Research Article

**Durinskia baltica** (Dinophyceae), a newly recorded species and genus from China, and its systematics

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**Abstract** A freshwater dinoflagellate was identified as *Durinskia baltica* (Levander) Carty & Cox by morphological characteristics, with the plate formula: Po, x, 4’, 2a, 6’, 5c, 4s, 5”, 2’’. *Durinskia* was a newly recorded dinoflagellate genus for China with two anterior intercalary plates and six characteristic precingular plates. Partial sequences of the small and large subunit ribosomal DNA and internal transcribed spacer sequences for the dinoflagellate cells were obtained from field samples. Molecular phylogenetic results indicated *Durinskia* species could cluster into a monophyletic group, which were distinct from *Peridinium* species. According to morphological and molecular evidence, it was agreed that the genus *Durinskia* was separated from the genus *Peridinium*, which could be a polyphyletic group. In addition, *D. baltica* was an infrequent diatom-harboring dinoflagellate which was known to possess an endosymbiotic diatom or diatom-like alga. The phylogenetic analyses indicated that *D. baltica* had a close affinity with *Peridiniopsis penardii* and *P. niei*, common freshwater bloom-forming species in China.

**Key words** China, *Durinskia*, *Durinskia baltica*, new record, phylogeny.

The dinoflagellates are an important group of phytoplankton in marine and freshwater environments, exploiting autotrophic, heterotrophic, mixotrophic, parasitic, and symbiotic modes of nutrition (Murray et al., 2005; Zhang et al., 2011). The cell of the genus *Durinskia* Carty & Cox is spherical, ovoid, or elliptical (plate formula: Po, x, 4’, 2a, 6’, 5c, 4s, 5”, 2’’). Based on tabulation features, the genus *Durinskia* separated from *Peridinium* was established by Carty & Cox (1986) to accommodate *Durinskia baltica*. The type species *D. baltica* has been reported in marine and freshwater habitats, occurring in the Baltic Sea, Central Europe, and USA (Carty & Cox, 1986; Popovský & Pfister, 1990). The genus is one of the few known diatom-harboring dinoflagellate species. According to endosymbiotic phylogeny, their diatom endosymbiont may originate from a pennate diatom (Inagaki et al., 2000).

In 2005, 2009, and 2010, we examined the dinoflagellate obtained from environmental samples in China and identified it as *D. baltica*. It is a new record of this dinoflagellate for China. This paper deals with phylogenetic affinities of *Durinskia* and other genus based on small subunit ribosomal DNA (SSU rDNA) sequences, partial large subunit ribosomal DNA (LSU rDNA) sequences, and internal transcribed spacer (ITS) sequences. Finally, we discuss the morphology and taxonomy of various species in this genus.

1 Material and methods

1.1 Collection and preservation

Samples were collected at several small freshwater ponds in Zhongshan, Guangdong Province, China, in July 2005 (A; 22°38’29”N, 113°21’15”E), in Xiantao, Hubei Province, China, in November 2009 (B; 30°21’22”N, 113°32’21”E), and in Qingdao, Shandong Province, China, in October 2010 (C; 36°21’32”N, 120°10’12”E) (Fig. 1). Samples were preserved in 10% formalin and 90% ethanol for morphological and molecular analyses. Ethanol-fixed samples were then frozen at −20 °C until analysis.

1.2 Morphospecies identification

For observation of the thecal plates, formalin-fixed cells were stained with 0.1% Fluorescent Brightener 28 (Sigma, Poole, UK) and observed using epifluorescence microscopy (DM5000B; Leica, Wetzlar, Germany). The method has been described in detail by Fritz & Triemer (1985) and Liu et al. (2008). Ethidium bromide-stained cells were prepared by a common method for observation of nuclei. We also observed cells for differential interference contrast using the Leica DM5000B microscope. Micrographs were taken with a Leica DFC320 digital camera.
1.3 Cell isolation and DNA extraction

Several cells (~10) of *Durinskia baltica* were isolated from ethanol-fixed field samples from Xiantao and Qingdao under an inverted microscope (CKX41; Olympus, Tokyo, Japan) for each polymerase chain reaction (PCR) amplification. These cells were then transferred to sterile distilled water droplets three times to facilitate removal of ethanol, then placed in a 200-μL thin-walled PCR tube containing a drop of mineral oil. Samples were then frozen at −20 °C until analysis. DNA extraction was treated with proteinase K. Details of this method were described by Ki et al. (2005).

