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Morphology and molecular phylogeny of *Pleurosira nanjiensis* sp. nov., a new marine benthic diatom from the Nanji Islands, China

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Abstract

A new marine benthic diatom, *Pleurosira nanjiensis* sp. nov., is described from the rocky intertidal zone of the Xiaochaiyu Island of the Nanji Islands in China. Its morphology was examined with light and scanning electron microscopy. Molecular phylogeny was reconstructed based on SSU rRNA and *rbc*L gene sequences. *Pleurosira nanjiensis* differs from congeners in possession of a combination of morphological features including the domed valve with broadly lanceolate, elliptical or circular valve outline, two elevated marginal ocelli, two (rarely three) rimportulae, and radiate striae.

Key words: *Pleurosira nanjiensis*, rocky intertidal area, marine diatom, Nanji Islands, new species

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1 Introduction

Trevisan (1848) erected the genus *Pleurosira* Meneghini for members of a subgenus in *Melosira* Agardh, which has cylinder cells connected by mucilage. Based on the redefinition of Compère (1982), *Pleurosira* is characterized by a cylindrical frustule, circular to broadly elliptical valve with clear separation between the usually flat valve face and a vertical mantle, the possession of poroid areolae, 2–4 marginal ocelli and 2–15 labiate processes located about midway between the valve centre and margin.

To date, the genus *Pleurosira* contains five species including four varieties and one forma (Compère, 1982; Guiry and Guiry, 2017). Among these, two species were reported only from freshwater environments. *Pleurosira laevis* f. *laevis* (Ehrenberg) Compère inhabits both freshwater and brackish environments and *P. laevis* f. *polymorpha* Compère dwells in both brackish and marine environments (Compère, 1982), while *P. inusitata* (Hohn and Hellerman) Desianti and Potapova and *P. socotrensis* var. *bengalensis* Compère have been reported only from brackish waters (Compère, 1982; Desianti et al., 2015).

In terms of distribution, *P. indica* Karthick and Kociolek, *P. socotrensis* (Kitton) Compère and three varieties of *P. socotrensis* were found from tropical Asia (Compère, 1982; Karthick and Kociolek, 2011). *Pleurosira inusitata* and *P. minor* were described from North and South America, respectively (Metzeltin et al., 2005; Desianti et al., 2015). Only *P. laevis* have been reported worldwide. Among these, *P. laevis*, *P. socotrensis* and *P. minor* were also reported in China (Pei et al., 2008; Liu et al., 2011). In the present study, we describe a new species of *Pleurosira* from a rocky intertidal zone in the East China Sea and its phylogenetic position is investigated with DNA sequencing. The morphological delimitation of the genus *Pleurosira* is also discussed.

2 Materials and methods

2.1 *Sample collection, cultivation and morphological observation*

Samples of benthic diatoms were collected from the lower rocky intertidal zone of the Xiaochaoyu Island (27°25.348′N, 121°05.459′E) of the Nanji Islands on Chinese coast of the East China Sea on 15 May 2015. Single cells were isolated from the samples and transferred to F/2 medium. Clonal cultures were established and maintained at 20–23°C, with 20–30 μmol photons m^{-2} s^{-1} from cool-white fluorescent tubes. The photoperiod was 14:10 light:dark (L:D).

Cleaned frustules were prepared by the bleach solution method (Nagumo and Kobayasi, 1990) and were mounted on a glass slide with Mountmedia (Wako Pure Chemical Industries, Ltd. Osaka, Japan). Nikon Eclipse 80i light microscopes (LM) equipped with differential interference contrast (DIC) were used for LM observation. For scanning electron microscope (SEM) observation, vegetative cells were fixed with 2.5% glutaraldehyde before cleaning. Cleaned frustules were air-dried and coated with

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osmium for SEM observation. A Hitachi S-3400 was used for SEM observation.

Terminology follows Anonymous (1975), Ross and Sims (1971), and Compère (1982).

