Cryptogamie, Algologie, 2012, 33 (3): 245-263

Diversity and seasonal occurrence of *Skeletonema* (Bacillariophyta) species in Xiamen Harbour and surrounding seas, China

Haifeng GU [a], Xiaodong ZHANG [b,c], Jun SUN [b] & Zhaohe LUO [a]*

[a] Third Institute of Oceanography, Xiamen, 361005, P. R. China

[b] Institute of Oceanology, Chinese Academy of Sciences, Qingdao, 266071, P. R. China

[c] Graduate School of the Chinese Academy of Sciences, Beijing, 100039, P. R. China

Summary – *Skeletonema costatum* like cells are recorded globally and often generate a huge biomass when blooming. In the present study we performed a taxonomic and geographic survey of the species diversity in *Skeletonema* along the Chinese coastline. We concentrated our efforts on Xiamen Harbour, where we followed the annual cycle of the generic diversity by collecting 51 water samples from 2006 to 2010. A total of 687 strains of *Skeletonema* were established and they were identified as *S. ardens*, *S. costatum*, *S. dohrnii, S. grevillei, S. menzelii*, and *S. tropicum*. *S. dohrnii* is a winter-spring species, whereas other species tend to appear in summer and autumn. *S. costatum* is the single species present throughout the year. To assess how the species overcome the period in which they are not detectable in the water column we also sampled the surface sediments and either isolated resting stages and incubated them into cultures, or we generated monoclonal strains by incubating the sediment and isolated whatever emerged from it. The above six species except *S. grevillei*, and *S. pseudocostatum* as well, were detected this way, implying that the species produce resting stages. Resting cells of *S. costatum* and *S. tropicum* were actually observed in the sediment.

China / resting cells / resting stages / *Skeletonema* / seasonal occurrence / Xiamen Harbour

Abbreviations: AIC, Akaike's Information Criterion; FP, fultoportula; IFPP, intercalary fultoportula process; LSU, large subunit; RP, rimoportula; SEM, scanning electron microscopy; TEM, transmission electron microscopy; TFP, terminal fultoportula; TFPP, terminal fultoportula process; TRP, terminal rimoportula; TRPP, terminal rimoportula process.

INTRODUCTION

The diatom genus *Skeletonema* Cleve is common in tropical to temperate coastal marine habitats where it often constitutes a major component of phytoplankton blooms (Gallagher, 1980; Itakura *et al*., 1997; Aubry *et al*., 2004; Huang *et al*., 2007). The genus includes several genetically and morphologically distinct species (Medlin *et al.,* 1991; Sarno *et al*., 2005; Zingone *et al*., 2005; Sarno *et al*., 2007), and as of now, eleven have been described. Apart from *S. costatum*

* Corresponding author: haifenggu@yahoo.com

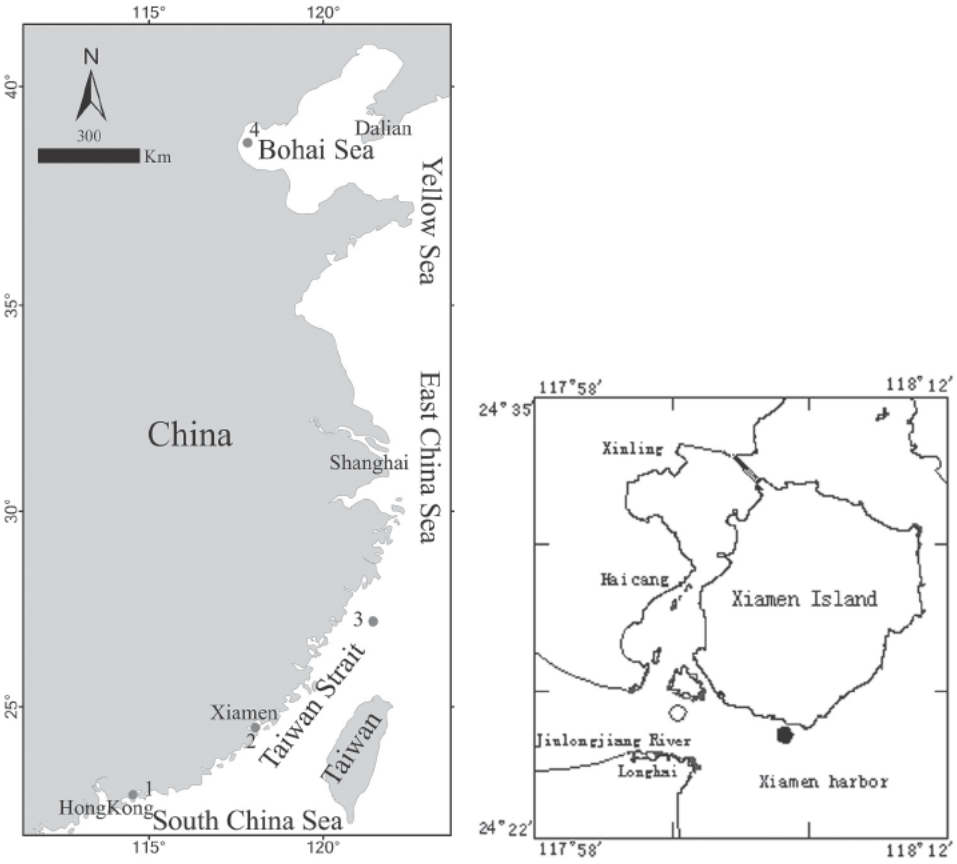
sensu stricto (Greville) Cleve *emend*. Zingone *et* Sarno there are: *S. ardens* Sarno *et* Zingone, *S. dohrnii* Sarno *et* Kooistra, *S. grethae* Zingone *et* Sarno, *S. grevillei* Sarno *et* Zingone, *S. japonicum* Zingone *et* Sarno, *S. marinoi* Sarno *et* Zingone, *S. menzelii* Guillard, Carpenter *et* Reimann, *S. pseudocostatum* Medlin *emend*. Zingone *et* Sarno, *S. subsalsum* (Cleve) Bethge, and *S. tropicum* Cleve. The taxonomic status of some of these species still needs to be confirmed, for instance by means of geographical sampling of species believed to be distinct when sampled only in Europe or the North Atlantic (*S. dohrnii* and *S. marinoi*; Godhe *et al*., 2006; Kooistra *et al*., 2008). Others, such as *S. menzelii* and *S. tropicum,* apparently consist of several cryptic species (Kooistra *et al*., 2008).

Skeletonema species participate in seasonal blooms and are often not detectable in the water column for the remainder of the year. In a few places, *Skeletonema* has been recorded in the plankton throughout the year (Gallagher, 1980; Hobson & McQuoid, 1997; Itakura *et al*., 1997; Huang *et al*., 2007), but such cases probably constitute series of different species, occurring one after the other (Sarno *et al*., 2005, 2007; Kooistra *et al*., 2008; Borkman & Smayda, 2009). Seven *Skeletonema* species have been reported in Chinese coastal waters (Chen & Gao 1993; Chen *et al*., 2007; Kooistra *et al*., 2008), but this information is based on sampling of opportunity; not on regular surveys and their seasonal occurrence remains to be determined.

Kooistra *et al*. (2008) showed that *Skeletonema* species possess extensive, almost cosmopolitan, distribution patterns, though with some exceptions. These authors recorded *S. grethae* along the Atlantic coast of the USA, but nowhere else. We assess if our increased sample effort uncovers this species in Xiamen Harbour, a site climatologically comparable to locations along the US-East coast where *S. grethae* seems to be common in summer (Kooistra *et al*., 2008; Borkman & Smayda, 2009).

The seasonal occurrence of *Skeletonema* species in the plankton raises the question where they remain the rest of the year. *Skeletonema* is known to survive as resting stage in bottom sediment (Itakura *et al*., 1997; McQuoid & Godhe 2004; Patil & Anil 2008). *Skeletonema*-blooms lead to an increase in the abundance of resting stages in the sediment (Patil & Anil, 2008) suggesting that blooms seed the benthic "seed-bank," which in its turn "seeds" subsequent blooms. Such resting stages are morphologically similar to the planktonic stage (Itakura *et al*. 1992) and can be detected directly through screening of sediment samples in microscopy or through genetic and morphological identification of monoclonal cultures raised from them. In the present study we explore the taxonomic diversity of *Skeletonema* resting stages in sediment samples to assess if this diversity reflects the *Skeletonema* diversity in the overlying water over the year.

