Phytotaxa 195 (1): 053–064
www.mapress.com/phytotaxa/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

http://dx.doi.org/10.11646/phytotaxa.195.1.3

Morphology and phylogeny of *Halamphora yongxingensis sp. nov.* (Bacillariophyta), a new marine benthic diatom isolated from Yongxing Island, South China Sea

HAI-YING JIANG[1,2], CHAO-QUN HU[1*], HAI-PENG YANG[3], LV-PING ZHANG[1], PENG-FEI PENG[4], PENG LUO[1], ZHE ZHAO[1] & JIAN-JUN XIA[1]

[1] *Key Laboratory of Tropical Marine Bio-resources and Ecology, Guangdong Provincial Key Laboratory of Applied Marine Biology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, 510301, China.*
[2] *Guangdong Public Laboratory of Wild Animal Conservation and Utilization, Guangdong Entomological Institute/South China Institute of Endangered Animals, Guangzhou, 510260, China*
[3] *Guangzhou DEAOU Biotechnology Technology Co., Ltd., Guangzhou, 510663, China*
[4] *South China Sea Marine Engineering Surveying Center, State Oceanic Administration, Guangzhou, China*
* *Corresponding author. E-mail: cqhu@scsio.ac.cn.*

Abstract

Halamphora yongxingensis sp. nov., a marine benthic diatom isolated from an intertidal reef around the Yongxing Island, South China Sea (16° 58' 43.3" N, 112° 14' 41.7" E), is described in this study on the basis of light and electron microscopy. This diatom is also compared with related taxa such as *Halamphora subturgida* (Hustedt) Levkov and *Amphora subtropica* Wachnicka & Gaiser. In addition, phylogenetic analyses based on 18S rDNA and *rbc*L gene were also conducted. The results revealed that *H. yongxingensis* was clustered into the *Halamphor*a clade, closely related to *Halamphora montana* (Krasske) Levkov. We discuss morphological differences between *H. yongxingensis* and *H. montana*.

Key words: 18S rDNA, diatom, *Halamphora yongxingensis*, phylogeny, *rbc*L, South China Sea, taxonomy

Introduction

The genus *Halamphora* (Cleve) Levkov (2009:165) was originally introduced as a subgenus of *Amphora* Ehrenberg ex Kützing (1844:107) but elevated to genus recently by Levkov (2009). *Amphora sensu lato* is a large genus widely distributed in continental, estuarine and marine environments. Cleve (1895) split *Amphora* into nine subgenera (*Amblyamphora, Amphora, Archiamphora, Calamphora, Cymbamphora, Diplamphora, Halamphora, Oxyamphora* and *Psammamphora*) based on frustule and valve outline, raphe position, stria type and girdle band striation. In the genus *Halamphora*, the chloroplast is H-shaped. Valve is asymmetric to the apical axis and symmetric to the transapical axis. The valve mantle is deep on the dorsal margin and shallow on the ventral margin. The dorsal fascia is usually absent. Proximal raphe ends are straight or dorsally deflected. In the internal view, the proximal raphe ends terminate at a single fused helictoglossa. Most of *Halamphora* species occur in marine or brackish water habitats (Spaulding 2011).

Benthic diatoms are widely used in aquaculture, such as abalone and sea cucumber culture, acting as inductors for larvae settlement and serving as the main food during the early juvenile stage (Kawamura *et al*. 1995, Ito & Kitamura 1997, Jouuchi *et al*. 2007). But different diatoms often exhibit profound differences in the effects of settlement and growth (Courtois de Viçose *et al*. 2010, Roberts & Lapworth 2001). Therefore, we need to know more detailed information on classification of the diatoms which were used as baits. Molecular tools play important roles in delimitation of diatom species and better understanding the phylogenetic relationships among diatom taxa (Kooistra *et al*. 2004, Rimet *et al*. 2011). Most molecular analyses have been conducted based on nuclear ribosomal DNA, mitochondrial genes (e.g. cytochrome oxidase subunit I (*cox*1) gene and cytochrome b (*cob*) gene) and chloroplast genes (e.g. the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbc*L) gene) (Evans *et al*. 2007, Alverson 2008).

When screening bait for *Stichopus horrens* Selenka (1867:316) in Yongxing Island, we found a new benthic

Accepted by David Williams: 29 Oct. 2014; published: 22 Jan. 2015

diatom that could be easily cultured and was good bait for tropical *S. horrens* juveniles in practice. The analysis showed that this diatom could meet the nutrition demand of *Stichopus* well (Zhou 2013). This work was part of a large programme on breeding tropical sea cucumber.

Halamphora yongxingensis sp. nov., a new benthic diatom species, is described in this study. Detailed valve structure is presented with light microscope (LM) and scanning electron microscope (SEM) images. We also provide 18S-ITS rDNA, *rbc*L gene sequence and molecular phylogenetic analyses as supplements for morphological classification.

Material and methods

Physico-chemical parameters of the sampling site

Benthic diatom samples were collected by scrubbing the intertidal reef around Yongxing Island, the Xisha Islands in the South China Sea (16° 58' 43.3" N, 112° 14' 41.7" E) on 24 September 2010. The samples were brought back to laboratory and cultured in f/2 medium (Guillard & Ryther 1962) with 30 $mg \cdot L^{-1}$ Na_2SiO_3. A single diatom cell was isolated carefully by micro-pipette under microscope and then propagated step by step. In addition, the optimal light intensity was 49–99 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the best temperature was 25–30℃, the optimal salinity was 30–35‰. The best concentrations of $NaNO_3$, NaH_2PO_4 and $Na_2SiO_3 \cdot 9H_2O$ were 75 $mg \cdot L^{-1}$, 44 $mg \cdot L^{-1}$, 150 $mg \cdot L^{-1}$, respectively (Zhou *et al*. 2012).

Water temperature, salinity, pH, electrical conductivity (EC_{25}) and dissolved oxygen (DO) were measured *in situ* with YSI 6920 multiprobe (YSI Ltd., U.S.A.). Concentration of dissolved inorganic nutrients: silicate ($Si\text{-}SiO_3^{2-}$), phosphate ($P\text{-}PO_4^{3-}$), nitrate ($N\text{-}NO^{3-}$) and ammonium ($N\text{-}NH^{4+}$) were measured by QuAAtro Segmented Flow Analysis (SFA) systems (SEAL Analytical Ltd., U.K.). The results are presented in Table 1.

TABLE 1. Water physico-chemical parameters of sampling site.