1.4 Polymerase chain reaction amplification and DNA sequencing

Three sets of PCR primers were used for PCR amplification of SSU rDNA (uP18f, 5′-AACCTGGTTGACTAGTTC-3′; uP18r, 5′-TGATCCAGGTCAATGAC-3′), partial LSU rDNA (domains D1–D2) (DinFi, 5′-GCATAAGTAMGYGGWGG-3′; DinRi, 5′-CCGTGGTTTCAAGACGGGT-3′) (Logares et al., 2007), and ITS rDNA (ITS1, 5′-TCCCTCCGCTATTGATATGC-3′; ITS4, 5′-TCCTCCGTTATTGATATGC-3′) (White et al., 1990). The PCR amplifications were as follows: 5 μL template DNA (as above), 1× PCR buffer, 0.25 μmol/L dNTP, 0.4 μmol/L each primer, and 0.65 U Taq DNA polymerase (ExTaq; Takara, Dalian, China) in 25 μL total volume reactions. The SSU PCR started with 5 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 56 °C, 2 min at 72 °C, ending with a final hold of 7 min at 72 °C. The LSU PCR temperature profile was equivalent to the SSU except for the annealing temperature of 52 °C. The ITS PCR temperature profile was equivalent to the LSU PCR. All PCR amplicons were cleaned using AxyPrep DNA Gel Extraction Kit (Axygen Biotechnology, Hangzhou, China), then some PCR products were cloned into pMD18-T vector (Takara). All clones
were sequenced by universal sequencing primer M13. The products were run on an ABI 3700 sequencer (Applied Biosystems, Foster City, CA, USA). Sequences were deposited in GenBank under the Accession Nos. GU999528–GU999529 and HQ588942–HQ588944.

1.5 Phylogenetic analyses

The SSU, LSU, and ITS sequences representing some dinoflagellates were downloaded from GenBank. *Noctiluca scintillans* was used as an outgroup in SSU phylogeny, *Perkinsus marinus* was used as an outgroup in LSU and ITS phylogenies. These taxa were used for phylogenetic analyses, including *Peridinium, Peridiniopsis, Scrippsiella, Pentapharsodinium, Kryptoperidinium, Prorocentrum, Ceratium, Pfiesteria, Cryptoperidiniopsis, Ensiculifera, Karenia,* and *Karalodinium*. The SSU, LSU, and ITS alignments consisted of 30 sequences with 1577 characters, 25 sequences with 459 characters, and 28 sequences with 527 characters, respectively. Edited sequences were aligned using Clustal X (v. 1.83), and resulting alignments were edited manually by SEA VIEW (v. 4.32) (Thompson et al., 1997). We analyzed sequence identity percentages and genetic distance by MEGA (v. 4.0.0.4103) (Kumar et al., 2004).

Phylogenies were estimated using maximum likelihood (ML) and Bayesian inference (BI) as implemented in PAUP 4.0 (v. 4.0b10) (Swofford, 2002) and MrBayes (v. 3.1.2) (Huelsenbeck & Ronquist, 2001). The program Modeltest (v. 3.07) was used to explore the model of sequence evolution that best fits the dataset by the hierarchical likelihood ratio test (Posada & Crandall, 1998). The evolutionary model used in ML and BI analyses for the SSU phylogeny was TrN + I + G, whereas the model selected for ITS phylogeny was GTR + G, whereas the model selected for ITS phylogeny was GTR + G + I. In ML analysis, a heuristic search option with random addition of sequences (100 replicates) and the tree bisection and reconnection branch-swapping algorithm were used for tree searching. Bootstrap analysis with 100 replicates of the dataset for ML was calculated to estimate statistical reliability. All Bayesian Markov Chain Monte Carlo analyses were run with seven Markov chains (six heated chains, one cold) for 1 000 000 generations and trees were sampled every 100 generations. The first 1000 trees (burn-in samples) were discarded, and the remaining samples were used to construct a Bayesian consensus tree and to infer posterior probability. Bootstrap values and posterior probabilities for some clades obtained across the phylogenies were presented on the nodes.

