, Kunsan National University, Gunsan 54150, Republic of Korea
[4]Institute of Ecological Sciences, School of Life Sciences, South China Normal University, Guangzhou 510631, China
[5]Institute of Agriculture and Food Research and Technology (IRTA), Sant Carles de la Ràpita, E-43540, Spain
[6]Royal Botanic Garden Edinburgh, Edinburgh EH3 5LR, Scotland, UK
[7]UTEX Culture Collection of Algae, Department of Molecular Biosciences, University of Texas at Austin, 205 W. 24th St. MS C0930 Austin, Texas 78712, United States
[8]Both authors contributed equally to this work
*Author for correspondence: kbs3603@naver.com

Background and aims – The diatom genus *Simonsenia* has been considered for some time a minor taxon, limited in its distribution to fresh and slightly brackish waters. Recently, knowledge of its diversity and geographic distribution has been enhanced with new species described from brackish-marine waters of the southern Iberian Peninsula and from inland freshwaters of South China, and here we report novel *Simonsenia* from fully marine waters.
Methods – New isolates of *Simonsenia* species were obtained from marine waters, the littoral zone of the Korean Yellow Sea coast and the Gulf of Mexico in Corpus Christi (Texas), and documented in LM, SEM and with DNA sequence data (plastid-encoded *rbc*L and *psb*C). Phylogenetic trees of raphid diatoms were constructed to assess the relationships of the new species and of the genus as a whole.
Key results and conclusions – Two novel species of *Simonsenia* (*S. eileencoxiae* and *S. paucistriata*) are described and a further putative taxon is characterized morphologically. The molecular phylogeny of the new *Simonsenia* species and previously sequenced species supports both the monophyly of the genus and its place within the Bacillariaceae. The *Simonsenia* clade clusters with clades composed of *Cylindrotheca*, *Denticula* and some *Nitzschia* spp. (including *N. amphibia*, *N. frustulum*, *N. inconspicua*). Hence *Simonsenia* is firmly positioned within the Bacillariaceae by molecular phylogenies, confirming its position within this group based on the possession of a canal raphe and its ultrastructure, and rejecting its classification within the Surirellaceae. Morphological data from the new *Simonsenia* species is typical for the genus, with a "simonsenioid" canal raphe type supported over the valve face with fenestral braces, alar canals connecting the canal raphe with the cell lumen, and the presence of fenestrae between the alar canals externally. Our results indicate unequivocally that the biogeography and the biodiversity of *Simonsenia* remain highly underestimated.

Key words – Bacillariaceae, diatoms, Gulf of Mexico, *Simonsenia*, molecular phylogeny, morphology, new marine species, Yellow Sea.

Plant Ecology and Evolution is published by Meise Botanic Garden and Royal Botanical Society of Belgium
ISSN: 2032-3913 (print) – 2032-3921 (online)

INTRODUCTION

Simonsenia Lange-Bert. and its generitype species (*S. delognei* (Grunow in Van Heurck) Lange-Bert.) were distinguished from the Nitzschiaceae (= Bacillariaceae) on the basis of electron microscopy (SEM and TEM) observations of alar canals connecting the raphe canal with the cell lumen (Lange-Bertalot 1979). For years, this genus has received little attention beyond diatom floristics (Krammer & Lange-Bertalot 1988, Werum & Lange-Bertalot 2004, Kelly et al. 2005, Żelazna-Wieczorek 2011, Kociolek 2012, Witkowski et al. 2014). More recently, the establishment of the first monoclonal cultures and sequencing of their DNA (Witkowski et al. 2015) has reignited some interest in the molecular phylogeny and ecology of this genus.

The discovery of alar canals connecting the raphe canal with the cell lumen in *Simonsenia* was significant, as alar canals are a distinct character of the sect. Robustae in the genus *Surirella* Turpin (Lange-Bertalot 1979, Krammer & Lange-Bertalot 1988, Ruck & Kociolek 2004, Ruck et al. 2016), which belongs to the family Surirellaceae, and not to the Bacillariaceae. The use of molecular markers in reconstructing the phylogeny of *Simonsenia aveniformis* Witkowski, Ana Gomes & Gusev, however, supported the classification of *Simonsenia* in the Bacillariaceae (Witkowski et al. 2015). More specifically, *rbc*L sequence data suggest *Simonsenia* is sister to *Cylindrotheca* Rabenh. and an unresolved clade of *Denticula* Kütz. and some taxa in the *Nitzschia* sect. Lanceolatae (Witkowski et al. 2015), with the Surirellaceae many nodes away. The phylogenetic distance between the Bacillariales and Surirellales was also documented in other molecular studies (Theriot et al. 2010, Ruck & Theriot 2011, Ruck et al. 2016). These molecular data suggest that despite the morphological similarity between the alar canals of *Simonsenia* and *Surirella* sect. Robustae (recently transferred into *Iconella* Jurilj in Ruck et al. 2016), these have evolved independently. Despite the morphological similarity of the alar canals and fenestrae, *Simonsenia* has a canal raphe clearly elevated and positioned at the valve margin, with the cell lumen closed by distinct fibulae (Lange-Bertalot 1979, Witkowski et al. 2014, 2015, You et al. 2016) – characters that are evidently absent in *Surirella* (Krammer & Lange-Bertalot, 1988, Round et al. 1990, Ruck & Kociolek 2004). With these differences in mind, Witkowski et al. (2015) compared the ultrastructure of the canal raphe across diatoms and proposed a third, "simonsenioid" canal raphe-type to distinguish from the "nitzschioid" and "surirelloid" types (e.g. Krammer & Lange-Bertalot 1988, Round et al. 1990, Ruck & Kociolek 2004). Thus, while *Simonsenia* is strongly nested within Bacillariaceae based on the molecular phylogeny, it is morphologically distinct from its phylogenetic sister taxa.

Regarding the ecology of *Simonsenia*, newly-described species and reports are showing a distribution in a wider range of habitats for the genus than expected (Noga et al. 2014, Witkowski et al. 2014, 2015, You et al. 2016). Detailed research has suggested that *S. delognei* is not limited in its distribution, but is in fact a widely distributed species in Europe and beyond. Although usually with low abundance, *S. delognei* occurs in the riverine networks of large regions, e.g. in the United States (Kociolek 2012), France (Bey M.-Y.& Ector L. 2013), SE Poland (Noga et al. 2014), NE Spain (R. Trobajo, unpublished observations) and Anatolia (Witkowski et al. 2014), in standing water bodies (Jüttner et al. 2010), and in isolated springs (Werum & Lange-Bertalot 2004, Żelazna-Wieczorek 2011). Geographically, it has been reported from Europe (e.g. Werum & Lange-Bertalot 2004, Kelly et al. 2005, Jüttner et al. 2010, Żelazna-Wieczorek 2011, Noga et al. 2014, Cantonati et al. 2017), the Middle East (Witkowski et al. 2014), the United States (Kociolek, 2012) and continental China (You et al. 2016). Furthermore, sampling performed in marine/brackish-waters (Witkowski et al. 2015) and in inland waters (You et al. 2016) has doubled the number of species known. The description of *S. aveniformis* from higher salinity areas of transitional waters (exceeding 15 psu) of southern Iberia expanded the ecological range known for the genus, which was previously regarded as a freshwater and slightly brackish-water taxon (*S. delognei*, *S. delicatula*). Records and descriptions of taxa found in southwestern continental China (*S. delognei* and *S. maolaniana* Q.You & Kociolek in You et al. 2016) expanded its known range to include karstic habitats.

In the present article, we show that the geographic distribution of the genus *Simonsenia* is even wider, and report it from the Western Pacific, along the Yellow Sea coasts of Korea and from the Taiwan Strait in East China, and from the Western Atlantic Ocean along the coast of the Gulf of Mexico. We also describe two new species to science, increasing the number of *Simonsenia* species known to six. Two of the treated taxa were grown in culture and one observed only in a fresh sample. The molecular phylogeny is based on sequences of the plastidic genes *rbc*L and *psb*C (Texas strain) or *rbc*L alone (Korea strain). The Texas strain established here as *S. paucistriata* sp. nov. and the Korean species from the fresh sample are morphologically very similar. We therefore described the former specimens as a new species and treated the Korean specimens as *S.* cf. *paucistriata* until we will be able to establish a clonal culture and sequence its DNA.

MATERIALS AND METHODS

Sampling areas (table 1)

Yellow Sea coast, Korea – Samples were collected at two locations from the intertidal zone of Shinan-Gun and Padori, situated at the Korean Yellow Sea coast (fig. 1). Sampling in Shinan-Gun was conducted by scraping attached diatoms off plastic tubes which supported and pumped sea water into tanks for fish farming. At the coast of Padori small pebbles and seaweeds were collected in December 2016. The western coast of Korea is mainly occupied by tidal flats composed of mud, clay and fine sand, but the coast at Padori is covered with small pebbles and rocks.

Gulf of Mexico, Corpus Christi, Texas – Sampling in Corpus Christi at Fish Pass on Mustang Island (fig. 1) was performed with a 20-µm mesh size plankton net in December 2014. Fish Pass is a narrow, shallow, 2–3 m wide channel that connects the Texas intercoastal waterways to the open Gulf of Mexico during wet seasons. Typically, only the intercoastal opening to the pass is wet all year round. The bottom

Table 1 – **Sampling sites and environmental data.**
ND – not determined.