Sampling Site	Collection time	Latitude	Longitude	Temperature	Salinity
Yongxing Island	Sep. 2010	16° 58' 43.3" N	112° 14' 41.7" E	29.4 ℃	35‰

TABLE 1. Water physico-chemical parameters of sampling site (continued).

pH	DO	EC_{25}	$Si\text{-}SiO_4^{4-}$	$P\text{-}PO_4^{3-}$	$N\text{-}NO_3^-$	$N\text{-}NH_4^+$
8.1	6.49 $mg \cdot L^{-1}$	50.73 $mS \cdot cm^{-1}$	0.038 $mg \cdot L^{-1}$	0.012 $mg \cdot L^{-1}$	0.176 $mg \cdot L^{-1}$	0.078 $mg \cdot L^{-1}$

Microscopy

The living diatoms were observed for chloroplast information. To clean the diatoms, materials were treated with hot H_2O_2 and HCl according to European standard EN 13946:2003 (CEN 2003). Samples were boiled in 30% H_2O_2 to remove the organic matter, and then treated with 10% HCl to remove calcium carbonate, followed by washing five times with deionized water. Slides for light microscopy were mounted with Naphrax® (Brunel Microscopes Ltd., U.K.) and examined using Olympus BX51 microscope with a 100× oil immersion objective (NA =1.30) (Olympus Optical Co. Ltd., Japan). For SEM examination, the cleaned sample was dried in dry oven, mounted onto aluminium stubs, coated with Platinum and examined under Hitachi S-3400N (Hitachi Ltd., Japan).

Terminology used in this study follows Krammer & Lange-Bertalot (2012) and the website: http://westerndiatoms.colorado.edu/ (Spaulding 2011).

DNA extraction, PCR amplification and phylogenetic analyses

DNA was extracted using TIANamp Marine Animals DNA Kit DP324 (Tiangen Biotech Co., Ltd., Beijing). Primers used for amplification were listed in Table 2. The ITS forward primer ITSF was designed according to 18S rDNA of *H. yongxingensis* and the reverse primer ITSR was designed from the homologous sequence data of 28S rDNA of diatoms which were available in GenBank nucleotide database. The PCR conditions were 94℃ for 4 min, followed by 35 cycles of 94℃ for 1 min, 40℃ for 1 min, 72℃ for 1.5min, and a final elongation step at 72℃ for 8 min. PCR products

were sequenced by Shanghai Life Technologies Biotechnology Co., Ltd. (Shanghai, China). All the sequences were assembled using ContigExpress program in the Vector NTI Advance® 11.5 (Shanghai Life Technologies Biotechnology Co., Ltd., Shanghai, China) and the new sequences were submitted to GenBank.

TABLE 2. Primers used to amplify the sequences of 18S rDNA, ITS rDNA and *rbc*L gene.

Gene	Primer Name	Primer Sequences	References
18S rDNA	18N5	5'-TGG TGC CAG CAG CCG CGG TA-3'	Chen *et al.* (2002)
	18N11R	5'-CTC AGT AAG CTT GAT CCT TCC GCA GGT TCA CC-3'	
ITS rDNA	ITSF	5'-GTG GCA TTA GGT TGT CGT-3'	This study
	ITSR	5'-TTC GCT GCG TTC TTC ATC-3'	
*rbc*L gene	*rbc*L40+	5'-GGA CTC GAA TYA AAA GTG ACC G-3'	Ruck & Theriot (2011)
	*rbcL*1444-	5'-GCG AAA TCA GCT GTA TCT GTW G-3'	

After sequencing, 1236 bp of 18S rDNA and 1578 bp of 5.8S rDNA-ITS rDNA were obtained. As 18S rDNA and 5.8S rDNA-ITS rDNA sequences partially overlapped, they were assembled to generate a new sequence (2374 bp) and submitted to GenBank (JF834543.1). Meanwhile, 1330 bp of *rbc*L gene of *H. yongxingensis* was obtained and submitted to GenBank (JX173069).

The obtained 18S rDNA-ITS rDNA and *rbc*L gene sequencs of *H. yongxingensis* were aligned with related sequences retrieved from GenBank, using Clustal W implemented in MEGA 4.0 (Kumar *et al*. 2008). The Neighbour-joining (NJ) trees were constructed using MEGA 4.0 (Kumar *et al*. 2008) with 1000 bootstraps and Kimura 2-parameters substitution model. The GTR + I + G model was selected as the best-fit model for Maximum Likelihood (ML) analysis by the Akaike information criterion (AIC) using Modeltest 3.7 (Posada & Crandall 1998, Posada & Buckley 2004). ML trees were constructed by PhyML 3.0 (Guindon *et al*. 2010) with 1000 bootstraps. The GTR+ I + G model was selected as the best-fit model for Bayesian inference (BI) by AIC using MrModeltest 2.2 (Nylander 2004). The BI trees were constructed by MrBayes 3.1.2 (Huelsenbeck & Ronquist 2005) using Markov Chain Monte Carlo (MCMC).

For the phylogenetic analyses, initially we selected 100 sequences with the highest similarity to examine the phylogenetic position of *H. yongxingensis*. Later, a smaller subset was selected considering relatedness of sequences. The final dataset of 18S rDNA and *rbc*L gene alignments consisted of 49 and 37 taxa, respectively, including *H. yongxingensis* and the outgroup taxon *Cyclotella meneghiniana* Kützing (1844:50) (AY496206.1), a centric diatom.

