Identification of a marine woloszynskioid dinoflagellate
Biecheleriopsis adriatica and germination of its cysts from southern Chinese coasts

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ABSTRACT
A strain of small-sized dinoflagellates, isolated from the culture of sediment incubation collected from the coastal areas in southern China, has been identified under microscopical observation and rDNA sequence. Surface sediments from two sea areas in the southern Chinese coastal waters were incubated for 20 and 40 days, and germinated vegetative cells were observed. The cells were identified as species in the Suessiaceae based on the morphological characteristics, ultrastructural features of the cell, as well as its swimming behavior. The studied strain clusters into a well-supported clade together with six sequences of Biecheleriopsis adriatica in the phylogenetic tree based on the large subunit (LSU) rDNA sequence. Therefore, the strain has been identified as B. adriatica based on morphological observation and phylogenetic analysis. B. adriatica was the dominant dinoflagellate species in the germinated phytoplankton community from both sea areas, which contributed 50%–83% to the total germinated dinoflagellates averagely. However, B. adriatica has not been reported in previous phytoplankton surveys, and was probably ignored or misidentified due to its small size and thin wall. The frequent and abundant occurrence of B. adriatica in the germinated phytoplankton community of many sea areas of the southern Chinese coastal waters suggests its wide and abundant distribution in these sea areas.

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Introduction
Dinoflagellates are one of the major components of marine phytoplankton assemblages, including a lot of harmful algal bloom (HAB) species (Moestrup et al., 2004). About 3000 taxa of dinoflagellates have been reported up to now (Guiry and Guiry, 2014). However, the actual biodiversity and biogeography of these microalgae are still mostly underestimated, particularly those of the unarmoured and thin-walled dinoflagellates because commonly used fixatives such as formaldehyde and Lugol’s solution impair preservation of their cell morphology and ultrastructure and make their identification problematic.

Woloszynskioid is a group of thin-walled dinoflagellate species (Fensome et al., 1993), and is known to occur mainly in...
freshwater environments (Hansen and Flaim, 2007; Moestrup et al., 2008). Cells of woloszynskioid species are characterized by being covered by small, thin amphiesmal plates, too numerous to be described using the Kofoidian system of plate terminology (Moestrup et al., 2009a). Morphological diversity of this group has been recently examined, and a lot of species have been clarified, and several new genera have been created based on their cellular morphology and phylogenetic relationship (Lindberg et al., 2005; Hansen et al., 2007). Moestrup et al. (2009a, 2009b) defined two new marine genera Biecheleria and Biecheleriopsis, which were previously grouped into Woloszynskia and Gymnodinium, respectively. Takahashi et al. (2014) described the morphology of six strains of Biecheleria and Biecheleriopsis from the Japanese coasts, and inferred their phylogenetic positions based on ribosomal DNA (rDNA) sequences. More recently, Jang et al. (2015) reported the presence of Biecheleriopsis adriatica in Korean coastal waters. It seems that the marine woloszynskioids distribute more widely than expected.

In previous studies, we observed massive occurrence of a small (<10 μm), unarmored/thin-walled dinoflagellate in the majority of cultures from incubations of sediments collected from the coastal areas in southern China (Kang et al., 2009; Wang et al., 2013). It occurred abundantly in the coastal waters of Daya Bay and the South China Sea (Wang et al., 2001). It was identified as Gymnodinium corii previously; however it is more likely to be the woloszynskioid species B. adriatica after detailed morphological observation and phylogenetic analysis. In this study, a strain from the culture of sediment incubation was isolated, and identified on the basis of microscopic observation and phylogenetic analysis of the large subunit (LSU) rDNA sequences. Meanwhile, the cultured phytoplankton from the surface sediments from two typical sea areas in southern China was observed. The aim of this study is to correctly define the species, and to know its distribution in the southern Chinese coastal waters through sediment germination.

