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

LI Yuhang1, NAGUMO Tamotsu3, XU Kuidong1, 2, 4*

1 Department of Marine Organism Taxonomy and Phylogeny, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
2 Laboratory for Marine Biology and Biotechnology, Pilot National Laboratory for Marine Science and Technology (Qingdao), Qingdao 266237, China
3 Department of Biology, School of Life Dentistry at Tokyo, The Nippon Dental University, Tokyo 102-8159, Japan
4 University of Chinese Academy of Sciences, Beijing 100049, China

Received 14 August 2017; accepted 30 November 2017

© Chinese Society for Oceanography and Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

A new marine benthic diatom, *Pleurosira nanjiensis* sp. nov., is described from the rocky intertidal zone of the Xiaochayiu 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 rbcL 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) rimpportulae, and radiate striae.

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


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 Helleman) 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 (Mettelzin 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...
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 ITS2 small subunit rDNA (SSU rDNA) sequence and the chloroplast encoded large subunit of RUBISCO (rbcL) 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 H2O. Primers SSU1 and ITS1DR were used to amplify SSU rDNA (Martin et al., 1988; Edgar and Theriot, 2004). Primers rbcL6+ and rbcL1444- were used to amplify rbcL gene (Alverson et al., 2007; Buck 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 rbcL gene sequences included 3 036 positions. The concatenated alignment of SSU rDNA and rbcL gene sequences partitioned by different gene, and in the case of rbcL, 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 codon of rbcL (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 107 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 RWiTy (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 nanjensis 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 rbcL gene sequences of Pleurosira nanjensis have been deposited in GenBank with the accession numbers MF578764 and MF578765, respectively.

Description: Cells form zig-zag colony (Figs 1a, b). Plastids are discoïd. 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 (Figs 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 copula has a dentate margin (Fig. 3c).

3.2 Phylogenetic analysis
The combined SSU rDNA and rbcL gene sequences information in GenBank, with strong nodal support (ML bootstrap=100%, Posterior probability=1.00). The genus Odontella Agardh is polyphyletic (Fig. 4). The Pleurosira clade is clustered with Odontella aurita (Lynghbye) Agardh, the generic type of Odontella, but with relatively lower support (ML bootstrap=79%, Posterior probability=0.94). Odontella obtusa Kützing, which resembles P. nanjensis to some extent, is sister to Triceratium dubium Brightwell and T. dicroutum 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 nanjensis 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 Chang, 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-
iscaceae, Ashworth et al. (2013) suggested that the morphologic-
al features such as the valve perforation and the position of rimo-
portulae and ocelli are important for discriminating the eupodi-
cacean diatoms (see Fig. 6 in Ashworth et al., 2013). Like congen-
ers, *P. nanjiensis* has the rimoportulae present about the midway be-
tween 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 molecu-
lar phylogenetic analysis support the assignment of *P. nanjiensis*
to the genus *Pleurosira*.

*Pleurosira nanjiensis* differs from congeners by a combina-
tion of morphological features, including the domed valve with
broadly lanceolate, elliptical or circular valve outline, two elev-
ated marginal ocelli, two (rarely three) rimoportulae, and radiate
striae (Table 1). As mentioned above, *P. nanjiensis* resembles all
the *Pleurosira* species that inhabits brackish or marine environ-
ment, namely *P. laevis* f. *polymorpha*, *P. inusitata* and *P. sco-
trensis* 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 nanji-
ensis* differ from *P. inusitata* in the radiate striae (vs. irregular are-
olae 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 (Desian-
ti 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-
Tellaria, *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. 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).
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 *rbcL*) 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.
clear separation between the valve face and mantle. However, the ocellar elevations in *P. nanjiensis* are blunted rounded and the cingulum is composed of six bands, while in *O. obtusa* the ocellar 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 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.

**Acknowledgements**

The authors thank Cai Houcai, Chen Wandong and all the staff of the Nanji Islands National Marine Natural Reserve Research Institute for their help in sample collection.

**References**


Desianti N, Potapova M, Beals J. 2015. Examination of the type material 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


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