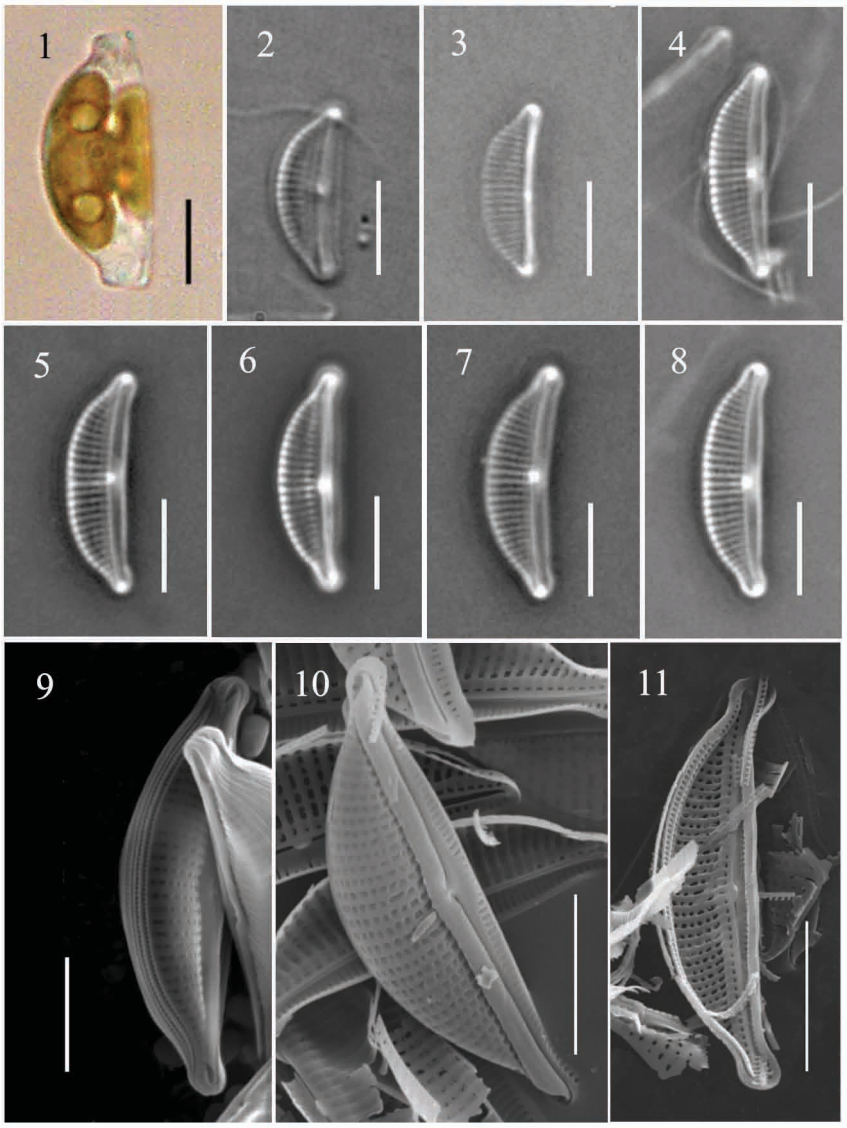
New species description

Division **Bacillariophyta**
Class **Bacillariophyceae** Haeckel 1878 *emend.* D.G. Mann in Round *et al*. 1990
Order **Thalassiophysales** D.G. Mann in Round *et al*. 1990
Family **Catenulaceae** Mereschkowsky 1902 in Round *et al*. 1990
Genus ***Halamphora*** (Cleve) Levkov 2009
Halamphora yongxingensis Jiang & Hu *sp. nov.* (Figs 1–23)

Cells solitary, chloroplast single and H-shaped (Fig. 1). Frustule elliptic with gently protracted, truncate ends, 9.2–18.2 µm long, 5.5–9.7 µm wide. Valves semi-elliptical, with ends protracted subcapitate to capitate, slightly deflected ventrally, with dorsal margin convex, with ventral margin more-or-less straight, 2.7–4.5 µm wide. Central nodule conspicuous and small. Dorsal striae nearly parallel at the valve center, becoming radiate near the poles, 21–30 in 10 µm, uniseriate, with small and round areolae 24–34 in 10 µm. Ventral striae composed of a single row of small elongated areolae, not interrupted (Figs 14, 20), 30–45 in 10 µm throughout (unresolved with LM).

In SEM external valve view (Figs 14–18), central area is expanded on the ventral side only, dorsal fascia absent. The raphe branches are straight, close to ventral margin. Distal raphe fissures are dorsally deflected. Proximal raphe fissures are slightly dorsally bent but nearly straight, central pores dilated (teardrop-shaped), forming an obtuse angle

at the centre (Fig. 16). Conopeum is well developed on the dorsal side of the valve, broadens slightly at the poles, reaching the dorsal valve margin and then tapers abruptly, forming a V-shape at the ends (Fig. 18). In internal view, a single row of elongated areolae close to the raphe-sternum is delimited by an internal longitudinal rib and continuous at the centre (Figs 19, 20). Internal proximal raphe ends are fused onto the flanks of a tongue-like protruding central helictoglossa (Fig. 20). The distal raphe ends terminate before the valve apices and the poorly developed helictoglossa present at the internal distal raphe ends (Fig. 21). Girdle bands are numerous, with two rows of ovoid or round poroids both on the dorsal and ventral side, 48–52 areolae in 10 μm on the dorsal side (Figs 22, 23).



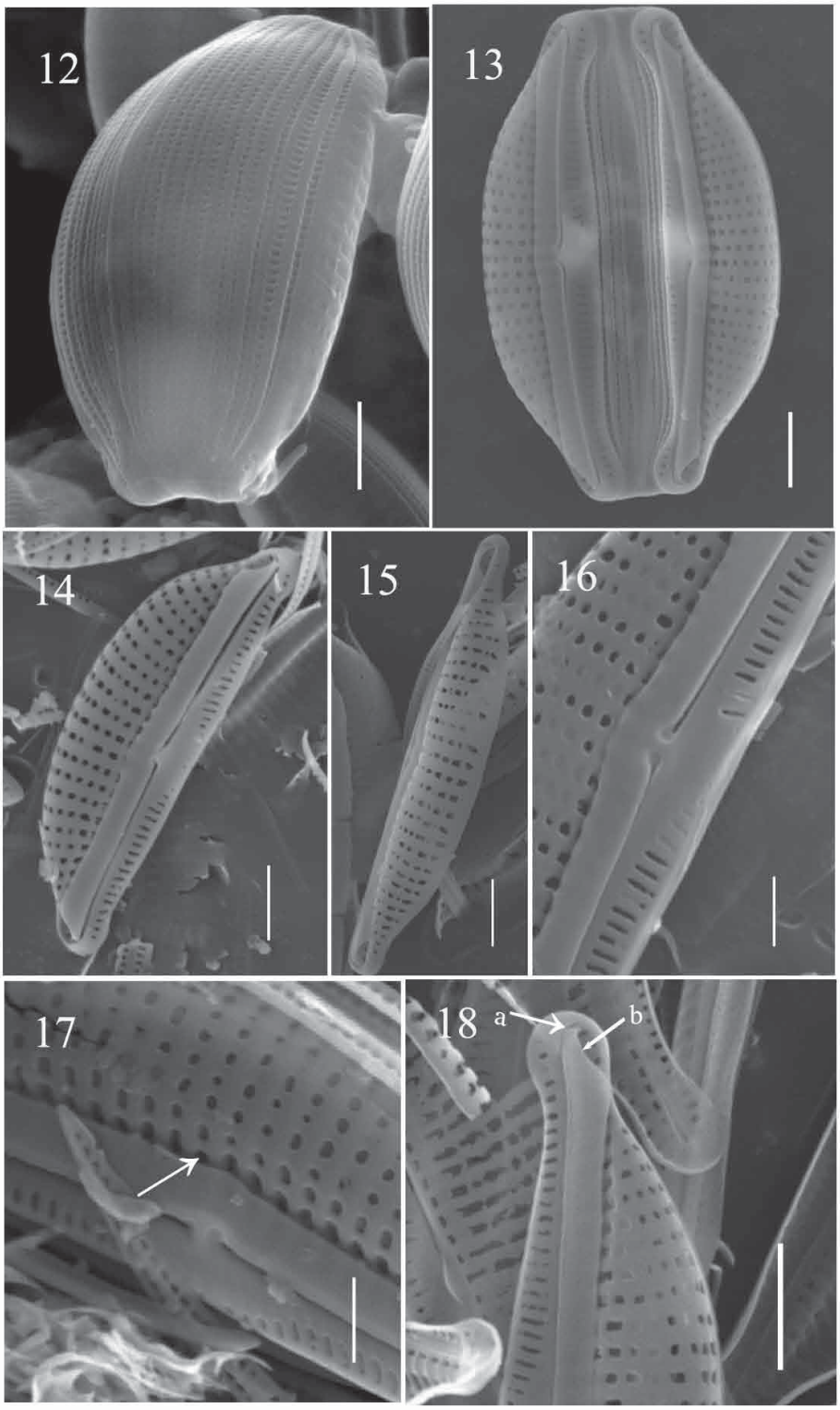
FIGURES 1–11. *Halamphora yongxingensis sp. nov.*

Fig. 1. Untreated material showing the plastid structure. LM micrographs. Scale bar = 5 μm.