2 Results

2.1 Description

**Durinskia** Carty & Cox, 1986

The general plate tabulation of this genus is Po, x, 4’, 2a, 6”, 5c, 4s, 5’”, 2’”’. Dinoflagellate with an apical pore surrounded by a pore plate and with a small ventral canal plate, eyespot present, with golden chloroplasts, theca ornamented with pores, freshwater or marine.

**Durinskia** belongs to Dinophyceae, Peridiniales, Peridiniaceae. It is identified as a new record of genus for China. Until now, only three species of this genus have been reported and *D. baltica* is the only species observed in our samples from China.


Cells are globular or ovoid, and slightly dorsoventrally flattened. The epithea is nearly equal in size to the hypotheca, or slightly larger than the hypotheca. Cells measure 20.0–32.5 μm (mean value = 27.6 ± 2.9, n = 30) in length, 17.5–27.5 μm (mean value = 23.7 ± 2.7, n = 30) in width. Apical pore complex is present. The cingulum is ca. 3–5 μm wide, and slightly displaced about a half of its own width. The sulcus is narrow and extends to the antapex. Numerous discoid yellowish brown chloroplasts are present near the cell surface. A rectangular red eyespot is situated close to the sulcus. The nucleus is large and situated in the center part of cell. The thecal plates are thin, smooth, and seem to have no ornamentations. The plate tabulation is Po, x, 4’, 2a, 6”, 5c, 4s, 5’”, 2’”’. (Figs. 2, 3). The apical pore plate (Po) is small and circular in shape. The canal plate (x) is also small and rectangular in shape. The arrangement of the epithecal plates is asymmetric. The apical plate 1’ is rhombic; plate 2’ is irregular pentagonal; plate 3’ is four-sided and relatively small; plate 4’ is pentagonal. The anterior intercalary plate 1a is very small and five-sided in shape, whereas plate 2a is relatively large and six-sided. The precingular plates 1’’, 4’’, and 6’’ are four-sided, whereas plates 2’’, 3’’, and 5’’ are five-sided. The cingulum is composed of five plates. The cingular plate 1c is very short, contacting with plate 1’ on the epitheca and 1’” on the hypotheca. The sulcus consists of four plates. The posterior sulcal plate Sp is relatively large and rhombic in shape. The posterior accessory sulcal plate Spa is rather small and surrounded by the other three sulcal plates. The arrangement of the
hypothecal plates is nearly symmetric. The hypotheca is composed of five postcingular plates (5") and two antapical plates of similar sizes (2""). Postcingular plates 1", 2", 4", and 5" are four-sided whereas plate 3" is five-sided. The antapical plates 1"" and 2"" are five-sided. The shapes and relative positions of thecal plates are described in Fig. 4.

Ecology: *D. baltica* occurred from July to October, suggesting it was a warm species. All of our specimens were collected from small freshwater ponds that were usually rich in humus. In our survey, *D. baltica* was never very abundant and usually accompanied by a small amount of other dinoflagellates such as *Peridinium umbonatum*. In our materials from Xiantao and Zhongshan, the euglenoids, including *Euglena* spp., *Lepocinclis* spp., *Phacus* spp., and *Trachelomonas* spp., usually became the important contributors to phytoplankton biomass. In our material from Zhongshan,
some green algae, including *Scenedesmus* spp. and *Pe-diastrum* spp., were the important contributors to phytoplankton biomass.

Distribution: Small freshwater ponds, pools; Zhongshan, Guangdong Province (July 2005); Xiantao, Hubei Province (November 2009); Qingdao, Shandong Province (October 2010).

Vouchers of specimens examined: GD-2005–01, from a pond, Zhongshan, Guangdong Province, collected by Guo-Xiang Liu on July 7, 2005; HB-2009–08a, from a pond, Xiantao, Hubei Province, collected by Qi Zhang on November 6, 2009; SD-2010–06a, from a pond, Qingdao, Shandong Province, collected by Qi Zhang on October 29, 2010. These specimens are deposited in the Freshwater Algal Herbarium (HBI), Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei, China.

### 2.2 Sequence similarity of *Durinskia* taxa

Estimates of sequence similarity provided a simple measure of relationship between these *Durinskia* taxa. The SSU rDNA sequence from *D. baltica* (Xiantao) had 98.7% and 95.9% sequence similarity with sequences of Accession Nos. AF231803 (*D. baltica*) and AB271107 (*D. capensis*) obtained from GenBank, respectively. The sequence similarity percentage based on partial LSU and ITS rDNA between *D. baltica* from Xiantao and Qingdao was 97.8% and 94.4%, respectively.