As study site we have chosen Xiamen Harbour because of its location in the Strait of Taiwan, a transition zone between tropical and temperate zones, which might allow discovery of species typical for cool water in winter and of warm-water-species in summer. We performed a monthly survey of the *Skeletonema* diversity in the plankton at a sampling site in Xiamen Harbour from 2006 to 2010. In addition, we obtained sediment samples of occasion in Xiamen Harbour and at three additional sampling sites along the Chinese coast from 2005 to 2009 (spanning different climates; see Fig. 1), to increase the probability of uncovering all of the species present in the region. Phylogenetic analyses were conducted with the sole purpose of identifying the position of the Chinese strains in the tree topology relative to strains from elsewhere (see *e.g.*, Kooistra *et al.* 2008).



Figs 1-2. Sampling stations. **1.** Sample stations along the coast of China: 1, Daya Bay; 2, Xiamen Harbour; 3, Taiwan Strait; 4, Bohai Sea. **2.** Xiamen Harbour. The black dot denotes the station where water samples were taken. The white dot denotes the station where sediment samples were taken.

MATERIALS AND METHODS

Collection sites

Xiamen Harbour (Fig. 2) is a semi-protected embayment with a surface of 275 km^2 located in the Strait of Taiwan between the South and East China Sea (Fig. 1). The Jiulongjiang River discharges around 1.48×10^{10} m^3 freshwater into Xiamen Harbour every year. Seawater temperatures in Xiamen Harbour range between 13°C in January and 30°C in July. Salinity is fairly stable over the year between 29.7 and 32.5 PSU, with a low in June (around 25.0 PSU) due to a peak in the river discharge. The yearly seawater temperature range at a sample station located at a more northern location in the Strait of Taiwan is similar to that in Xiamen Harbour. A sample station in the Bohai Sea, located in Northern China,

shows a seawater temperature range from 0°C in February to 21°C in August. A sample station in Daya Bay, situated in the South China Sea, ca 50 km to the East of Hong Kong, exhibits a yearly seawater temperature range from 17°C in February to 31°C in September (geographical locations of sites, see Fig. 1; geographical coordinates and sample dates of the sites, see Table 1).

Culture isolation and maintenance

Thirty-nine seawater samples were gathered at a fixed station (0.5 m deep) in Xiamen Harbour (Fig. 2) from February 2006 to December 2009. Monoclonal cultures were established by isolating individual chains of *Skeletonema* from these samples immediately upon arrival in the laboratory, using drawn-out Pasteur pipettes and several washes with droplets of sterile seawater. Only a few strains were established as cultures from a single sample and identified on the basis of both morphology and genetics (Table 2). To clarify their temporal distribution quantatively, 12 water samples were collected at a monthly interval in 2010. A minimum of 52 chains (maximum: 68) were isolated and established into cultures, and identified genetically. The *Skeletonema* cell density in the water sample of December, 2010 is rather low, however, we managed to isolate 27 chains. Cultures were maintained in f/2 medium at 20°C, with an irradiance of 90 µmol photons·m^{-2}·s^{-1} and a light: dark cycle of 12h: 12h, from now on called "standard culture conditions."

Surface sediment samples were collected in Xiamen Harbour from a site 13 m deep as well as from sample sites in the Strait of Taiwan, the Bohai Sea and Daya Bay (Table 1) from 2005 to 2009 using a grab sampler. The sediment samples were stored in the dark at 4°C for at least one month until further treatment to ensure that anything growing up from this material consisted of resting stages and not of actively growing, planktonic chains that accidentally happened to be located on the bottom. Approximately 3 g of wet sediment was mixed with 20 ml filtered seawater and sonicated for 2 min (100 watts) to dislodge detritus particles. The watery slurry was filtered through a 100-µm sieve and subsequently through a 20-µm sieve and the 20-100 µm fraction was resuspended in 1 ml filtered seawater. *Skeletonema* resting cells were isolated from this material as described above for chains and observed in LM. These resting stages were exposed to culture condition to assess their germination capacity, and if germination was successful, to identify them morphologically and genetically. The watery slurry was incubated also directly in a 96 well plate under standard culture conditions. In those wells in which *Skeletonema* appeared, only one chain per well was isolated and brought into monoclonal culture to avoid possible pseudo-replication. Those chains always yielded successful isolates (Table 3).

Microscopy

For light microscopy, vegetative cells were examined and photographed using an Olympus BX51 light microscope (Olympus, Tokyo, Japan) with a digital camera (Qimaging, Burnaby, Canada) at 400× magnification using phase contrast.

For electron microscopy, culture samples were treated with 100% sulphuric acid (H_2SO_4) and subsequently rinsed several times with distilled water. For transmission electron microscopy (TEM), a drop of liquid containing the rinsed frustules was placed on a Formvar-coated copper grid and left to dry in the air. The grids were examined under a JEOL JEM-100 transmission electron

Table 1. Sediment sample date and location along the coast of China

Sample date	*Region*	*Sample location*	*Depth (m)*
10/03/2005	Taiwan Strait	121.426°E, 27.208°N	52
27/09/2009	Taiwan Strait	121.426°E, 27.208°N	52
16/05/2007	Bohai Sea	117.833°E, 38.750°N	14
14/08/2007	Bohai Sea	117.833°E, 38.750°N	14
14/08/2008	Xiamen Harbour	118.051°E, 24.450°N	13
14/11/2008	Xiamen Harbour	118.051°E, 24.450°N	13
09/07/2008	Daya Bay	114.548°E, 22.677°N	15

Table 2. Strains established from the water samples taken in Xiamen Harbour from 2006 to 2009

Species	*Strain*	*Sample date*	*Species*	*Strain*	*Sample date*
S. ardens	SCXM32	23/07/2006	*S. dohrnii*	SCXM43	24/12/2006
	SCXM33	30/07/2006		SCXM46, 47	14/01/2007
	SCXM35	13/08/2006		SCXM48, 49	10/02/2007
	SCXM39	09/09/2006		SCXM50	12/03/2007
	SCXM41	15/10/2006		SCXM54	07/04/2007
	SCXM45	24/12/2006		SCXM55, 56	12/05/2007
	SCXM65	09/12/2007		SCXM57	17/06/2007
S. costatum	SCXM24	22/04/2006		SCXM59	23/07/2007
	SCXM34	30/07/2006		SCXM60, 61	19/08/2007
	SCXM37	27/08/2006		SCXM64, 67	09/12/2007
	SCXM38	09/09/2006		SCXM68	24/01/2008
	SCXM40	15/10/2006		SCXM69, 70	02/03/2008
	SCXM44	24/12/2006		SCXM78-81	14/12/2009
	SCXM62	16/09/2007	*S. grevillei*	SCXM76	16/08/2009
	SCXM63	09/12/2007	*S. menzelii*	SCXM72	09/10/2008
	SCXM73	09/10/2008		SCXM75	16/08/2009
	SCXM77	14/09/2009	*S. tropicum*	SCXM30	25/06/2006
S. dohrnii	SCXM14	12/02/2006		SCXM31	02/07/2006
	SCXM17	12/03/2006		SCXM42	22/10/2006
	SCXM20,21	09/04/2006		SCXM53	12/03/2007
	SCXM23	22/04/2006		SCXM58	23/07/2007
	SCXM25,26	08/05/2006		SCXM66	09/12/2007
	SCXM28	05/06/2006		SCXM71	14/08/2008
	SCXM29	18/06/2006		SCXM74	15/07/2009

microscope (JEOL, Tokyo, Japan). For scanning electron microscopy (SEM), a drop was placed on a glass cover-slip, left to dry in the air, and coated with platinum, and examined under a LEO 1530 Gemini scanning electron microscope (Zeiss/LEO, Oberkochen, Germany).