2.2 *DNA extraction, sequencing and phylogenetic analysis*

Diatom pellets obtained by centrifuging the liquid cultures for 5 min at 1 000 *g*. Total DNA was extracted using Plant Genomic DNA Kit (Tiangen Biotech Co., China). Partial fragments of 18S small subunit rDNA (SSU rDNA) sequence and the chloroplast encoded large subunit of RUBISCO (*rbc*L) gene were amplified by polymerase chain reaction (PCR). The volume of each PCR reaction was 25 μL, containing 2.0 μL template DNA, 12.5 μL of 2× EasyTaq PCR SuperMix polymerase (TransGen Biotech, China), 0.5 μL of each primer (10 mmol/L) and sterile distilled H_2O. Primers *SSU*1 and ITS1DR were used to amplify SSU rDNA (Medlin et al., 1988; Edgar and Theriot, 2004). Primers *rbc*L 66+ and *rbc*L 1444– were used to amplify *rbc*L gene (Alverson et al., 2007; Ruck and Theriot, 2011). The PCR cycles for the two markers followed Alverson et al. (2007). PCR products were purified using the TIANgel Midi Purification Kit (Tiangen Biotech Co., China) and sequenced by an ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

The sequences were aligned using MAFFT v. 7 and further modified manually in Mesquite v. 3.2 (Katoh and Standley, 2013; Maddison and Maddison, 2017). Highly variable regions in which the alignment could not be determined unambiguously were excluded before phylogenetic analysis. The final alignment of a concatenated alignment of SSU rDNA and *rbc*L gene sequences included 3 036 positions. The concatenated alignment of SSU rDNA and *rbc*L gene sequences partitioned by different gene, and in the case of *rbc*L, by codon position. The GTR+G+I model was selected under the AICc criterion by Partitionfinder 2 for all partitions except the GTR+G model for the second condon of *rbc*L (Lanfear et al., 2017)

Maximum likelihood (ML) analyses were performed with RAxML v8.0.0 (Stamatakis, 2014). The reliability of internal branches was assessed using a non-parametric bootstrap method with 1 000 replicates. Gaps were treated as missing data. Bayesian inference (BI) analyses were carried out with MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). The programs ran for 10^7 generations with trees sampled every 1 000 generations and the first 25% of trees were discarded as burn-in. Convergence was judged based on the average standard deviation of split frequencies (all less than 0.01) and the ESS values (more than 4 000) analyzed in the R Package RWTY (Warren et al., 2017). The remaining trees were used to generate a consensus tree and calculate the posterior probabilities of all branches using a majority-rule consensus approach. FigTree v1.4.2 and Adobe illustrator CS6 were used to view and edit trees for publication.

3 Results

3.1 *Morphological description*

***Pleurosira nanjiensis* Yuhang Li, Nagumo and Kuidong Xu sp. nov.**

Diagnosis: Valve domed with broadly lanceolate, elliptical or circular outline. Valve length 15.5–46.8 μm, width 13.0–31.1 μm, pervalvar height 13.5–15.2 μm. Valve with external spins. Two ocelli, ocellate elevation blunt widely rounded. Poroid areolae with domed cribra. Two (rarely three) rimoportula about midway between centre and margin. Six bands. Striae radiated 15–21 per 10 μm, 14–18 pores per 10 μm on bands.

Holotype: Holotype slide MBM285985 has been deposited in the Marine Biological Museum, Chinese Academy of Sciences (MBMCAS) at Qingdao, China

Type locality: Xiaochaiyu Island, Nanji Islands, Wenzhou City, Zhejiang Province, China.

Etymology: Named after the type locality Nanji Islands.

Distribution and ecology: The species is currently known only from the type locality, where it inhabited the rock surface in the lower intertidal zone.

Gene sequences: The SSU rDNA and *rbc*L gene sequences of *Pleurosira nanjiensis* have been deposited in GenBank with the accession number MF578764 and MF578765, respectively.

Description: Cells form zig-zag colony (Figs 1a, b). Plastids are discoid. Valves are domed (rarely flat in culture, Fig. 2b arrow) with broadly lanceolate, elliptical or circular outline (Figs 1c–h and 2a–f). Valve is 15.5–46.8 μm long, 13.0–31.1 μm wide, and 13.5–15.2 μm high (Figs 1c–k). Two conspicuous blunt and robust ocellate elevations are present at two poles (Fig. 2c arrows). Each ocellus is surrounded by a thin hyaline area (Fig. 2g). On the external valve surface, spins are randomly distributed (Fig. 2g arrow). Poroid areolae are occluded by domed cribra externally (Fig. 2e arrow) and round internal foramina (Fig. 3b) in different size. Striae are radiated, 15–21 per 10 μm (Figs 1c–h and 3a–b). Usually two, rarely three small rimoportulae are positioned on both sides of the axis passing through the ocelli, about midway between the centre and the margin (Figs 1d, g–h, 2d and 3a–b arrows). The valve constricts near the margin (Figs 1j and 2h arrow). The cingulum is composed of six porous open bands in mature individuals, 14–18 per 10 μm (Figs 2b and 3b). The valvocopula has a dentate margin (Fig. 3c).