Locality	Temperature (°C)	Salinity (psu)	Date	Latitude	Longitude
Shinan-Gun	21.1	33.1	Jun. 2016	35°02′42.61″N	126°10′57.51″E
Padori	17.1	32	Dec. 2016	36°44′15.00″N	126°07′49.70″E
Fish Pass, Mustang Island, Corpus Christi	ND	c. 28–34	Dec. 2014	27°40′48″N	97°10′22.8″W

of Fish Pass is composed of fine sediments, and the subtidal and intertidal vegetation is dominated by reeds.

Isolation and cultures

Monoclonal cultures were obtained by successive dilution using micro-pipettes. Single cells of *Simonsenia* were isolated from samples collected from Padori into 24-cell culture plates (ISO 13485, SPL life sciences, Korea) using a Pasteur pipette (Pre-sterilized & Pre-plugged, Poulten & Graf, England) under an inverted microscope (TS100, Eclipse, Nikon, Japan). Subsequently a single isolated *Simonsenia* cell was cultured at 20°C, 32 psu salinity and with a light:dark cycle of 12:12 h. Successfully cultivated strains in cell culture plates were first placed in glass tubes for several days and progressively increased in volume by using 125 mL, 250 mL and 500 mL bottles (PC bottle, Nalgene). For the *Simonsenia* clone from the Gulf of Mexico, a single cell was isolated into a Petri dish (50 mm diameter) containing 5 mL 35 psu f/2 culture medium, through a glass dropper by operating the dropper teat by hand under the inverted microscope (Nikon Eclipse TS100, Japan). The isolated cell was rinsed with sterile culture medium three to four times to remove contaminants and was then placed close to a north-facing window or in a batch incubator at 18°C under a 16:8 h light:dark cycle, illuminated with 50 µmol photons m^{-2} s^{-1} of white light.

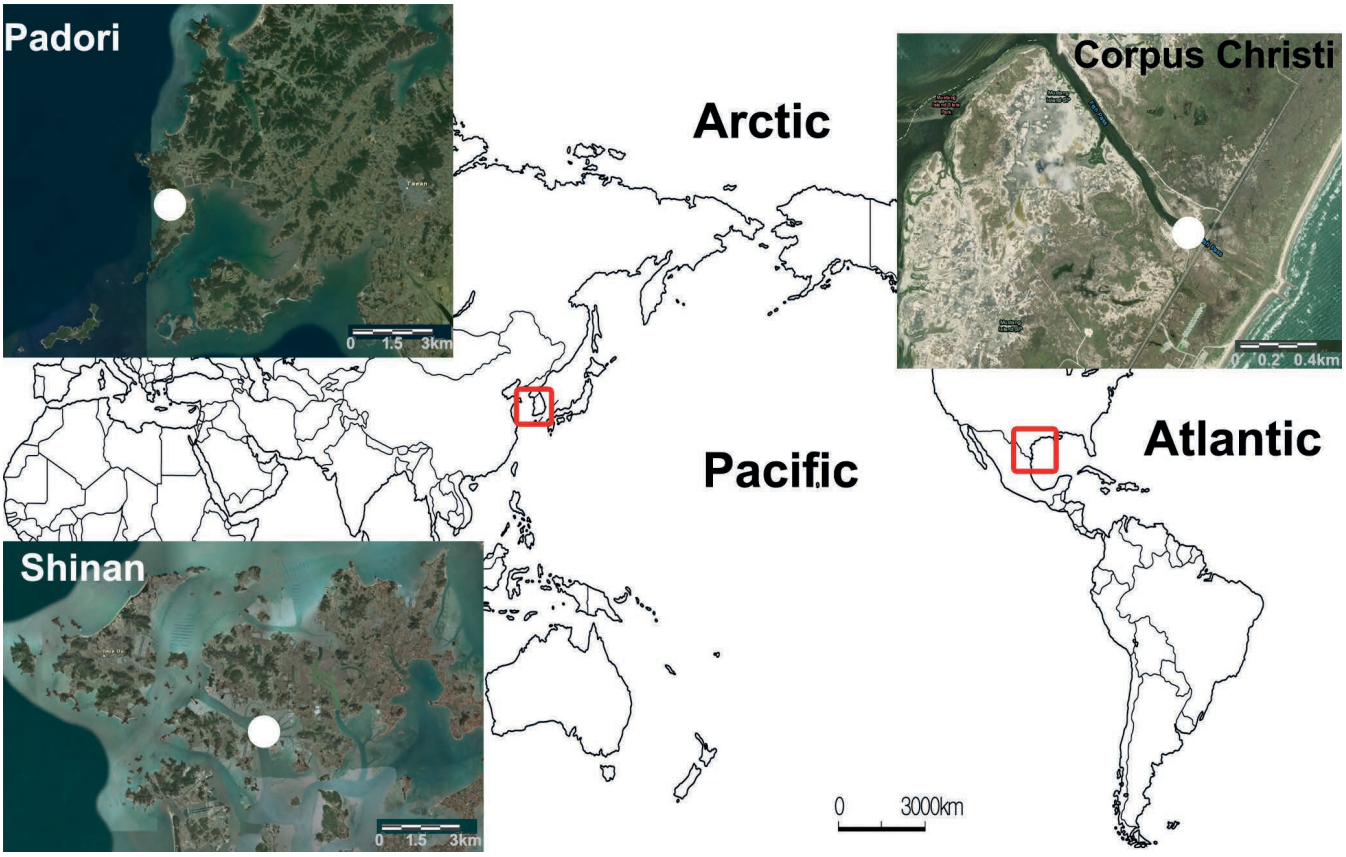

Figure 1 – Map showing the sampling area of Padori and Shinan-Gun at the Yellow Sea coast**,** Korea, and at Fish Pass, Mustang Island, Corpus Christi, Gulf of Mexico, United States. World map downloaded from National Geographic Information Institute (http://www.ngii.go.kr/en). Maps of Padori, Shinan-Gun and Fish Pass from Google Maps (© Google 2018). These images are not covered by the terms of the Creative Commons licence of this publication. For permission to reuse, please contact the rights holders (National Geographic Information Institute, http://www.ngii.go.kr/en; and Google).

Scanning electron microscopy

For scanning electron microscopy (SEM) of the Korean clone, the preparation of the material followed the protocol of Hasle & Fryxell (1970). Clean frustules were washed with distilled water to remove salts. They were coated with platinum in an ion sputter E-1045 (40 s) and examined with a field emission SEM (S-4800+EDS, Horiba: EX-250 at 5kV) at the Joint Experiment Practice Center of Kunsan University. The clone from the Gulf of Mexico was prepared according to Witkowski et al. (2014) and TEM observations were performed at Warsaw University of Technology, SEM at Faculty of Material Science and Engineering, and at the Podkarpacie Innovative-Research Centre of Environment, University of Rzeszów, Poland with a Hitachi SU 8010.

DNA extraction and PCR

For the *Simonsenia* clone from Korea, the primers used for PCR amplification and sequencing of the plastidic gene (*rbc*L) were DP*rbc*L1 and DP*rbc*L7 (Jones et al. 2005), with PCR conditions following Stepanek et al. (2016). The genomic DNA was extracted using the genomic DNA AccuPrep® Genomic DNA extraction kit (Bioneer, Daejeon, Korea) according to the manufacturer's instructions. The *rbc*L volume of each PCR was 50 µL : 2 µL (20 ng) purified DNA template; 5 µL 10× Takara Taq buffer (includes 50 mM Tris-Acetate-EDTA); 2 µL Dream Taq DNA polymerase (5U μL^{-1}); and double distilled water (DDW) to a final volume of 50 µL. PCR conditions for *rbc*L were as follows: 210 s at 94°C followed by 36 cycles × 50 s at 52°C, and 90 s at 72°C and final extension of 900 s at 72°C (Thomas, 2016). PCR products were sent to Genotech company (Daejeon, Korea) and sequenced after purification.

As for the clone from the Gulf of Mexico, two plastidic genes (*rbc*L and *psb*C) were sequenced, using the amplification and sequencing primers listed in Li et al. (2018). The genomic DNA of the clone was extracted using High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany) following the protocol for isolation of nucleic acids from bacteria or yeasts according to the manufacturer's instructions. The volume of each PCR was 25 µL: 10–20 ng DNA template; 2.5 µL 10× Dream Taq buffer including 20 mM $MgCl_2$ (Thermo Fisher Scientific, USA); 0.5 µL 5 mM Ultrapure dNTPs Set (EURx, Gdansk, Poland); 0.5 µL each primer (10 µM); 0.15 µL 5 U/µL Dream Taq DNA polymerase (Thermo Fisher Scientific, USA); and sterile ddH_2O to a final volume of 25 µL. PCR conditions for *rbc*L and *psb*C were as follows: 94°C for 2 min, 35 cycles of 94°C for 15 s, 55°C for 15 s, 72°C for 1 min 15 s, and a final extension at 72°C for 7 min. The PCR products were purified and then sequenced with Sanger method using BigDye Terminator version 3.1 chemistry and ABI3730 x1 sequence by the oligo.pl DNA Sequencing Laboratory IBB PAS, Warsaw, Poland.

Phylogenetic analyses

To obtain the tree inferred from concatenated three-gene dataset, firstly, the three-gene (SSU+*rbc*L+*psb*C) sequences of each species were merged into one sequence in Mesquite by order. For the taxa missing one of the genes either SSU or *psb*C, an empty space was left. In this case, the sequence of each gene was aligned in the Mesquite. For the *Simonsenia* strain from Korea *rbc*L gene has been sequenced, whereas for the Texas strain *rbcL* and *psb*C.