DNA extraction, PCR amplification and cycle sequencing

Cells in late-exponential growth phase were collected by centrifugation. Total genomic DNA was extracted using a cetyl trimethyl ammonium boomide (CTAB) procedure (Kooistra *et al.*, 2003) and used as template for amplifying

Table 3. Strains established from the sediment sampled at stations (Table 1) along the coast of China

Strains	*Species*	*Sample date*	*Sample location*
SCND01, 04	*S. costatum*	10/03/2005/	Taiwan Strait
SCND02, 03	*S. tropicum*	10/03/2005/	Taiwan Strait
SCND05	*S. costatum*	27/09/2009/	Taiwan Strait
SCND06	*S. dohrnii*	27/09/2009/	Taiwan Strait
SCBH01	*S. dohrnii*	16/05/2007	Bohai Sea
SCBH02	*S. costatum*	16/05/2007	Bohai Sea
SCBH03	*S. ardens*	14/08/2007	Bohai Sea
SCBH04	*S. dohrnii*	14/08/2007	Bohai Sea
SCBH 05, 06	*S. costatum*	14/08/2007	Bohai Sea
SCDY01	*S. tropicum*	09/07/2008	Daya Bay
SCDY02, 03	*S. pseudocostatum*	09/07/2008	Daya Bay
SCDY04	*S. menzelii*	09/07/2008	Daya Bay
SC04, 05, 08, 09	*S. ardens*	14/08/2008	Xiamen Harbour
SC06	*S. costatum*	14/08/2008	Xiamen Harbour
SC02, 03	*S. dohrnii*	14/08/2008	Xiamen Harbour
SC01,07	*S. tropicum*	14/08/2008	Xiamen Harbour
SC11, 13	*S. ardens*	14/11/2008	Xiamen Harbour
SC15	*S. costatum*	14/11/2008	Xiamen Harbour
SC10, 12	*S. dohrnii*	14/11/2008	Xiamen Harbour
SC14	*S. tropicum*	14/11/2008	Xiamen Harbour

approximately 800 bp of the LSU rDNA gene using the primers of D1R (5'-ACCCGCTGAATTTAAGCATA-3') and D3Ca (5'-ACGAACGATTTGCAC GTCAG-3'). In case this primer pair did not yield satisfying products, we used D1R and D2C (5'-CCTTGGTCCGTGTTTCAAGA-3') (Scholin *et al.*, 1994). The PCR protocol was as follows: initial denaturation for 3.5 min at 94°C, followed by 35 cycles of 50 s denaturation at 94°C, 50 s annealing at 45°C, and 80 s extension at 72°C, plus a final extension of 10 min at 72°C. PCR products were purified using a purification kit (Lulong, Xiamen, China) and sequenced in both directions using the ABI Big-Dye dye-terminator technique (Applied Biosystems, Foster city, USA), according to the manufacturers recommendations. For some strains the PCR product was cloned into a Bluescript vector and two or three clones were picked up for sequencing. The sequence data was initially evaluated using the BLAST program (Altschul *et al*., 1997) against published sequences in GenBank. Multiple alignments of the sequences were performed using the ClustalX package (Thompson *et al*., 1997).

Phylogenetic analysis

The sequence data set included 67 sequences (see Table 4 and Table 1 in Kooistra *et al.*, 2008), spanning the hyper-variable 5'-end of the nuclear encoded large subunit rRNA gene region, i.e., the partial LSU rDNA. The alignment contained 650 positions including a few where gaps needed to be introduced in some of the sequences. As outgroup sequences we utilized those of *Minidiscus spinulosus* Gao, Cheng et Chin and *M. comicus* Takano.

Table 4. List of new sequence data used for phylogenetic analyses, including geographic origin and accession numbers. Other sequence data see table 1 in Kooistra *et al.*, 2008

Species	*Locality of isolation*	*Strain*	*GenBank accession number*
Minidiscus comicus	Xiamen Harbour	SC72	JQ657759
Minidiscus comicus	Xiamen Harbour	MCXM01	JQ657758
M. spinulosus	Bohai Sea	SSND12	JQ657760
Skeletonema ardens	Xiamen Harbour	SCXM32	JQ657733
S. ardens	Xiamen Harbour	SCXM35	JQ657734
S. ardens	Xiamen Harbour	SCXM41	JQ657735
S. ardens	Xiamen Harbour	SCXM65	JQ657736
S. ardens	Bohai Sea	SCBH03	FJ809890
S. costatum	Xiamen Harbour	SCXM24	JQ657737
S. costatum	Xiamen Harbour	SCXM34	JQ657738
S. costatum	Xiamen Harbour	SCXM38	JQ657739
S. costatum	Xiamen Harbour	SCXM40	JQ657740
S. costatum	Xiamen Harbour	SCXM63	FJ809891
S. dohrnii	Xiamen Harbour	SCXM17	FJ809892
S. dohrnii	Xiamen Harbour	SCXM56	JQ657741
S. dohrnii	Xiamen Harbour	SCXM60	JQ657742
S. dohrnii	Xiamen Harbour	SCXM61	JQ657743
S. dohrnii	Bohai Sea	SCBH04	JQ657744
S. grevillei	Xiamen Harbour	SCXM76	JQ657745
S. grevillei	Xiamen Harbour	SCXM566	JQ657746
S. grevillei	Xiamen Harbour	SCXM547	JQ657747
S. grevillei	Xiamen Harbour	SCXM572	JQ657748
S. menzelii	Xiamen Harbour	SCXM72	FJ809893
S. menzelii	Xiamen Harbour	SCXM75	JQ657749
S. menzelii	Daya Bay	SCDY04	JQ657750
S. pseudocostatum	Daya Bay	SCDY02	JQ657751
S. pseudocostatum	Daya Bay	SCDY03	JQ657752
S. tropicum	Daya Bay	SCDY01	JQ657753
S. tropicum	Xiamen Harbour	SCXM30	JQ657754
S. tropicum	Xiamen Harbour	SCXM42	JQ657755
S. tropicum	Xiamen Harbour	SCXM53	JQ657756
S. tropicum	Xiamen Harbour	SCXM66	JQ657757

Phylogenetic analyses were conducted in MEGA 4 (Tamura *et al.*, 2007) using the maximum parsimony (MP) and neighbor-joining (NJ) method. The MP tree was obtained using the Close-Neighbor-Interchange algorithm with search level 3 in which the initial trees were obtained with the random addition of sequences (10 replicates). All positions containing gaps and missing data were eliminated from the dataset. There were a total of 522 positions in the final dataset, out of which 327 were parsimony informative. One thousand replicates were performed in unweighted parsimony bootstrap analysis.

For NJ analysis, we used the AIC-selected model (Kimura 2-parameter) to compute dissimilarity values, and these were used to construct a tree. The robustness of tree topology was conducted using bootstrap with 1000 replications.

RESULTS

A total of six *Skeletonema* species were identified among 687 (628 in 2010) strains established from water samples collected in Xiamen Harbour. Seventeen of these strains were keyed out as *S. ardens*, 262 as *S. costatum*, 311 as *S. dohrnii*, 15 as *S. grevillei*, 18 as *S. menzelii*, and 64 as *S. tropicum*. Species of *Skeletonema* displayed regular seasonal occurrence.

A total of six *Skeletonema* species were identified among 31 strains grown from resting stages collected from sediment samples (Table 3). Fourteen of these strains were from Xiamen Harbour and were found to belong to: *S. ardens, S. costatum, S. dohrnii* and *S. tropicum.* Six cultures raised from the Strait of Taiwan were identified as *S. costatum*, *S. dohrnii* and *S. tropicum.* Two samples from the Bohai Sea (taken in May and August) showed the presence of *S. ardens, S. costatum* and *S. dohrnii*, and one sample from Daya Bay (taken in July) revealed the presence of *S. menzelii, S. pseudocostatum* and *S. tropicum.*