3.2 *Phylogenetic analysis*

The combined SSU rDNA and *rbc*L gene analysis showed that *Pleurosira nanjiensis* sp. nov. formed a robust clade with *P. laevis* and *P. laevis* f. *polymorpha*, the only two *Pleurosira* species have sequences information in GenBank, with strong nodal support (ML bootsrap=100%, Posterior probability=1.00). The genus *Odontella* Agardh is polyphyletic (Fig. 4). The *Pleurosira* clade is clustered with *Odontella aurita* (Lyngbye) Agardh, the generitype of *Odontella*, but with relatively lower support (ML bootstrap=79%, Posterior probability=0.94). *Odontella obtusa* Kützing, which resembles *P. nanjiensis* to some extent, is sister to *Triceratium dubium* Brightwell and *T. dictyotum* Sims and Ross.

4 Discussion

In Compère's definition of *Pleurosira*, the valve face is usually flat and distinctly separated from the vertical valve mantle, and the ocelli are not elevated from the general valve face. *Pleurosira nanjiensis* sp. nov. possesses a domed valve and ocellate elevations and thus are deviated from the generic diagnosis. Nonetheless, Compère (1982) ever described *P. laevis* f. *polymorpha* with a domed valve and elevated ocelli, a form that can tolerate higher salinity than *P. laevis* f. *laevis*. Likewise, a domed valve and elevated ocelli were reported in *P. inusitata* and *P. socotrensis* var. *benalensis*, both of which were found from brackish environments (Compère, 1982; Desianti et al., 2015). Moreover, these characters also occurred in the freshwater species *P. socotrensis*, when it was cultured in enriched seawater medium (Li and Chiang, 1979). Thus, both the ocellate elevation and the domed valve shape are likely associated with the saline environments these species inhabit, and should not be used to define the genus *Pleurosira*.