Figs 2–11. Valve view showing the outline of the valve and size and shape variation. Fig. 8. Holotype. Fig. 9. The longest one. Fig. 10. The widest one. Fig. 11. The one with densest dorsal striae. Figs 2–8. LM micrographs. Figs 9–11. SEM micrographs. Scale bar = 5 μm.

Type:—CHINA. Guangdong: Yongxing Island, the Xisha Islands in the South China Sea, 16° 58’ 43.3” N, 112° 14’ 41.7” E, collected from the intertidal reef around Yongxing Island, 24 September 2010, slide preserved at Marine Biodiversity Collection of South China Sea (SCSMBC, Guangzhou, Guangdong province), holotype slide SCSMBC 001618 (represented by valve in Fig. 8), isotype slide SCSMBC 001619 and specimen SCSMBC 001620 (preserved in 100% ethanol).

Etymology:—The species was derived from the type locality, Yongxing Island, the Xisha Islands in the South China Sea.



FIGURES 12–18. *Halamphora yongxingensis sp. nov.*,SEM micrographs.

Fig. 12. Frustule in dorsal view. Note the valve mantles and the girdle bands. Scale bar = 2 μm.

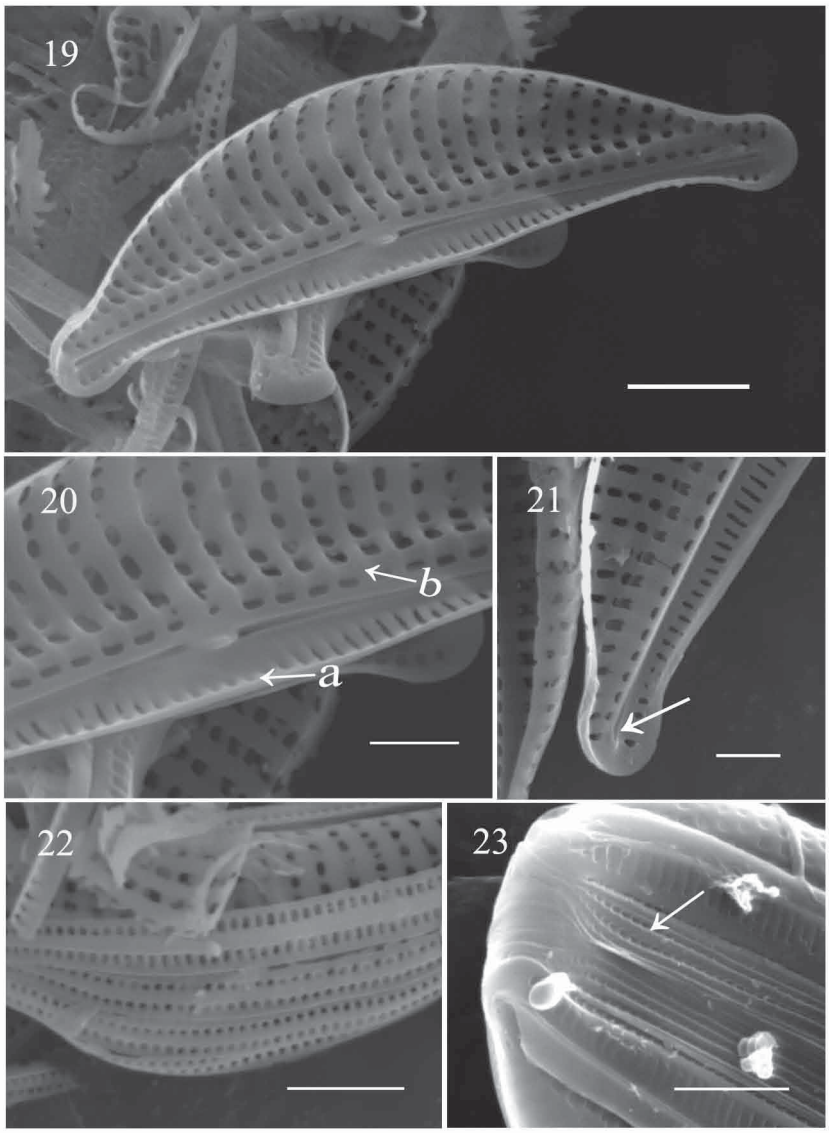
Fig. 13. Frustule in ventral view. Note the small central area, girdle bands and uniseriate areolae on the valve dorsal striae. Scale bar = 2 μm.

Fig. 14. Valve in external view showing the uniseriate dorsal striae, continuous ventral striae, the narrow conopeum and the raphe. Scale bar = 2 μm.

Fig. 15. Valve in external view showing the high mantle on dorsal side. Scale bar = 2 μm.

Figs 16, 17. Central part of the valve in external view. Scale bar = 1 μm. Fig. 16. Note the proximal raphe fissures bent dorsally, tear-drop-shaped central pores. Fig. 17. Note the dorsal striae composed of uniseriate areolae throughout (arrow).

Fig. 18. Detail of the valve apex in external view. Note the distal raphe fissure bent dorsally (arrow a). Also note the conopeum broadens at the poles and then tapers abruptly, forming a V-shape at the ends (arrow b). Scale bar = 2 μm.



FIGURES 19–23. *Halamphora yongxingensis sp. nov.*,SEM micrographs.

Fig. 19. Valve in internal view. Scale bar = 2 μm.

Fig. 20. Central part of the valve in internal view. Note the proximal raphe ends terminate at a tongue-like expansion, the continuous striae on the ventral side of the valve (arrow a). A single row of elongated areolae close to the raphe-sternum is delimited by an internal longitudinal rib (arrow b) and not interrupted at the centre. Scale bar = 1 μm.

Fig. 21. Valve apex in internal view showing distal raphe ending at a poorly developed helictoglossa (arrow). Scale bar = 1 μm.

Fig. 22. Girdle bands on dorsal side of the frustule composed of two rows of ovoid or round poroids. Scale bar = 2 μm.