1. Materials and methods

1.1. Sediment sampling

Ten stations were established in Zhelin Bay and seven stations in the Guishan Island sea area in the southern Chinese coastal waters (Fig. 1). Triplicate sediment samples were collected by a box-shaped piston corer in Zhelin Bay in November 2008 and in the Guishan Island sea area in January 2009. The top sediments (0–2 cm) from three boxes collected during each sampling were put together in a tightly sealed bag. These samples were filled with N2 and kept in the dark at 4°C for at least six months. Every time subsamples were extracted, the sediment slurry was mixed manually to reduce sampling variability.

1.2. Algal culture

Algal culture was established by germinating phytoplankton in Z6 from the Zhelin Bay. Two milliliters of the sieved sediment samples (20–125 μm) was suspended in 30 mL of modified f/2 culture media (35.7 μmol/L NO3−-N, 2.38 μmol/L PO43−-P), prepared in autoclaved, filtered, aged local seawater (32 PSU). Incubation was carried out at 25°C, 100 μmol photons

Fig. 1 – Sampling stations off the coast of Guishan Island (A) and in Zhelin Bay (B).
m$^{-2}$-sec$^{-1}$ with a 12:12 light/dark cycle. Single cells were isolated from the sediment cultures after 20 days incubation with a micropipette into f/2-Si media (Guillard, 1975). The culture was maintained in the media at 20°C, 100 μmol photons m$^{-2}$-sec$^{-1}$ with a 12:12 light/dark cycle.

1.3. Morphological characterization

1.3.1. Light microscopy (LM)
Live and fixed vegetative cells were observed and measured using a Leica-LEitz DMI 25 inverted microscope (Leica Microsystems GmbH, Wetzlar, Germany), and micrographs were taken with a ProgRes C10 Plus digital camera (JENOPTIK Laser, Optik, Systeme GmbH, Germany). Measurements were made at ×400–600 using the Video Test Size 5.0 image analysis software (JENOPTIK Laser, Optik, Systeme GmbH).

1.3.2. Scanning electron microscopy (SEM)
Cultured samples were fixed with 2.5% glutaraldehyde (Sigma Aldrich) for 2 hr, and filtered onto an Isopore filter (3 μm pore size, Millipore, USA). The filters were subsequently washed with distilled water twice (10 min) and dehydrated in an ethanol series (15%, 20%, 30%, 50%, 70%, 80%, 95%), for 15 min each, followed by two rinses in 100% ethanol for 30 min each, and then in 100% ethanol:100% isooamy acetate (V:V = 1:1) and 100% isooamy acetate for 30 min each. Samples were critical point-dried in liquid CO$_2$. Filters were subsequently glued to SEM-stubs with colloidal silver, sputter coated with gold–palladium, and examined with a Philips XL-30 SEM (Philips, Holland).

1.4. DNA extraction, polymerase chain reaction (PCR) amplification, sequencing, and phylogenetic analyses

Approximately 10 mL cell culture was collected at the exponential growth phase by centrifugation at 3000 rpm for 10 min. Genomic DNA extraction was performed using the DNeasy Plant Kit (Qiagen, Valencia, CA, USA), according to the manufacturer’s instructions. The extracted DNA was immediately frozen at −80°C. Partial sequences D1–D2 of LSU rDNA were amplified using primers D1R (5′-ACCCGCT GAATTTAAGCATA-3′) and D2C (5′-CCCCTTGGTCCGTGTTTC-3′) (Scholin et al., 1994). All primers used were obtained from Sangon Biotech (Shanghai) Co., Ltd., China.

PCR reactions were performed using a Veriti® 96-Well Thermal Cycler (ABI, USA) and PCR reagents (Gibco-BRL) as recommended by the manufacturers. PCR amplification conditions were: 95°C (5 min), 94°C (30 sec), 57°C (45 sec) and 72°C (1 min) for 30 cycles. A final extension at 72°C for 10 min was included in the amplification. The results were analyzed by electrophoresis at 100 V with a 1.0% agarose gel containing ethidium bromide and visualized under UV illumination. Purification and sequencing were carried out by an external service (Shanghai Biotechnic Inc., China) using the internal primers and an ABI PRISM 3730 Autosequencer (Applied Biosystems). The sequences were submitted to the International Nucleotide Sequence Database Collaboration at NCBI (GenBank accession no. GU477610).