Morphological and molecular identification

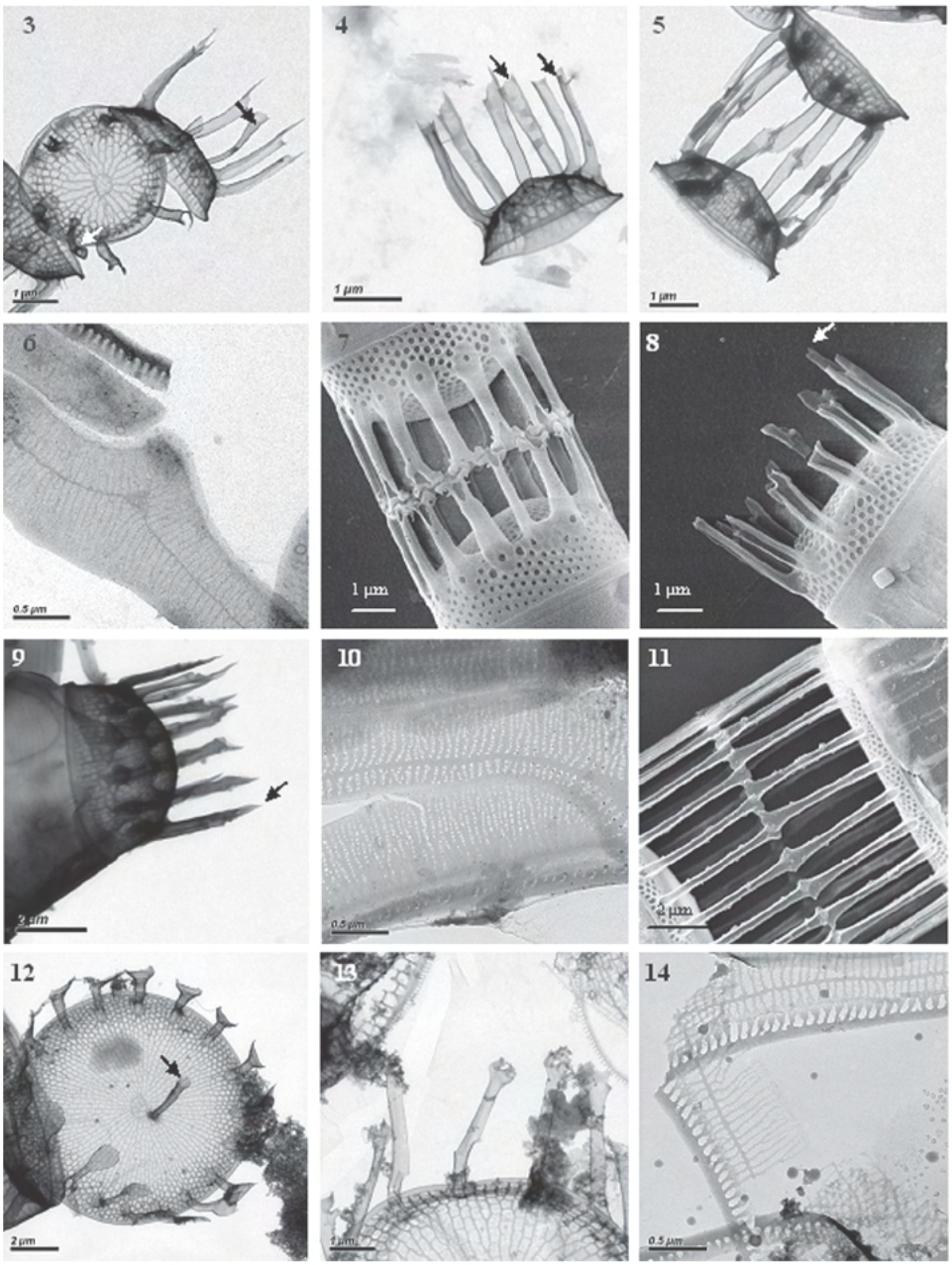
Vegetative cells of the Chinese strains of *S. ardens* are 2-6 μm in diameter, forming relatively short chains composed of less than 20 cells in culture. Each cell contains one or two plastids. The TRP lies just inside the marginal ring of TFPs and has a long process that is wider and obliquely truncated at its tip, with a spoutlike teapot end (Fig. 3). The TFPPs are open with a markedly distal spine, and occasionally flat and flared tips were also observed (Figs 3-4). The IFPPs have narrow, pointed distal ends, often ending in a spine (Fig. 5). The transverse ribs in the copulae are interspersed by hyaline areas (Fig. 6). The strains of *S. ardens* from Xiamen Harbour shared identical LSU rDNA sequences, and showed identity with the LSU of strain CCMP794 collected from Singapore. Strain SCBH03 from the Bohai Sea differed from these sequences in five positions (Table 4 and Fig. 27).

Vegetative cells of the Chinese strains of *S. costatum* are 7-13 μm in diameter, contain 1-2 plastids, and form long (up to 200 cells) and coiled chains in culture. Each IFPP generally connects to two IFPPs of the adjacent valve (1:2 junctions) (Fig. 7). The TRP is located near the valve margin and has a relatively long external process (Fig. 8). The tips of the TFPPs are generally claw

Figs 3-6. TEM of *Skeletonema ardens*. **3.** Strain SCXM35, terminal valve showing the long marginal TRPP (arrows). **4.** Strain SCXM35, terminal valve showing flat and flared TFPPs (arrows). **5.** Strain SCXM35, intercalary valves with 1:1 junctions. **6.** Strain SCXM65, cingular band with thin transverse ribs interspaced by hyaline areas. [Scale bars: 1 μm (Figs 3-5) and 0.5 μm (Fig. 6)]. ▶

Figs 7-10. SEM and TEM of *Skeletonema costatum*. **7.** Strain SCXM37, SEM of intercalary valves with 1:2 junctions. **8.** Strain SCXM44, SEM of terminal valve showing the long marginal TRPP (arrow). **9.** Strain SCXM44, TEM of terminal valve showing spine like TFPPs (arrow). **10.** Strain SCXM63, TEM of cingular band with thin transverse ribs interspaced by rows of pores. [Scale bars: 1 μm (Figs 7 and 8), 2 μm (Fig. 9) and 0.5 μm (Fig. 10)].

Figs 11-14. SEM and TEM of *Skeletonema dohrnii*. **11.** Strain SCXM47, SEM of intercalary valves with 1:2 junctions. **12**. Strain SCXM69, TEM of terminal valve showing the long subcentral TRPP (arrow). **13**. Strain SCXM69, TEM of terminal valve showing claw like TFPPs (arrow). **14.** Strain SCXM69, TEM of cingular band with thin transverse ribs interspaced by hyaline areas. [Scale bars: 2 μm (Figs 11 and 12), 1 μm (Fig. 13) and 0.5 μm (Fig. 14)].



like, but sometimes like a spine (Figs 8-9). The copulae have a central longitudinal ridge and transverse branching ribs that are interspaced by rows of small pores (Fig. 10). The *S. costatum* strains shared identical LSU rDNA sequences and were identical to that of strain B202 from United States (Table 4 and Fig. 27).

Vegetative cells of the Chinese strains of *S. dohrnii* are 3-10 μm in diameter, each contain 1-2 plastids, and often form straight or curved colonies in culture (maximum observed: 104 cells). The IFPPs of adjacent cells align and link with their counterparts in a 1:1 fashion (1:2-linkage in strain SCXM47). The IFPPs interlock with a plain joint without intricate knots or knuckles (Fig. 11). The TFPPs are split and possess flat and flared tips, though claw like TFPP were also observed (strain SCXM56) (Figs 12-13). The TRP is subcentral and has a long tubular process that is at times cup-shaped at its apex (Fig. 12). The copulae are ornamented with transverse branched ribs, interspaced with a hyaline area (Fig. 14). The *S. dohrnii* strains shared identical LSU rDNA sequences (Table 4), and were identical to that of strain NIES-223 from Japan (Fig. 27).

Vegetative cells of the Chinese strains of *S. grevillei* are 3-5 μm in diameter, contain 1-2 plastids, and form colonies of up to 95 cells in culture. The distal ends of the IFPPs are narrow and connect to those of the adjacent valve in a 1:1 fashion by a knuckle like joint (Fig. 15). The TRP lies close to the valve face margin near the marginal ring of TFPPs. The TFPPs are irregularly truncated at their tips, which bear one or two small lateral spines (Fig. 16). The copulae are flanked by thin, transverse branching ribs, interspersed with a hyaline area (Fig. 17). The *S. grevillei* strains SCXM76, SCXM566 shared identical LSU rDNA sequences, and differed from strains SCXM547 and SCXM572 in three positions (Table 4). The latter two strains differed from strain CCMP1685 in four positions (Fig. 27).

Vegetative cells of the Chinese strains of *S. menzelii* are 5-10 μm in diameter, contain 1-2 plastids, and occur solitary or in pairs in culture. The fultoportular processes (FPs) have open and long processes (Fig. 18) with two satellite pores in their basal part (Fig. 19). The rimoportular process (RP) is situated on the border of the central area and has a long tubular process (Fig. 18). The copulae possess thin, transverse ribs interspersed with a hyaline area (Fig. 20). The Chinese strains of *S. menzelii* shared identical LSU sequences (Table 4), and differed from the strain Naos13 from Gulf of Panama only in one position (Fig. 27).