Fig. 23. Frustule pole showing the girdle bands on ventral side composed of two rows of ovoid or round poroids (arrow). Scale bar = 2 μm.

Phylogenetic analyses

All the phylogenetic trees based on 18S rDNA (Fig. 24) consisted of four distinct clades, Surirellales and Rhopalodiales clade, Naviculales clade and two *Amphora* clades. *Amphora* 1 (*Halamphora*) clade and Naviculales clade were sisters. *Halamphora yongxingensis* was located in *Amphora* 1 clade with high bootstrap values and posterior probability (97/90/1.00). In *Amphora* 1 clade, *H. yongxingensis* first clustered with *Halamphora montana* (Krasske) Levkov (2009:207) (AJ243061.1) with support values of 50/78/1.00 (NJ/ML/BI), then merged with *Halamphora cf. capitellata* Frenguelli (1938:303) (AJ535158.1), *Halamphora normanii* (Rabenhorst) Levkov (2009:208) (AM501958.1), *Halamphora coffeaeformis* (C. Agardh) Levkov (2009:179) (FR865481.1, HM805019.1, HQ912602.1) in order. The phylogenetic trees based on *rbc*L gene (Fig. 25) also showed the similar results: *Amphora* species formed into two monophyletic groups and *H. yongxingensis* was located in *Halamphora* clade with a sister taxon *H. montana*.

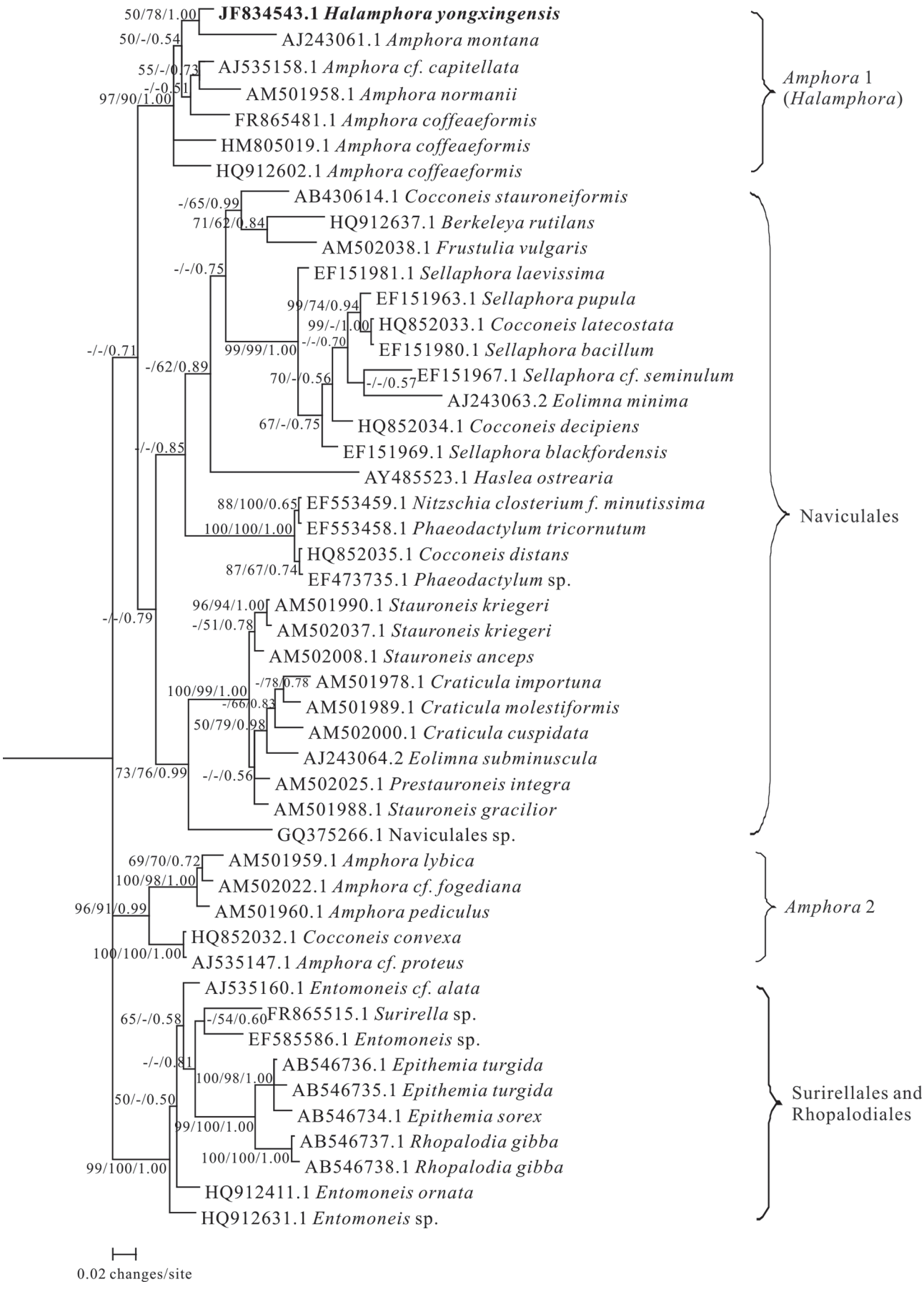


FIGURE. 24. Molecular phylogenetic tree based on 18S rDNA sequences (1184 positions included). The tree shown was resulted from Bayesian inference using GTR + I + G model. Outgroup taxon was excluded. Nodal support values (NJ/ML/BI) greater than 50% (NJ, ML) and 0.50 (BI) were shown. Both of NJ and ML bootstrap analyses were performed with 1000 replicates.