Based on a phylogenetic study of Biddulphiaceae and Eupod-

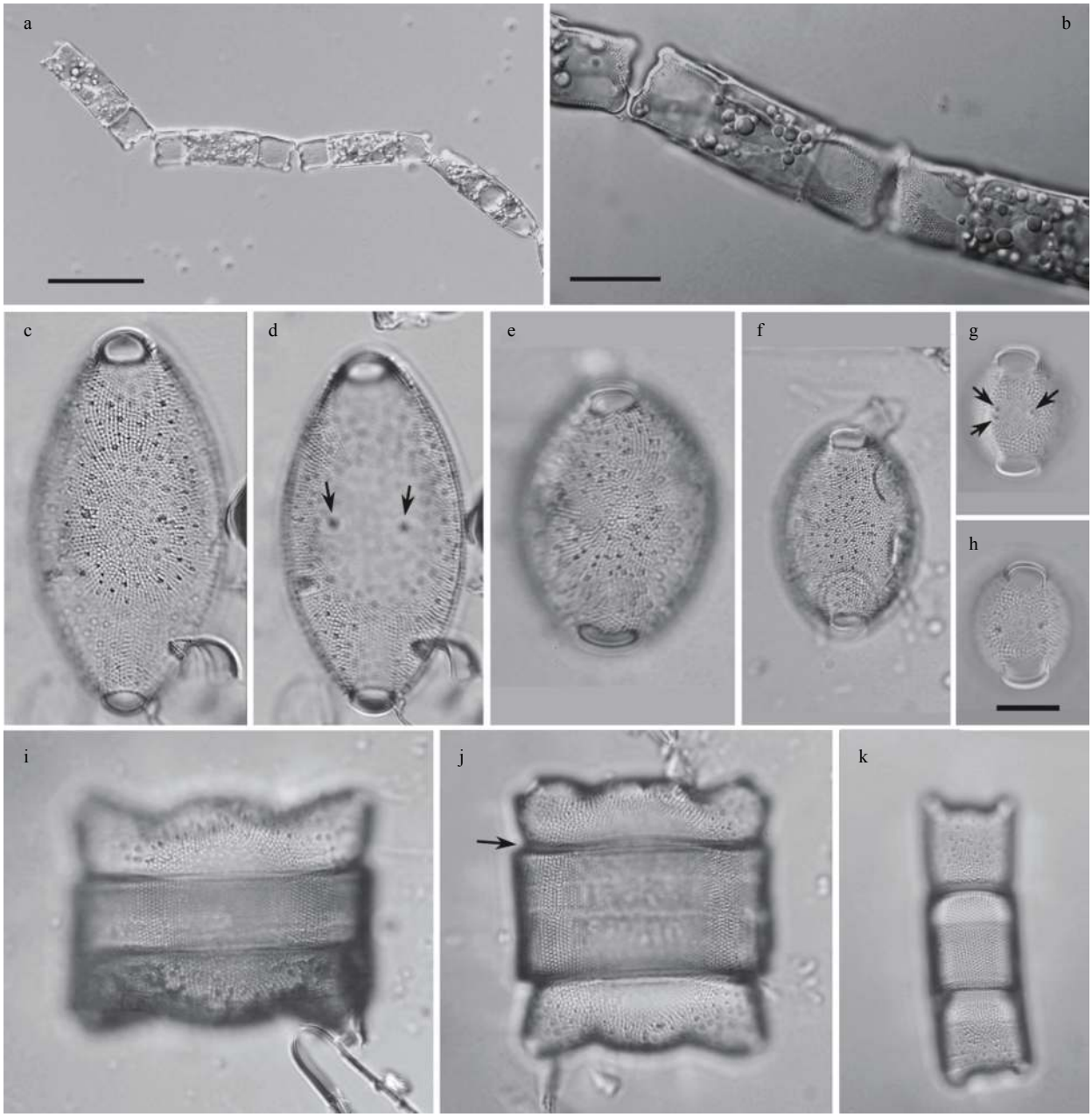

Fig. 1. LM micrographs of *Pleurosira nanjiensis*. Scale bar: 30 μm (a), 20 μm (b) and 10 μm (c–k). a–b. Zig-zag clone of *P. nanjiensis*, c–h. variation of valves from broadly laceolate to circluar, note the two or three rimoportulae (arrows), and i–h. variations of frustule in girdle view.

iscaceae, Ashworth et al. (2013) suggested that the morphological features such as the valve perforation and the position of rimoportulae and ocelli are important for discriminating the eupodiscacean diatoms (see Fig. 6 in Ashworth et al., 2013). Like congeners, *P. nanjiensis* has the rimoportulae present about the midway between the valve centre and margin on both side of the valve, and the simple valve perforation which is open in round forma internally. These important features together with the result of the molecular phylogenetic analysis support the assignment of *P. nanjiensis* to the genus *Pleurosira*.

Pleurosira nanjiensis differs from congeners by a combination of morphological features, including the domed valve with broadly lanceolate, elliptical or circular valve outline, two elevated marginal ocelli, two (rarely three) rimportulae, and radiate striae (Table 1). As mentioned above, *P. nanjiensis* resembles all the *Pleurosira* species that inhabits brackish or marine environment, namely *P. laevis* f. *polymorpha*, *P. inusitata* and *P. socotrensis* var. *benalensis*, in possessing a domed valve or elevated ocelli. However, *P. nanjiensis* has 2 or 3 rimoportulae, whereas there are 6–15 in *P. socotrensis* var. *benalensis*. *Pleurosira nanjiensis* differ from *P. inusitata* in the radiate striae (vs. irregular areolae in *P. inusitata*), and differ from *P. laevis* f. *polymorpha* in the broadly lanceolate valve outline. Furthermore, the two species can also be separated by the DNA sequences dissimilarity (Desianti et al., 2015). In addition, Metzeltin et al. (2005) described *P. minor* with a more or less broadly lanceolate outline, but the species has no domed valve and elevated ocelli.