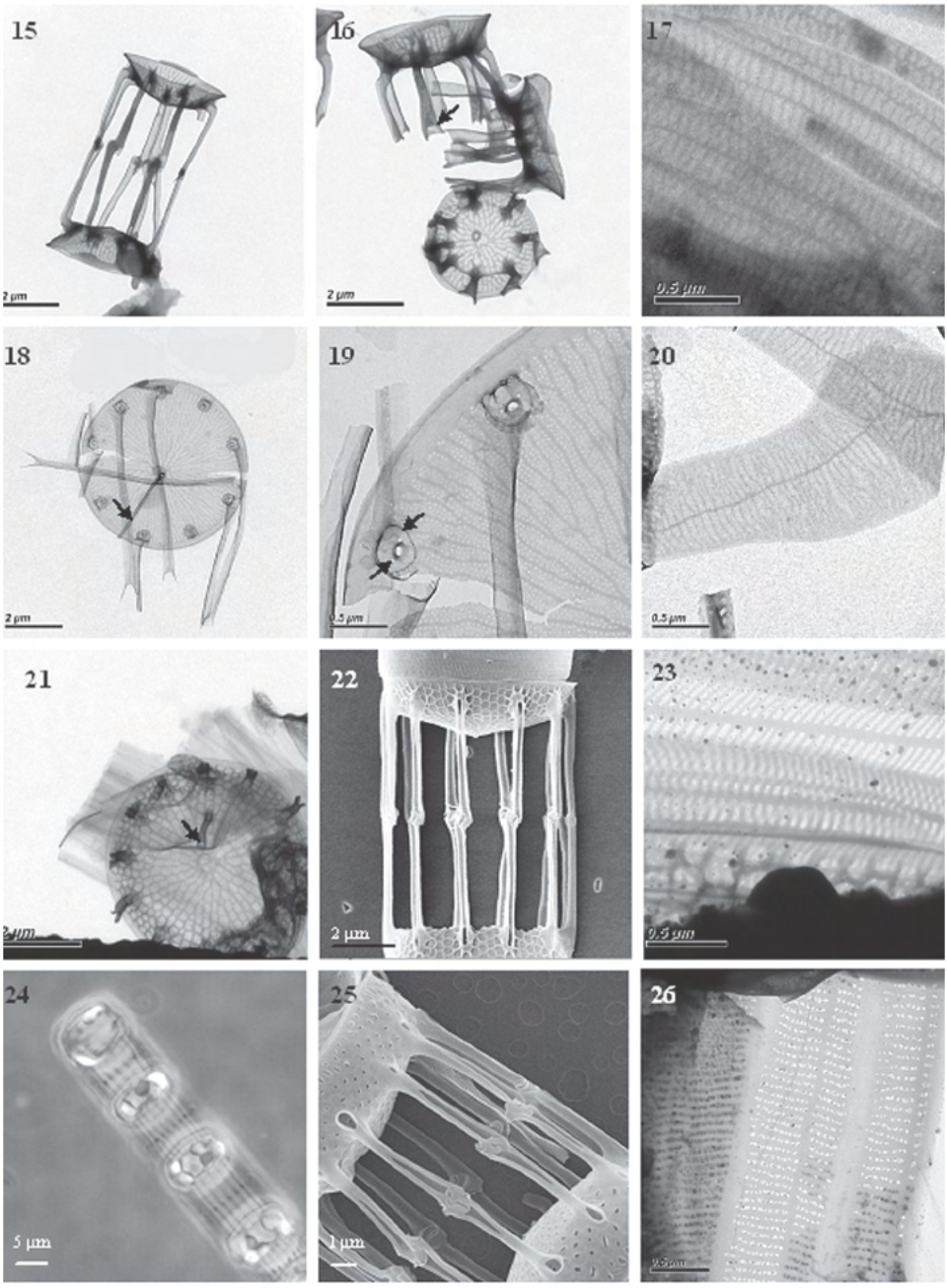
Vegetative cells of the two Chinese strains of *S. pseudocostatum* are 4-7 μm in diameter, contain 1-2 plastids, and form relatively short chains (maximum 18 cells) in culture. The TRP is subcentral and has a long process. Each TFPP has a narrow, claw-like (Fig. 21) or spiny tip. The IFPPs connect to

Figs 15-17. TEM of *Skeletonema grevillei* (strain SCXM09). **15.** Intercalary valves with 1:1 junctions. **16.** Terminal valve showing irregularly truncated TFPPs. **17.** Cingular band with thin transverse ribs interspaced by hyaline areas. [Scale bars: 2 μm (Figs 15 and 16) and 0.5 μm (Fig. 17)]. ▶

Figs 18-20. TEM of *Skeletonema menzelii* (strain SCXM72). **18.** Valve with subcentral RPP (arrow) and long FPPs with two spines. **19.** Detail of valve showing a FP with two satellite pores (arrows). **20.** Cingular band with thin transverse ribs interspaced by hyaline areas. [Scale bars: 2 μm (Fig. 18) and 0.5 μm (Figs 19 and 20)].

Figs 21-23. SEM and TEM of *Skeletonema pseudocostatum* (Strain SCDY02). **21.** TEM of terminal valve showing the subcentral TRPP (arrow). **22.** SEM of intercalary valves with 1:1 junctions. **23.** TEM of cingular band with thin transverse ribs interspaced by hyaline areas. [Scale bars: 2 μm (Figs 21 and 22) and 0.5 μm (Fig. 23)].

Figs 24-26. LM, SEM and TEM of *Skeletonema tropicum*. **24.** Strain SCXM30, LM of colony with numerous chloroplasts in each cell. **25**. Strain SCXM42, SEM of intercalary valves with 1:1 junctions. **26.** Strain SCXM71, TEM of cingular band with thin transverse ribs interspaced by rows of pores. [Scale bars: 5 μm (Fig. 24), 1 μm (Fig. 25) and 0.5 μm (Fig. 26)].



those of adjacent cells by a knuckle-like connection in a 1:1-fashion (Fig. 22). Transverse ribs are interspaced by hyaline area in the copulae (Fig. 23). The LSU sequences of the two Chinese strains SCDY02 and SCDY03 of *S. pseudocostatum* (Table 4) were identical to the one of the Italian strain B140 (Fig. 27).

Vegetative cells of the Chinese strains of *S. tropicum* are 4-15 μm in diameter, contain 2-8 plastids (Fig. 24), and form long, straight chains in culture (maximum 65 cells). The TRP has a long, external, trumpet-shaped process, located midway between the annulus and the valve margin. The IFPPs interlock like a knuckle (Fig. 25). The junction is generally of the 1:1 type, but occasionally 1:2 junctions are seen (Fig. 24). In the copulae, the transverse ribs are interspaced by rows of pores (Fig. 26). The tips of the TFPPs are truncated or claw like. The LSU sequences of *S. tropicum* strains from China (Table 4) were identical to those of strains CCMP788 and SZN-B144 from the USA and Italy (Fig. 27).

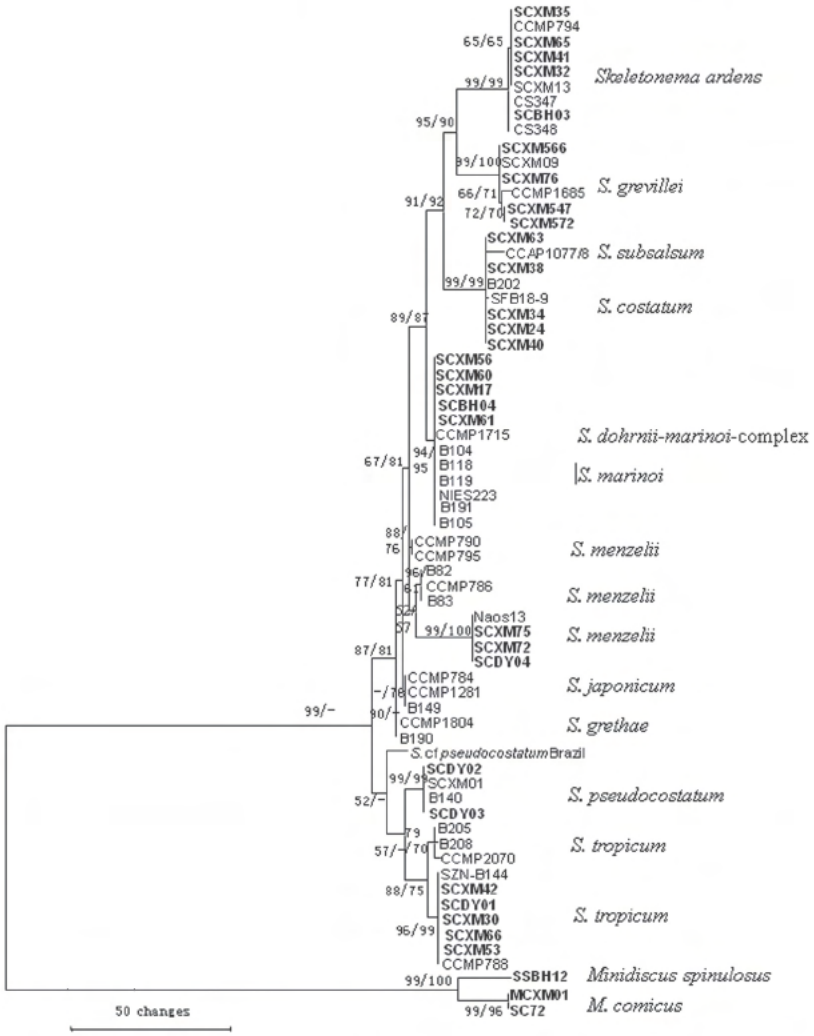


Fig. 27. Phylogeny of *Skeletonema* inferred from partial large subunit rDNA sequence based on MP and NJ. Two species of *Minidiscus* were selected as an outgroup. Bootstrap values > 50% are shown to the left of internal nodes. The first numbers are from MP analyses (1000 replications), and the second are from NJ analyses (1000 replications).