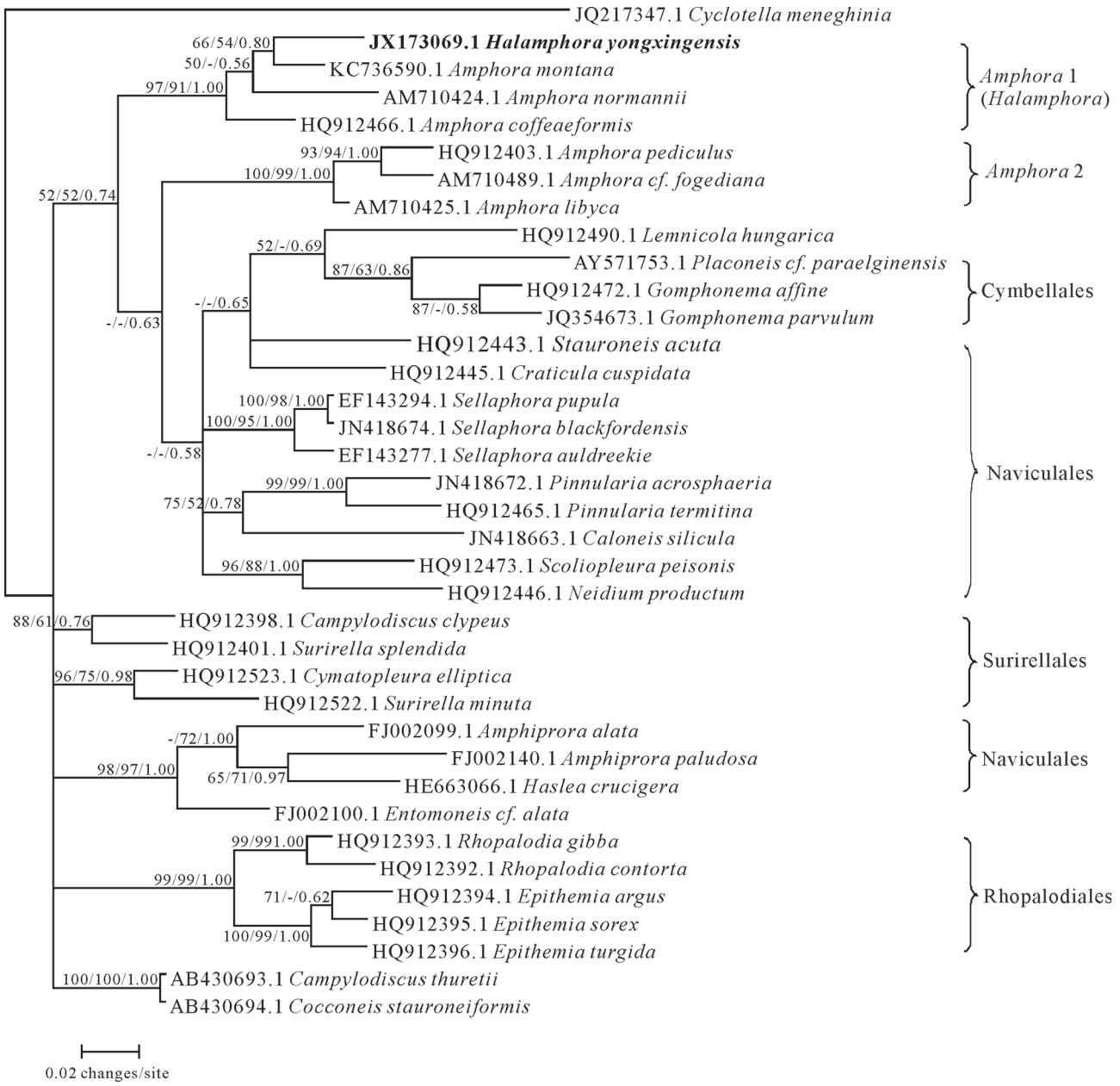


FIGURE 25. Molecular phylogenetic tree based on *rbc*L gene sequences (692 positions included). The tree shown was resulted from Bayesian inference using GTR + I + G model. Nodal support values (NJ/ML/BI) greater than 50% (NJ, ML) and 0.50 (BI) were shown. Both of NJ and ML bootstrap analyses were performed with 1000 replicates.

Discussion

Halamphora yongxingensis from Yongxing Island is morphologically similar to *Halamphora subturgida* (Hustedt) Levkov (2009:231) in terms of valve outline, range of size (however, *H. yongxingensis* is a little smaller) (Table 3), conopeum morphology, striae density, areolae arrangement and the shape of the proximal raphe ends. Both of the species have a longitudinal rib near the raphe-sternum, but the longitudinal rib of *H. yongxingensis* is continuous while that of *H. subturgida* is interrupted at valve centre. *Halamphora subturgida* has a broad ventral axial area while *H. yongxingensis* has a narrower ventral side. The two species also differ in the density of girdle band areolae in dorsal view (more dense in *H. yongxingensis*) and the obviously thicken virgae in internal view of *H. subturgida*. In addition, *H. yongxingensis* is a benthic marine diatom whereas *H. subturgida* is tropical freshwater species (Sala *et al*. 2006).

TABLE 3. Morphometric data of *Halamphora yongxingensis* compared with related species.

Species	Length (μm)	Width (μm)		Dorsal striae (no. in 10μm)		Ventral striae (no. in 10μm)		Valveareolae (no. in 10μm)	Girdle band areolae (no. in 10μm)	Source of data
		Frustule	Valve	Centre	Poles	Centre	Poles			
Halamphora yongxingensis	9.2–18.2	5.5–9.7	2.7–4.5	21–30	-	30–45	-	24–34	48–52	this paper
Halamphora subturgida (Hustedt) Levkov	14–25.2	7–11	2.9–4.5	18–24	20–27	22–34	28–36	21–30	32–38	Sala *et al.* (2006)
Amphora subtropica Wachnicka & Gaiser	16–35	-	4–7	16–20	18–23	34–40	-	-	-	Wachnicka & Gaiser (2007)
Halamphora salinicola Levkov & Diaz	20–34	-	2.5–3.7	21–26	-	-	-	-	-	Levov (2009)
Halamphora holsatica (Hustedt) Levkov	26–47	15–25	7–9	14–15	-	16–18	-	15–18	-	Levov (2009)
Halamphora subholsatica (Krammer) Levkov	32–46	-	5.5–7.8	13–16	-	22–24	-	20–24	-	Levov (2009)
Halamphora tenuissima Hustedt	9.4–9.7	-	2.2–2.3	34–36	40–42	47–55	55–60	57–81	57–58	Clavero (2000)
Halamphora montana (Krasske) Levkov	12–20	-	3–4.5	36	-	-	-	-	-	Krasske (1932)

Halamphora yongxingensis might be confused with *Amphora subtropica* Wachnicka & Gaiser (2007:407) when analyzed with LM as the similar outline but the former is obviously shorter and narrower than the latter. *Halamphora yongxingensis* also differs from *A. subtropica* based on the density of dorsal striae (more dense in *H. yongxingensis*) (Table 3). Additional differences between these two species can be observed with SEM (compare Figs 68–70 in Wachnicka & Gaiser 2007 and Figs 14, 16). However, Wachnicka & Gaiser (2007) didn't show the information of internal view and girdle band of *A. subtropica*.

Halamphora yongxingensis resembles *Halamphora salinicola* Levkov & Diaz (2009: 220), but the latter species is semi-lanceolate and longer (L=20–34 µm). According to Levkov (2009), the areolae of *H. salinicola* are larger and elliptical in vegetative cells while the areolae in *H. yongxingensis* are small and round.

In general, *H. yongxingensis* can be differentiated from *Halamphora holsatica* (Hustedt) Levkov (2009:196) by its lower valve size and obviously higher density of dorsal and ventral striae. More differences can be observed under SEM with respect to morphology of conopeum at the valve apex in external view (compare Fig.18 with Fig. 10 in Sar *et al*. 2003). In *H. yongxingensis*, the conopeum broadens at the poles and then tapers abruptly, forming a V-shape at the ends (Fig. 18), which is not observed in *H. holsatica*. Furthermore, the dorsal areolae of *H. yongxingensis* are small and round, while in *H. holsatica*, areolae are large and elongated. Internally the single row of dorsal areolae close to the raphe of *H. yongxingensis*, which is delimited by an internal longitudinal rib, is continuous while that of *H. subturgida* is interrupted at the centre.