A maximum likelihood tree was estimated using a three-gene dataset including 119 raphid diatom taxa (electronic appendix 1), with two araphid diatoms – *Tabularia* cf. *tabulata* and *Ctenophora pulchella* (Ralfs ex Kütz.) D.M.Williams & Round – as the outgroups. Within the three-gene dataset, all of the taxa were represented by *rbc*L sequences, and 77 taxa by SSU sequences or *psb*C sequences. The total length of the alignment was 4338 base pairs (bp), in which 1–1757 bp were SSU sequences, 1758–3230 bp were *rbc*L sequences and 3231–4338 bp were *psb*C sequences. For the ML analyses, the SSU data were partitioned by paired and unpaired sites based on a secondary structural alignment of the SSU primary sequences using a 23-diatom model provided by Edward C. Theriot and the program SSU-align v. 0.1 (Nawrocki 2009), where the ambiguous sites with a PP (posterior probability) less than the default of 0.9 were removed to limit the influence of uninformative "noise" on the analyses. The protein-coding *rbc*L and *psb*C genes were partitioned by codon position. Alignment of *rbc*L and *psb*C primary sequences was performed using BioEdit v. 7.2.5 (Hall 1999) using the "ClustalW multiple alignment" function (electronic appendix 2). After alignment, the secondary structural alignment of SSU sequences was uploaded into Mesquite v. 3.04 (Maddison & Maddison, 2015), followed by the corresponding aligned *rbc*L and *psb*C data for each species (electronic appendix 2). Phylogenetic trees were estimated with 1000 bootstrap replicates using the rapid bootstrap analysis in RAxML v. 8.1 (Stamatakis 2014), with a GTR+G+I model applied to each partition. The best-scoring ML tree was chosen as the final tree and the bootstrap values were added to the corresponding nodes on the tree diagram.

RESULTS

Two new species of *Simonsenia* were isolated into clonal culture, whereas the third taxon was only observed in SEM. Morphological descriptions follow for all three taxa, with formal taxonomic descriptions for the two taxa in culture. Before we treat the taxa in detail, we make one general comment on their gross morphology. Whereas the species isolated from Padori beach has a strongly eccentric raphe canal as in previously described *Simonsenia* species (e.g. in *S. aveniformis*, Witkowski et al. 2015), in the Texas strain and in the Korean specimens from Shinan-Gun the raphe canal is only weakly displaced from the valve centre. However, all three taxa possess a distinct distal and proximal valve mantle free of areolation which is positioned either proximally or distally to raphe canal. For the position of the mantle in relation to raphe canal see Mann (1978: text figure 1, and 1986). For the terminology used in describing *Simonsenia* ultrastructure refer to fig. 2L (exterior view of a complete frustule) & 2M (valve, interior view).

Simonsenia eileencoxiae B. Kim, J.-G.Park & Witkowski, **sp. nov.**

Figs 2 & 3

Type material – Yellow Sea, western coast of Korea, Taean County, from sand and small stones of the Padori Beach in Taean-Gun (36°44′15.00″N, 126°07′49.70″E), Dec. 2016, *Byoung-Seok Kim & Jong-Gyu Park* s.n. (holo-: BM, slide 101955, depicted in fig. 2H; iso-: SZCZ, diatom collection of Andrzej Witkowski, Palaeoceanology Unit, Faculty of Geosciences, University of Szczecin, slide SZCZ25984).

Description: LM – Fig. 2A–K. Details of frustule and valve difficult to resolve in LM, except for the transapical ribs and striae, and the raphe canal, which is positioned marginally (fig. 2E). Sometimes portulae are visible in LM (fig. 2C, G & H). Frustules very small, rectangular in girdle view with rounded apices, with two simple fore-and-aft plastids, typical for *Simonsenia* and *Nitzschia* (fig. 2A & B). Valves lanceolate with protracted apices, 7–13 µm in length and 1.9–2.1 µm in width (n = 15).

Description: SEM – Figs 2L, M & 3. Valves lanceolate with protracted apices and a valve face that is regularly undulate externally and internally, with elevated virgae and depressed striae. Valve mantle narrow, devoid of areolation (fig. 2L & M). Canal raphe strongly eccentric, proximal (central) endings absent, distal ones present as strongly bent terminal fissures externally, internally as helictoglossae (fig. 2M). Canal raphe with solid outer wall (figs 2L, 3A–B & F–H), opening to the interior by a series of round portulae (the inner openings of alar canals: figs 2M, 3D–E). Regular, porous plates occur between neighbouring portulae (fig. 3D–E & I), which subtend the canal raphe, closing the cell surface and function as fibulae; there are 11–12 in 10 µm. Canal raphe supported externally over valve surface by cylindrical braces (fenestral bars, 26–28 bars in 10 µm, marked *fb* in fig. 2L; cf. also fig. 3A–C, arrowhead in fig. 3B) and perforated with relatively large hymenate pores in lower part, facing the perforated plates closing the valve surface (marked *pp* in fig. 2L, but cf. also fig. 3I). Transapical striae parallel, bi- to multiseriate (on the apices) and occurring only on valve face; they are resolvable in LM, with c. 26–28 in 10 µm (figs 2M, 3B & D). Transapical ribs (virgae) rectangular and straight near the distal margin. Areolae small, circular, occluded by hymenes (arrow in fig. 3E); likewise hymenate pores are present on the fibulae (fig. 3I). Girdle composed of open porous bands (white arrow in fig. 3F–H), each girdle band containing two rows of transapically elongate pores.

Etymology – This species is dedicated to Dr. Eileen Cox, NHM London, in appreciation of her contribution to research on the taxonomy and biology of diatoms.

Distribution and autecology – *Simonsenia eileencoxiae* is only known with certainty from the type habitat, the beach of Padori, on the Yellow Sea coast of Korea. However, Cheng et al. (1993, reproduced also in Gao et al. 2012) illustrated a diatom from Xiamen Harbor on the Fujian coast in the West Pacific, which they identified as *Denticula subtilis* Grun. This illustration may be the first record of *S. eileencoxiae* (Cheng et al. 1993: fig. 256), but without confirmatory observations or DNA data, there remains some doubt. The salinity of the holotype habitat, Padori beach, was 32 psu, and the substrate was composed of small pebbles. Hence this taxon appears to be fully marine and is epilithic.

Simonsenia paucistriata Chunlian Li, Ashworth & Witkowski, **sp. nov.**

Fig. 4

Type material – Gulf of Mexico, Mustang Island near Corpus Christi, Texas, plankton net sample from Fish Pass (27°40′48″N, 97°10′22.8″W), Dec. 2014, *Matt Ashworth* s.n. (holo-: BM, slide 101956, depicted in fig. 4D; iso-: SZCZ, diatom collection of Andrzej Witkowski, Palaeoceanology Unit, Faculty of Geosciences, University of Szczecin, slide SZCZCH_839).

Description: LM – Fig. 4A–H. Frustules very small, rectangular in girdle view with rounded corners. Valves lanceolate with acute apices, 12–13 µm in length and 1.8–2.3 µm in width (n = 20). Details of frustule and valve not resolvable in LM, except for the canal raphe and alar canals/fenestrae (fig. 4A–H).

Description: SEM and TEM – Fig. 4I–P. Valves lanceolate with an undulate valve face. Canal raphe somewhat displaced from the valve centre towards the proximal valve margin, proximal central raphe endings absent, distal raphe endings bent externally (arrow in fig. 4J). Canal raphe composed of a solid outer wall (fig. 4I–L & P), communicating with the cell interior by a series of alar canals, which open internally by elliptic portulae (arrows in fig. 4M, but cf. also fig. 4N & O), with the spaces between neighbouring portulae filled in by perforated plates (the fibulae: 6–7 in 10 µm; these subtend the canal raphe and close the cell surface). The space between alar canals is perforated by only a few pores, usually one. Canal raphe supported over valve surface by external braces (fenestral bars, 12–14 in 10 µm; arrow in fig. 4L, but cf. also fig. 4I–K & P). Canal raphe circular in cross section, with the surface within fenestrae pointing towards the valve surface perforated by a row of small pores. Transapical striae (13–14 in 10 µm) apparently triangular, depressed below the two neighbouring transapical ribs and composed of irregularly arranged areolae; transapical ribs (virgae) likewise 13–14 in µm, also triangular and multiply forked near the distal mantle (arrowheads in fig. 4P). Areolae small, circular; occlusions not observed. Girdle composed of open bands (arrow in fig. 4O).

Etymology – This species name refers to the poorly developed striae composed of few areolae, observed with difficulty even in SEM and TEM.

Distribution and autecology – *Simonsenia paucistriata* is known only from the type locality: Fish Pass, Mustang Island near Corpus Christi, Gulf of Mexico, Texas, USA. The salinity of the holotype habitat ranges between 28 and 34 psu. While samples were collected with a plankton net, this area is shallow (< 3 m depth), and it is possible the specimens were tychoplanktonic and the taxon might be benthic.