The sequence generated from the studied strain was aligned with other 46 LSU rDNA sequences downloaded from GenBank, including 21 sequences in the Suessiaceae, three sequences in the Borghelliaceae, and 23 sequences attributable to other dinoflagellate orders, Gonyaulacales, Peridineales, Prorocentrales, Dinophysiales, Gymnodiniales. Species names and GenBank accession numbers are given in the phylogenetic tree. Phylogenetic analysis was conducted with neighbor-joining (NJ) methods using molecular evolutionary genetics analysis (MEGA) software v5.05 (Tamura et al., 2011). Bootstrap support (BS) values were estimated with 1000 replicates for NJ analyses. The genetic distances were calculated by MEGA software v5.05.

1.5. Germination of surface sediments from coastal waters of southern China

1.5.1. Sediment treatment and incubation
For incubation, 1.0 g of untreated wet sediments was placed into 100 mL culture flasks, and the flasks were filled with 40 mL modified f/2 media (Guillard, 1975), prepared in autoclaved, filtered, aged offshore local seawater. The nitrogen, phosphorus and silica concentrations in the media were modified to 35.7 μmol/L, 2.38 μmol/L and 35.7 μmol/L, respectively, which correspond to the nutrient concentrations in local sea waters (Wang et al., 2009). Other elements were at the same levels as in the f/2 culture media. For every sampling station, three replicate flasks were incubated for 20 and 40 days, respectively. As the water temperatures in local sea water were between 20°C and 30°C with an annual mean of ca. 25°C (Wang et al., 2009), incubation was carried out at 25 ± 1°C. Irradiance was 100 μmol photons m$^{-2}$-sec$^{-1}$, produced by fluorescent tubes with a 12:12 LD photoperiod. After incubation, 30 mL of culture was pipetted from the flasks without stirring up the sediment. The samples were preserved with 1% Lugol’s iodine, and concentrated to 5 mL by sedimentation.

1.5.2. Observation of germinated phytoplankton
Germinated phytoplankton species were identified and counted from aliquots of 0.5–1.0 mL sub-samples by a Leica DMIRB microscope. Observation was at 400× magnification. The identification of germinated phytoplankton was generally based on the light microscopic observation, and on electric scan microscope when necessary. At least three observations were made for each sample, and generally over 1000 cells and at least a minimum of 200 cells were observed in each sample with confidence limits ±6% and ±14%, respectively (Anderson and Throndsen, 2003). The abundance of germinated phytoplankton is reported as cells/g of wet weight of sediment.

2. Results

2.1. Cell morphology

The cells are yellow brownish (Fig. 2A). The cells are oval in outline, dorsoventrally flattened (Fig. 2A), with a length of 10.3–15.1 μm (average of 12.1 ± 1.4 μm, n = 20) and a width of 6.3–9.5 μm (average of 7.7 ± 1.5 μm, n = 20). The epicone and hypocone are similar in length. The epicone is rounded. The hypocone appears rounded and slightly bilobate due to the sulcus extension until the antapex. The nucleus is round
and large in the centre of the cells (Fig. 2A). One or two yellow-brownish chloroplasts are present around the cell periphery (Fig. 2A). Cells swim fast in a straight line, rotating around the apical axis. Cells stop swimming a few minutes after slide preparation, and lose their original shape.