MP analysis generated 473 most parsimonious trees. One of these trees was illustrated in Fig. 27. Trees differed only among one another in the branching patterns in clades encompassing single species. The phylogenetic tree based on NJ analysis differed from them only slightly.

Temporal distribution of *Skeletonema* in the plankton of Xiamen Harbour

Thirty-nine water samples were collected in Xiamen Harbour from 2006 to 2009 and chains of *Skeletonema* were observed in all but a few of these. *Skeletonema tropicum,* which is easily recognized because of its multiple plastids per cell, appeared in low numbers at the end of June and bloomed in July or August of the years 2006-2009. This species was also detected in the plankton in October 2006, and in March and December of 2007 (Table 2).

Twelve water samples were collected from Xiamen Harbour in 2010 at a monthly interval and chains of *Skeletonema* were observed in all of them. Two species were recovered from five samples; three species were isolated from two samples, four were collected from four samples, and five from the sample taken in August.

Skeletonema dohrnii remained to be dominant in water samples collected from January to June (> 80%) and it also appeared in December, with relatively low percentage (around 7%). *S. costatum* was present throughout the year. It comprised less than 20% of *Skeletonema* assemblage from January to June and more than 40% from July to December. *S. tropicum* was recorded in all samples except those collected from February to May. It generally accounts for less than 10% of *Skeletonema* assemblage, but in August, September and December, it contributes more than 20%.

Skeletonema ardens was recorded from samples collected from July to October. *S. grevillei* was detected in August and September, and *S. menzelii* appeared in January, July, August and October. None of them exceeded 20% of *Skeletonema* assemblage in a single sample (Fig. 28).

Resting stages of *Skeletonema* in the sediment along the coast of China

All abovementioned *Skeletonema* species except *S. grevillei*, and *S. pseudocostatum* as well, were also recovered from sediment samples gathered along the Chinese coast. Resting cells of *Skeletonema* were observed directly in these samples by means of LM. Their morphology differed from that of planktonic material in that they were composed of relatively short chains of several cells, which showed more robust frustules and condensed cytoplasmic masses (Fig. 29). We isolated ten such chains and succeeded in giving rise to *S. costatum* (strains SCBH05 and SCND05) and *S. tropicum* (strain SCND01).

A second kind of resting cells of *Skeletonema* was observed in sediment samples collected in Xiamen harbour, Daya bay and Taiwan Strait. These resting stages often consist of three or four cells each possessing a group of cytoplasmic masses (Fig. 30). Around ten such resting stages were isolated and incubated in standard culture conditions, but germination failed. Such kind of resting stage were also encountered from a water sample collected in Xiamen harbour in December, 2010 and two of them gave rise to *S. tropicum* strains successfully (Fig. 31).

Strains of four species: *S. ardens*, *S. costatum*, *S. dohrnii* and *S. tropicum,* were recovered by incubating sediment samples collected in Xiamen Harbour in August and November, 2008. It should be noted that vegetative cells of *Skeletonema* were not detected in the plankton in November 2008.

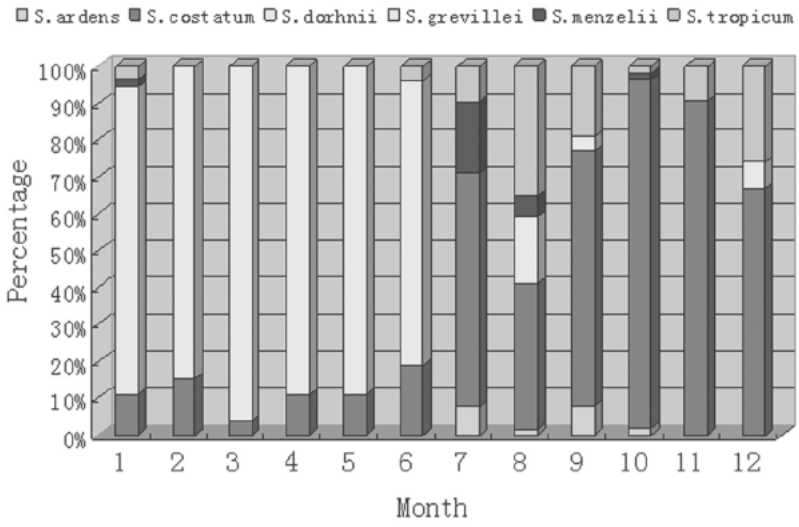
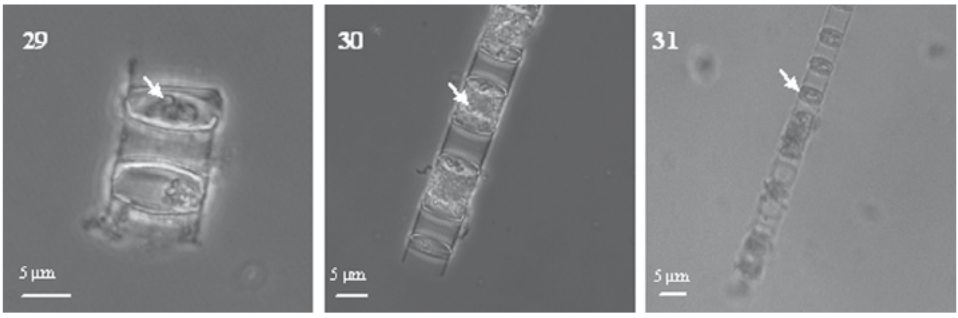


Fig. 28. Relative abundances of *Skeletonema* in Xiamen Harbour, 2010.



Figs 29-31. LM of *Skeletonema* resting cells. **29.** Resting cells of *Skeletonema costatum* with condensed cytoplasmic masses (arrow), producing strain SCND05. **30.** Resting cells of *Skeletonema* with a group of cytoplasmic masses (arrow). **31.** A resting cell of *Skeletonema tropicum*, generating new vegetative cells (arrow). [Scale bars: 5 μm].

Strains of *S. costatum*, *S. dohrnii* and *S. tropicum* were recovered also by incubation of sediment samples collected in Taiwan Strait in March 2005 and September 2009. The sediments collected at the sample station in the Bohai Sea in May and August 2007 yielded *S. ardens*, *S. costatum* and *S. dohrnii*. And the sediment collected at the sample station in Daya Bay, July 2008 gave rise to cultures of *S. menzelii*, *S. pseudocostatum* and *S. tropicum*.

DISCUSSION

Morphology and sequence data

The *Skeletonema* specimens collected in Xiamen Harbour and at other sites along the Chinese coast belong to species already described in Sarno *et al.*

(2005, 2007) and Zingone *et al*. (2005). *Skeletonema ardens* from the Chinese coast conforms to the description in Sarno *et al*. (2007), though the distal end of the TFPPs is occasionally claw-like instead of spiny. Chinese strains of *S. costatum* and *S. grevillei* conform to the descriptions in Zingone *et al*. (2005) and Sarno *et al*. (2007), though in the case of *S. costatum,* the TFPPs show both claw-like and spiny tips. Our strains of *Skeletonema costatum* are morphologically identical to that of strain SK-XM, but differ from the strain SK-DH in that the latter possess both 1:1 and 1:2 linkage (Chen *et al*., 2007), implying that linkage type can be plastic. The copulae of the Chinese specimens of *S. dohrnii* are conform those described in Sarno *et al*. (2005). The latter authors distinguished *S. dohrnii* from its close relative, *S. marinoi* by means of the ultrastructure of the copulae. *Skeletonema dohrnii* was described as possessing copulae with transverse, branched ribs, interspaced by hyaline areas, whereas in *S. marinoi* these branched ribs were interspaced by rows of pores (Sarno *et al*., 2005), but this distinction seems not as clear-cut as these authors proposed because Ellegaard *et al*. (2008) occasionally observed *S. dohrnii*-type copulae in strains identified genetically as *S. marinoi* (Ellegaard *et al*., 2008), and copulae with transverse ribs interspaced by rows of pores were observed in some cells of *S. dohrnii* (Sarno *et al*., 2005, 2007). The TFPPs of the Chinese specimens of *S. dohrnii* possess not only flat and flared tips as described in Sarno *et al*. (2005), but also claw like ends. *Skeletonema pseudocostatum* and *S. tropicum* from the Chinese coast are conform the description in Sarno *et al*. (2005), though also in these species TFPPs possess both spiny and claw like tips. *Skeletonema menzelii* from the Chinese coast conforms to the description in Sarno *et al*. (2005). Our results show that intraspecific ultrastructural details are similar between geographically distant sites (such as the Gulf of Naples and Xiamen Harbour). The claw-like TFPP tips in the Chinese species and the absence of such claws in the descriptions in Sarno *et al*. (2005, 2007) and Zingone *et al*. (2005) may simply be due to plasticity, or constitute subtle phenotypical differentiation between geographically distant populations.