Halamphora yongxingensis can be differentiated from *Halamphora subholsatica* (Krammer) Levkov (2009:228) by valve size (L=32–46 µm, B=5.5–7.8 µm in *H. subholsatica*) and ventral striae (interrupted at the center of *H. subholsatica*). With respect to the dorsal stria structure, although the striae of *H. subholsatica* are uniseriate, a recessed pore plate containing 4 poroids in each areola can be observed externally under SEM. In addition, *H. yongxingensis* can also be distinguished by its tapered ends of conopeum as opposed to inflated in *H. subholsatica* (compare Fig. 18 with Fig. 228:5 in Levkov 2009).

Halamphora yongxingensis can be easily distinguished from *Halamphora tenuissima* Hustedt (1955:14) by its obviously lower density of dorsal and ventral striae. Besides, *H. tenuissima* also has denser dorsal areolae than *H. yongxingensis* (Table 3).

The most similar taxa *H. subturgida* and *A. subtropica* were not included in the phylogenetic trees due to the lack of sequences in NCBI database. According to phylogenetic trees based on 18S rDNA and *rbc*L gene sequences, *H. yongxingensis* was closely related to *H. montana* (Krasske) Levkov (2009:207). But *H. yongxingensis* could be easily distinguished from its sister taxon *H. montana* based on morphological features. The most obvious difference is that *H. montana* possesses a semi-stauros at central area (Krasske 1932), which is not present in *H. yongxingensis*.

Morphologically, the new species *H. yongxingensis* clearly corresponds to the description of the genus *Halamphora*, such as frustule biraphid, wedge-shaped, valve asymmetrical to the apical axis, raphe eccentric, positioned along ventral margin, dorsal fascia absent, girdle bands numerous, the proximal raphe ends terminate at a single fused helictoglossa internally. Furthermore, phylogenetic analyses based on 18S rDNA and *rbc*L gene sequences also suggested that this new species belongs to *Halamphora*. As for the taxonomical level of *Halamphora*, Sar (2010) didn't agree with Levkov (2009) and argued that the limits among *Halamphora*, *Amphora sensu latoand Amphora sensu stricto* had been established without sufficient precision. According to the phylogenetic trees, *Amphora sensu lato* was split into two monophyletic clades (Figs 24–25). And *H. yongxingensis* was located in *Amphora* clade 1 which contained *H. coffeaeformis*, the type species (lectotype) of the genus *Halamphora* (Levkov 2009). Moreover, when investigated the monophyly of *Amphora sensu lato* and of Cleve's subgenera, Stepanek & Kociolek (2014) found *Halamphora* to be monophyletic and separated from *Amphora sensu stricto*. Therefore, we prefer to agree with Levkov (2009), subgenus *Halamphora* should be raised as genus and the new species *H. yongxingensis* belongs to the genus *Halamphora*.

Acknowledgements

We are grateful to Prof. Linda K. Medlin (Alfred Wegener Institute, Germany) for providing micrographs of *Halamphora capitellata* (AJ535158.1), and to Xutao Wang for laboratory instrument support. We thank Dr. Ting Chen for the critical reading. Thank also goes to the anonymous reviewers for their instructive comments which greatly improved this manuscript. We also thank the editor Dr. Patrick Kociolek for his patience and advice. This study was funded by the National Key Technologies R&D Program (2009BAB44B02), Marine Fisheries Science and Technology Promotion Project of Guangdong Province (A201100D01, A201101D02), the Key Projects in the National Science & Technology Pillar Program during the 12th Five-year Plan Period (2012BAD18B03), Guangdong Province Program (2012B091100272, 2012B061500012).

References

Alverson, A.J. (2008) Molecular systematics and the diatom species. *Protist* 159: 339–353. http://dx.doi.org/10.1016/j.protis.2008.04.001

Archibald, R. & Schoeman, F. (1984) *Amphora coeffeaeformis* (Agardh) Kützing: a revision of the species under light and electron microscopy. *South African Journal of Botany* 3: 83–102.

Boyer, C.S. (1927) Supplement: Synopsis of North American Diatomaceae, Part II.-Naviculatae, Surirellatae. *Proceedings of the Academy of Natural Sciences of Philadelphia* 79: 229–583.

CEN (2003) *Water quality - Guidance standard for the routine sampling and pretreatment of benthic diatoms from rivers*. European Standard EN 13946:2003. Comité European de Normalisation, Geneva, 13 pp.

Chen, Y.Q., Wang, N., Zhang, P., Zhou, H. & Qu, L.H. (2002) Molecular evidence identifies bloom-forming *Phaeocystis* (Prymnesiophyta) from coastal waters of southeast China as *Phaeocystis globosa*. *Biochemical Systematics and Ecology* 30: 15–22. http://dx.doi.org/10.1016/s0305-1978(01)00054-0

Clavero, E., Grimalt, J.O. & Hernández-Mariné, M. (2000) The fine structure of two small *Amphora* species. *A. tenerrima* Aleem & Hustedt and *A. tenuissima* Hustedt. *Diatom Research* 15(2): 195–208. http://dx.doi.org/10.1080/0269249x.2000.9705495

Cleve, P.T. (1895) Synopsis of the Naviculoid Diatoms, Part II. *Kongliga Svenska-Vetenskaps Akademiens Handlingar* 27: 1–219.

Courtois de Viçose, G., Viera, M., Bilbao, A. & Izquierdo, M. (2010) Larval settlement of *Haliotis tuberculata coccinea* in response to different inductive cues and the effect of larval density on settlement, early growth, and survival. *Journal of Shellfish Research* 29: 587–591. http://dx.doi.org/10.2983/035.029.0306

Evans, K.M., Wortley, A.H. & Mann, D.G. (2007) An assessment of potential diatom "barcode" genes (*cox*1, *rbc*L, 18S and ITS rDNA) and their effectiveness in determining relationships in *Sellaphora* (Bacillariophyta). *Protist* 158: 349–364. http://dx.doi.org/10.1016/j.protis.2007.04.001

Fourtanier, E. & Kociolek, J.P. (2011) *Catalogue of Diatom Names*, on-line version, California Academy of Sciences, San Francisco, USA. Available from: http://research.calacademy.org/research/diatoms/names/index.asp (accessed 20 October 2012).

Frenguelli, J. (1938) Diatomeas del Querandinense Estuariano del Río Matanza en Buenos Aires. *Revista del Museo de La Plata* 1: 291–314.

