Multigene Assessment of Biodiversity of Diatom (Bacillariophyceae) Assemblages from the Littoral Zone of the Bohai and Yellow Seas in Yantai Region of Northeast China with some Remarks on Ubiquitous Taxa

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ABSTRACT


Diatoms are important contributors to the benthic microeukaryote flora. This manuscript lays the foundation for future metagenomic and environmental sequencing projects off coastal China by curating diatom DNA sequences from the Yantai region of the Bohai and Yellow Seas (Northeast China). These studies are based on cultures established from samples collected in different seasons from marine littoral and supralittoral zones in 2013 and 2014. Thirty-six diatom strains were cultured successfully and identification of these clones was determined by light and scanning electron microscopy (LM and SEM) and DNA sequencing of the nuclear-encoded small subunit ribosomal RNA (SSU) and chloroplast-encoded rbcL and psbC genes. The strains primarily represent raphid pennate genera, such as Amphora, Amphora (Oxyamphora), Caloneis, Diploneis, Halamphora, Navicula, Nitzschia, Particellus, Pleurosigma, Sirella and Tryblionella. When the DNA markers from these strains were analyzed in a multi-genus phylogeny, we found that some clones-particularly within the genera Amphora, Navicula and Nitzschia—show greater than expected genetic diversity despite their very similar morphology and morphometrics. We also compared the molecular and morphological identities of several seemingly ubiquitous marine littoral taxa in the genera Amphora and Nitzschia from the Indian Ocean and Atlantic Ocean, the Red Sea and Adriatic Sea to their Yellow Sea counterparts.

ADDITIONAL INDEX WORDS: Diatoms (Bacillariophyta), Bohai Sea, Yellow Sea, littoral zone, metagenomics, multigene approach (rbcL, psbC, SSU), biodiversity, biogeography, ubiquitous taxa, Red Sea, Adriatic Sea.

INTRODUCTION

Despite the length of the Chinese coast, the diatom flora of the marine littoral zone is rather poorly studied, particularly the Shandong Province and northeast part of China (reviewed in Park et al., 2012). Other than some early diatomological surveys (e.g. Meister, 1935), only a few large scale studies focused on diatom identification have resulted in the publication of floristic monographs with numerous microphotographs, including electron microscopic images (Gao et al., 2003; Jin et al., 1985, 1991). Some recent efforts have been undertaken to study the species composition and biogeography of selected genera of marine benthic diatoms; Protococelia and Rhopalodia in Li et al. 2009, two marine Gyrosigma spp. and two Nitzschia described as new for science in Liu et al. (2015a,2015b, 2015c, respectively). However, most of these efforts are concentrated in the southern Chinese coast.
Hence we have undertaken our research in the north east as we believe the potential for new discoveries in this region must be very high.

The development of molecular methods has revolutionized how diatoms are studied. DNA sequencing has drastically affected the study of phylogeny, evolution and systematics of the diatoms at the species and population level (Medlin and Kazmarska, 2004; Souffreau et al., 2011; Sorhannus, 2007; Theriot et al., 2010). Extensive studies of morphology and molecular markers resulted in discoveries of cryptic species and species complexes in marine planktonic diatoms such as Skeletonema (e.g. Kooistra et al., 2008; Sarno et al., 2007) and Pseudo-nitzschia (e.g. Landholm et al., 2002, 2012).

With the increased sequencing of diatom molecular markers, the potential for using these markers to simplify or even automate diatom identification has also become more viable though DNA barcoding. The use of markers such as coxl (Evans et al., 2007), rbcL (Hamsher et al., 2011) and rDNA (e.g. Moniz and Kazmarska, 2009; Ruggiero et al., 2015; Zimmermann et al., 2014) has been proposed for barcoding purposes. Tests of these methods have yielded good results in differentiating closely-related species. Advances in high-throughput sequencing have made it possible to sequence the barcode markers from hundreds of microorganisms present in a sample of water or benthos (“metabarcoding”) in a fraction of the time required for experts in the taxonomy of microorganisms to identify and characterize these assemblages by microscopy. The main drawback with this approach is the need for a well-curated database of voucher sequences, present only in the most rudimentary form for diatoms. GenBank, for example, offers sequences for maybe five hundred identified species of freshwater and marine diatoms (out of an estimated 100,000 species), but these sequences are supported by little metadata or voucher images to confirm their identity or habitat. To fully realize the potential of metabarcoding and environmental sequencing, we need a database populated with imaged vouchers and environmental metadata, such as salinity, temperature, nutrient levels, pH and oxygen content and saturation, to infer community structure and ecology.

In this paper we provide, for the first time, both morphological and molecular data on species composition and diversity of diatom assemblages of the coastal zone of the Bohai and Yellow Seas. The data come from LM and EM observations and multigene sequencing of diatom taxa collected and cultured from the study area. We have focused on some of the smallest diatoms (below or slightly exceeding 10 μm), which are either too small or too finely silicified to withstand any traditional sample processing and observation by LM.

**MATERIALS AND METHODS**

**Study area**

Bohai Sea, also known as Bohai Gulf, is the innermost gulf of the Yellow Sea. The Bohai Sea is bounded by the Changshan Islands chain between the Liaodong and Shandong Peninsulas. The average sea surface temperature (SST) of the Bohai Sea reaches its minimum in February (~1.5–3.6 °C) and its maximum in August (24–27 °C). In the past 31 years, the SST has increased 0.48 °C and has continued to rise at a rate of 0.01 °C/yr (Lin, Su, and Xu, 2000). There is also an uptrend in the salinity of surface water according to recent observations (Ju, 2011; Yu et al., 2012).

The circulation in the Bohai Sea is weak, where surface flow velocity varies from 3 to 15 cm/s. In general, exterior sea water (with generally higher salinity and temperature) enters the sea across the northern part of the Changshan Islands chain, and flows out of the sea along the north coast of the Shandong Peninsula (Yu et al., 2012). The Yellow River also contributes to the circulation. As a result, in summer, the SST and salinity of Dalian sites on the two sides of Liaodong Peninsula are distinct, and those in the sampling sites along the northern coasts of Shandong Peninsula vary gradually from west to east; average SST decreases from 27°C to 25°C and average salinity increases from 28 to 30.5 psu. Sampling sites are shown in Figure 1.

The western coastal current of the Yellow Sea continues to flow along the coast of the Shandong Peninsula, and then flows southward (Guan, 1994; Yu et al., 2012). But the currents influence little on the surface seawater of our Qingdao sites. Where we sampled in Qingdao, the SST ranges from 23.2 °C to 28.7 °C and the salinity ranges from 29.2 to 30.9 psu (Wang et al., 2011).

As for the surface seawater, the Qingdao sites and the sites on the north coast of the Shandong Peninsula belong to two different water masses; coastal waters and the Yellow Sea water mass. In summer, the Dalian sites can be marginally under the enlarged sphere of influence of the Yellow Sea water mass (Yu et al., 2012).

![Figure 1. Map displaying the location of the study area](https://bioone.org/journals/...)

Figure 1. Map displaying the location of the study area.
Table 1. Location of selected sampling sites in the Yellow Sea with the environmental measurements

<table>
<thead>
<tr>
<th>Site</th>
<th>Yantai Binhai Road holoturoidae aquaculture</th>
<th>Muping YIC Station (5)</th>
<th>Muping YIC Station (6)</th>
<th>Muping sea water (7)</th>
<th>Muping microbial mat (8)</th>
<th>Horse Island</th>
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<td>37°27′7″</td>
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<td>37°27′21.71″</td>
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<td>27.44</td>
<td>32.38</td>
<td>29.45</td>
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<tr>
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**Sampling**

Marine littoral diatoms from the Yantai region were sampled three times, in June 2013, October 2013 and September 2014. In 2014 only the coast in Yantai (including Muping and Horse Island) was sampled, whereas in 2013 samples were collected at Yantai, Muping, Laizhou, Chang Dao Island and Laizhou Bay. We sampled various substrates at all locations: sediment (either sand or mud), small gravel, rock scrape and seaweeds. In Yantai we also sampled aquaculture enclosures (containing molusk and holothurians) and microbial mats on the benthos in Muping (Figure 1). Samples were collected in test tubes filled with seawater with some free space for aeration. Immediately after sampling, living diatoms were confirmed by LM. Altogether 31 samples were collected in Muping, Yantai, Horse Island and Chang Dao in June 2013 and September 2014, and six samples in Laizhou Bay in October 2013 (Table 1). During 2014, environmental data (water temperature, conductivity, salinity dissolved oxygen, oxygen saturation, and pH) were measured from each site by means of YSI model 556MPS Probe.

**Isolation, culturing and microscopy**

Samples containing living diatoms were stored in natural light. For diatom isolation, a small volume of the field samples was transferred into plastic petri dishes and enriched with 1/2 culture medium (Guillard, 1975) with the salinity of the medium matching the field measurements. After approximately three weeks of enrichment, single living diatom cells were isolated by micropettes under the inverted microscope using the capillary tube technique (Andersen and Kawachi 2005). Diatom cells were isolated into fresh plastic petri dishes filled with culture medium, labeled and placed into a growth chamber at 18°C under a 12 h light–12 h dark cycle, illuminated by 50 μmol m² s⁻¹ of white light. During a one to two week period, inoculated petri dishes were checked for diatom growth and any potential contamination. Live cells were photographed in counting chambers of an inverted Nikon TS300 microscope (Nikon Corporation, Tokyo, Japan) equipped with Nikon (Nikon Corporation, Tokyo, Japan) x100 PlanAPOchromatic oil immersion lens (n.a. = 1.40) with differential interference contrast (DIC) to document chloroplast structure.

In order to document valve ultrastructure by LM and EM, samples were cleaned of organic material by boiling the cultured cell suspension in 30 ml of 30% hydrogen peroxide for a few hours to remove the cell content, followed by adding ca. 10 ml of 10% HCl to remove calcium carbonate. After oxidation, cleaned samples were successively rinsed with deionized water. The diatom suspension was pipetted onto ethanol-cleaned cover slips and left to air dry. Naphrax® (Brunel Microscopes Ltd., Willshire, U. K.) was used as a mounting medium. LM observations of cleaned material were conducted with a Zeiss Axio Imager 2 (Carl Zeiss Microscopy GmbH, Jena, Germany) using a DIC 100x oil immersion objective (n.a. = 1.46).

Ultrastructural observations were made with scanning and transmission electron microscopy (SEM and TEM, respectively). For SEM examinations, a drop of the cleaned sample was filtered onto Nucleopore Whatman polycarbonate membranes (Fisher Scientific, Schwerte, Germany). Filters were air-dried overnight, mounted on aluminum stubs and coated with gold-palladium (SEM) or osmium (TEM). SEM observations were made at the Goethe University in Frankfurt using a Hitachi S–4500, the Yantai Institute of Coastal Zone Research (YIC) in Yantai using a Hitachi S–4800, and at the Warsaw University of Technology, Faculty of Materials Science and Engineering using a Hitachi SU 8000 and SEM/STEM S–5500 in which the specimens were simultaneously observed in scanning and transmission mode (all instruments made by Hitachi Ltd., Tokyo, Japan).