In SEM, the epicone appears elliptical to rounded (Fig. 2B–E). The hypocone appears clearly asymmetrical when cells are observed in either ventral (Fig. 2B, D) or dorsal (Fig. 2C) view. The cingulum is rather wide, and is located in the median portion of the cell. The cingulum is descending displaced by approximately once its own width (Fig. 2B, D). The sulcus is deeply invaginated until it reached the epicone, and enlarging towards the posterior end (Fig. 2B, D). The sulcus was therefore slightly sigmoid (Fig. 2B, D) as the lower right of the epicone had a narrow fingerlike projection into the sulcus. The transverse flagellum is conspicuous and long (Fig. 2E).

2.2. Phylogenetic analysis

A phylogenetic tree (Fig. 3) was constructed based on the partial LSU rDNA sequences. The LSU rDNA sequence of the studied strain clusters within a well-supported (99% bootstrap support) clade including six B. adriatica sequences, here called the Biecheleriopsis clade. The sequences attributed to Gymnodinium pygmaeum, G. cori and Protodinium simplex clade in the Biecheleriopsis clade, and thus these species, as already pointed out by Moestrup et al. (2009a, 2009b) and Takahashi et al. (2014), should be referred to as B. adriatica. The genetic distances between our sequence and the other six sequences of B. adriatica are less than 0.010 (Table 1). Our sequence (GU477610) is closest to the type species of B. adriatica (EU857537). The genetic distances are between 0.084 and 0.094 to species in genus Biecheleria, and 0.114–0.358 to other species in the order Suessiales. Therefore, our strain has been identified as B. adriatica based on morphological observation and phylogenetic analysis.

The Biecheleria clade, which includes Biecheleria, Gymnodinium and Wołoszyńska, is resolved as a close sister (99%) to the Biecheleriopsis clade, even though the bootstrap support within the Biecheleria is moderate (77%). Other genera within the Suessiaceae including Symbiodinium, Polarella, and Pelagodinium form a larger phylogenetic group with low bootstrap support (<50%). Suessiaceae and Borghiellaceae are sisters in the order Suessiales with low support (54%). Suessiales are clearly distinct from the other dinoflagellate orders: the Gymnodiniales, Peridiniinales, Dinophysiales, Prorocentrales, and Gonyaulacales (Fig. 3). It should be noted that some sequences including our sequence that attributed to either Gymnodinium or Protodinium clustering within the Suessiales are likely to be wrongly identified.

2.3. Germinated phytoplankton

All sediment samples from the two bays contained phytoplankton resting cells, which germinated into vegetative cells during the incubation. Altogether, 85 taxa were recorded, but the number of species was likely to be higher because not all taxa were identified to species level. Sixty nine taxa were recorded in cultures of samples from ten stations of Zhelin Bay, and 74 taxa from seven stations of Guishan Island sea area. Diatoms and dinoflagellates were the most diverse groups, of which 56 and 20 taxa were observed, respectively. Vegetative cells of chlorophytes, dictyochophyte, euglenophytes, haptophytes, and raphidophytes occurred as well.

The diatoms Chaetoceros, Pseudo-nitzschia, Skeletonema and Thalassiosira were dominant. B. adriatica was the most abundant dinoflagellate species. Bloom-forming species such as Alexandrium tamarense, Gymnodinium catenatum, Karenia mikimotoi, and Scrippsia trochoidea were also common. The species composition of germinated phytoplankton was dominated by diatoms, dinoflagellates and other groups such as haptophytes and raphidophytes in samples from Zhelin Bay after 20 days incubation. However, species in the other phytoplankton groups including Chrysocromulina sp., Phaeocystis globosa, and Heterosigma akashiwo, were common in most stations after 40 days incubation (Fig. 4A, B). The phytoplankton community structures were comparable after 20 days and 40 days of incubation in samples from the Guishan Island sea area, which were quantitatively dominated by diatoms and dinoflagellates (Fig. 4C, D).
Fig. 3 – Maximum likelihood (ML) tree of Biecheleriopsis adriatica DY based on D1–D2 regions of LSU rDNA sequences of the order Suessiales (sequences inside the red rectangle) and other dinoflagellate orders. Numbers at the nodes indicate percentage bootstrap support (BS) from 1000 replicates. Bootstrap values >50% are shown at nodes from top. The accession numbers follow the name of the species. Sequence derived from this study is highlighted in yellow box. Names in brackets indicate the synonyms of these sequences in the GenBank. The names presented in the ML tree are according to Moestrup et al. (2009a, 2009b) and Takahashi et al. (2014). LSU: large subunit, rDNA: ribosomal DNA.
Table 1 – The genetic distances among sequences in the order Suessiales, the distances of our sequence (GU477610) to sequences in genus Biechleriopsis are highlighted in bold font.