Pleurosira nanjiensis is also similar to the generitype of *Odon-*

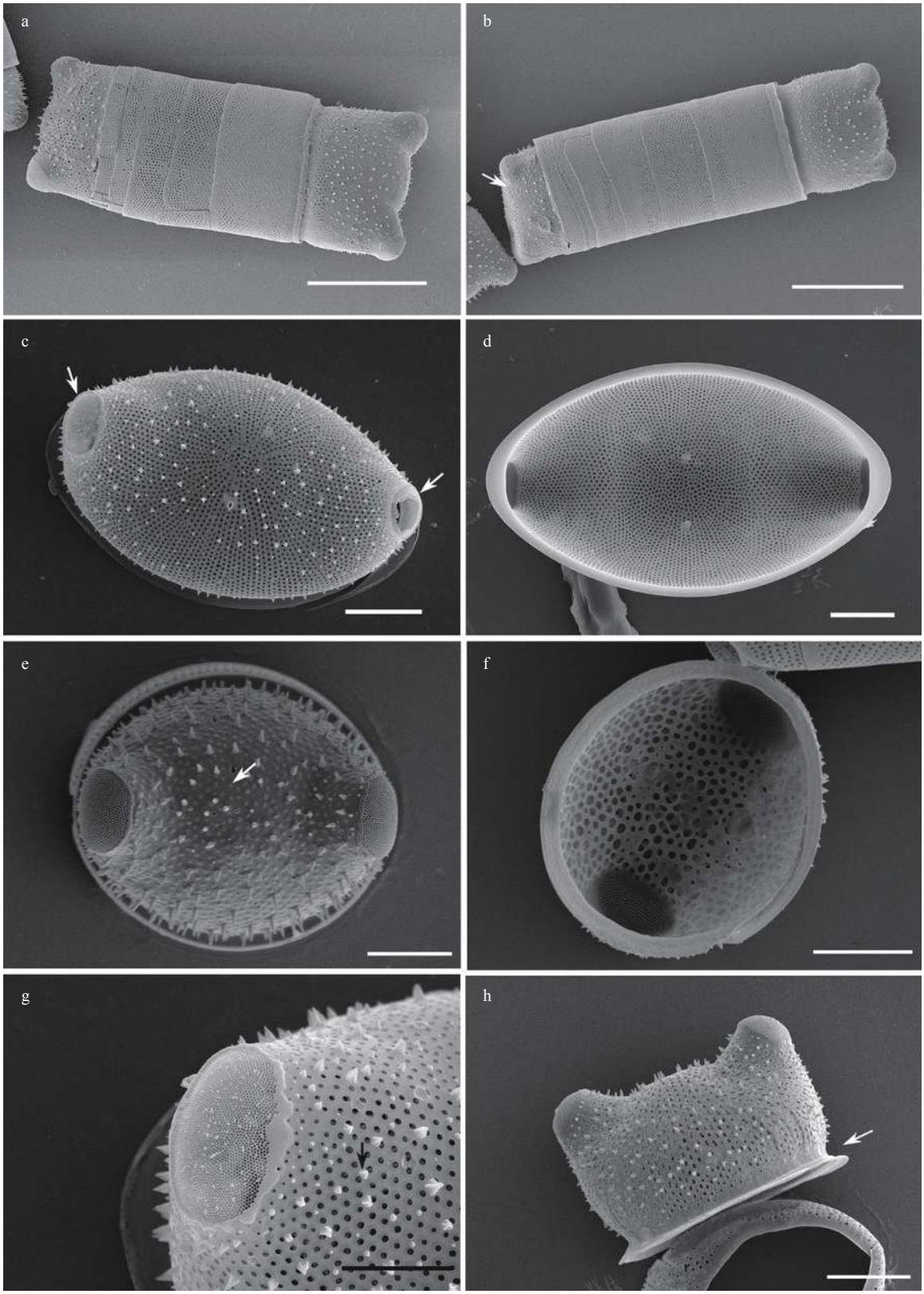

Fig. 2. SEM micrographs of *Pleurosira nanjiensis*. Scale bars: 20 μm (a, b), 10 μm (c, d, h), and 5 μm (e–g). a–b. Frustules of *P. nanjiensis*, note the valve with flat face (arrow); c–d. external and internal view of a relative large valve with broadly lanceolate outline, note the two ocellate elevations (arrows); e–f. external and internal view of a relative small valve with circular outline, note the domed cribra of areoale (arrow); g. ocellate elevation, note the spines on the external valve surface; and h. valve constrict near the valve margin (arrow).