**DNA extraction and PCR amplification**

The protocol for DNA extraction and PCR amplification follows Li et al. (2015). Depending on cell density, several milliliters of cell suspension from an exponentially growing culture were centrifuged for 15 min at 8,000 rpm to create a cell pellet. Genomic
DNA was extracted from these pellets using the Genomic DNA NucleoSpin® Plant II Kit (Macherey-Nagel, Germany) according to the manufacturer’s instructions. The small subunit (SSU) of the nuclear ribosomal RNA and two chloroplast genes rbcL and psbC were amplified using the primers and the protocols described in Theriot et al. (2010). PCR amplification of the D1/D2 regions of the nuclear 28S rDNA was done using the primers and protocols as described in Scholin et al. (1994). PCR products were visualized in 1% agarose gel (Maximus, Poland) and then purified using Exonuclease I & Polar-BAP (EURx, Gdańsk, Poland) protocol. PCR products were sent to oligo.pl DNA Sequencing Laboratory IBB PAS, Warsaw, Poland for Sanger sequencing using BigDye Terminator v. 3.1 chemistry and an ABI3730 xl sequencer.

Phylogenetic analyses

Maximum likelihood (ML) analysis was performed with a concatenated three-gene (SSU, rbcL and psbC) dataset (Table 2).

Ingroup taxa included our new additions plus rapid taxa from the three-gene dataset of Theriot et al. (2010). We chose Ctenophora pulchella (Ralls ex Kützing) D. M. Williams & Round and Tabularia cf. tabulata (C. Agardh) Snoeij as outgroups as they occur in the clade sister to raphids in Theriot et al. (2010).

The secondary structure alignment of SSU primary sequences was performed by SSU-align using covariance models (Nawrocki 2009). Ambiguous sites with a posterior probability (PP) less than the default of 0.9 were removed. The dataset was partitioned by gene, codon position (in case of chloroplast markers) and paired/unpaired sites (in case of SSU markers) with a GTR+G+I model. Support for clades were evaluated by performing 1000 bootstrap replicates using rapid Bootstrap analysis in RAxML v8.1 (Stamatakis, 2014). The best-scoring ML tree was chosen as the final tree and bootstrap values were added to the corresponding nodes. Results of the phylogenetic analysis are illustrated in Figure 2.

Table 2. GenBank accession numbers of SSU rDNA, rbcL and psbC sequences derived from species used in the genetic analysis. Data of the newly sequenced species are marked in bold.

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Figure 2. Phylogenetic tree based on Maximum Likelihood reconstruction of our concatenated, 3–gene dataset (nuclear-encoded ribosomal RNA and chloroplast-encoded rbcL and psbC). Bootstrap values included over corresponding nodes; values < 50% not included.
RESULTS

Although attempts were made to culture cells from all sites, 36 clones were successfully isolated from eight out of 37 samples (Table 3). The most successful cultures were isolated from the shallow water sediments at Laizhou Bay at the Bohai Sea site (LB 2; 6 cultures), while the Horse Island (Yellow Sea) sample (H1 1) from small stones covered with green biofilm in the surf zone yielded only a single culture.

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<td>+++</td>
<td>+</td>
<td>SZCZCH1658</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3. Light microscopic images of the diatom clones from the Bohai and Yellow Sea. LM, DIC. Figs a–c: Nitzschia trapezaformis Li Ch., Witkowski & Yu sh. sp. nov. Figure d: Nitzschia nanodissipata Li Ch. & Witkowski sp. nov. Figure e: Caloneis cf. westii. Figure f: Diploneis cf. parva. Figure g: Cocconeis cf. macarenica. Figure h: Nitzschia cf. volendinostrata. Figure i: Tryblionella gaona Witkowski & Li Ch. Figure j: Orizaformis holocuta Witkowski, Li Ch. & Asworth. Figure k: Sarcella cf. fastuosa. Figure l: Navicula sp. Figs. m–p: Navicula zhengii Witkowski & Li Ch. sp. nov., in Figure 30 illustrated is auxospore of N. zhengii from wild population. Figure q: Kolbeisina sinica Witkowski & Li Ch. sp. nov. Figs s, t: Halamphora cf. tenerima Hustedt. Figure u: Navicula hippoantafallax Witkowski & Li Ch. sp. nov. Figure v: Nitzschia aurariae Cholnoky. Scale bar in Figure k = 10 μm.
Table 4. Comparison of clone SZCZCH283—Cocconeis cf. mascara

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Length μm</th>
<th>Width μm</th>
<th>Central area</th>
<th>Striae in LM resolved(SV)</th>
<th>Striae in LM resolved(RV)</th>
<th>Stria density in 10 μm(SV)</th>
<th>Stria density in 10μm(RV)</th>
<th>Shape of areolae (RV)</th>
<th>Arealae versus virgae width</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. neothumens var. marina</td>
<td>10–13</td>
<td>5–7</td>
<td>Very small, at one side</td>
<td>+</td>
<td>+</td>
<td>20–26</td>
<td>26–32</td>
<td>Rectangular</td>
<td>Same width</td>
</tr>
<tr>
<td>C. mascara</td>
<td>6,1–10</td>
<td>4,4–6</td>
<td>Rather small</td>
<td>+</td>
<td>–</td>
<td>26–32,5</td>
<td>24–32,5</td>
<td>Circular</td>
<td>Narrower</td>
</tr>
<tr>
<td>Cocconeis cf. mascara</td>
<td>6–12,5</td>
<td>4–6,5</td>
<td>Distinct</td>
<td>+</td>
<td>+</td>
<td>21–28</td>
<td>25–28</td>
<td>Oblong</td>
<td>Narrower</td>
</tr>
</tbody>
</table>

RV-raphe valve; SV-sternum valve

Table 5. Comparison of clone SZCZCH662—Cocconeis cf. cupulifera with established taxa

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Length μm</th>
<th>Width μm</th>
<th>Central area</th>
<th>Striae in LM resolved(SV)</th>
<th>Striae in LM resolved(RV)</th>
<th>Stria density in 10 μm(SV)</th>
<th>Stria density in 10μm(RV)</th>
<th>Stema (RV) shape</th>
<th>Special features</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. cupulifera</td>
<td>6.2–8.4</td>
<td>4–5</td>
<td>Very small, at one side</td>
<td>+</td>
<td>–</td>
<td>13–20</td>
<td>49 in the middle, 65 at the margin</td>
<td>Circular</td>
<td>Round cupules on the SV surface</td>
</tr>
<tr>
<td>(Riaux-Gobin et al. 2011)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. cf. cupulifera</td>
<td>6.0–9.3</td>
<td>3.5–5.8</td>
<td>In SEM very small, at one side</td>
<td>+</td>
<td>–</td>
<td>26–32</td>
<td>38–44</td>
<td>Rectangular</td>
<td>Broad to very broad, elliptic-lanceolate, convex</td>
</tr>
<tr>
<td>(this study)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Shallow grooves separate the striae on SV</td>
</tr>
</tbody>
</table>

RV-raphe valve; SV-sternum valve

Bohai Sea sites

Laizhou Bay

Laizhou Bay is located within the Bohai Sea proper. Measured water salinity has oscillated between 29 – 30 psu, with temperatures in the shallow water zone reaching 26°C in summer. Oxygen content at Laizhou stations was low, ranging from 4, 8–5, 4 mg/L, whereas oxygen saturation ranged from 71–80 % (Table 1). Laizhou Bay cultures originated from a water sample (LB1) taken from the beach area. Two strains of apparently the same species, similar to Nitzschia daviiformis Hustedt (SZCZCH970 and 971) – see Krammer & Lange-Bertalot (1988), were isolated from this sample. Examination of live samples revealed this species to be one of the most abundant taxa in our collections. Based on morphological and molecular data illustrating the differences from N. daviiformis we propose the description of a new species based on these clones, N. traheiformis Li, Ch., Witkowski & Yu Sh. sp. nov. (Figure 3a–c; 5). The new species differs from N. daviiformis by having smaller valve width (3,0 – 4,7 versus 5–7 μm) and a coarser striaation, 32–34 versus 40 in 10 μm, respectively (cf. Krammer and Lange-Bertalot 1988, see taxonomic section).

Sample LB 2 was collected from medium-grained sediment in very shallow water (5 cm). This sampling site contained Nitzschia sp. sect. Dissipatae (SZCZCH974), Caloneis cf. westii (W. Smith) Hendey (clones SZCZCH1001 and 1002, Figure 3e, Hendey 1964, Figure 44, 5–10, 45; 1–13), a third strain of Nitzschiatraheiformis (SZCZCH972, Figure 3b), Diploneis cf. parca (A. Schmidt) Boyer (SZCZCH102, Figure 3f) and Cocconeis sp. (SZCZCH283, Figure 3g). The latter clone resembles two taxa: Cocconeis neothumens Krammer var. marina de Stefano, Marino & Mazella (De Stefano, Marino and Mazella, 2000) and Cocconeis mascara Krammer & Riaux-Gobin & Compère (Riaux-Gobin and Compère, 2008). As shown in the Table 4, Cocconeis sp. (SZCZCH283) size data and ultrastructure indicates that it is more similar to Cocconeis mascara, though not identical—the two taxa differ in terms of areola shape, which is circular in C. mascara and oblong in our clone. Although the stria density in raphe valve(=RV) overlaps, in C. mascara they are not resolvable under LM (26–35, 5 versus 21–28 in 10 μm in C. mascara and our clone, respectively). No molecular data is available for Caloneis sp. (SZCZCH1001) at this time.

Clone SZCZCH974 is described below as Nitzschia nanodissipata Li & Witkowski sp. nov. (Figure 12). This species is easily assigned to Nitzschia sect. Dissipatae exemplified by N. dissipata (Kützing) Rabenhorst due to raphe position and the irregular fibulae, despite its very small size. Indeed it can even be identified in LM as N. dissipata, however, its marine habitat (salinity exceeding 25 psu), valve width below 3 μm, stria density slightly exceeding 50 in 10 μm and fibula density 12–14 in 10 μm indicate that we are dealing with different species (see Manoylov, 2010 for comparison with N. dissipata).

A clone that we tentatively identified as Diploneis cf. parca based on similarity in gross morphology has a much smaller size than D. parca (12–15 μm in length and 6–7 μm in width in our taxon, versus 20–33 μm in length and 10–17 μm in width in D. parca). Another difference between our clones and D. parca concerns stria density, 23–25 in 10 μm in our clone versus 16–17 in D. parca (Gerloff and Helmeke, 1975; Witkowski, et al., 2000). Although we successfully extracted DNA from the Diploneis cf. parca clone, amplification was unsuccessful. We will delay any taxonomic decision until we manage to amplify and sequence the amplicons.