Simonsenia* cf. *paucistriata

Fig. 5

Description: SEM – (Currently we only have SEM images.) The size and ultrastructure of the specimens resemble *S. pau-*

cistriata. Frustules are rectangular in girdle view with rounded ends, valves are lanceolate with acute apices, 11–14 µm in length and 2.1 µm in width (n = 3). This diatom is similar to *S. paucistriata* with its lanceolate valve shape and undulating valve face (fig. 5C). Canal raphe somewhat displaced from the valve centre towards the proximal mantle (fig. 5A & B); proximal central raphe endings absent (fig. 5B), distal raphe endings bent in the same direction. The canal raphe has a diameter similar to that of *S. paucistriata*. Portulae round, 6–7 in 10 µm (arrowheads in fig. 5F, but cf. also fig. 5E, G & H); the plates closing the valve surface (i.e. the fibulae) are perforated with a very few pores, usually one. Canal raphe supported over the valve surface by external braces (fenestral bars, 12–13 in 10 µm; fig. 5A–C), which are very similar to those in *S. paucistriata*. The transapical striae are triangular and depressed below the transapical ribs (virgae, cf. fig. 5C),

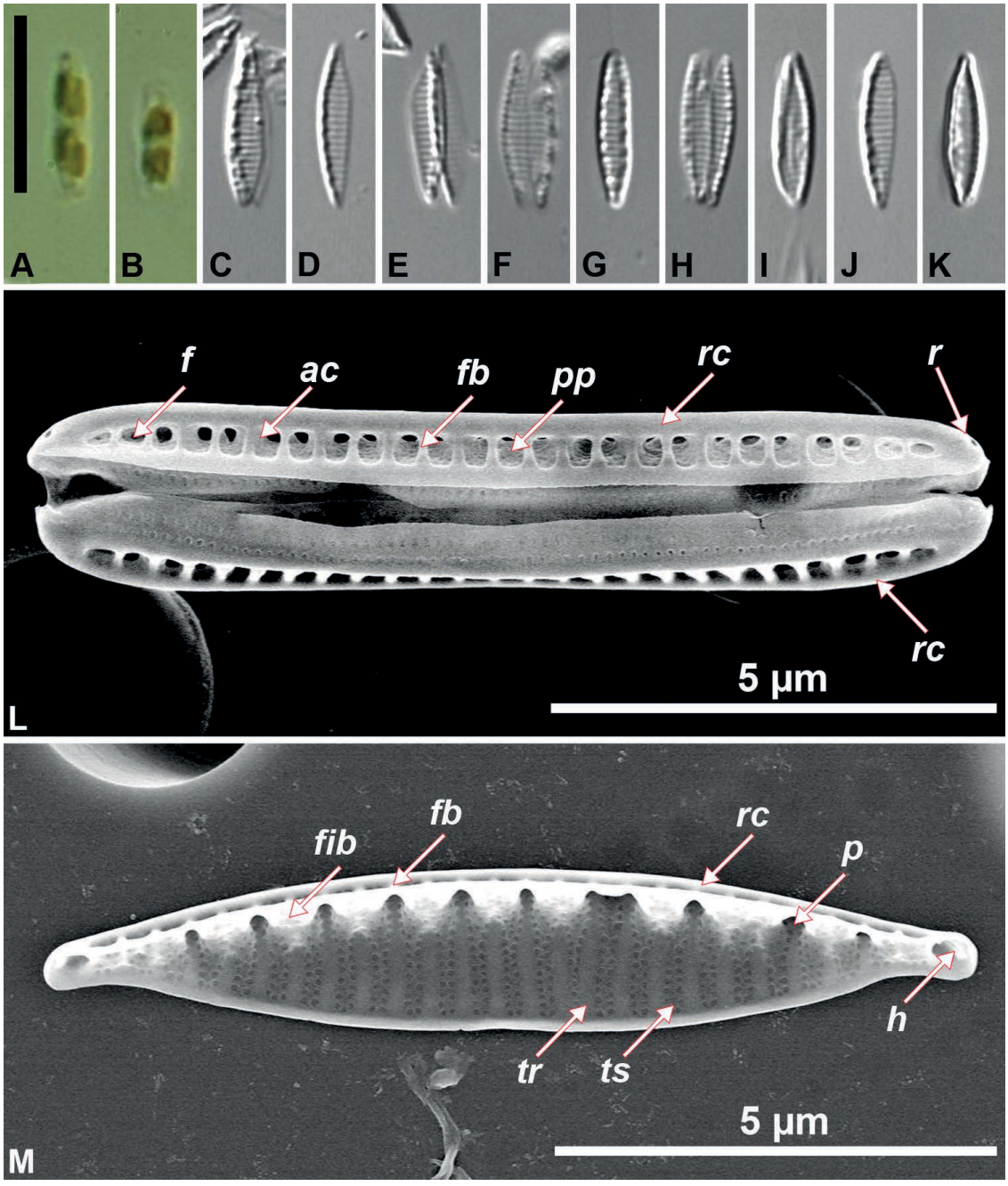

Figure 2 – *Simonsenia eileencoxiae*: A & B, LM, live specimens from the culture illustrating the position and shape of the plastids; C–K, LM, series of specimens illustrating the shape of the valves. Specimen illustrated in H is selected as holotype. Note the presence of the transapical ribs (C–H & J) and the raphe canal (E); L & M, SEM; L, external view of the whole frustule with particular features most characteristic for *Simonsenia* marked with abbreviations: *ac* – alar canal; *f* – fenestrae; *fb* – fenestral bar; *pp* – perforated plate (fibulae); *rc* – raphe canal; *r* – raphe slit. M, internal view of the valve with selected characters marked: *fb* – fenestral bar; *fib* – fibulae (perforated plate); *h* – helictoglossae; *p* – portulae (opening of alar canal); *rc* – raphe canal; *tr* – transapical ribs; *ts* – transapical striae. Scale bar A–K = 10 µm.

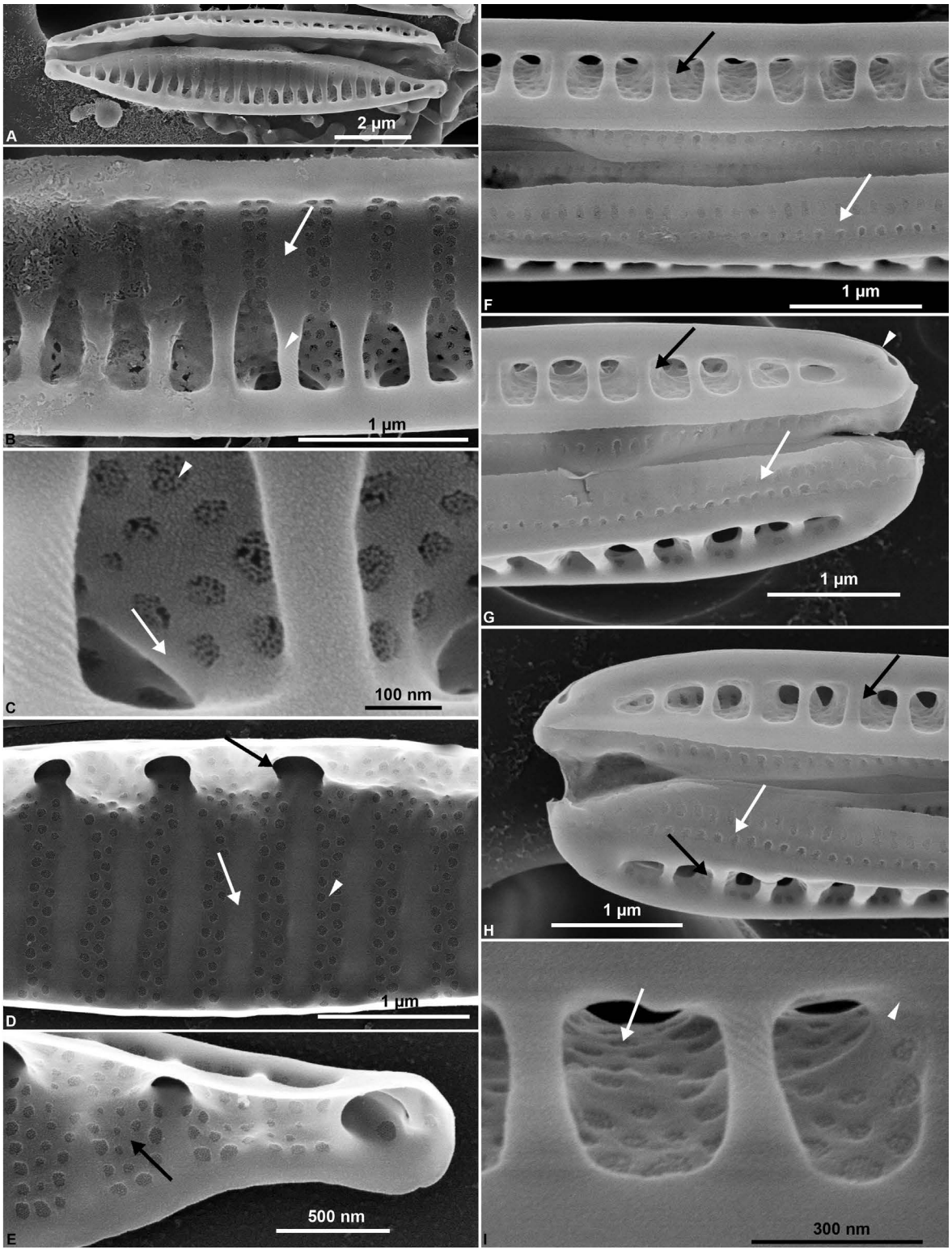

Figure 3 – *Simonsenia eileencoxiae* in SEM: A, the two valves of a frustule detached; B & C, close-ups of the specimen illustrated in A. Note a change in the shape of transapical ribs (rectangular on the valve face, white arrow), and cylindric as fenestral braces (arrowhead) in B. Close-up of the fenestra with alar canals (arrow), note the areolae with hymenate occlusions (arrowhead) in C; D & E, internal valve view illustrating position of the portulae and alternating biseriate transapical striae (arrowhead) and virgae (white arrow) and openings of the portulae (black arrow) in D. Internal view of the valve near the apex, note the multiseriate striae (arrow) in E; F–I, close-ups of the specimen illustrated in L. Note the valvocopula with two series of transapically elongate poroids (white arrows) in F–H illustrating the central and apical parts of the same frustule. Note the position of the alar canals (black arrows); I, close-up of the fenestrae. Note the perforations of the plate closing the valve surface within the fenestrae (arrow) and the contact of alar canal with the raphe canal (arrowhead).