### 2.3 Phylogenetic results

The two analytical methods yielded more or less the same topology, but only the ML trees are presented. Most phylogenies had weakly defined backbone topologies but several well-supported internal clades (Fig. 5). In all phylogenies, there was always a highly supported clade comprising freshwater species of *Peridinium* sensu stricto group, which was composed of *P. willei*, *P. cinctum*, *P. gatunense*, *P. volzii*, *P. bipes*, and so on. In SSU, LSU, and ITS phylogenies, all *Durinskia* species, including *D. capensis* and *D. baltica* from various collections, could cluster into a monophyletic clade with high support values (bootstrap probability

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of 2 and 0–1 anterior intercalary plates, respectively (Carty & Cox, 1986).

Because the name *Durinskia baltica* has been changed several times, it is necessary to have a brief historical retrospect. Levander (1892) originally described a species in freshwater habitat considered to be *Glenodinium cinctum* Ehrenberg. In 1894 he revised it as *Glenodinium balticum* (Levander, 1894). Lemmermann (1910) transferred it to *Peridinium* and referred it in connection with the freshwater subgenus *Cleistoperidinium*, a group lacking an apical pore (in fact, this species has an apical pore). Schiller (1937) transferred it to the subgenus *Orthoperidinium*, a group with four-sided apical plate 1’ and considered that it was a synonym for *P. dybowskii* depicted by Woloszynska (1917). *Peridinium balticum* was transferred to the genus *Peridiniopsis* redefined by Bourrelly (1968), and renamed *Peridiniopsis balticum* (Levander) Bourrelly. Carty & Cox (1986) considered that it was different from *Peridinium* and *Peridiniopsis* based on its two anterior intercalary plates and six precingular plates and therefore constructed the new genus *Durinskia* to accommodate it. There were some minor plate tabulation variations in a small number of cells during fusions and divisions (Chesnick & Cox, 1985).

So far there are three species of the genus *Durinskia*. The first member is *D. baltica*, the type species of the genus. The second species is *Durinskia capensis* Pienaar, Sakai & Horiguchi (Pienaar et al., 2007). This species occurred in tidal pools at Kommetjie, Cape Province, South Africa, resulting in very characteristic orange-red water. The obviously displaced cingulum and relatively large plate 1a in *D. capensis* were different from the first species (Pienaar et al., 2007). The third species is *Durinskia oculata* (Stein) Gert Hansen & Flaim. Hansen & Flaim (2007) described this dinoflagellate from Ampola Lake, Trentino Province, Italy. The tabulation of this species is identical with *D. baltica*.

There are still some taxonomic problems about *D. oculata* waiting to be resolved. Hansen & Flaim (2007) considered that their material fits well with Stein’s (1883) original illustrations of *Glenodinium oculatum* Stein, with respect to the shape of cell, chloroplast, and eyespot. However, Stein (1883) did not provide any information with respect to plate tabulation. The tabulation was later depicted by Woloszynska (1917) for a species considered to be *Peridinium oculatum* (Stein) Woloszynska (=*G. oculatum*). The tabulation illustrated by Woloszynska was different from the material from Hansen & Flaim (2007) by reason of having one intercalary plate and seven precingular plates. The tabulation illustrated by Woloszynska was similar to a species depicted considered to be *Peridiniopsis oculatum* (Stein)
Bourrelly (=*G. oculatum* Stein) depicted by Popovský & Pfiester (1990). However, the tabulation of *G. oculatum* illustrated by Lindemann (1926) was similar to the material from Hansen & Flaim. Hansen & Flaim (2007) agreed with the description from Lindemann and made a new combination *D. oculata*. They considered *Peridinium dybowskii*, *D. baltica*, and *D. oculata* as separate species – *P. dybowskii* depicted by Woloszyńska (1917) usually occurred in fresh waters and was furnished with numerous transversely orientated pores on the thecal plates; *D. baltica* usually occurred in brackish waters and had more smooth thecal plates; *D. oculata* usually occurred in fresh waters and was more globular in shape with a more minute eyespot. Nevertheless, more convincing evidence is needed to support that the three names represent three varied species.