Both strains of Caloneis sp. isolated from Laizhou samples resembled C. westii in valve length; however, detailed morphological comparisons showed marked differences between the two taxa. Our strains have distinctly smaller valve width (13–15 μm versus 20–28 μm in C. westii) and much denser striae (26–28 in 10 μm) than in C. westii (12–14 in 10 μm). Fur-
thermore the central raphe endings are different, strongly bent on one side (Figure 3e).

The phylogenetic analysis illustrated in Figure 2 shows relationships of the strains from Laizhou Bay. Cocconeis cf. muscarenae is grouped within a clade of Achnanthidaeae with Planothidium sp. (clone SZCZCH26) from Corpus Christi, Texas. A small taxon, which in terms of gross morphology and ultrastructure evidently belongs in Cocconeis, is fairly distant from the cluster of Cocconeis spp. downloaded from GenBank (Cocconeis placenta Ehrenberg—UTEX FD23 and Cocconeis sp. Ehrenberg—ECT3901) in the phylogenetic tree (Figure 2). This is not a new observation; in previously-published phylogenetic trees, Cocconeis stauroeformis has been in a distant position from Cocconeis placenta. For example, in Witkowski et al. (2014) Cocconeis stauroeformis was on a relatively long branch that was sister to a large clade composed of Surrillales and Rhopalodiales. The three clones of N. traheaformis clustered together in our tree with high support values (Figure 2; bootstrap support value [bw] = 99%). Nitzschia nanodissipata (SZCZCH974) is sister to a clade formed by three very small clones of Nitzschia sp. sect. Dissipate. They originated from the Yellow Sea (SZCZCH845, Figure 3h), the Red Sea coast of Saudi Arabia (KSA0039) and the Mozambique coast of the Indian Ocean (SZCZP96). The Yellow Sea clone is sister (bw = 90%) to the clones from the Indian Ocean and the Red Sea. The two latter clones formed a clade with rather high support (bw 97%) and maybe conspecific (Figure 2). Our Caloneis cf. westii (strain SZCZCH1002) is sister to two Caloneis sp. sequences from GenBank, and these three samples are sister to Pinnularia termitina from GenBank (UTEX LB FD484). This clade is sister to a clade that includes P. brebi-ssoi (UTEX FD274) and 3 Caloneis samples from GenBank (Figure 2).

Yellow Sea sites

Water salinity in Yellow Sea sites was slightly higher than those in the Bohai Sea, ranging from 30.6–31.16 psu at the open coast (Muping, Horse Island) to 29.7 psu at the tidal flat in the entrance area to Horse Island. Summer temperatures ranged from 20°C at the open coast to 22.2°C in the artificial pond of Yantai Institute of Coastal Zone Research CAS (YIC) field station to 23.4°C at the tidal flat (low tide). Measured oxygen content at some sites (holothuroidean aquaculture, pond at YIC Station) was low, between 5.47 and 6.83 mg/L, while the open coast in Muping was 8.95 mg/L. The maximum oxygen content has been measured in shallow waters of the Horse Island surf zone (Table 1).

Chang Dao Island

Only two sites sampled on Chang Dao Island (CD5, from sand and CD9, from a rock scrape near the cliff area) yielded clones, with four each. The following clones were isolated from CD5: Surirella cf. fastuosa (Ehrenberg) Kützing (SZCZCH189, Hendey, 1964), a very small unidentified Navicula sensu stricto species (SZCZCH965), Orizaformis holarctica Witkowski, Li & Ashworth (SZCZCH111, Li et al., 2015, Figure 3j) and Trybli- nella sp. (SZCZCH97, Figure 3i; 7), which we describe here as a new species; T. gaoana Witkowski & Li sp. nov. (see “Taxonomic Recommendations” below). One of a few clones that has not been observed under SEM is Navicula s. s. (SZCZCH965), which we prefer to identify as Navicula sp. pending further study (Figure 3i). The four cultures isolated from CD9 (SZCZCH96, SZCZCH98, SZCZCH99 and SZCZCH100) were identified as belonging to the genus Navicula, and identical in terms of quantitative size data and morphology. Three clones (SZCZCH96, SZCZCH98, SZCZCH99) were grouped with bw support of 100% and nested inside a larger clade containing clone SZCZCH100, also with bw support of 100%. These Navicula clones were abundant in the wild sample, and spontaneous auxosporation was observed (Figure 3o). Based on their unique morphology and molecular monophyly we describe these clones as a distinct species, Navicula zhengii Witkowski & Li sp. nov. (see “Taxonomic Recommendations” below).

The best studied of the Yellow Sea diatoms is Orizaformis holarctica, which has been characterized in terms of both morphology and molecular phylogeny and has a geographic range across the Holarctic marine realm of the Pacific Ocean. Strains of O. holarctica have been isolated from Chang Dao Island, Hokkaido Island (CIMP143; ex “Bellerochea malleus”) in Japan and from the Monterey Bay region in California. Our Surirella cf. fastuosa clone is similar in morphology to S. fastuosa in terms of size as the valves (82–93 μm in length and 52–61 μm in width), valve length/width ratio (1.5:1) and the number of transapical ribs (1–2 in 10 μm; Figure 3k) (Hendey 1964, Ruck 2010). However, we still have some doubts about its identity due to the molecular results, which position S. cf. fastuosa sister to a clade including Surirella minutu/Cymatospleura elliptea with a weak support (bw = 43%, Figure 2). Our molecular data show Trybli- nella gaoana clone SZCZCH97 is sister to T. apiculata from GenBank (bw = 100%).

Muping

Muping site 2 (MP2) is an artificial pond located within the Yantai Institute of Coastal Zone Research (YIC) Field Station, filled with seawater from the nearby Yellow Sea. As a result of this semi-isolation, it has slightly higher water temperatures than the nearby Yellow Sea coastal waters; 22.2°C versus 20.2°C. Salinities are not different between the pond and sea, and oscillate between 30 and 31 psu during the summer. Small stones and sand from shallow water were sampled from this pond, but the cultures were established only from sand collections. At this site a very small monoraphid species was isolated (SZCZM123, Figure 3r; 8) which is morphologically similar to Kolbesia amoena, but we recognize it as a new species; Kolbesia sinica Witkowski & Li Ch. sp. nov. (see ”Taxonomic Recommendations” below). On the phylogenetic tree (Figure 2) K. sinicagrouped with Karayeva plaenensis var. gessneri. In the taxonomic section we discuss the generic affinity of Ka. plaenensis var. gessneri, along with the description of Ko. sinica.

Two Halamphora taxa (Halamphora spp. SZCZCH101; Figure 3s and SZCZCH975; Figure 3t), a small undetermined Navicula sp. (SZCZCH703; Figure 3t) and the common marine coastal species Nitzschia auraruae (SZCZCH969) were also isolated from this site. The Halamphora spp. clones have somewhat different valve outlines, i.e. obtuse (SZCZCH975) versus capitate apices (SZCZCH101). However, their sizes are very similar; valve length is 9.8–11.7 μm versus 13–14 μm, while stria density is 22–28 in 10 μm and 26 in 10 μm in SZCZCH101 and SZCZCH975, respectively. In SEM, both taxa have striae composed of double rows of small areolae and resemble Amphora tenerinna Husttedt (Clavero et al., 2000). Similar in LM is also our Halam-
phora clone SZCZH623 (Figure 4a) isolated from a microbial mat at Muping; however, SEM reveals areolae composed of single rows of areolae. Our Halamphora clones form a clade with H. cofaeiformis (7977–AMP1H01) from GenBank, although with low support (lv = 64%). Likewise, relationships among our Halamphora clones received low lv support; this clade needs further morphological and molecular work to resolve relationships. The fourth Navicula sp. (SZCZH703, Figure 3u; 6) is relatively small, with the valve length of 14–16 μm and width of 4.7 – 5.6 μm, and bears rather coarse striation (18 – 19 in 10 μm) easily resolvable in LM. Morphologically, it can be mistaken for small Hippodonta spp. Lange-Bertalot, Metzeltin & Witkowski. Our observations of samples from the marine littoral zone show that Hippodonta sp. from these habitats are usually robust and have resolvable structural characters, like a low striae density in LM (Witkowski et al., unpublished observations). Careful examination in SEM and comparison with similar established taxa (e.g. N. perminuta Grunow) suggest that this Navicula strain is a new species. We describe it here under the name N. hippocontafallax Witkowski & Li Ch. sp. nov. (see “Taxonomic Recommendations” below). Navicula hippocontafallax (SZCZH703) is sister to the Navicula tripunctata/N. cavi/Navicula cryptocephala/Navicula reinhardtii clade with low bootstrap support (lv = 47%). Finally, clone SZCZH969 shows features typical of Nitzschia aarauiae in LM (Figure 3v), but we have not confirmed the identity with SEM. DNA sequence data (Figure 2) places this strain sister to another clone of Nitzschia aarauiae (SZCZH966; Figure 4b), which was isolated from Muping research station.

Three and two clones were isolated from samples collected at Muping 3 (MP3) and Muping 6 (MP6), respectively. Both samples were taken from exposed microbial mats with dense growth of diatoms and cyanobacteria at the coast in front of YIC field station. Both sites have been impacted by a small stream discharging into the sea, resulting in a decrease in salinity (23.42°C) and increase in water temperature (32.38°C). Dissolved oxygen content was rather low at 6.63 mg/L. There were several clones that appear to be undescribed taxa in these samples. One of these, clone SZCZH968, fell with in the Stauroneidaceae the molecular phylogeny (Figure 2) and our literature search suggests that this clone represents an undescribed genus and species. Its valves are very small and not easily resolvable under LM, with a valve size of 12–14 μm in length and 2.7 – 3.5 μm in width, and the transapical striae are parallel throughout with 30–36 in 10 μm. The most characteristic feature of this clone is the raphe sternum, distinct even in LM and appearing slightly elevated above the valve surface in SEM (Figure 4e; 8). This clone is in a basal position of Stauroneidaceae clade, sister to Parlibellus/Fistulifera and Stauroneis/Cricatula (Figure 2), although bootstrap values are very low within this clade. Here we establish a new genus based on the morphology of clone SZCZH968 and name it Sternimirus shandongensis Witkowski & Li Ch. gen. et sp. nov. (see “Taxonomic Recommendations” below). It differs from Stauroneis by the absence of a staurus. (Round et al. 1990, Lange-Bertalot, 2001; this study).

Amphora sp. SZCZH697 is in a clade with one species of Amphora subgenus Oxyamphora Cleve. Our literature search suggests that this clone represents a new species, based on morphology and morphometric data. Its size is small and the structure of the valve barely resolvable under LM, hence we describe it under the name Amphora vixivisibilis Li & Witkowski sp. nov. (Figure 4d, 10). Interestingly, clone SZCZH967 clusters with Amphora laevisissima from GenBank (7314–AMPHO85) at the basal part of the tree and is sister to the whole clade of Bacillariaceae and Achnanthaceae spp. (Figure 2). Stepanek and Kociolek (2014) have already shown that Amphora as a genus is polyphyletic and requires a thorough revision based on frustule and chloroplast as well as DNA sequence data.