tella, *O. aurita*, in the presence of a domed cribra. However, *O. aurita* has centrally located rimoportulae with externally spine-like tubes. *Pleurosira nanjiensis* is also similar to *Odontella obtusa* in the presence of 2 or 3 rimoportulae, 2 ocelli, and with no

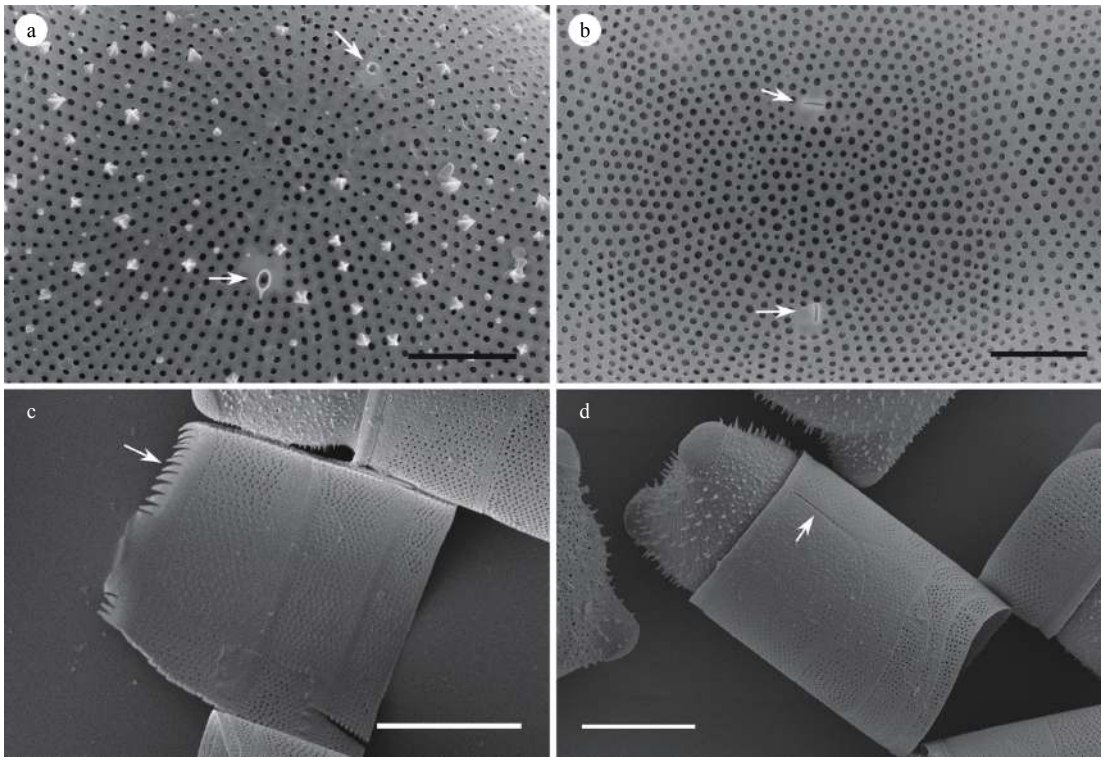

Fig. 3. SEM micrographs of *Pleurosira nanjiensis*. Scale bars: 5 μm (a, b) and 10 μm (c, d). a. External openings of two rimoportulae (arrows), b. two small rimoportulae on internal valve face (arrows), c. valvocopula with dentate edge (arrow), and d. opened valvocopula (arrow).

Fig. 4. Two-gene (SSU rRNA and *rbc*L) phylogenetic tree resulted from Maximum likelihood (ML) and Bayesian inference (BI) showing the positions of *Pleurosira nanjiensis* (bold characters). Nodal support for branches in the ML and BI trees is marked in order (ML/BI). Only bootstrap values over 50% are shown on the tree. All branches are drawn to scale. The scale bar corresponds to four substitutions per 100 nucleotide positions.