Results of the genetic identification show that our Chinese strains are generally genetically identical or highly similar to their con-specifics from elsewhere. The exceptions are *S. ardens* and *S. grevillei*; sequences of our Chinese strains of *S. ardens* are close to those of strain CCMP794, but the sequence of strain SCBH03 from the Bohai Sea differs at five positions from the sequences of the other strains, and the sequence of *S. grevillei* strains differ from that of CCMP1685 in four positions. Whether these genetic differences represent cryptic species diversity in *S. ardens* and *S. grevillei* has to await the availability of morphological and genetic information of more strains of these morpho-species. The Chinese strains within morphologically perceived species show micro-variation among their sequences to the same extent as is already present among the sequences of strains from elsewhere, challenging the explanation that this variation is related to geography.

Seasonality and biogeography

Skeletonema dohrnii is a most frequently recorded species in Xiamen Harbour. The species is detected here from December to August next year, but it remains dominant only in winter and spring. This occurrence accords with its appearance in the Gulf of Naples in winter (Mediterranean Sea; Sarno *et al*., 2005) and in higher latitudes or cooler waters in spring and early summer (see table 1 in Kooistra *et al*., 2008).

In contrast, *Skeletonema ardens*, *S. costatum*, *S. grevillei* and *S. tropicum* appear to be species typical of the summer and autumn. *S. ardens* is reported from Singapore and from the Gulf of Carpentaria in Australia (Sarno *et al*., 2007), which are both typically tropical environments, supporting the view that *S. ardens* is a tropical species that can occur also in the warm season of warm-temperate regions, such as the Strait of Taiwan. *S. grevillei* appeared in the warm season of Xiamen harbour, and of Arabian Sea as well (Kooistra *et al*., 2008), suggesting that this is, *as S. ardens*, a tropical species that occurs also in the warm season of warm temperate regions.

Skeletonema costatum s. s. is recorded outside China from tropical to warm-temperate waters such as the Indian River Lagoon (Florida), the Lagoa dos Patos (Southern Brazil), the mouth of the Rio de la Plata (Uruguay; see Kooistra *et al*., 2008) and Goa (India; personal communication, Ravi Naik) suggesting it is a tropical species that occurs also in the warm season of warm temperate regions. The same explanation seems to hold for *S. tropicum,* which is encountered also in the Mediterranean Sea during the late summer and autumn (Kooistra *et al*., 2008).

Skeletonema menzelii is mostly recorded from Xiamen Harbour in summer and autumn, however, this species comprises several morphologically identical, but genetically different species. Interpretation of any seasonal patterns in *S. menzelii* has to await detailed information on its cryptic diversity and on the distribution patterns of each of these cryptic species. *S. pseudocostatum* has been recorded in Xiamen harbour in March, 2004 (Kooistra *et al.*, 2008), but disappeared thereafter. It might escape detection due to low density or just be extinct regionally. It is worth noting that our results are mainly based on one years' intense observation, seasonal occurrence of *Skeletonema* species might vary among different years.

Skeletonema marinoi, S. grethae and *S. japonicum* were never detected in our sample effort. Absence from our collection is of course no proof of absence from Xiamen harbour and surrounding waters. *Skeletonema grethae* is commonly encountered along the Atlantic coast of the US and seems there a typical warm water species. It has never been detected anywhere else except for an unconfirmed record near Seattle. Our failure to detect it even in the summer period in Xiamen Harbour implies that the species is either so rare that it escapes detection, or that it does not occur here at all. *Skeletonema marinoi* is common in Europe (Godhe *et al.*, 2006; Ellegaard *et al*., 2008; Kooistra *et al*., 2008*)* where it is found in dense blooms in the northern Adriatic in winter (Sarno *et al*., 2005) and in the spring blooms in western and northern European coastal waters, often near river outflows. The distribution pattern of *S. japonicum* in Kooistra *et al*. (2008) suggests that this species is typical of cold upwelling or in regions with cold winters. Xiamen Harbour does not experience such conditions.

Resting stages

The present study reports resting cells of *S. tropicum* and *S. costatum s. s.* for the first time. We did not actually record resting cells of *S. ardens, S. dohrnii*, *S. menzelii* and *S. pseudocostatum*, but have only gathered indirect evidence of their existence by means of germination experiments performed on small aliquots of the sediment in culture conditions. Resting cells of these species might be overseen in LM because of their possible small size.

Our finding that resting stages of *Skeletonema* germinated also outside the periods in which the species were found in detectable numbers in the plankton of Xiamen Harbour (in August and November) suggests that these resting stages

are able to germinate at any time during the year as long as they are incubated under conditions favourable for germination. This finding corroborates similar results by McQuoid & Godhe (2004). Itakura *et al*. (1992) observed that resting cells of *Skeletonema* from the sediment germinated within three hours, whereas those from the water body did not geminate, suggesting that newly-formed resting stages need a dormancy period before they are able to germinate, but that once this dormancy period is over, they can germinate rapidly when exposed to favourable conditions. McQuoid *et al*. (2002) found that *Skeletonema* resting stages can survive for several decades (In laboratory experiments by Lewis *et al*. (1999) these stages have been shown to survive in the dark at 5°C for as long as 73 months). Such long survival periods guarantee that the sediment always contains resting stages ready to germinate if triggered.

The regular appearance of *S. dohrnii* in Xiamen Harbour in the winter and spring might be explained by the hypotheses that it was transported from other geographical areas, or just remained in the water column at very low numbers. Germination of resting stages triggered by short day-length in combination with low temperature is also highly possible. The environmental conditions could be worse for competitors and therefore the population of *S. dohrnii* reached high abundance. According to Hobson (1981) and Hargraves & French (1983) photoperiod is an important factor triggering germination of resting stages, and Eilertsen *et al*. (1995) and McQuoid & Hobson (1995) state that photoperiod in combination with temperature controls germination of resting stages and subsequent growth of vegetative cells. Resting stages have to be resuspended in the water column, into the light, to permit germination. Light is an absolute necessity for growth and its intensity is a factor triggering germination of diatom resting stages (Hollibaugh *et al*., 1981). Turbulence-driven resuspension of resting stages into the water column, and their subsequent germination might contribute to the irregular occurrence of phytoplankton species in shallow water (Roman & Tenore, 1978; Ishikawa & Furuya, 2004). High turbulence conditions occur frequently in Xiamen Harbour in winter and early spring, and may explain the occasional occurrences of *Skeletonema* species (*S. costatum* and *S. tropicum*) in the plankton in very low densities outside their typical blooming season.

In conclusion, our more intense sampling effort in Xiamen Harbour and our samples of occasion, obtained from geographically distant sites along the Chinese coastline, showed a high *Skeletonema* diversity, but it did not reveal any species new to science or to this area. The latter suggests that the biogeographical study of Kooistra *et al*. (2008) covers the species diversity, at least as far as the temperate zones are considered. However, *Skeletonema* diversity of truly tropical coastal regions still remains to be explored. Our failure to detect *S. grethae* along the Chinese coastline further supports (but does not prove) the hypothesis that the latter species is not cosmopolitan. Last but not least, the fact that strains of almost all *Skeletonema* species (except *S. grevillei*) detected along the Chinese coastline could be obtained by incubating bottom sediments gathered outside the periods of these species' occurrences in the phytoplankton suggests that most of them persist as resting stages in bottom sediments.