From the second microbial mat collection (MP6), we isolated another Nitzschia aarauiae clone (SZCZH966, Figure 4b). The gross morphology and size data thoroughly conform to the description of N. aarauiae by Krammer & Lange-Bertalot (1988). Its phylogenetic position (Figure 2) is within the Bacillariaceae, forming a clade with another clone of Nitzschia aarauiae, which is sister to Denticula kuetingii Grunow. We also isolated Pleurosigma cf. stuxbergii Grunow (SZCZH973, Figure 4h) from this collection. This species shows some characteristics of P. stuxbergii, such as the crossing angle of oblique striae systems (ca. 51°); however, it is much smaller than P. stuxbergii (cf. Cleve and Grunow, 1880). This strain forms a clade with another species representing Pleurosigma, Gyrosigma acuminatum (Kützing) Rabenhorst from GenBank (UTEX FD317, Figure 2).

Aquaculture in Yantai

We also sampled aquaculture enclosures dedicated to raising holothur ioidians located very close to the sea coast, south of Binghai Rd. (Figure 1). The environmental data for the enclosures was the same as for the open coast, with a salinity of 30.2 psu and water temperature of 25.44°C. Four clones were isolated from these enclosures; two clones of Amphora helenensis Giffen (SZCZH581, 582, Figure 4e; f, Giffen 1973), one monoraphid diatom (SZCZH662, Figure 4g) identified here as Cocconeis cf. cu-pulifera Riaux-Gobin, Romero, Compère & Al Handal (Riaux-Gobin et al., 2011, see our Table 4) and a single clone of Nitzschia sp. (SZCZH658, Figure 4i) apparently belonging in sect. Dubiae (Krammer and Lange-Bertalot, 1988). For comparative purposes, two additional strains of A. helenensis were added to the dataset for phylogenetic analysis, including one from the Adriatic Sea (SZCZH774, 4j) and one from Namibia (SZCP12, 4k). The Yellow Sea strains identified as Amphora helenensis conform to the species description by Giffen (1973). Valve length ranged between 12.9-19.5 μm and the width from 2.9-4.3 μm, and stria density was 18-21 in 10 μm, slightly exceeding those given by Giffen (17-20 in 10 μm, Giffen 1973). LM examination of the clones revealed the presence of a hyaline strip crossing the striae on the dorsal side and central area developed only at the ventral side, the most characteristic feature of A. helenensis. These strains form two subclades, sister to A. pediculus (Kützing) Grunow ex. A. Schmidt from GenBank (9491-AMPHO08) in the phylogenetic tree (Figure 2). One subclade consists of one of the clones isolated from aquaculture enclosures (SZCZH582) and A. helenensis (SZCP12) from Namibia, though with low support (lv <50%). The second subclade contains the Adriatic Sea clone (SZCZH774), sister to the two Yellow Sea clones (SZCZH95; Figure 4i, isolated from Yantai public Beach [Y21] and SZCZH704; Figure 4m.
isolated from Horse Island biofilm on small stones) (Figure 2). There is substantial genetic diversity represented in this clade, which could suggest cryptic speciation.

The Nitzschia sp. sect. Dubiaea train (SZCZCH658) isolated from this collection formed a clade with the N. dubiformis Hustedt from GenBank—(AB430696 for rbcL, and AB430616 for SSU, Figure 2). These two taxa are sister to a clade formed by our three clones of Nitzschia trabeaformis (SZCZCH970, 971, 972, Figure 2). Although strain SZCZCH658 resembles N. dubiformis in gross morphology, it is much larger (83–86 μm versus 40–50 μm in length) and has much coarser striation (26 in 10 μm versus 40 in 10 μm).

The last clone isolated from aquaculture is a monoraphid diatom identified as Cocconeis cf. cupulifera (SZCZCH662; Figure 4g). This species has some ultrastructural characters similar to representatives of Cocconeis, such as the internal central raphe endings bent in opposite directions, and groups with other Cocconeis clones (SZCZP67 and UTSA0056) in the phylogenetic tree (Figure 2). Cocconeis cf. cupulifera has a convex raphe valve, while the sternal valve is concave, which is opposite from other described Cocconeis spp. (e.g. Kramer and Lange-Bertalot, 1991; Round et al., 1990). Clone SZCZCH662 also shows a certain degree of similarity in the raphe valve to some representatives of Psaenothrix Buchtyarowa & Round 1996, e.g. P. levanderi (Hustedt) Buchtyarowa & Round 1996 (Potapova, 2010a), but differs from either Psaenothrix or Cocconeis in having a raphe valve with a hyaline band running close and parallel to the margin. The sternal valve of C. cf. cupulifera similarly to C. cupulifera has simple marginal processes (cf. Riaux-Gobin et al., 2015). Since we have not observed the girdle bands and have at least one more clone with similar ultrastructure, we refrain from any taxonomic decisions of this taxon until more observations and molecular data are available.

**Public beach in Yantai, Moon Bay**

Three clones were isolated from Yantai (Y11) public beach in Moon Bay, which has a very flat sandy bottom; a very small Nitzschia sect. Dissipateae (SZCZCH845; Figure 3h); Entomoneis sp. (SZCZM496; Figure 4i); and the Amphora helenensis (SZCZCH95) clone mentioned above. The Entomoneis clone is notable, in that it appears to be one of the smallest representatives of the genus ever recorded, with the apical axis ranging from 12 to 20 μm and a stria density of 50 in 10 μm (Osada and Kobayasi 1990).

Clone SZCZCH845 seems closely related to another very small Nitzschia sp. sect. Dissipateae (SZCZCH974) clone that we have described here as Nitzschia nanodissipata (Figure 2). In terms of shape, stria and fibulae density clone SZCZCH845 seems to belong to the same taxon as clones from the Red Sea (KSA0039) and Indian Ocean (SZCZP36); genetically, the three are monophyletic as well, with clone sister (bv = 90%) to a clones KSA0039 and SZCZP36. Based on morphological and molecular data, we describe these clones as Nitzschia volvendirostrata Ashworth, Đahek & Witkowski (see "Taxonomic Recommendations" below; Figure 13). We should also note that all the Nitzschia sp. sect. Dissipatiae in this analysis (including Nitzschia martiana and Nitzschia sp. KSA0035 are monophyletic with a high degree of support (bv = 96%).

An additional sample from fine sand of Moon Bay at Yantai public beach (Y11) yielded two clones: Parlibellus sp. (SZCZCH75, Figure 4p) and a peculiar Halamphora sp. (SZCZH452, Figure 4r). Clone SZCZCH75 is included in Parlibellus based on the frustule morphology and the two deeply lobed plastosids (Figure 4s) typical for the genus (Cox 1988). Also typical for Parlibellus is the girdle of the frustule, which is broad and composed of numerous perforated bands. To the best of our knowledge, none of the established Parlibellus taxa possess dorsiventral frustules present in clone SZCZCH75; thus, we describe this clone as a new species: P. harffiana Witkowski, Li Ch., & Yu sp. nov. (Figure 9). On the three gene phylogenetic tree, P. harffiana is placed within the Stauroneidaeae (Figure 2, but see also Nakov et al., 2014); this is a different placement than the morphology-based classification of Parlibellus E. J. Cox (Cox, 1988), in which Parlibellus is included in the family Berkeleyaceae D. G. Mann in Round et al. (1990), which is represented in our tree by Berkeleya rutinals (ECT3616) and Climaconeis ridulene (ECT3724). Parlibellus harffiana is sister (bv <50%) to a small clade formed by two Parlibellus strains—one of which is from Guan and the second one from Santa Rosa in Costa Rica (Figure 2).

**Halamphora clone SZCZH452 (Figure 4r, 11) has a valve structure typical for Amphora sensu stricto, with dorsal striae composed of a few solitary areolae and central area developed on a relatively broad ventral side. However, it has girdle bands that are perforated with a row of solitary puncta. The phylogenetic analysis placed clone SZCZH452 within the Halamphora clade (Figure 2). Contrary to the "citrus fruit-like" frustules of Amphora sensu stricto and Halamphora (Levko 2009), SZCZH452 has frustules resembling Catenula or Eucynoma. The two valves of a given frustule are dorsiventral, but their valve face is flat, and the perforated girdle bands do not show compression on the ventral side (Figure 11d). Taking into account our literature search and its position on the phylogenetic tree we describe this clone as a new species of Halamphora; *H. catenulafalsa* Witkowski & Li Ch. sp. nov. (Figure 11).

**Horse Island**

Horse Island is connected via a bridge to the mainland and is located east of Yantai. Salinity in this site is the same as other Yellow Sea sites; however, due to the rocky bottom, water is very well mixed and oxygenated (11.73 mg/l O₂), transparent and warm (29.45°C). Although several samples were taken, the only clone isolated from this location originates from small stones covered with biofilm; Amphora helenensis (SZCZCH704, Figure 4m, already discussed above).

**Taxonomic Recommendations**

As previously discussed, some clones isolated for this study represent significant genetic diversity within certain clades (Navicula, Nitzschia dubiformis, Amphora). While it is possible these strains represent interspecific genetic diversity among cryptic species, at this time we cannot detect any morphological or ultrastructural character that supports naming new species from these clones. Therefore, until we have more data these identifications must remain provisional but we feel they must be included in this manuscript to demonstrate some of the issues that must be resolved for metagenomic surveys to accurately describe an environment.
Figure 4. Light microscopic images of the diatom clones from the Bohai and Yellow Sea. LM, DIC. Figure a. *Halamphora* sp. Figure b. *Nitzschia aurantiaca*. Figure c. *Sternimitus shandongensis* Witkowski & Li Ch. gen. et sp. nov. Figure d. *Amphora sxxvisibilis* Li Ch. & Witkowski. Figs e, f. *Amphora helenensis* Giffen. Figure g. *Cocconeis cf. cupulifera*. Figure h. *Pleurosigma cf. stuxbergii*. Figure i. *Nitzschia cf. dubiformis* Hustedt. Figs j-m. *Amphora helenensis*. Figure n. *Cocconeis* sp. SZCZP67. Figs o, t. *Entomoneis* sp. Figs p, s. *Parlibellus harffiana* Witkowski, Li Ch. & Yu Sh. sp. nov. Figure r. *Halamphora catenulafalsa* Witkowski & Li Ch. sp. nov.

Scale bar in Figure h = μm.
Figure 5. *Navicula zhengii* Witkowski & Li Ch. sp. nov. illustrated in SEM. Figure a. External valve view, note the presence of Voigt discordance pointing a secondary valve side (arrow) and strongly bent apical raphe end (arrowhead). Figure b. Internal valve view. Figure c. Close up of the valve apex exterior, note rather small, but distinctly hooked towards secondary valve side raphe apical end. Figure d. Close up of the internal side of the valve apex, note the oblique position of the raphe slit (arrowhead).
Naviculaceae Kützing 1849

*Navicula* Bory de St. Vincent

*Navicula zhengii* Witkowski & Li Ch. sp. nov. (Figure 3m–p, 5a–d)

Diagnosis: Valves lanceolate with very slightly offset, acutely rounded apices, 10.2–22.5 μm μm in length (15–21 μm in culture), 3.9–5.5 μm in width. Axial and central area barely recognizable in LM. Raphe straight, filiform, external central raphe endings distinct, approximate each other, apical raphe endings in LM not observed. Transapical striae in LM easily resolvable, slightly radiate, becoming slightly convergent before apices, 21–25 in 10 μm.