Table 1. Comparison of *Pleurosira nanjiensis* sp. nov. with *P. laevis* f. *laevis*, *P. laevis* f. *polymorpha*, *P. inusitata*, *P. socotrensis* var. *benalensis* and *Odontella obtusa*

Characteristics	*P. nanjiensis*	*P. laevis* f. *laevis*	*P. laevis* f. *polymorpha*	*P. inusitata*	*P. socotrensis* var. *benalensis*	*O. obtusa*
Length/μm	15.5–46.8	30–130	50–150	24.4–78.5	160–250	26–78
Width/μm	13.0–31.1	n.d.	n.d.	18.7–52.6	170–270	
Height/μm	13.5–15.2	n.d.	n.d.	n.d.	n.d.	29–81
Striae	radiate	radiate	radiate	irregular	irregular at cenre, radiate near marign	
Stria density per 10 μm	15–21	n.d.	n.d.	12–16	n.d.	8–11
Valve outline	broadly lanceolate, elliptical or circular	broadly elliptical	elliptical to subcircular.	elliptical	elliptical	convex, elliptical to lanceolate, inflated central area
Valve face	domed (rarely flat in culture)	flat	more or less domed valve	domed	flat	domed
Ocelli	blunt wide rounded	flat	elevated	elevated	elevated	blunt slightly acute
Areolae	poroid, closed by an external cribrum	porous	porous	poroid, closed by an external cribrum	n.d.	poroid, closed by an external cribrum
External ridges over the valve surface	absent	absent	absent	absent	absent	present
Rimoportula	2–3	2, more rarely 3–4, very rarely none.	2, more rarely 3–4, very rarely none.	2	6–15	2–3
Cingulum	6 bands	3 bands	n.d.	n.d.	n.d.	5 bands
Habitats	low tide zone, marine, epilithic or epipelic	brackish	marine and brackish	brackish	brackish	marine littoral and planktonic
References	present study	Compère (1982), Johnson and Rosowski (1992)	Compère (1982)	Desianti et al. (2015)	Compère (1982)	Hustedt (1930), Lavigne et al. (2015)

clear separation between the valve face and mantle. However, the ocellate elevations in *P. nanjiensis* are bluntly rounded and the cingulum is composed of six bands, while in *O. obtusa* the ocellate elevations are acute and there are only five bands. Moreover, *P. nanjiensis* has no network of ridge surrounding the areolae on the external valve surface as in *O. obtusa* (Lavigne et al., 2015). The phylogenetic tree also suggested *O. obtusa* is closed related to *Triceatium* rather than *P. nanjiensis* (Fig. 4). Nonetheless, the species status of *O. obtusa* needs to be confirmed because the original description and illustrations by Kützing (1844) are rather simple and many subsequent descriptions of the species are inconsistent.

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References

Alverson A J, Jansen R K, Theriot E C. 2007. Bridging the rubicon: phylogenetic analysis reveals repeated colonizations of marine and fresh waters by thalassiosiroid diatoms. Molecular Phylogenetics and Evolution, 45(1): 193–210, doi: 10.1016/j.ympev.2007.03.024

Anonymous. 1975. Proposals for a standardization of diatom terminology and diagnoses. Nova Hedwigia, Beiheft, 53: 323–354

Ashworth M P, Nakov T, Theriot E C. 2013. Revisiting Ross and Sims (1971): toward a molecular phylogeny of the Biddulphiaceae and Eupodiscaceae (Bacillariophyceae). Journal of Phycology, 49(6): 1207–1222, doi: 10.1111/jpy.12131

Compère P. 1982. Taxonomic revision of the diatom genus *Pleurosira* (Eupodiscaceae). Bacillaria, 5: 165–190

Desianti N, Potapova M, Beals J. 2015. Examination of the type materials of diatoms described by Hohn and Hellerman from the Atlantic Coast of the USA. Diatom Research, 30(2): 93–116, doi: 10.1080/0269249X.2014.1000020

Edgar S M, Theriot E C. 2004. Phylogeny of *Aulacoseira* (Bacillariophyta) based on molecules and morphology. Journal of Phycology, 40(4): 772–788, doi: 10.1111/(ISSN)1529-8817

Guiry M D, Guiry G M. 2017. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org [2015-05-07/2017-12-09]