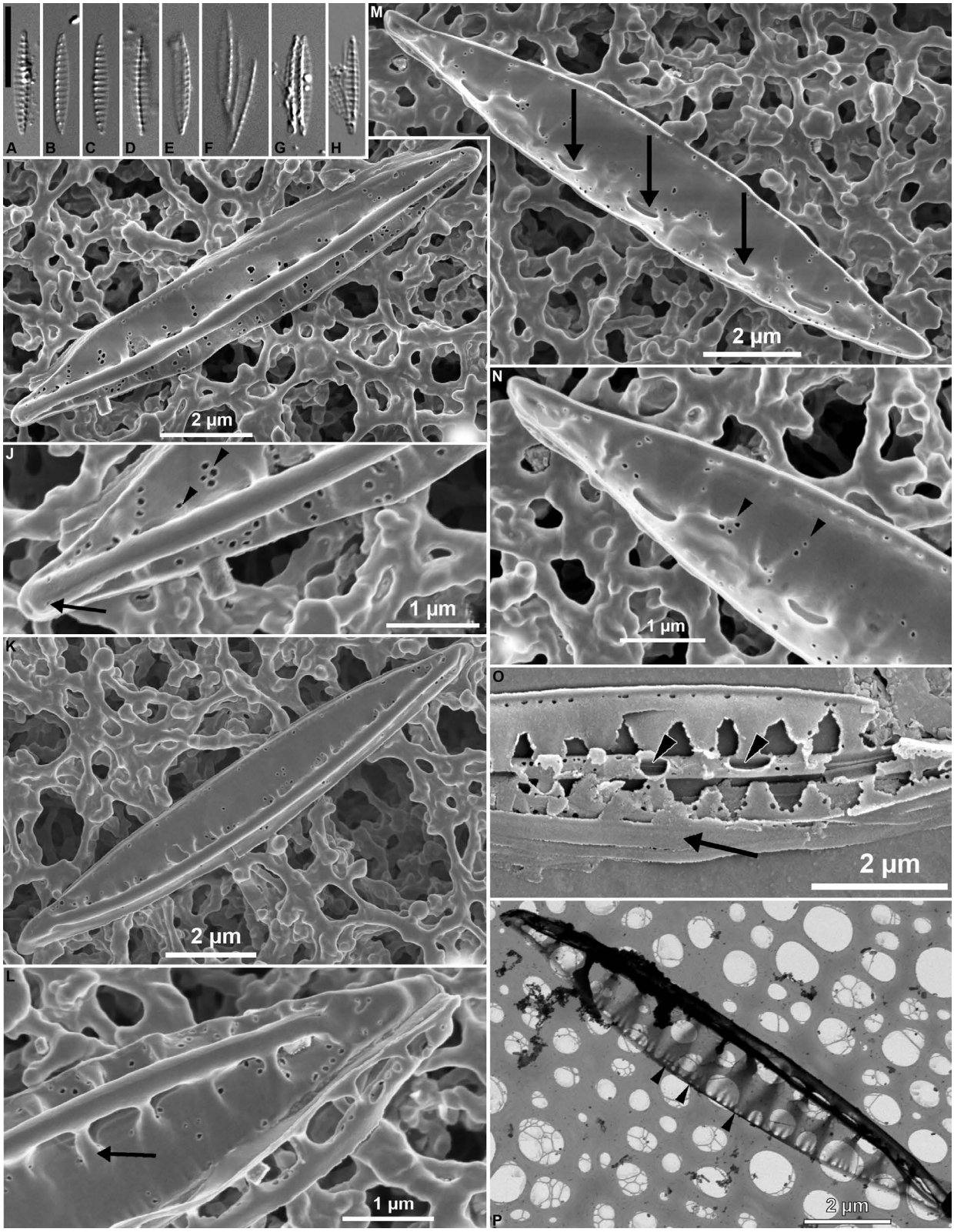

Figure 4 – *Simonsenia paucistriata*: A–H, LM, specimen illustrated in D is selected as the holotype; I–O, SEM; I–L, valve exterior of the whole specimens. Note the position of slightly eccentric raphe canal suspended over the fenestral braces (arrow) in L; J, close-up of the valve apex, note the sparse areolae (arrowheads) and bent apical raphe end (arrow); M & N, internal view of the valve showing the shape and position of portulae (arrows) and sparse areolation (arrowheads); O, internal view of partly broken specimen, note the girdle band (arrow) and opening of the alar canals (portulae) broken from the raphe canal (arrowheads); P, the whole specimen illustrated in TEM, note the presence of multiply forked transapical ribs along the distal margin (arrowheads).

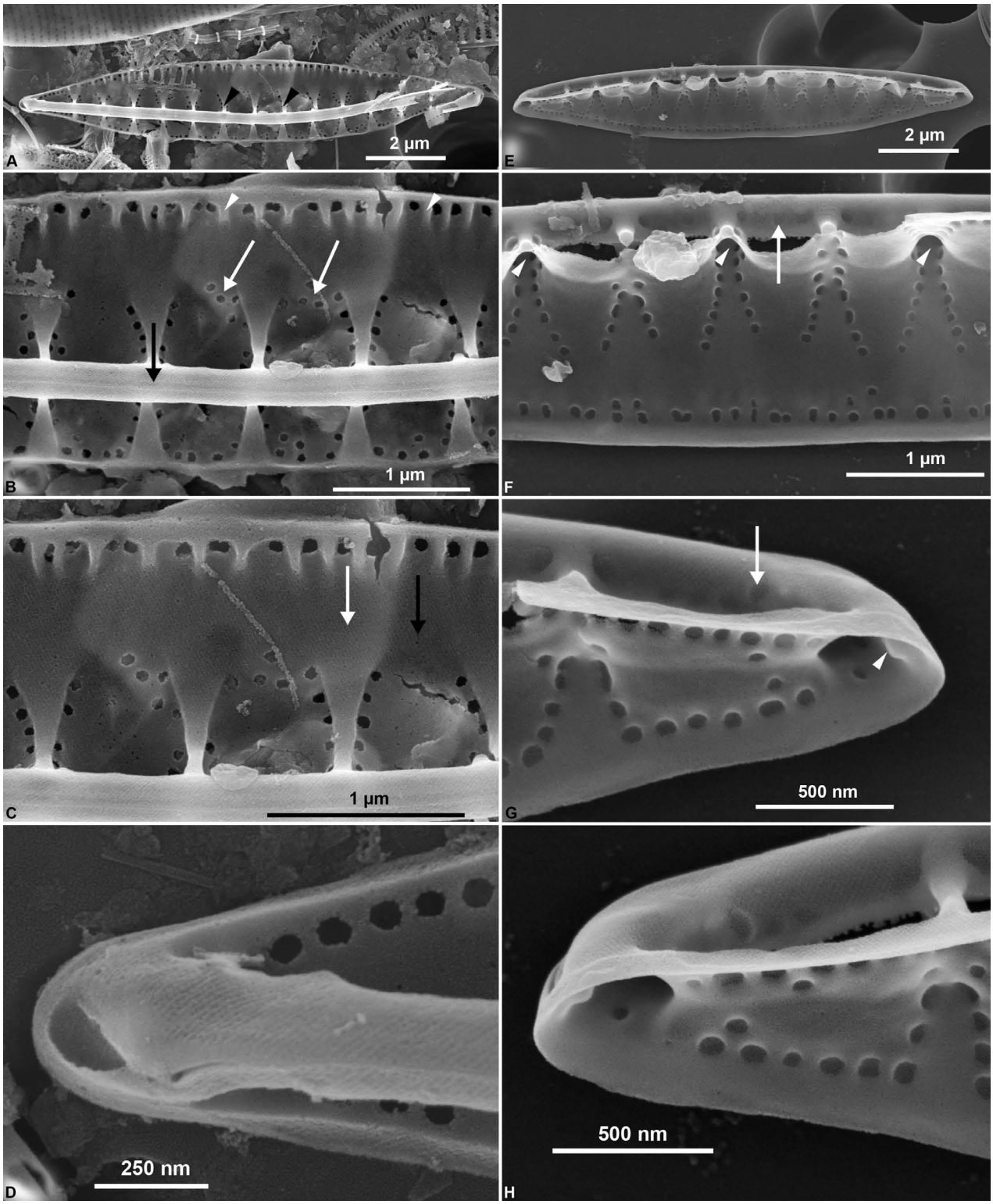

Figure 5 – *Simonsenia* cf. *paucistriata* in SEM: A, external valve view of the whole specimen. Note slightly eccentric canal raphe suspended on fenestral braces (arrowheads); B–D, close-ups of the specimen illustrated in A; B, external view of the valve central part. Note the sparse areolation of the transapical striae (white arrows) and the triangular shape of the transapical ribs, which are multiply forked near the distal valve mantle (arrowhead) and raphe slit (black arrow) in B. Note the elevated transapical ribs (white arrow) and depressed position of the transapical striae (black arrow) in C. The close up of the valve apex with bent external terminal raphe end in D; E, internal valve view; F–H, series of close-ups of specimen illustrated in E. Note the internal surface of the raphe canal (arrow) and the openings of the portulae (arrowheads) in F; G–H, close-ups of the internal view at the valve apices. Note the opening of the raphe canal with small helictoglossa in it (arrowhead) and the areolation on the internal surface of the raphe canal (arrow) in G.