Guillard, R.R. & Ryther, J.H. (1962) Studies of marine planktonic diatoms: I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. *Canadian Journal of Microbiology* 8: 229–239. http://dx.doi.org/10.1139/m62-029

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* 59: 307–321. http://dx.doi.org/10.1093/sysbio/syq010

Guiry, M.D. & Guiry, G.M. (2012) *AlgaeBase*, National University of Ireland, Galway. Available from: http://www.algaebase.org (accessed 25 October 2012).

Huelsenbeck, J.P. & Ronquist, F. (2005) Bayesian analysis of molecular evolution using MrBayes. *In*: Nielsen, R. (Ed.) *Statistical methods in molecular evolution.* Springer, New York, pp. 183–232. http://dx.doi.org/10.1007/0-387-27733-1_7

Ito, S. & Kitamura, H. (1997) Induction of larval metamorphosis in the sea cucumber *Stichopus japonicus* by periphitic diatoms. *Hydrobiologia* 358: 281–284. http://dx.doi.org/10.1023/A:1003165811962

Jouuchi, T., Satuito, C. & Kitamura, H. (2007) Sugar compound products of the periphytic diatom *Navicula ramosissima* induce larval settlement in the barnacle, *Amphibalanus amphitrite*. *Marine Biology* 152: 1065–1076. http://dx.doi.org/10.1007/s00227-007-0753-6

Kützing, F.T. (1844) *Die kieselschaligen Bacillarien oder Diatomeen*. Zu finden bei W. Köhne, Nordhausen, 152 pp.

Kawamura, T., Saido, T., Takami, H. & Yamashita, Y. (1995) Dietary value of benthic diatoms for the growth of post-larval abalone *Haliotis discus hannai*. *Journal of Experimental Marine Biology and Ecology* 194: 189–199. http://dx.doi.org/10.1016/0022-0981(95)00099-2

Kooistra, W.H.C.F., Forlani, G., Sterrenburg, F.A.S. & Stefano, M.D. (2004) Molecular phylogeny and morphology of the marine diatom *Talaroneis posidoniae gen. et sp. nov.*(Bacillariophyta) advocate the return of the Plagiogrammaceae to the pennate diatoms. *Phycologia* 43: 58–67. http://dx.doi.org/10.2216/i0031-8884-43-1-58.1

Krammer, K. & Lange-Bertalot, H. (2012) *Bacillariophyceae,* Chinese edition, translated by Liu, W., Zhu, Y.S. & Huang, Y.Y. Sun Yat-sen

University Press, Guangzhou, 493 pp.
Krasske, G. (1932) Beiträge zur Kenntnis der Diatomeenflora der Alpen. *Nova Hedwigia* 72: 92–135.
Kumar, S., Nei, M., Dudley, J. & Tamura, K. (2008) MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. *Briefings in Bioinformatics* 9: 299–306. http://dx.doi.org/10.1093/bib/bbn017
Levkov, Z. (2009) *Amphora sensu lato*. *In*: Lange-Bertalot, H. (ed.) *Diatoms of Europe*, vol.5 A.R.G. Gantner Verlag K.G., Ruggell, pp. 1–916.
Nylander, J.A.A. (2004). *MrModeltest 2.2*. Available from: http://www.abc.se/~nylander/mrmodeltest2/mrmodeltest2.html
Posada, D. & Crandall, K.A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818. http://dx.doi.org/10.1093/bioinformatics/14.9.817
Posada, D. & Buckley, T.R. (2004) Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology* 53: 793–808. http://dx.doi.org/10.1080/10635150490522304
Rimet, F., Kermarrec, L., Bouchez, A., Hoffmann, L., Ector, L. & Medlin, L.K. (2011) Molecular phylogeny of the family Bacillariaceae based on 18S rDNA sequences: focus on freshwater *Nitzschia* of the section *Lanceolatae*. *Diatom Research* 26: 273–291. http://dx.doi.org/10.1080/0269249x.2011.597988
Roberts, R.D. & Lapworth, C. (2001) Effect of delayed metamorphosis on larval competence, and post-larval survival and growth, in the abalone *Haliotis iris* Gmelin. *Journal of Experimental Marine Biology and Ecology* 258: 1–13. http://dx.doi.org/10.1016/s0022-0981(00)00346-4
Round, F.E., Crawford, R.M. & Mann, D.G. (1990) *The diatoms: biology and morphology of the genera.* Cambridge University Press, Cambridge, 747 pp.
Ruck, E. C., & Theriot, E. C. (2011) Origin and evolution of the canal raphe system in diatoms. *Protist* 162: 723–737. http://dx.doi.org/10.1016/j.protis.2011.02.003
Sala, S.E., Sar, E.A., Hinz, F. & Sunesen, I. (2006) Studies on *Amphora* subgenus *Halamphora* (Bacillariophyta): the revision of some species described by Hustedt using type material. *European Journal of Phycology* 41: 155–167. http://dx.doi.org/10.1080/09670260600556609
Sar, E.A., Sala, S.E., Hinz, F. & Sunesen, I. (2003) Revision of *Amphora holsatica* (Bacillariophyceae). *European journal of phycology* 38: 73–81.
Sar, E.A. (2010) Book reviews. *Diatom Research* 25: 227–228. http://dx.doi.org/10.1080/0269249X.2010.9705844
Selenka, E. (1867) Beiträge zur Anatomie und Systematik der Holothurien. *Zeitschrift Für Wissenschaftliche Zoologie* 17: 291–374.
Spaulding, S. (2011) *Diatoms of the United States*. Available from: http://westerndiatoms.colorado.edu/taxa/genus/halamphora (accessed 27 October 2012).
Stepanek, J.G. & Kociolek, J.P. (2014). Molecular phylogeny of *Amphora sensu lato* (Bacillariophyta): An investigation into the monophyly and classification of the amphoroid diatoms. *Protist* 165: 177–195. http://dx.doi.org/10.1016/j.protis.2014.02.002
Wachnicka, A.H. & Gaiser, E.E. (2007) Characterization of *Amphora* and *Seminavis* from South Florida, USA. *Diatom Research* 22: 387–455. http://dx.doi.org/10.1080/0269249x.2007.9705722
Zhou, Y.C., Hu, C.Q., Peng, P.F., Luo, P. & Zhang, L.P. (2012) The optimal culture of benthic diatom (*Amphora* sp.), a tropical sea cucumber (*Stichopu horrens*) bait. *Marine Sciences* 36: 40–47. [In Chinese]
Zhou, Y.C., Peng, P.F., Hu, C.Q., Zhong, M., Chen, Y.F., Zhao, Z. & Xia, J.J. (2013) Analysis of nutritional value and observation of biological characteristics in microalga *Amphora* sp. as food for sea cucumber *Stichopus horrens*. *Journal of Tropical Oceanography* 32: 59–64. [In Chinese]