Hustedt F. 1930. Die Kieselalgen Deutschlands, Österreichs und der Schweiz unter Berücksichtigung der übrigen Länder Europas sowie der angrenzenden Meeresgebiete Teil 1. In: Rabenhorst L, ed. Kryptogamen Flora von Deutschland, Österreich und der Schweiz Vol 7. Leipzig: Akademische Verlagsgesellschaft Leipzig, 847–849

Johnson L M, Rosowski J R. 1992. Valve and band morphology of some freshwater diatoms. V. variations in the cingulum of *Pleurosira laevis* (Bacillariophyceae). Journal of Phycology, 28(2): 247–259, doi: 10.1111/j.0022-3646.1992.00247.x

Katoh K, Standley D M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution, 30(4): 772–780, doi: 10.1093/molbev/mst010

Karthick B, Kociolek J P. 2011. Four new centric diatoms (Bacillariophyceae) from the Western Ghats, South India. Phytotaxa, 22: 25–40, doi: 10.11646/phytotaxa.22.1

Kützing F T. 1844. Die Kieselschaligen Bacillarien Oder Diatomeen. Nordhausen: Zu finden bei W. Köhne, 137

Lanfear R, Frandsen P B, Wright A M, et al. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution, 34(3): 772–773

Lavigne A S, Sunesen I, Sar E A. 2015. Morphological, taxonomic and nomenclatural analysis of species of *Odontella*, *Trieres* and *Zygoceros* (Triceratiaceae, Bacillariophyta) from Anegada Bay

(Province of Buenos Aires, Argentina). Diatom Research, 30(4): 307–331, doi: 10.1080/0269249X.2015.1110536

Li Chiawei, Chiang Y. 1979. A euryhaline and polymorphic new diatom, *Proteucylindrus taiwanensis* gen. et sp. nov. British Phycological Journal, 14(4): 377–384, doi: 10.1080/00071617900650431

Liu Qi, Wu Bo, Liu Yan, et al. 2011. Preliminary studies on diatoms from Chongming East Beach. Plant Science Journal (in Chinese), 29(5): 570–579

Maddison D R, Maddison W P. 2017. Mesquite: a modular system for evolutionary analysis. Version 3.2. http://mesquiteproject.org [2017-01-18/2017-06-07]

Medlin L, Elwood H J, Stickel S, et al. 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. Gene, 71(2): 491–499, doi: 10.1016/0378-1119(88)90066-2

Metzeltin D, Lange-Bertalot H, García-Rodríguez F. 2005. Diatoms of Uruguay. Compared with other taxa from South America and elsewhere. In: Lange-Bertalot H, ed. Iconographia Diatomologica. Annotated Diatom Micrographs. Vol 15. Taxonomy-Biogeography-Diversity. Koenigstein: A R G Gantner Verlag K G, 736

Nagumo T, Kobayasi H. 1990. The bleaching method for gently loosening and cleaning a single diatom frustule. Diatoms, 5: 45–50

Pei Guofeng, Liu Guoxiang, Hu Zhengyu. 2008. *Pleurosira laevis* (Ehrenberg) Compere, a new record freshwater diatom from China. Journal of Wuhan Botanical Research (in Chinese), 26(5): 458–460

Ronquist F, Huelsenbeck J P. 2003. MrBayes 3: bayesian phylogenetic inference under mixed models. Bioinformatics, 19(12): 1572–1574, doi: 10.1093/bioinformatics/btg180

Ross R, Sims P A. 1971. Generic limits in the Biddulphiaceae as indicated by the scanning electron microscope. In: Heywood V H, ed. Scanning Electron Microscopy: Systematic and Evolutionary Applications. London: Academic Press, 155–177

Ruck E C, Theriot E C. 2011. Origin and evolution of the canal raphe system in diatoms. Protist, 162(5): 723–737, doi: 10.1016/j.protis.2011.02.003

Stamatakis A. 2014. RAxML Version 8: a tool for Phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics, 30(9): 1312–1313, doi: 10.1093/bioinformatics/btu033

Trevisan V B A. 1848. Saggio Di Una Monografia Delle Alghe Coccotalle. Padova: Seminario, 96

Warren D L, Geneva A J, Lanfear R. 2017. RWTY (R We There Yet): an R package for examining convergence of Bayesian phylogenetic analyses. Molecular Biology and Evolution, 34(4): 1016–1020