|    | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   | 13   | 14   | 15   | 16   | 17   | 18   | 19   | 20   | 21   | 22   | 23   |
|----|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| 1  | Biechleriopsis adriatica DY GU477610 | 0.000 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 2  | Biechleriopsis adriatica EU857537    | 0.002 | 0.000 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 3  | Gymnodinium com AF318226             | 0.002 | 0.002 | 0.000 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 4  | Biechleriopsis adriatica AB858354    | 0.010 | 0.007 | 0.009 | 0.000 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 5  | Biechleriopsis adriatica AB858355    | 0.009 | 0.005 | 0.007 | 0.002 | 0.000 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 6  | Biechleriopsis adriatica AB858356    | 0.010 | 0.007 | 0.009 | 0.002 | 0.000 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 7  | Biechleriopsis adriatica AF060901    | 0.010 | 0.007 | 0.009 | 0.002 | 0.000 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 8  | Biecheleria pseudopalustris AF260402 | 0.094 | 0.082 | 0.085 | 0.087 | 0.085 | 0.085 | 0.000 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 9  | Biecheleria pseudopalustris AF260402 | 0.094 | 0.082 | 0.085 | 0.087 | 0.085 | 0.085 | 0.000 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 10 | Gymnodinium corii AF318226           | 0.002 | 0.000 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 11 | Biecheleria pseudopalustris AF260402 | 0.094 | 0.082 | 0.085 | 0.087 | 0.085 | 0.085 | 0.000 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 12 | Biecheleria pseudopalustris AF260402 | 0.094 | 0.082 | 0.085 | 0.087 | 0.085 | 0.085 | 0.000 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 13 | Biecheleria pseudopalustris AF260402 | 0.094 | 0.082 | 0.085 | 0.087 | 0.085 | 0.085 | 0.000 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 14 | Symbiodinium micromphalum AF060896   | 0.260 | 0.251 | 0.251 | 0.258 | 0.258 | 0.258 | 0.221 | 0.215 | 0.213 | 0.212 | 0.000 |      |      |      |      |      |      |      |      |      |      |      |      |
| 15 | Symbiodinium micromphalum AF060896   | 0.260 | 0.251 | 0.251 | 0.258 | 0.258 | 0.258 | 0.221 | 0.215 | 0.213 | 0.212 | 0.000 |      |      |      |      |      |      |      |      |      |      |      |      |
| 16 | Symbiodinium micromphalum AF060896   | 0.260 | 0.251 | 0.251 | 0.258 | 0.258 | 0.258 | 0.221 | 0.215 | 0.213 | 0.212 | 0.000 |      |      |      |      |      |      |      |      |      |      |      |      |
| 17 | Polarella glacialis AY036081         | 0.117 | 0.088 | 0.088 | 0.086 | 0.088 | 0.088 | 0.084 | 0.084 | 0.085 | 0.091 | 0.082 | 0.241 | 0.245 | 0.261 | 0.000 |      |      |      |      |      |      |
| 18 | Polarella glacialis FJ939578         | 0.117 | 0.088 | 0.088 | 0.086 | 0.088 | 0.088 | 0.084 | 0.084 | 0.085 | 0.091 | 0.082 | 0.241 | 0.245 | 0.261 | 0.000 |      |      |      |      |      |      |
| 19 | Polarella glacialis AY036081         | 0.117 | 0.088 | 0.088 | 0.086 | 0.088 | 0.088 | 0.084 | 0.084 | 0.085 | 0.091 | 0.082 | 0.241 | 0.245 | 0.261 | 0.000 |      |      |      |      |      |      |
| 20 | Polarella glacialis FJ939578         | 0.117 | 0.088 | 0.088 | 0.086 | 0.088 | 0.088 | 0.084 | 0.084 | 0.085 | 0.091 | 0.082 | 0.241 | 0.245 | 0.261 | 0.000 |      |      |      |      |      |      |
| 21 | Polarella glacialis AY036081         | 0.117 | 0.088 | 0.088 | 0.086 | 0.088 | 0.088 | 0.084 | 0.084 | 0.085 | 0.091 | 0.082 | 0.241 | 0.245 | 0.261 | 0.000 |      |      |      |      |      |      |
| 22 | Polarella glacialis FJ939578         | 0.117 | 0.088 | 0.088 | 0.086 | 0.088 | 0.088 | 0.084 | 0.084 | 0.085 | 0.091 | 0.082 | 0.241 | 0.245 | 0.261 | 0.000 |      |      |      |      |      |      |
| 23 | Polarella glacialis AY036081         | 0.117 | 0.088 | 0.088 | 0.086 | 0.088 | 0.088 | 0.084 | 0.084 | 0.085 | 0.091 | 0.082 | 0.241 | 0.245 | 0.261 | 0.000 |      |      |      |      |      |      |
| 24 | Polarella glacialis FJ939578         | 0.117 | 0.088 | 0.088 | 0.086 | 0.088 | 0.088 | 0.084 | 0.084 | 0.085 | 0.091 | 0.082 | 0.241 | 0.245 | 0.261 | 0.000 |      |      |      |      |      |      |
B. adriatica occurred in cultures of all samples except those from stations Z5 and Z7 (Fig. 5A). It was more abundant in samples from the Guishan Island sea area (Fig. 5B). The maximum cell numbers were 135 cells/g in Zhelin Bay and 716 cells/g in Guishan Island sea area after 20 days incubation, and 64 cells/g and 307 cells/g after 40 days incubation, respectively. It contributed 0%–88% of total phytoplankton (Fig. 6) with an average of 13.7% and 38.6% after 20 days incubation, and 7.6% and 17.6% after 40 days incubation in Zhelin Bay and Guishan Island sea area, respectively. Its maximum contribution to the total germinated dinoflagellates was up to 100%, with averages from 50%–83%. The results indicated that B. adriatica was the dominant dinoflagellate species in the germinated phytoplankton community in both sea areas.