Holotype: slide no. SZCZCH99, deposited in Palaeoceanology Unit, Faculty of Geosciences, University of Szczecin, leg. A. Witkowski June 26th 2013

Isotype: slide BM 101818 in the Natural History Museum, London, UK.

Habitat: Chang Dao island near Yantai, China, cliff rock in Geopark, Yellow Sea; 37°57.17′ N 120°44.04′ E, rock scrape.

Etymology: this species is dedicated to Professor Zheng Zhichang from Guangzhou Marine Geological Survey (GMGS) who is an esteemed geologist in appreciation of his achievements in science and promotion of scientific cooperation between GMGS and University of Szczecin in Poland.

Morphology: Valve external surface flat, with shallow steep mantle (Figure 5a, b). Girdle bands narrow, plain. Transition between the valve face and the mantle rather steep. Axial area very narrow, very slightly broadening into a small but distinct rhombic central area (Figure 5a). Raphosternum slightly elevated in the valve middle, slightly bent along apical axis. External central raphe endings slightly expanded, tear drop-like. Apical raphe endings bent in the same direction to form a short hook (Figure 5a, c). Transapical striae composed of slit-like areolae arranged in rows parallel along the apical axis, prolonged onto the mantle consequently in all clones, 45–50 in 10 μm. Internal valve flat, mantle shallow. Internal central raphe endings slightly expanded, simple (Figure 5b). Raphe internally terminating in a small and simple helictoglossa at the apices (Figure 5d). Striae forming areolae positioned in shallow grooves between the two neighbouring virgae.

Comparison with similar established taxa; *Navicula zhengii* sp. nov. is unlikely to be mistaken with any previously established small *Navicula* species. Small *Navicula* from brackish-water and marine environements, e.g. *N. paul-schulzii* Witkowski & Lange-Bertalot, *N. alexandrae* Lange-Bertalot, Bogaczewicz-Adamczak & Witkowski, *N. bozenae* Lange-Bertalot, Witkowski & Zgrundo all posses more robust striation and distinct central area with external central endings distant from each other (e.g. Witkowski, 1994; Lange-Bertalot et al., 2003).

*Navicula hippocontafallax* Witkowski & Li Ch. spec. nov. (Figures 3u, 6a–d)
Diagnosis: Valves linear with parallel margins and broadly rounded apices, 14.4–16 μm in length, 4.7–5.8 μm in width. Axial area in LM barely resolvable, central area in a form of relatively narrow fascia developed as a result of shortening of a single stria in the valve middle. External central raphe endings approximate, barely discernible in LM. Transapical striae relatively robust, more or less parallel, 18–20 in 10 μm.

Holotype: slide no. SZCZCH703, deposited in Palaeoceanology Unit, Faculty of Geosciences, University of Szczecin, leg. A. Witkowski, June 2013

Isotype: slide BM 101819 in the Natural History Museum, London, UK.

Habitat: Muping near Yantai, China, Yellow Sea, 37° 27’ 21.71” N 121° 42’ 10.38” E, water from the sediment.

Etymology: the specific epithet “hippodontaffalax” is derived from the similarity of the species in LM to some small Hippodonta spp.

Morphology: Valve face flat with relatively shallow mantle (Figure 6a). The transition from the valve face to the mantle is gradual, the mantle shallow and areolated. Axial area very narrow, somewhat broader at the valve primary side. Central area, fascia like, developed due to shortening of the solitary middle stria pair. External central raphe endings slightly expanded, drop-like, relatively distant from each other (Figure 6a). Apical raphe endings geniculate, strongly hooked in the same side. Internal central raphe endings small, very close each other (Figure 6b–d). Transapical striae in SEM parallel until the middle of the raphe, branch length becoming divergent towards the apices. Striae composed of closely spaced, apically elongate areolae, 50 in 10 μm (Figure 6b–d).

Comparison with similar established taxa: Navicula hippocmotaffalax sp. nov. shows some similarity to Hippodonta species, e. g. H. linearis (Østrup) Lange-Bertalot, Metzeltin & Witkowski. This species, however, is characterized by much coarser striation and occur in brackish-waters. N. hippocmotaffalax is unlikely to be confused with any established species of Navicula sensu stricto due to its relatively robust and parallel striation despite very small size. Its central area somewhat resembles N. perminuta Gronow, which has a different narrowly lanceolate shape, narrower valves (2–4 μm), and coarser areolae (33 in 10 μm, e. g. Lange-Bertalot, 2001). To some extent N. hippocmotaffalax resembles Navicula pseudotypes H. Kobayasi, which has been synonymized with Hippodonta pseudotypes (H. Kobayasi) Lange-Bertalot emend. Blanco in Blanco et al. (2012). The major difference between the two taxa is that N. hippocmotaffalax is apical raphe endings, which are simple in H. pseudotypes and geniculate in N. hippocmotaffalax. It is possible that the diatom identified as N. pseudotypes from the Xiamen harbour (Cheng et al., 1993, Figure 150, 151) actually represents N. hippocmotaffalax, though it is difficult to be certain based on the images presented.

Stauroneidaceae D. G. Mann 1990 in Round et al. (1990)

Kolbesia sinica Krzywda, Witkowski & Li Ch. sp. nov. (Figures 3r, 7a–d)

Figure 7. Kolbesia sinica Witkowski & Li Ch. sp. nov. illustrated in SEM. Figure a. Raphe valve external view, note the presence of strongly hooked apical raphe ends (arrowhead) and large areolae (macroareolae sensu Bukhtiyarova 2006) covered with thin silica flap (arrow) and slightly expanded, approximate external central raphe endings (black arrowhead). Figure b. Raphe valve internal view, note coaxial areolae central raphe endings (arrowhead). Figure c. Sternum valve external view, note the presence of sealed raphe slit (arrow). Figure d. Sternum valve internal view, note the presence of large areolae covered with thin silica flap (arrowhead).
Diagnosis: Valves linear with slightly set off, obtusely rounded apices, \( n = 54 \), 10.0–13.0 \( \mu \)m long, 3.3–4.1 \( \mu \)m broad. Sternum valve (SV); stertum very narrow, linear, slightly expanded in the middle. Transapical striae parallel in the middle becoming slightly radiate towards the apices, 31–34 in 10 \( \mu \)m. Raphe valve (RV); raphe straight, axial area very narrow linear, central area almost absent, transapical striae parallel in the middle becoming slightly radiate towards apices, 38–40 in 10 \( \mu \)m.

Holotype; slide no. SZCZM123 deposited in Palaeoceanology Unit, Faculty of Geosciences, University of Szczecin, leg. A. Witkowski, 24.06.2013.

Isotype; slide BM 101820 Natural History Museum, London, UK.

Type locality; Yellow Sea coast, Shandong Province, East China; 37°27′19″.37″ N 121°42′7.27″ E.

Habitat; sandy bottom of the artificial saltwater pond at the YIC field station in Muping.

Etymology; sinica (Latin = Chinese) referring to the country of origin of the holotype habitat.

Morphology; The complete frustule in girdle view has not been observed thus far, but the few loose girdle bands we have seen are non-perforated.

SV; Sternum valve surface slightly convex at the margin becoming flat towards the middle. The mantle is very shallow, the narrow stertum is progressively slightly expanding from apices towards the middle (Figure 7c). The transapical striae are composed of large macroareolae, occluded with thin hymenes (Figure 7d). At the valve surface and interior slightly below apices along both sides of the stertum a relatively broad lateral area is observed. Internally the stertum valve has shallow and structure less mantle. The stertum is very narrow, and indistinctly raised above the valve surface level (Figure 7d).

RV; Raphe valve external surface is slightly concave at the margin, and the stertum-sternum is slightly elevated above the valve surface. Axial area is very narrow and strictly linear, expanded in the middle to form an oblong central area. The raphe is filiform and straight. The external central raphe endings are in a form of a small drop, positioned close to each other, and the apical raphe ends are hooked in the same side (Figure 7a). Transapical striae are parallel to slightly radiate and are positioned in shallow grooves separated by somewhat elevated virgae. The striae are composed of large macroareolae, occluded with thin hymenes (Figures 7). Similar to SV, the surface of the RV bears a lateral area. Internally the raphe valve is flat with shallow mantle (Figure 7a). Internal central raphe endings are slightly expanded, close to each other and coaxial, the raphe terminates in a very small and indistinct helictoglossa (Figure 7b).

The newly described species is known thus far only from the type habitat, the Bohai Sea coast at Muping, Yantai, Shandong Province in East China.

Comparison with similar established taxa; In terms of gross morphology, the species resembles Madinithidium Witkowski, Desrosiers & Riaux-Gobin in Desrosiers et al. (2014), however, they differ significantly in the ultrastructure of the striae. In Madinithidium, both valves have striae with large membrane-like occlusions, whereas in Kolbesia sinica (clone SZCZM123) occlusions are apparently structureless (Figure 7a; d). In the phylogenetic tree, Kolbesia sinica is sister to Karayevia ploenusis var. gessneri, and this pair is sister to a clade composed of Schizostauron sp. and Astatiella sp. The Muping monoraphid culture is morphologically very different than Karayevia ploenusis var. gessneri (Potapova, 2010b), due to its capitate valves versus only slightly set off apices in a new species and robust valve structure which allows to recognize easily the striation of both valves under LM. The striae of the RV in K. ploenusis var. gessneri are radiate and easily recognisable in LM whereas in K. sinica they are first visible under electron microscope and parallel throughout Karayevia ploenusis var. gessneri in our opinion shall be transferred into Kolbesia because its morphology is significantly different from Karayevia. The two genera Kolbesia and Karayevia have completely different striaion (Round and Bukhtiyarova, 1996). In Karayevia, both valves have striae composed of small circular areolae, whereas in Kolbesia the striae are formed of macroareolae which are covered externally with a sicula flap hence the two transapically elongate small areolae are visible in electron microscope (cf. Snoeijis, 1993, in Achnanthes amoena Hustedt, Round & Bukhtiyarova 1996; Potapova 2010c in Karayevia amoena (Hustedt) Bukhtiyarova).

Sternimirus Witkowski & Li gen. nov.

Frustules rectangular with rounded corners, with a few bands. Chloroplasts unknown. Valves linear to linear elliptic with obtusely rounded apices. Valve face slightly domed. Raphe stertum relatively robust, axial area narrow in LM, distinguishable. Raphe filiform, straight, external central endings somewhat expanded, approximate, apical raphe endings terminate at apices in a form of geniculate hook in the same side. Transapical striae very dense but resolvable in LM, in SEM composed of rectangular areolae, which are elongate about the transapical axis. Internal central raphe endings simple, very slightly bent in the same side.