Acknowledgements. We greatly acknowledge Wiebe H.C.F. Kooistra for writing of an early version of this paper. We thank Yan Wang and Defu Xiong at Jinan University for providing sediment samples from the Bohai Sea and Daya Bay. Li Gu at Third Institute of Oceanography was thanked for help with TEM examination. Diana Sarno of the Service for Identification of Marine Phytoplankton at the Stazione Zoologica Anton Dohrn is thanked for critical evaluation of our strain identifications. The project was supported by Science and Technology Department of Community projects of China (200905004-3 and

201005015-1) and the Public Science and Technology Research Funds Projects of Ocean (Grant No. 200905004–3). Culture and sequencing was carried out at the Third Institute of Oceanography in Xiamen.

REFERENCES

ALTSCHUL S.F., THOMAS L.M., ALEJANDRO A.S., ZHANG J., MILLER W. & LIPMAN D.J., 1997 — Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic acids research* 25: 3389-3402.

AUBRY F.B., BERTON A., BASTIANINI M., SOCAL G. & ACRI F., 2004 — Phytoplankton succession in a coastal area of the NW Adriatic, over a 10-year sampling period (1990-1999). *Continental shelf research* 24: 97-11.

BORKMAN D.G. & SMAYDA T., 2009 — Multidecadal (1959-1997) changes in *Skeletonema* abundance and seasonal bloom patterns in Narragansett Bay, Rhode Island, USA. *Journal of sea research* 61: 84-94.

CHEN G.F., WANG G.C., ZHANG B.Y. & FAN X.L., 2007 — Morphological and phylogenetic analysis of *Skeletonema costatum*-like diatoms (Bacillariophyta) from the China Sea. *European journal of phycology* 42: 163-175.

CHEN Z.D. & GAO Y.H., 1993 — Nanodiatoms from Xiamen Harbour (I). *Acta phytotaxonomica Sinica* 31: 197-200.

EILERTSEN H.C., SANDBERG S. & TOLLEFSEN H., 1995 — Photoperiodic control of diatom spore growth: a theory to explain the onset of phytoplankton blooms. *Marine ecology progress series* 116: 303-307.

ELLEGAARD M., GODHE A., HARNSTROM K. & MCQUOID M., 2008 — The species concept in a marine diatom: LSU rDNA-based phylogenetic differentiation in *Skeletonema marinoi/dohrnii* (Bacillariophyceae) is not reflected in morphology. *Phycologia* 47: 156-167.

GALLAGHER J.C., 1980 — Population genetics of *Skeletonema costatum* (Bacillariophyceae) in Narragansett Bay. *Journal of phycology* 16: 464-474.

GODHE A., MCQUOID M.R., KARUNASAGAR I., KARUNASAGAR I. & REHNSTAM-HOLM A.S., 2006 — Comparison of three common molecular tools for distinguishing among geographically separated clones of the diatom *Skeletonema marinoi* Sarno et Zingone (Bacillariophyceae). *Journal of phycology* 42: 280-291.

HARGRAVES P.E. & FRENCH F.W., 1983 — Diatom resting spores: significance and strategies. *In*: Fryxell G.A. (ed.) *Survival Strategies of the Algae*. New York, Cambridge University Press, pp. 49-68.

HOBSON L.A., 1981 — Seasonal variations in maximum photosynthetic rates of phytoplankton in Saanich Inlet, Vancouver Island, British Columbia. *Journal of experimental marine biology and ecology* 52: 1-13.

HOBSON L. & MCQUOID M.R., 1997 — Temporal variations among planktonic diatom assemblages in a turbulent environment of the southern Strait of Georgia, British Columbia, Canada. *Marine ecology progress series* 150: 263-274.

HOLLIBAUGH J.T., SEIBERT D.R.L. & THOMAS W.H., 1981 — Observations on the survival and germination of resting spores of three *Chaetoceros* (Bacillariophyceae) species. *Journal of phycology* 17: 1-9.

HUANG C.J., WANG C., DONG Q.X. & LIU X.P., 2007 — Population dynamics of *Skeletonema costatum* in Zhelin Bay of Eastern Guangdong. *Acta ecologica Sinica* 27: 142-151 (in Chinese with English abstract).

ISHIKAWA A. & FURUYA K., 2004 — The role of diatom resting stages in the onset of the spring bloom in the East China Sea. *Marine biology* 145: 633-639.

ITAKURA S., IMAI I. & ITOH K., 1992 — Morphology and rejuvenation of *Skeletonema costatum* (Bacillariophyceae) resting cells from the bottom sediments of Hiroshima Bay, the Seto Inland Sea, Japan. *Bulletin of plankton society of Japan* 38: 135-145 (in Japanese with English abstract).

ITAKURA S., IMAI I. & ITOH K., 1997 — "Seed bank" of coastal planktonic diatoms in bottom sediments of Hiroshima Bay, Seto Inland Sea, Japan. *Marine biology* 128: 497-508.

KOOISTRA W.H.C.F., DESTEFANO M., MANN D.G., SALMA N. & MEDLIN L.K., 2003 — The phylogenetic position of *Toxarium*, a pennate-like lineage within centric diatoms (Bacillariophyceae). *Journal of phycology* 39: 185-197.

KOOISTRA W.H.C.F., SARNO D., BALZANO S., GU H., ANDERSEN R. A. & ZINGONE A., 2008 — Global diversity and biogeography of *Skeletonema* species (Bacillariophyta). *Protist* 159: 177-193.

LEWIS J, HARRIS A., JONES K. & EDMONDS R., 1999 — Long-term survival of marine planktonic diatoms and dinoflagellates in stored sediment samples. *Journal of plankton research* 21: 343-354.

MCQUOID M.R. & HOBSON L.A., 1995 — Importance of resting stages in diatom seasonal succession. *Journal of phycology* 31: 44-50.

MCQUOID M.R., GODHE A. & NORDBERG K., 2002 — Viability of phytoplankton resting stages in the sediments of a coastal Swedish fjord. *European journal of phycology* 37: 191-201.

MCQUOID M.R. & GODHE A., 2004 – Recruitment of coastal planktonic diatoms from benthic versus pelagic cells: variation in bloom development and species composition. Limnology and oceanography 49: 1123-1133.

MEDLIN L.K., ELWOOD H.J., STICKEL S. & SOGIN M.L., 1991 — Morphological and genetic variation within the diatom *Skeletonema costatum* (Bacillariophyta): evidence for a new species, *Skeletonema pseudocostatum. Journal of phycology* 27: 514-524.

PATIL J.S. & ANIL A.C., 2008 — Temporal variation of diatom benthic propagules in a monsoon-influenced tropical estuary. *Continental shelf research* 28: 2404-2416.

ROMAN M.R. & TENORE K.R., 1978 — Tidal resuspension in Buzzards Bay, Massachusetts. I. Seasonal changes in the resuspension or organic carbon and chlorophyll a. *Estuarine and coastal marine science* 6: 37-46.

SARNO D., KOOISTRA W.H.C.F., MEDLIN L.K., PERCOPO I. & ZINGONE A., 2005 — Diversity in the genus *Skeletonema* (Bacillariophyceae). II. An assessment of the taxonomy of *S. costatum*-like species with the description of four new species. *Journal of phycology* 41: 151-176.

SARNO D., KOOISTRA W.H.C.F., HARGRAVES P.E. & ZINGONE A., 2007 — Diversity in the genus *Skeletonema* (Bacillariophyceae). III. Phylogenetic position and morphology of *Skeletonema costatum* and *Skeletonema grevillei*, with the description of *Skeletonema ardens* sp. nov. *Journal of phycology* 43: 156-170.

SCHOLIN C.A., HERZOG M., SOGIN M. & ANDERSON D.M., 1994 — Identification of group- and strain-specific genetic markers for globally distributed *Alexandrium* (dinophyceae). II. Sequence analysis of a fragment of the LSU rRNA gene. *Journal of phycology* 30: 999-1011.

TAMURA K., DUDLEY J., NEI M. & KUMAR S., 2007 — MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular biology and evolution* 24: 1596-1599.

THOMPSON J.D., GIBSON T.J., PLEWNIAK F., JEANMOUGIN F. & HIGGINS D.G., 1997 — The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic acids research* 24: 4876-4882.

ZINGONE A., PERCOPO I., SIMS P.A. & SARNO D. 2005 — Diversity in the genus *Skeletonema* (Bacillariophyceae). I. A reexamination of the type material of *S. costatum* with the description of *S. grevillei* sp. nov. *Journal of phycology* 41: 140-150.