Fig. 4 – Quantitative proportions of different groups of germinated phytoplankton. A, B: samples from Zhelin Bay; C, D: samples off the coast of Guishan Island; A, C: 20 days incubation; B, D: 40 days incubation.

Fig. 5 – Cell number of germinated Biecheleriopsis adriatica in Zhelin Bay (A) and off the coast of Guishan Island (B) after 20 days (the white column) and 40 days (the black column) incubation.
In this study, a dinoflagellate species isolated from the germinated phytoplankton after incubation of the surface sediments from Zhelin Bay, was analyzed using morphological and molecular methodologies. The external characteristics (shape, size, color, epical and hypoconal flanges) and ultrastructural features of the cell, as well as its swimming behavior, indicated that the dinoflagellate basically coincided with the description of the type species of *B. adriatica* (Moestrup et al., 2009b) and those from Japanese coastal waters (Takahashi et al., 2014). Given the difficulty in identifying these small-sized *Gymnodinium*-like species by light microscopy, the strain was wrongly identified as *G. corii* in our previous studies (Kang et al., 2009; Wang et al., 2013). Some other species were originally defined in *Gymnodinium* or other genera in Gymnodiniales and have been examined and clarified into Suessiales recently (Lindberg et al., 2005; Hansen et al., 2007; Moestrup et al., 2008, 2009a, 2009b; Siano et al., 2010). Phylogenetic analyses inferred from LSU rDNA sequences support this conclusion, showing that our sequence closely clusters in the Biecheleriopsis clade together with other six sequences of *B. adriatica* including the type species (EU857537) described in Moestrup et al. (2009b) and three strains (AB858351-AB858353) from Japanese coastal waters (Takahashi et al., 2014). The Biecheleriopsis clade is phylogenetically distant from the Gymnodiniales, but being a member of the order Suessiales (Fig. 3). According to the morphological characteristics and rDNA sequence, the strain in this study was defined as *B. adriatica*.