12–14 in 10 µm; the apex of the triangle is pointing into the proximal mantle. Each stria is composed of irregularly arranged areolae (arrows in fig. 5B, but see also fig. 5C), which continue over surface of portulae into the alar canal and up to the canal raphe. Transapical ribs (virgae) elevated above valve surface and likewise triangular, multiply forked near distal valve margin (arrowheads in fig. 5B). Areolae small, circular, with hymenate occlusions. Girdle not observed thus far.

Comments – The major difference observed between the SEM morphology of this taxon and that of *S. paucistriata* from Texas is the more regular areolation. The areolae are positioned around the valve margin and mark the contact between the transapical ribs and the striae. This could, however, result from the fact that the Yellow Sea specimens examined here originated from a fresh sample and are much more strongly silicified, while the weak silicification in the Gulf of Mexico specimens might be an artifact of culture conditions. Because of the lack of DNA data from the Yellow Sea specimens, we currently refer to it as *S.* cf. *paucistriata* until more data are available.

Distribution and autecology – *Simonsenia* cf. *paucistriata* is only known so far from samples scraped off tubes belonging to enclosures used for fish aquaculture in Shinan-County in Korea. The salinity of the sampling habitat was measured at 33.1 psu.

Molecular phylogeny of *Simonsenia*

The DNA sequence data supported the placement of *Simonsenia* within the family Bacillariaceae and the monophyly of the genus itself (bootstrap value, bv = 81%), with *S. paucistriata* from the USA sister to *S. aveniformis* and *S. eileencoxiae* from Korea. The *Simonsenia* clade is sister to a clade consisting of some taxa of *Nitzschia* (including *N. amphibia* Grunow, *N. frustulum* (Kütz.) Grunow and *N. inconspicua* Grunow), *Denticula*, *Fragilariopsis* and *Pseudo-nitzschia*, but support for this relationship is very low (bv = 54%). Within this clade, monophyletic *Pseudo-nitzschia* (bv = 96%), grouped with *Fragilariopsis cylindrus*, and this assemblage was sister to the group consisting of *N. aurariae*, *N.* cf. *pusilla* (the identification of this sequence in Genbank as *N. frustulum* was revised by Rovira et al. 2015), *N. valdestriata*, *Nitzschia* sp. (SZCZCH658), *N. amphibia* and *N. inconspicua*, but with low support (fig. 6).

DISCUSSION

The molecular phylogeny and morphology of the genus *Simonsenia* was described and discussed in detail in Witkowski et al. (2014, 2015), and nothing in the current study contradicts the conclusions in those articles. To avoid repetition, we will focus here on the comparison of the new taxa with known species. It is noteworthy that the molecular phylogeny reveals that *S. eileencoxiae* has a closer relationship with *S. aveniformis* than with *S. paucistriata*, which has 92.6% *rbc*L sequence similarity with *S. eileencoxiae*. Morphologically, the differences between *S. eileencoxiae* and *S. paucistriata* are obvious: the position of the canal raphe is marginal in *S. eileencoxiae* and *S. aveniformis* (Witkowski et al. 2015), but slightly displaced towards the valve centre in *S. paucistriata* and *S.* cf. *paucistriata*. *Simonsenia delognei* and *S. delicatula* also have marginal raphe systems. The second novel morphological character for the genus present in *S. paucistriata* and *S.* cf. *paucistriata* is the undulate valve face with triangular striae and transapical ribs. The presence of multiply forked transapical ribs also seems to be unique to *S. paucistriata*, since it was not observed in *S. aveniformis*, *S. delicatula*, *S. delognei* and *S. maolaniana*, where the transapical ribs and striae are linear. The morphological characters that define *S. paucistriata* and *S.* cf. *paucistriata* have not been observed in any of the known freshwater (cf. Lange-Bertalot 1979, Kociolek 2012, Witkowski et al. 2014, You et al. 2016) or brackish/marine (Witkowski et al. 2015) *Simonsenia* taxa.

When compared to established taxa, *S. eileencoxiae* to some extent resembles *S. delognei*, as the two taxa overlap in terms of valve length and width. They differ, however in the shape of the valve apices and the densities of the fenestral bars and transapical ribs (cf. table 2). The two taxa are similar in terms of the origins of the fenestral braces, each being born from one transapical rib (table 2). However, the braces differ in shape, being rectangular on the valve face and becoming cylindrical as a support of the canal raphe in *S. eileencoxiae*, but cylindrical for their whole extent in *S. delognei*. The pore sizes on the fibulae vary significantly, with smaller pores in *S. delognei*; the areolae in the biseriate striae of *S. delognei* are located roughly opposite each other but alternate in *S. eileencoxiae* (Lange-Bertalot 1979, Witkowski et al. 2014, this paper). *Simonsenia eileencoxiae* would be difficult to confuse with *S. delicatula* and *S. aveniformis*. In the case of *S. aveniformis*, the major differences are the forked transapical bars (virgae) and the fact that single fenestral bars originate from two transapical bars, whereas in *S. eileencoxiae* the transapical bars are not forked. In *S. delicatula*, the transapical and fenestral bars are much finer and cylindrical (though slightly flattened) unlike in *S. eileencoxiae*, where they are thick and rectangular, in part confined to the valve face. In addition, close to the distal valve mantle, the transapical bars in *S. delicatula* are forked (Witkowski et al. 2015).

Simonsenia paucistriata bears no strong similarity to any established species. The raphe canal is suspended on the fenestral braces and slightly displaced as opposed to being strongly eccentric like in other taxa. No other species of *Simonsenia* so far has been characterized by triangular striae and transapical ribs. The transapical ribs are further multiply forked along valve distal mantle, which has not been recorded thus far. The transapical bars in *S. delicatula* are also forked (Witkowski et al. 2015), but always dichotomously, not multiply as in *S. paucistriata*.

Among the new species there are distinct differences in ultrastructure (cf. table 2). The most significant difference (also visible in LM) is the position of the canal raphe. In *S. eileencoxiae* the canal raphe has a strong marginal position close to the proximal mantle and resembles *S. delognei* (cf. Witkowski et al. 2014, 2015). In *S. paucistriata* and *S.* cf. *paucistriata* the canal raphe is slightly displaced from the valve midline and the proximal valve mantle is relatively broad when compared to the distal mantle and hence can be

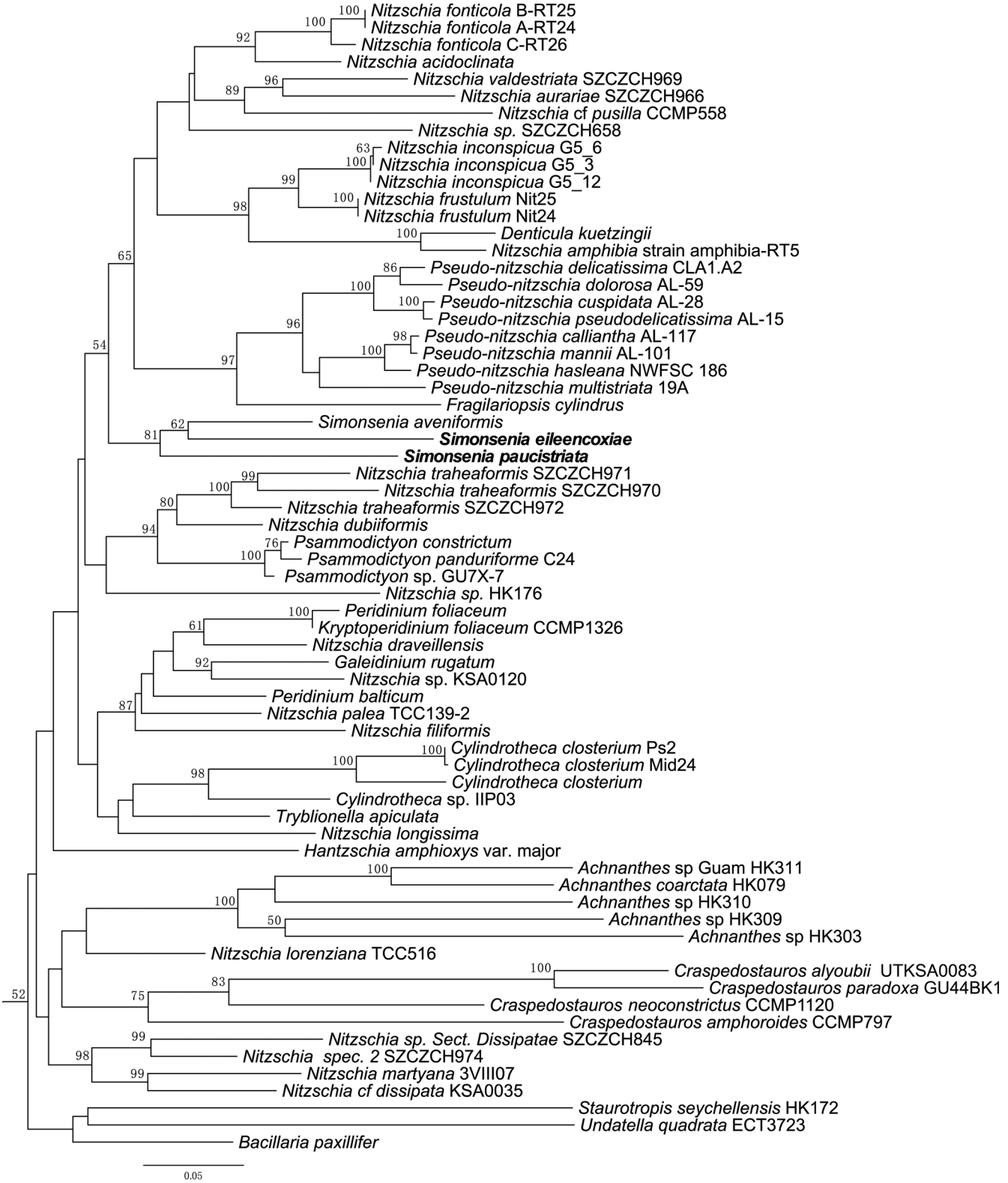

Figure 6 – Phylogenetic tree reconstructed from concatenated three gene (SSU+*rbc*L+*psb*C) data set. Taxa described in this paper as new to science are in bold type.