Type species; Sternimirus shandongensis Witkowski & Li Ch. sp. nov.

Etymology; the name of the new genus is derived from Latin name of stertum and mirus-Latin ‘surprising’, in reference to valve shape of new genus.

Habitat; observed in marine environment of Bohai Sea, the sandy bottom of shallow part of an artificial pond from YIC research station at Muping, Yellow Sea coast, Shandong Province in East China.

Sternimirus shandongensis Witkowski & Li Ch. sp. nov. (Figure 4c, 8a, b)

Diagnosis; Valves linear with obtusely round apices, 12–14 \( \mu \)m in length, 2.7–3.5 \( \mu \)m in width. Raphe stertum narrow linear but distinct even in LM and in SEM slightly elevated above the valve surface. Axial area very distinct, narrow and linear, central area missing likely due to elevated raphe-sternum. Raphe simple, slit-like. External central raphe endings simple, very slightly expanded, close to each other. Transapical striae parallel throughout, resolvable under LM, 30–36 in 10 \( \mu \)m.

Holotype; slide SZCZH968 deposited in Palaeoceanology Unit, Faculty of Geosciences, University of Szczecin, leg. A. Witkowski, 24.06.2013.

Isotype; slide BM 101821 Natural History Museum, London, UK.

Type locality; The Bohai Sea coast, Shandong Province, East China; 37°27′19″.37″ N 121°42′7.27″ E.

Habitat; sandy bottom of the artificial saltwater pond at the YIC field station in Muping.
Etymology: *shandoongensis* derived from the name of the province viz. Shandong, East China in which the Yellow Sea coast has been studied.

**Figure 8.** *Sternimirus shandoongensis* gen. et sp. nov. Witkowski & Li Ch. illustrated in LM and SEM. Figure a. A series of valves illustrated in LM, note a distinct stement and central nodule and parallel striae. Scale bar x3000. Figure b. An aggregate of valves showing external and internal views. Note strongly hooked apical raphe ending (arrow) and transapically elongate areolae (arrowhead)

**Morphology:** 

Valves linear with obtusely round apices, valve surface slightly domed. Raphe sternum narrow linear, slightly elevated above the valve surface. Axial area very distinct, narrow and linear, central area missing likely due to elevated raphe-sternum. Central nodule very well distinguished, rectangular. Raphe simple, slit-like. External central raphe endings simple, very slightly expanded, close to each other (Figure 8b). Apical raphe endings terminate below the apices hooked in the same side. Internally, proximal raphe endings are small and very slightly bent in one side, whereas distally raphe terminates in a very small helictoglossa (Figure 8b). Transapical striae parallel throughout, under SEM composed of transapically elongate areolae, 40 in 10 μm.

Comparison with established taxa: Due to its very small size *S. shandoongensis* can be misidentified as several marine monoraphid taxa, such as the recently established *Madinitihidium* Witkowski, Desrosiers & Riaux-Gobin. The absence of a SV, usually associated with the RV in wild samples should help to distinguish bi-raphid *Sternimirus* taxa from monoraphid taxa. Other genera, such as *Eolimna* occur only in freshwater, or like *Fistulifera* (Lange-Bertalot, 2001), which has a very characteristic raphe sternum and even much more delicate structure. Tiny *Chamaepinnularia*, which also may be confused with *Sternimirus*, usually have a much more coarse valve structure and wide lanceolate axial area. Interestingly *Sternimirus* appears to have apically elongate areolae and this is typical for *Stauroneis* taxa (Round et al., 1990).

Comment: in the recent sampling a second undescribed yet species of *Sternimirus* has been isolated, successfully grown and sequenced. LM and EM and three gene data of the latter species place it in a small clade with *S. shandoongensis* (Witkowski & Li unpublished observations).

*Parlibellus harffiana* Witkowski & Li Ch. & Yu sp. nov. (Figure 4p, s, 14a-c)

**Diagnosis:** Frustules rectangular in girdle view with rounded corners. Chloroplasts two per cell. Valves dorsiventral, semi lunate, with broadly rounded apices, 8.5–16.5 μm in length, 3.5–5.0 μm in width. Axial and central areae in LM barely discernible, central nodule distinct, external central raphe endings distinct, slightly expanded. Transapical striae parallel throughout becoming slightly radiate towards apices, 20–22 in 10 μm.

**Holotype:** slide no. SZCZCH75, deposited in Palaeoceanology Unit, Faculty of Geosciences, University of Szczecin, leg. A. Witkowski June 27, 2013

**Isotype:** slide BM 101822 Natural History Museum, London, UK.

**Habitat:** Yantai public beach, sand from the very gently sloped splash zone.

**Etymology:** This species is named in honour of Professor Jan Harff, distinguished geologist whose scientific interest has focused on coastal processes of the Bohai and Yellow Seas and the Baltic Sea.

**Morphology:** Frustules in girdle view rectangular with rounded apices, girdle composed of numerous punctated bands (Figure 9a). Valve surface flat, with the transition between the valve face and the mantle gradual. Axial area asymmetric, very narrow, along the valve ventral side, somewhat broader along the dorsal one, becoming broader towards the valve centre and with a small, apically elongate central area. Raphe external central endings slightly expanded relatively distant from each other. Apical raphe endings terminate in apices in a form of short, ventrally-deflected side hooks. In some specimens a very narrow canoepum-like structure positioned along the raphe has been observed (Figure 9b). Transapical striae composed of oblong areolae, 25–30 in 10 μm, in some specimens closed with externally positioned flap like occlusions (Figure 9b). The areolae are externally positioned in a shallow grooves. Internally valve surface flat, with relatively shallow mantle. Raphe simple slit-like, internal central endings distinct, somewhat expanded, at apices terminate is a small and simple helictoglossa (Figure 9c). Transapical striae forming areolae positioned internally into distinct grooves.

Comparison with similar established taxa: *Parlibellus harffiana* can only be compared with some Lunella Snoeij species and *Lunella ghalebii* Witkowski, Lange-Bertalot & Metzeltin in parti-
cular. The taxa in question differ, however, in terms of size data.

**Catenulaceae Mereschkovsky**

*Amphora vixvisibilis* Li Ch. & Witkowski sp. nov. (Figure 4d, 10a–d)

Diagnosis: Valves slightly lunate, dorsi-ventral, with straight to slightly undulate ventral margin, gently bent dorsal margin and obtusely rounded apices. Valves 13.1–15.2 μm long, 2.8–4.8 μm in width. Raphe sternum slightly bent towards dorsal margin, positioned close to the ventral margin. Transapical striae not resolvable in LM; in SEM, 43–46 in 10 μm.

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**Figure 9. Parlibellus karfiuna** Witkowski, Li Ch. & Yu Sh. sp. nov. illustrated in SEM. Figure a. External view of two complete frustules, note the perforated girdle band (arrow). Figure b. External valve view, note the presence of relatively large central nodule (arrow) and apical raphe endings terminating on the valve mantle beyond an apex (arrowhead). Figure c. Internal valve view.

**Figure 10. Amphora vixvisibilis** Li Ch. & Witkowski sp. nov. illustrated in SEM. Figure a. External view of the valve, note long strongly bent apical raphe ends (arrowhead). Figure b. Internal view of the valve, note short and simple apical internal raphe ends terminating in a small helictoglossa (arrowhead). Figure c. Close-up of the valve centre external view of specimen, illustrated in Figure a. Figure d. Close-up of the valve internal view of the specimen illustrated in Figure b.
Figure 11. Halamphora catenulafalsa Witkowski & Li Ch. sp. nov. Isotype; slide BM 101823 in the Natural History Museum, London, UK.

Etymology: the name of this species is derived from its transapical striae not visible under LM, “vixvisibilis” means “not visible”.

Morphology: While the whole frustule was not observed, the girdle appears to be composed of several open bands, each with three rows of small perforations. Axial area narrow, central area in a form of hyaline fascia present only at the dorsal side, spanning the space from the central node to the valve dorsal margin, slightly depressed below the valve general external surface (Figure 10a, c). Raphe composed of two branches, each slightly bent towards the valve dorsal side. External central raphe endings approximate each other, barely expanded and bent towards the ventral side, apical endings strongly bent and positioned in a distinct groove (Figure 10a, c). Internally central raphe endings bent towards the dorsal side and apically terminate in very small helictoglossae (Figure 10b). The transapical striae (only visible in SEM) on dorsal valve side parallel for almost the entire length except at apices where they become slightly convergent, 43–46 in 10 μm. At the ventral side, striae significantly denser, 53–56 in 10 μm. Transapical striae composed of small oblong areolae, up to 60 in 10 μm at the dorsal side and up to 80 in 10 μm at the ventral side (Figure 10d).

Comparison with similar established taxa; All described taxa belonging in Amphora subgen. Oxyamphora from marine environments are significantly larger than our species. Recently Edlund et al. (2009) described Amphora soninkhishigae Edlund, Shimneman & Levkov from saline lakes in Mongolia in Amphora subgen. Oxyamphora, but this taxon is somewhat larger, has a different valve shape and a higher stria density (ca. 50 in 10 μm) than our clone.

_Halamphora catenulafalsa_ Witkowski & Li sp. nov. (Figures 4r, 11a–d)

Diagnosis: Frustules rectangular in girdle view with rounded apices. Valves dorsiventral, semi-lunate, dorsal margin strongly bent, ventral margin straight to slightly convex, with obtusely rounded apices, 4.5–9.5 μm long, 3.1–5.2 μm in width. Raphe very close to ventral margin, axial area barely distinguishable, central area absent. Transapical striae on the dorsal side relatively coarse, parallel only in the valve middle, becoming convergent towards apices, 20–23 in 10 μm.

Holotype: slide no. SZCZCH452, deposited in Palaeoceanology Unit, Faculty of Geosciences, University of Szczecin, leg. A. Witkowski June 2013

Habitat: Yantai Institute of Coastal Zone Research, field station, microbial mat at the exposed sites, fine sand; 37°27’ 19.92” N 121°42’ 10.54” E

Isotype; slide BM 101824 in the Natural History Museum, London, UK.

Etymology: the name of this species is derived from Latin “falsa”-means false, in this case a false _Catena_.

Morphology: Frustules in girdle view observed under SEM show the presence of distinct bands perforated with a single row of relatively large puncta (Figure 11a, b). Valve surface flat, valves parallel to each other (Figure 11c, d), with no girdle compression at the ventral side as is typical for _Halamphora_ and _Amphora sensu strictio_ (Levkov, 2009). Raphe straight, relatively close to the ventral margin. External central raphe endings indistinct, very close each other. Apical raphe endings short, strongly
bent towards the dorsal side. At the dorsal side raphe slit is usually covered with a very narrow canalpeum. Axial area in SEM very narrow, central area mostly absent but a rectangular area present in some specimens at the ventral side. Transapical striae composed of solitary oblong areolae, which are positioned in shallow grooves, ca. 20 in 10 μm (Figure 11a, c). Virgae much broader than the striae (Figure 11c).