### 3.2 Phylogenetic position

According to sequence similarity based on SSU rDNA, there was a relatively high degree of sequence homology (98.7%) between the dinoflagellate from Xiantao and *D. baltica* isolated from Salton Sea, California, USA, an inland saline lake (UTEX1563, AF231803). Moreover, the sequence of *D. baltica* from Xiantao had 95.9% sequence similarity with a sequence of *D. capensis*. The results indicated the dinoflagellate sampled from Xiantao should belong in *Durinskia*, and furthermore, it was more closely related to *D. baltica* than to *D. capensis*. In addition, there was a relatively high degree of sequence homology (97.8%, 94.4%) between our materials isolated from Xiantao and Qingdao based on partial LSU and ITS rDNA. Considering faster substitution rates of LSU and ITS rDNA, it was acceptable intraspecific variability in rDNA of different geographical populations from our environment samples.

Most phylogenies had weak backbone topologies but several well supported internal clades. Our results strongly supported the polyphyly of the genus *Peridinium* and the monophyly of *Peridinium sensu stricto* group. When more and more genera had been separated...
from *Peridinium*, it was likely that this genus would only comprise the freshwater species of *Peridinium sensu stricto* group. In all phylogenies, all *Durinskia* species could cluster into a monophyletic group with high support values (BP = 100, PP = 1.00). In all phylogenies, the *Durinskia* species were distantly related to *Peridinium* species. Although the *Durinskia* species were closely related to several *Peridiniopsis* species (*P. penardii*, *P. niei*, and *P. kevei*) in LSU and ITS phylogenies, they did not fit with the genus *Peridiniopsis* with respect to plate tabulation. Moreover, all phylogenetic results showed that the genus *Peridiniopsis* species might be a polyphyletic group and the definition of the genus *Peridiniopsis* based on plate tabulation clearly seemed to be an oversimplification. Therefore, it was reasonable for erecting the genus *Durinskia*.

It was no surprise that *D. baltica* had a close affinity with *Peridiniopsis penardii* in our phylogenetic analyses, because the two species were the infrequent diatom-harbouring dinoflagellates known to possess an endosymbiotic diatom or diatom-like alga (Imanian & Keeling, 2007; Takano et al., 2008). The endosymbiont of *D. baltica* came from an original pennate *Nitzschia*-like diatom, whereas the endosymbiont of *P. penardii* came from an original centric *Thalassiosira/Skeletonema*-like diatom (Takano et al., 2008).

As well as these two dinoflagellates, others including *Kryptoperidinium foliaceum* (Stein) Lindemann (Jeffrey & Vesk, 1976; Dodge, 1983; Kempton et al., 2002), *Peridinium quinquecorne* Abé (Horiguchi & Pienaar, 1991), *Dinothrix paradoxa* Pascher (Horiguchi & Chihara, 1993), *Gymnodinium quadridibatum* (Horiguchi & Pienaar, 1994), *Durinskia capensis* (Pienaar et al., 2007), and *Galeidinium rugatum* Tamura & Horiguchi have been known to harbor chloroplasts from diatoms (Tamura et al., 2005). Takano et al. (2008) suggested that the host phylogeny was indicative of a monophyletic group of diatom-harboring dinoflagellates and there was a serial replacement of endosymbionts from an original pennate *Nitzschia*-like diatom to a centric diatom. In addition, our phylogenetic analyses also indicated that *D. baltica* was closely related to *P. niei*, but the endosymbiont has not yet been found in *P. niei* (Liu et al., 2008). The reasonable explanation was that the endosymbiont genome reduced genetic material gradually and available genes were transferred to a host nucleus, and finally the endosymbiont might have converted into the host organelle during the evolutionary history of *P. niei*.

In published reports, *D. baltica* occurred in both marine and freshwater habitats (Carty & Cox, 1986; 1987; Carty et al., 1987; Tóth, 1984; 1985).
Popovský & Pfiester, 1990). We have observed the species in freshwater habitats in our study, but the distribution of this species in China is still unclear, especially in the marine environment. However, it is unclear whether we can detect consistent morphological, physiological, and genetic variability of *D. baltica* from the two different habitats, and whether the variability could be used for distinguishing marine and freshwater populations.

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