Table 2 – ***Simonsenia*** **species morphological data.**
The three taxa treated in this paper are compared to the known species. ND – not determined. [1] difficult to observe due to paucity of areolae, but apparently where observed their number changes from one to several.

Taxon/ character	*S. eileencoxiae*	*S. paucistriata*	*S.* cf. *paucistriata*	*S. aveniformis*	*S. delognei*	*S. delicatula*	*S. maolaniana*
Source of information	This paper	This paper	This paper	Witkowski et al. 2015	Lange-Bertalot 1979, Witkowski et al. 2014	Mikhailov & Makarova 1983	You et al. 2016
Valve length (µm)	12–13, n = 15	12–13, n = 20	11–12, n = 3	7.5–13.7	8.0–17.2	12.0–22.0	8.9–25.4
Valve width (µm)	1.9–2.0	1.8–2.3	19–2.1	2.0–2.5	1.6–2.0	2.0–2.5	2.3–3.4
raphe position	marginal/ eccentric	near-central	near-central	marginal/ eccentric	marginal/ eccentric	marginal/ eccentric	marginal/ eccentric
Transapical ribs (in 10 µm)	24–26	13–14	12–14	50–60	16–22	18–20	13–17
Fenestral bars (in 10 µm)	24–26	13–14	12–13	24–25	16–22	18–20	13–17
Fibulae (in 10 µm)	11–12	6–7	6–7	c. 12	11	8–9	6–7
Fenestral bar position	born on each transapical rib	born on each transapical rib	born on each transapical rib	born from two adjacent transapical ribs	born on each transapical rib	born on each transapical rib	born on each transapical rib
Transapical ribs	simple	forked distally	forked distally	simple	simple	forked distally	simple
Striae	biseriate	multiseriate[1]	multiseriate[1]	Uniseriate	bi- to multiseriate	bi- to multiseriate	bi- to multiseriate
Valve face	undulate	strongly undulate	strongly undulate	± flat	undulate	undulate	undulate
Structure of the most advalvar bands	two rows of poroids	two rows of poroids	ND	two rows of poroids	a single row of poroids	ND	ND

observed even in LM as a distinct line suspended on braces when specimens are seen in girdle view. The fenestrae and sometimes the alar canals (fig. 4B–E) are likewise visible.

Although *Simonsenia* still contains a small number of species, our recent exploration of unexplored marine habitats, during which we have specifically focused on small and new species in Bacillariaceae, including *Simonsenia*, shows that its diversity is probably much higher. Indeed, other new *Simonsenia* species have already been observed in the marine littoral of the island of Martinique, along the east coast of the United States and in South Africa (Witkowski, unpublished observations).

Based on our current background on *Simonsenia* spp., marine habitats are much more promising in terms of diversity, but definitely not abundance. All the formally described marine species, including those in this paper, have only been observed from cultures (enrichment wells) where fresh samples were placed and cells divided for several days before isolation. The only exception is the taxon from the fish farm in Korea where, possibly due to eutrophic conditions, *Simonsenia* cf. *paucistriata* was abundant enough to be observable in SEM of natural populations. The other species have been barely observable in fresh samples.

Freshwater habitats are, in places, better studied and the presence of *Simonsenia* either with low or higher abundance has been reported from many freshwater riverine systems; it is sometimes abundant in springs (cf. Żelazna-Wieczorek 2012, Witkowski et al. 2014). With increasing records of *Simonsenia*, a more complete picture of its ecology and distribution is beginning to emerge. Of particular interest is the first record by You et al. (2016) of the occurrence of *S. delognei* in continental China, in karstic waters of Guizhou Province in Southwestern China. In fact, in areas where floristic studies were carried out regularly, *S. delognei* is frequently reported, its abundances are estimated, and in some cases the distribution maps and information were published (e.g. Kelly et al. 2005, Kociolek 2012, Noga et al. 2014, Cantonati et al. 2017, Jüttner et al. 2010, 2019). With these new records and descriptions, we now know that *Simonsenia* species can be found in freshwater, brackish-water and marine habitats. The current article suggests that brackish-water/marine environments might have more species of *Simonsenia* and the potential for finding new species is higher here than in freshwater habitats. In terms of substratum preference in marine environments, *Simonsenia* apparently occurs in the epilithon (*S. eileencoxiae*, *S. aveniformis*; Witkowski et al. 2015) and in the tychoplankton or epipsammically (*S. paucistriata* in Texas).

Just as the SEM quickly became a valuable tool for studying and documenting diatom diversity, allowing researchers a greater number and span of characters to explore and compare on the diatom frustule, the use of molecular markers for

documenting species has been a similar breakthrough, providing a new dataset of characters shared by all diatoms to use in describing, identifying and comparing taxa. In recent years, this has been particularly well documented in small celled, morphologically-indistinct taxa belonging to the Cymatosiraceae (Dąbek et al. 2017), Plagiogrammaceae (Li et al. 2015), Fragilariaceae (Li et al. 2016, 2018) and Bacillariaceae (Rovira et al. 2015, Witkowski et al. 2015). While the particular case of *Simonsenia* discussed in this manuscript did not "require" DNA sequence data to resolve the taxon from morphologically similar congeneric taxa, we have included the sequence data anyway as part of a "total evidence" approach to taxonomy.

We wish to stress that the inclusion of DNA sequence data is not part of any effort to craft a narrative where sequence data are required for taxon description or molecular markers being some sort of "superior" data type. Molecular markers are simply another tool in the diatomists' tool kit for taxonomy and identification, valued for their diagnostic utility across the diversity of diatoms; while some taxa might lack specific morphological structures for comparison (spines, pore fields, portulae), all diatoms possess certain molecular markers (ribosomal RNA and almost always RUBISCO). Additionally, molecular markers provide us a wealth of characters; a feature-rich frustule might provide us with a half-dozen or so characters to document and compare, while a single DNA sequence can provide hundreds or thousands of characters per taxon. However, much like SEM morphology and ultrastructure data, DNA sequence data suffer behind LM morphological data as the "bellwether" data for taxonomy simply because of their paucity; diagnostic utility for a data type in identification requires a large comparative database, and at this point in time, the 200+ years of documented LM morphology is still the largest pool for data for comparison. Likewise, access to SEM and DNA sequencing facilities is still not universal and can be cost prohibitive, limiting their effectiveness. Interpreting molecular markers for taxonomic decisions can fall into the same pitfalls that lead to ambiguity in evaluating morphological characters (be they LM or SEM) as we decide how much variation within characters of any data type can exist within/between species or genera. But for now, we will operate under the assumption that these are problems that can and will be addressed with time, effort and the relentless advance of technology augmenting the collection of all types of data. Even if DNA sequence data is not "required" for the identification of these *Simonsenia* species, we feel obligated to provide these data for future generations of researchers, much as past diatomists provided the LM and SEM morphological data we currently use as the foundation of diatom taxonomy.

SUPPLEMENTARY DATA

Supplementary data are available at *Plant Ecology and Evolution*, Supplementary Data Site (https://www.ingentaconnect.com/content/botbel/plecevo/supp-data), and consist of: (1) GenBank accession numbers of the species used in the phylogenetic reconstruction (pdf); (2) *Simonsenia* alignment (NEXUS file); and (3) phylogenetic tree *rbc*L partial gene sequences.

ACKNOWLEDGMENTS

Dr. Izabela Zgłobicka, Warsaw University of Technology, and dr. Łukasz Peszek, University of Rzeszów, are acknowledged for their help with TEM and SEM examination, Prof. Gao Yahui, Xiamen University, for sharing the image of *Denticula subtilis* Grunow *sensu* Cheng et al. (1993). Funding from Polish Ministry of Science Topical Subsidy to the University of Szczecin. Project Maestro from Polish National Science Centre (NCN) in Cracow grant no. N2012/04/A/ST10/00544 and a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR201839201 and NIBR201801205) are acknowledged.

REFERENCES

Bey M.-Y., Ector L. (2013) Atlas des diatomées des cours d'eau de la région Rhône-Alpes. DREAL Auvergne-Rhône-Alpes, Direction regionale de l'Environnement, de l'Aménagement et du Logement. Tome 6: 973–1182. Available from http://webissimo.developpement-durable.gouv.fr/IMG/pdf/Tome6_2013_print_cle1cb18a.pdf [accessed 29 Mar. 2019].

Cantonati M., Kelly M.G., Lange-Bertalot H. (2017) Freshwater benthic diatoms of Central Europe: over 800 common species used in ecological assessment. Schmitten-Oberreifenberg, Koeltz Botanical Books.

Cheng Z.D., Gao Y.H., Liu S.C. (1993) Nanodiatoms from Fujian Coast. Beijing, China Ocean Press. [in Chinese].