Comparison with established taxa: As discussed in the description, *Halamphora catenulafalsa* is unlikely to be confused with any established species either in *Halamphora* or in *Amphora sensu lato*, nor is it likely to be confused with a few described *Catenula* species. *Catenula* has short raphe branches with a prominent central nodule and apical raphe endings apart from the apices. Additionally, the striae of *Catenula* are either almost absent or composed of a few areolae (Witkowski et al., 2000). Small species from a recently-described group of taxa related to *Amphora pediculus* (Levkov, 2009) all are different from newly described species.

**Bacillariaceae Ehrenberg**

*Nitzschia nanodissipata* Li & Witkowski sp. nov. (Figures 3d, 12a–d)

Diagnosis: Frustules differ from many species in the genus due to the central position of the canal raphe. The girdle composed of bands perforated with a few rows of poroids. Valves lanceolate with apices very slightly set off and acutely rounded, 15–16.5 μm in length, 2.5–4.0 μm in width. Fibulae distinct, the distance between fibulae variable, seemingly the two median fibulae somewhat apart from each other, 8–14 in 10 μm. The transapical striae are not resolvable in LM.

Holotype: slide no. SZCH974, deposited in Paleooceanology Unit, Faculty of Geosciences, University of Szczecin, leg. A. Witkowski June 2013

Habitat: Laizhou Bay, site LB1, water sample; 37°27.362′ 121°42.215′

Isotype: slide BM 101825 in the Natural History Museum, London, UK.

Etymology: the specific epithet refers to the smaller size dimensions in comparison to similar *Nitzschia dissipata*; Greek “nanos” means dwarf.

Morphology: Valves lanceolate with apices very slightly set off and acutely rounded. Canal raphe in a more or less central position. A distinct canalpeum is attached to the canal raphe along its whole length. In SEM the whole length of canalpeum is marked with a single row of small poroids. The raphe does not possess the central nodule Figure 12a–c). The apical external raphe endings are strongly hooked, geniculate in the same side; on the internal side the raphe terminates apically in small helicosteglossae (Figure 12c, d). Internally the raphe is subtended with distinct fibulae; the distance between fibulae is variable, though the greatest distance is between the two median fibulae (Figure 12b). The transapical striae parallel throughout, slightly exceeding 50 in 10 μm, whereas the striae-forming areola density amounts to ca. 60 in 10 μm (Figure 12b).

Comparison with established taxa: Due to the central raphe position and the irregular fibulae this species is easily assigned to *Nitzschia sect.* Dissipatata exemplified by *N. dissipata* (Kützing) Rabenhorst, despite its very small size. Indeed, in LM it can be confused with *N. dissipata*; however, its marine habitat (salinity exceeding 25 psu), valve width below 3 μm, stria density slightly exceeding 50 in 10 μm and fibulae density 8–14 in 10 μm indicate that we are dealing with different species (Manoylov, 2010).

Figure 12. *Nitzschia nanodissipata* Li Ch. & Witkowski sp. nov. illustrated in SEM. Figure a. Valve external view, note the elevated, positioned in the centre canopiate (arrow) raphe bearing canal. Figure b. Valve internal view showing randomly distributed fibulae. Figure c. Close up of the valve apex, note strongly hooked raphe end (arrowhead). Figure d. Close up of the internal view of the valve apex

*Nitzschia volvendirostrata* Ashworth, Dąbek & Witkowski sp. nov. (Figure 13a–c)

Diagnosis: Frustules rectangular to nearly quadratic in smallest specimens, with rounded apices in girdle view. Girdle relatively broad. Valves linear to linear lanceolate with rostrate, broadly rounded apices, 7–11.5 μm in length and 3.0–3.5 μm in width. Raphe positioned on a keel with conopeum, however, only fibulae are recognizeable under LM. Fibulae are simple and irregularly distributed, 8–9 in 10 μm. Transapical striae not distinguishable under LM.

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Figure 13. *Nitzschia volvendirostrata* Ashworth, Dąbek & Witkowski sp. nov. illustrated in LM and SEM. Figure a. Specimens from Tofo sand in Mozambique, Indian Ocean. Figure b. Specimens from the Red Sea, the type habitat, the most distant specimen in the right side of Figure b is the holotype (arrowhead). Figure c. External and internal view of a specimen from the type habitat of the Red Sea, note randomly distributed fibulae. Figure d. External view of the specimen from Tofo sand. Figure e. Internal view of the specimen from Tofo sand.

Holotype: slide no. BM 101815 in the Natural History Museum, London, UK.

Isotype: slide no. SZCZ23112 deposited in Palaeoecology Unit, Faculty of Geosciences, University of Szczecin.


Etymology: this species is named after the “rolling pin” kitchen utensil which the gross morphology of frustules resembles. No phrase for “rolling pin” exists in Latin, so we base the name on “volvendis,” for “rolling.”

Morphology: Frustules with relatively broad girdle, composed of numerous bands perforated with small puncta. Raphe slightly eccentric, both external and internal central raphe endings missing (central nodule absent), while apical raphe endings strongly bent in the same direction (Figure 13d). Raphe positioned on an elevated keel and subvented with morphologically simple, irregularly-distributed fibulae, 8–9 in 10 μm (Figure 13c, e). Raphe keel with a narrow, apically oriented silica strip in the form of a canoeum. A single row of comparatively large areolae can be found on the keel, between the canoeum and valve surface (Figure 13d). Transapical striae parallel throughout, 52–54 in 10, composed of solitary rows of areolae, 80–90 in 10 μm (Figure 13c–e). The two clones from the Yellow Sea and Tofo Sand from the Mozambique coast of the Indian Ocean are identical in terms of morphology, while the Red Sea specimens are somewhat larger in size. The branch length in the phylogenetic tree based on sequence data is very long for the Yellow Sea (SZCZCH845) strain, therefore we keep it separate.

Comparison with similar established taxa: the new species is unlikely to be confused with any established species of *Nitzschia* spp. sect. Dissipatae. *Nitzschia nanodissipata* is larger than this species, has coarser striae and more robust areolae.

*Nitzschia traheaformis* Li Ch., Witkowski & Yu Sh. sp. nov. (Figure 3a, b, 14a–f)

Diagnosis: Frustules nitzchioid in shape, with marginal canal raphe, broad girdle and chloroplasts two per cell. Valves linear lanceolate with cuneate, slightly capitulate apices, 17–54 μm in length and 3.0–4.7 μm in width. Raphe-bearing margin constricted in the middle, whereas the opposite margin straight to very slightly constricted. Raphe strongly eccentric, the central nodule sometimes recognizable in LM. Transapical striae usually resolvable in LM, 32–34 in 10 μm, fibulae relatively coarse, 11–14 in 10 μm.

Holotype: slide no. SZCZCH971, deposited in Palaeoecology Unit, Faculty of Geosciences, University of Szczecin, leg. J. Harff & J. Deng, October 31st 2013

Habitat; Laizhou Bay, site LBI, water sample. 37°27.362’ 121°42.215’

Isotype: slide BM 101826 in the Natural History Museum, London, UK.

Etymology: the name of this species is derived from its shape resemblance to a sledge, Latin “trahea”, hence *traheaformis* means “similar to a sledge”.

Morphology: Raphe strongly eccentric, raphe bearing canal in SEM shows a row of relatively large poroids (Figure 14a, b, d, e). The central nodule in SEM clearly visible both internally and externally and the slightly expanded central endings are close to each other (Figure 14b–d). Apical raphe endings strongly bent in one side (Figure 14a, e). Raphe subtended with the fibulae which, aside from the center-most pair, are equidistantly distributed. Fibulae in a form of simple arches bridging the two sides of the valve (Figure 14f). Transapical striae parallel throughout,
composed of regularly distributed areolae that are located in shallow groves. Areolae circular in shape, 40–50 in 10 μm (Figure 14a–e).

Figure 14. *Nitzschia traeformis* Li Ch., Witkowski & Yu Sh. sp. nov. illustrated in SEM. Figure a. External view of the diagonally positioned specimen, note the eccentric position of the raphe bearing canal. Figure b. Close up of the valve middle part external view with central nodule (arrowhead). Figure c. Close up of the internal view of the valve. Figure d. Close up of the external view of the valve. Figure e. Close up of the valve apical part external view, note the strongly hooked raphe apical end. Figure f. Valve internal view showing the shape and position of the tabulae.

Comparison with established taxa; Our clones resemble *N. dubiformis* Hustedt in terms of valve outline, but distinctly differ from it morphometrically. The length range of our clones is 17–54 μm, which overlaps with *N. dubiformis* (reported as 40–50 μm), but the width range (3.0–4.7 μm) is smaller than in *N. dubiformis* (reported as 5–7 μm). The two taxa clearly differ, however, by the significantly lower number of stria (32–34 in 10 μm, resolvable in LM) and tabulae (11–14 in 10 μm) densities in *N. traeformis*; in *N. dubiformis* the stria density is 40 in 10 μm, while tabulae density is 16–18 in 10 μm (Krammer and Lange-Bertalot, 1988; Simonsen, 1987). Genetically, there is significant variation in the DNA sequences between this species and the *N. dubiformis* sequences published on GenBank, sup-
porting the designation of this clone as a different species.

*Trybionella gaoana* Witkowski & Li Ch. sp. nov. (Figure 3i, 15)

Diagnosis: Valves linear lanceolate, slightly constricted in the middle, with acutely rounded apices, 13–22 μm long, 4.9–6.5 μm in width (5.2–5.8 μm at the constriction). Transapical striae parallel in the middle becoming slightly radiate towards apices, 26–33 in 10 μm. The fibulae mostly equidistant with the central pair more distant from each other, 12–14 in 10 μm.

Holotype; slide no. SZCZCH97, deposited in Palaeoceanology Unit, Faculty of Geosciences, University of Szczecin, leg. A. Witkowski June 26th 2013

Isotype; slide BM 101827 in the Natural History Museum, London, UK.

Type habitat; the sediment of Wangfu Jiao Reef in the littoral zone of Chang Dao island near Yantai, China, Bohai Sea; 37° 57.17’ N 120°44’.04” E.

Etymology; this species is dedicated the esteemed diatomologist Professor Gao Yahui from Xiamen University in appreciation of his achievements in science and supervision of students.

Morphology; Frustules in girdle view flat, with a typical nitzschio- chloid marginal canal raphe. The girdle is composed of several open bands perforated with single row of small poroids. The valve surface of *T. gaoana* distinctly undulate with a canal raphe on one margin. (Figure 15a; b). Mantle perforated with one to two rows of areolae. In wild populations the distal valve margin is marked with a distinct rib-like silica deposition that marks the transition between the valve face and the mantle (Figure 15c). Raphe very strongly eccentric; external central raphe endings slightly expanded and form a small but distinct central nodule both externally and internally (Figure 15d). The fibulae are e-quidistant, 12–14 in 10 μm, plain, with the median pair farther apart than the others (Figure 15b; d). Transapical striae are developed at both margins of the valve, 26–33 in 10 μm (Figure 15a; b). The striae are composed of areolae which are more or less oval in shape, up to 50 in 10 μm (Figure 15c; d). The striae are interrupted by a very broad sterno (Figure 15a; b), but appear to persist on the sterna surface as ridges and warts (Figure 15c).