In the germination experiments, *B. adriatica* occurred in most cultures. It was also the dominant species (identified as *G. corii*) in germinated phytoplankton community during the incubation of surface sediments from other sea areas of the southern Chinese coastal waters (Kang et al., 2009; Wang et al., 2013). Therefore, this species should commonly occur in the water column of the southern Chinese coastal waters due to the wide distribution of its cysts. However, this species has not been reported in previous phytoplankton surveys in Chinese coastal waters. It is possible, however, that since the small-sized naked/thin-walled dinoflagellates are difficult to identify and are not usually determined to the species or genus level in routine monitoring, they might be ignored or misidentified. For example, *Gymnodinium* sp., which was similar in size and shape to *B. adriatica*, was abundant in water samples in Daya Bay of the southern Chinese coast in May 1998, but was not identified to species level (Wang et al., 2001). Based on the common occurrence of *B. adriatica* in germinated phytoplankton in this study and those from the Daya Bay, we presume that the *Gymnodinium* sp. reported by Wang et al. (2001) was *B. adriatica*.

Our sediments were stored in cold, dark, and anoxic conditions for six months before incubation in order to ensure that most of the resting stages went through the compulsory dormant stages, and to restrain the viability of vegetative diatoms and temporary resting stages. Therefore, the resting cysts in the cultures should develop from resting stages including *B. adriatica*. Moestrup et al. (2009b) observed the small spiny resting cyst of *B. adriatica*. The formation of cysts has been reported in genera of the order Suessiales as well, such as *Biecheleria* (Kremp et al., 2005; Siano et al., 2009), *Borghella* (Moestrup et al., 2008), and *Polarella* (Montresor et al., 1999). Even though resting cysts of *B. adriatica* were observed in neither sediments nor cultures in our study, this species is certainly cyst-forming.

*B. adriatica* is a nano-sized phytoplankton, which has the ability to multiply fast as do most of the small-sized species. Meanwhile, *B. adriatica* is a mixotrophic dinoflagellate (Moestrup et al., 2009b), and might be capable of utilizing organic nutrients and be tolerant to nutrient limitation as are many other dinoflagellate species (Yamaguchi et al., 2005; Wang et al., 2011). Its massive occurrences in the germinated phytoplankton community after 20 days and 40 days incubation in this study and our previous studies (Wang et al., 2013) support its competitive advantages in long-term incubation batch culture. The southern China coast has been one of the fastest developing regions in the world and eutrophication has greatly increased over the past two decades. The high nutrient levels and subtropical climate in this area provide favorable conditions for the growth of phytoplankton especially for the small-sized species (Wang et al., 2009). The frequent and abundant occurrence of *B. adriatica* in the germinated phytoplankton community of many sea areas of the southern Chinese coastal waters suggests its wide distribution. This species was reported in sea areas of Japanese and Korean coastal waters as well (Takahashi et al., 2014; Jang et al., 2015).

**Fig. 6** – The proportions of germinated Biecheleriopsis adriatica to total germinated phytoplankton in Zhelin Bay (A) and off the coast of Guishan Island (B) after 20 days (the white column) and 40 days (the black column) incubation.
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