Dąbek P., Ashworth M., Witkowski A., Li Ch., Bornman T.G., Gonçalves V., Park J., Khim J. (2017) Towards a multigene phylogeny of the Cymatosiraceae (Bacillariophyta, Mediophyceae) I: Novel taxa within the subfamily Cymatosiroideae based on molecular and morphological data. Journal of Phycology 53: 342–360. https://doi.org/10.1111/jpy.12501

Gao Y.H., Yang Q.L., Chen C.P. (2012) Marine diatoms in China's seas. In: Huang Z.G., Lin M. (eds) An illustrated guide to species in China's seas: 23–240. Xiamen, Ocean Press.

Hall T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Hasle G.R., Fryxell G.A. (1970) Diatoms: cleaning and mounting for light and electron microscopy. Transactions of the American Microscopical Society 89: 469–474. https://doi.org/10.2307/3224555

Jones H.M., Simpson G.E., Stickle A.J., Mann D.G. (2005) Life history and systematics of Petroneis (Bacillariophyta), with special reference to British waters. European Journal of Phycology 40: 61–87. https://doi.org/10.1080/09670260400024675

Jüttner I., Chimonides P.J., Ormerod S.J. (2010) Using diatoms as quality indicators for a newly-formed urban lake and its catchment. Environmental Monitoring and Assessment 162: 47–65. https://doi.org/10.1007/s10661-009-0775-2

Jüttner I. (2019) Simonsenia delognei (Grunow) Lange-Bertalot; 1979; 132. In: Jüttner I., Bennion H., Carter C., Cox E.J., Ector L., Flower R., Jones V., Kelly M.G., Mann D.G., Sayer C., Turner J.A., Williams D.M. (eds) Freshwater Diatom Flora of Britain and Ireland. Amgueddfa Cymru – National Museum Wales. Available from https://naturalhistory.museumwales.ac.uk/diatoms/browsespecies.php?-recid=2792 [accessed 22 Jul. 2018].

Kelly M.G., Bennion H., Cox E.J., Goldsmith B., Jamieson J., Juggins S., Mann D.G., Telford R.J. (2005) Common freshwater

diatoms of Britain and Ireland: an interactive key. CD-ROM. Bristol, Environment Agency. ISBN 978-1-84-432705-8.

Kociolek J.P. (2012) Simonsenia. In: Diatoms of North America. Retrieved July 19, 2018. Available from https://diatoms.org/genera/simonsenia [accessed 19 Jul. 2018].

Krammer K., Lange-Bertalot H. (1988) Bacillariophyceae, Teil 2: Bacillariaceae, Epithemiaceae, Surirellaceae. In: Ettl H., Gerloff J., Heynig H., Mollenhauer D. (eds) Süsswasserflora von Mitteleuropa, vol. 2 (2). Stuttgart & New York, G. Fischer.

Lange-Bertalot H. (1979) Simonsenia, a new genus with morphology intermediate between Nitzschia and Surirella. Bacillaria 2: 127–136.

Li Ch., Ashworth M.P., Witkowski A., Dąbek P., Medlin L.K., Kooistra W.H.C.F., Sato S., Zgłobicka I., Kurzydłowski K.J., Theriot E.C., Sabir J.S.M., Khiyami M.A., Mutwakil M.H.Z., Sabir M.J., Alharbi N.S., Hajarah N.H., Qing S., Jansen R.K. (2015) New insights into Plagiogrammaceae (Bacillariophyta) based on multigene phylogenies and morphological characteristics with the description of a new genus and three new species. PLoS ONE 10(10): e0139300. https://doi.org/10.1371/journal.pone.0139300

Li C.L., Ashworth M.P., Witkowski A., Lobban C.S., Zgłobicka I., Kurzydłowski K.J, Qin S. (2016) Ultrastructural and molecular characterization of diversity among small araphid diatoms all lacking rimoportulae. I. Five new genera, eight new species. Journal of Phycology 52: 1018–1036. https://doi.org/10.1111/jpy.12460

Li C.L., Witkowski A., Ashworth M.P., Dąbek P., Sato S., Zgłobicka I., Witak M., Khim J.S., Kwon C.-J. (2018) The morphology and molecular phylogenetics of some marine diatom taxa within the Fragilariaceae, including twenty undescribed species and their relationship to Nanofrustulum, Opephora and Pseudostaurosira. Phytotaxa 355: 1–104. https://doi.org/10.11646/phytotaxa.355.1.1

Maddison W.P., Maddison D.R. (2015) Mesquite: a modular system for evolutionary analysis, version 3.04. Available from: http://mesquiteproject.org [accessed 29 Mar. 2019].

Mann D.G. (1978) Studies in the family Nitzschiaceae (Bacillariophyta). PhD thesis, University of Bristol, Bristol, UK. Available from http://rbg-web2.rbge.org.uk/algae/publications_mann_thesis.html [accessed 29 Mar. 2019].

Mann D.G. (1986) Nitzschia subgenus Nitzschia (Notes for a monograph of the Bacillariaceae 2). In: Ricard M. (ed.) Proceedings of the 8th International Diatom Symposium; 215–226. Koenigstein, Otto Koeltz.

Mikhailov V.I., Makarova I.V. (1983) Simonsenia delognei and Simonsenia delicatula (Bacillariophyta) – the first representatives of the genus Simonsenia in the USSR. Botanicheskij Zhurnal 68: 1245–1246.

Nawrocki E.P. (2009) Structural RNA homology search and alignment using covariance models. PhD thesis, Washington University in Saint Louis, School of Medicine, St Louis, USA.

Noga T., Kochman N., Peszek Ł., Stanek-Tarkowska J., Pajączek A. (2014) Diatoms (Bacillariophyceae) in rivers and streams and on cultivated soils of the Podkarpacie Region in the years 2007–2011. Journal of Ecological Engineering 15: 6–25. https://doi.org/10.12911/22998993.1084168

Round F.E., Crawford R.M., Mann D.G. (1990) The diatoms. Biology and morphology of the genera. Cambridge, Cambridge University Press.

Rovira L., Trobajo R., Sato S., Ibáñez C., Mann D.G. (2015) Genetic and physiological diversity in the diatom Nitzschia inconspicua. Journal of Eukaryotic Microbiology 62: 815–832. https://doi.org/10.1111/jeu.12240

Ruck E.C., Kociolek P. (2004) Preliminary phylogeny of the family Surirellaceae – (Bacillariophyta). Bibliotheca Diatomologica, vol. 50. Berlin & Stuttgart, J. Cramer.

Ruck E.C., Theriot E.C. (2011) Origin and evolution of the canal raphe system in diatoms. Protist 162: 723–737. https://doi.org/10.1016/j.protis.2011.02.003

Ruck E.C., Nakov T., Alverson A.J., Theriot E.C. (2016) Phylogeny, ecology, morphological evolution, and reclassification of the diatom orders Surirellales and Rhopalodiales. Molecular Phylogenetics and Evolution 103: 155–171. https://doi.org/10.1016/j.ympev.2016.07.023

Stamatakis A. (2014) RAxML Version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stepanek J.G., Hamsher S.E., Mayama S., Jewson D.H., Kociolek J.P. (2016) Observations of two marine members of the genus Cymbellonitzschia (Bacillariophyta) from Tokyo Bay, Japan, with the description of the new species Cymbellonitzschia banzuensis. Phycological Research 64: 26–34. https://doi.org/10.1111/pre.12110

Theriot E.C., Ashworth M., Ruck E., Nakov T., Jansen R.K. (2010) A preliminary multigene phylogeny of the diatoms (Bacillariophyta): challenges for future research. Plant Ecology and Evolution 143: 278–296. https://doi.org/10.5091/plecevo.2010.418

Thomas E.W. (2016) Exploring species boundaries in the diatom genus Rhoicosphenia using morphology, phylogeny, ecology, and biogeography. PhD thesis, University of Colorado, Denver, USA. Available from https://scholar.colorado.edu/ebio_gradetds/87 [accessed 29 Mar. 2019].

Werum M., Lange-Bertalot H. (2004) Diatoms in springs from Central Europe and elsewhere under the influence of hydrogeology and anthropogenic impacts. In: Lange-Bertalot H. (ed.) Iconographia Diatomologica, vol. 13. Ruggell, A.R.G. Gantner.

Witkowski A., Barka F., Mann D.G., Li C.L., Weisenborn J.L.F., Ashworth M.P., Kurzydłowski K.J., Zgłobicka I., Dobosz S. (2014) A description of Biremis panamae sp. nov., a new diatom species from the marine littoral, with an account of the phylogenetic position of Biremis D.G. Mann et E.J. Cox (Bacillariophyceae). PLoS ONE 9: e114508. https://doi.org/10.1371/journal.pone.0114508

Witkowski A., Gomes A., Mann D.G., Trobajo R., Li C.H., Barka F., Gusev E., Dąbek P., Grzonka J., Kurzydłowski K.J., Zgłobicka I., Harrison M., Boski T. (2015) Simonsenia aveniformis sp. nov. (Bacillariophyceae), molecular phylogeny and systematics of the genus, and a new type of canal raphe system. Scientific Reports 5: 17115. https://doi.org/10.1038/srep17115

You Q., Kociolek J.P., Yu P., Cai M., Lowe R., Wang Q. (2016) A new species of Simonsenia from a karst landform, Maolan Nature Reserve, Guizhou Province, China. Diatom Research 31: 269–275. https://doi.org/10.1080/0269249X.2016.1227377

Żelazna-Wieczorek J. (2011) Diatom flora in springs of Łódź Hills (Central Poland). In: Witkowski A. (ed.) Diatom Monographs, vol. 13. Ruggell, A.R.G. Gantner.

Managing Editor: Ingrid Jüttner

Submission date: 6 Sep. 2018

Acceptance date: 11 Mar. 2019