Comparison with similar established taxa; *Trybionella gaoana* can be confused with *Nitzschia ligowskii* Witkowski, Lange-Bertalot, Brzezińska & Kociolek in Witkowski et al. (2004), which also likely belongs in *Trybionella*. The two taxa show, however, distinct differences in both LM and EM; for example, *Trybionella gaoana* valves are more panduriform in outline than those of *N. ligowskii*. The stria density of the two taxa barely overlaps, with 22–27 in 10 μm and 26–33 in 10 μm in *N. ligowskii* and *T. gaoana*, respectively. Finally, only a single row of relatively large areolae occur on the proximal mantle of *N. ligowskii*, whereas three rows of areolae occur in *T. gaoana*.

**DISCUSSION**

Our research on diatom assemblages of the Bohai and Yellow Sea has been conducted using two different methodologies; one classic (microscopy) and one more modern (molecular). First, we have performed a traditional observation of species composition of the diatom assemblages based on microscopy prepared from processed samples (e.g. Smol and Stoermer, 2010; Witkowski et al., 2004). Observations on these samples revealed the presence of species-rich diatom assemblages, though the small size of many of these taxa has made them difficult to identify through light microscopy alone. Small biraphid forms were dominant in these samples. Such species are often difficult to identify even to the generic level. Likewise abundant are monoraphid forms belonging in *Planothidium*, *Coconetes* and *Anorthonema*. Less numerous in terms of diversity, but abundant in terms of individuals, are cy-prymnotoid taxa such as *Plagiogrammospis cf. vanheurckii* (Grunow) Hasle, von Stosch & Syvertsen and one unidentified species tentatively assigned to *Minutocellus* Hasle, von Stosch & Syvertsen, with a strong similarity to *M. africana* Dañbe & Witkowski (Dañbe et al., 2014). The small size of many of these diatoms make cultures and molecular data all the more important for the long-term monitoring of Chinese coasts, replacing the need for specialists and time-consuming microscopy with quick and relatively-inexpensive DNA sequencing techniques, discussed below. Additionally, these cultures can be used to study the life cycle (e.g. Davydovych et al., in preparation), the eco-physiology of individual or groups of taxa (e.g. Scholz and Liebezeit, 2012) or even to catalog and harvest organic chemical compounds and cellular products (e.g. fatty acids) or carbohydrates for commercial purposes (Scholz and Liebezeit, 2013; Witkowski et al., 2013).

The use of molecular markers has also jump-started interest in the phylogeny, taxonomy and evolution of diatoms. In terms of phylogeny and evolution, the resolution of the diatom “tree of life” is increasing dramatically every year as more diverse taxa are sampled (e.g. Therriot et al., 2015). At the same time, intense research is ongoing in order to find the best DNA “barcode” for diatoms for quick and unskilled diatom identification, though a database of identified, vouchered and sequenced diatoms necessary for such an endeavor is still woefully inadequate (Zimmermann et al., 2014), particularly for marine littoral benthic diatoms. Although there are numerous examples of studies where particular taxonomic or phylogenetic issues regarding marine benthic diatoms have been addressed, these are typically restricted to smaller, more tractable clades and genera with limited geographic sampling (e.g. Ashworth et al., 2012, 2013; Kooistra et al., 2004, Sato et al. 2008a,b). Understanding the dispersal and genetic variability of diatom species is only possible with a diverse range of DNA sampling from across the globe, which can include severely undersampled but geographically vast sites such as coastal China (this study), the psychrophilic diatoms of the Arctic and Antarctic by Choi et al. (2008) or tropical coral reefs (Ashworth et al., 2012; Lobban, et al., 2011; Li et al., 2015). The study of Choi et al. used SSU and rbcL. and morphology (LM and SEM) to document the phylogenetic relationships between taxa inhabiting sea ice in the high latitudes of both hemispheres.

Here we present for the first time the phylogenetic position of many members of diatom assemblages from the marine littoral zone of China, with accompanying environmental metadata and LM and SEM documentation for each of 36 clones studied. In this way we provide an important reference data for future diatom studies in the Yellow Sea and probably far beyond in other sites in the same latitudinal and environmental bands. The diatom assemblages we studied are principally composed of large to medium sized epip- sammic and epiphytic forms (both rare in DNA databases which are largely populated by planktonic diatoms), which we identify
as Amphora helenensis, Caloneis cf. westii, Diploneis cf. parca, Nitzschia trapeformis or Surirella cf. fastuosa. Of these, A. helenensis, Caloneis cf. westii, N. trapeformis seem to represent "weedy" diatoms present in the most collection sites and readily adapting to culture conditions. (Witkowski et al., unpublished observations). Although their morphology is consistent in LM and SEM, all of A. helenensis and N. trapeformis reveal some degree of genetic variation far outpacing their morphological variation. For example, A. helenensis seems to include two taxa, the first one represented by clones SZCZH95 from sample Y21 and SZCZCH704 from Horse Island, which are genetically distant from clone SZCZH582 but are sister to another clone (SZCM774) isolated from the Adriatic Sea coast near Dubrovnik. This is very interesting because A. helenensis belongs in a group of very frequently observed (LM and SEM) diatom taxa (e.g. Witkowski et al., 2000). Our clones from Yellow Sea conform very well with size range of A. helenensis as outlined by in original description (Giffen, 1973) of a taxon from the western coast of South Africa in St. Helena Bay. They also show high degree of similarity between each other. The only difference between Yantai region clones and A. helenensis from the type habitat in South Africa is slightly higher stria density in Yellow Sea clones, 19–23 versus 17–20 in 10 μm respectively.

Even more interesting are four Navicula sensu stricto clones, which we could not identify to the species level. They all originate from sample no. 9 from Chang Dao Island. Three of them SZCZH96, SZCZCH98 and SZCZCH99 group together, whereas the fourth one, SZCZH100, is sister to this group with very high (1%=100%) support. A careful analysis of LM and SEM images revealed barely any morphological differences between the clones. All four clones show a morphology typical for Navicula sensu stricto, and to the best of our knowledge represent rather new species. There are barely any species with that tiny size and fine structure illustrated in any publication even as Navicula sp., hence our decision to establish a new species based on these clones (cf. N. zhengii).

Some species have been easily identified by microscopy, e.g. Nitzschia australiae and it will be interesting to compare their DNA sequence variation with the same taxa from distant locations. Our cultures also contain potentially undescribed monoraphid taxa, some of which never appear in samples processed for microscopy, e.g. Kolbesia sinica. Cocconeis cf. culiferas forms a clade (designated "Cocconeidae I" in Figure 2) with two other strains of Cocconeis (SZCZ96, UTSA0056). Cocconeis cf. murscarentica Riaux-Gobin (Riaux-Gobin Campère, 2008) forms a clade with Planthidium sp. and together with Lennicola hungarica is sister to Cocconeidae I. This is very interesting as C. cf. murscarentica possesses all of the structural and ultrastructural characteristics typical for Cocconeis Ehrenberg as exemplified by C. scutellum Ehrenberg the type species of the genus (Round et al., 1990). Would it mean that Cocconeis as a genus even in a very narrow sense is paraphyletic? The phylogeny of the other species C. cf. culiferas is surprising as this species morphology and ultrastructure place it in a genus related to former Achnathes sensu lato, now recognized as Psammothidium (e.g. Potapova, 2010a). Our C. cf. culiferas has convex RV and concave SV, which is the opposite in Cocconeis. Hence its position in Cocconeidae, despite the fact that its frustule does not conform with "cocconeid" shape is surprising. Unlike Cocconeis spp., monoraphid species Kolbesia sinica (clone SZCM123) from Muping is sister to Karayeva pleonensis var. gessneri and grouped with a cluster composed of monoraphid taxa of Schizostauron and Astarteilla and is related to Stauroneidaceae. Another interesting taxon within Stauroneidaceae is clone SZCZH102. This is a bi-raphid diatom of very small size, which is grouping with a clade composed of two small groups Stauroneis acuta/Cratrícula cuspidata and Parlibellus/Fistulifera. Parlibellus sp. in this clade originates from our culture and is interesting due to asymmetry of the frustule along apical axis. This feature can even be observed in LM (Figure 4p). It shows some resemblance to Lunella ghelabii Witkowski, Lange-Bertalot & Metzeltin 2000, however, the latter species has distinctly capitate apices, and the raphe sternum is very close to the ventral margin, whereas in our culture it is displaced towards the ventral margin. In addition, the Yellow Sea species has a smaller size and denser striation. Based upon above comparison we believe that clone SZCZH75 might represent a new species.

In this paper we present a number of small to very small taxa that usually escape routine diatomological analysis by microscopy. Using light and electron microscopy as well as molecular markers we have been able to document degrees of morphological and genetic variation, even within clones of benthic diatoms from the same sample. On the other hand we have shown that some clones from very distant locations suggest a truly global distribution of some benthic diatom species, as is the case with Nitzschia volvodirostrata from the Yellow Sea, Indian Ocean and the Red Sea, and A. helenensis from the Namibian coast and the Yellow Sea, thought the amount of genetic variation in the latter species means we cannot at this moment completely rule out "cryptic speciation" within identical morphotypes.

CONCLUSIONS

This paper reports the first stage of our research using a multigene approach in studying the diversity of diatom assemblages in the marine littoral of the Bohai and Yellow Seas. The further sampling and culture experiments are continued and involve larger areas across the Yellow Sea coasts. We provide, for the first time, both morphological and molecular data on species composition and the data result from LM and EM observations and multigene sequencing of diatom taxa collected and cultured from a part of the Bohai and Yellow Seas coasts. This study lays the foundation for future metagenomic and environmental sequencing projects off coastal China by curating DNA sequences of the Bohai and Yellow Seas. As we can see use of culture facilities significantly enhances the record of diatom taxa in littoral marine assemblages. Althought dominant are large cosmopolitan taxa, in culture tiny diatoms are grown which usually have no chance to be observed in processed samples. Many of these taxa are probably new to science. Morphological and molecular data shall make the comparison with established taxa easy. In just one region, Bohai and Yellow Sea coasts, we can estimate the number of novel taxa (genera and species) at ca. 20–30%.

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LITERATURE CITED


Ju, X., 2011. Comparative analyses on the characteristics of spatial distribution of the temperature and salinity of Bohai Sea, Yellow Sea and East China Sea in summer and winter. Paper presented at the FIO Symposium of physical oceanography and marine climatology, Qingdao.


Ruck, E. C., 2010. Phylogenetic systematics of the canal raphe
bearing orders Surirellales and Rhopalodiales (Bacillariophyta). University of Texas at Austin. 170 pp.


