Korean Journal of Environmental Biology

Original article

https://doi.org/10.11626/KJEB.2023.41.4.608

Korean J. Environ. Biol.
41(4) : 608-615 (2023)
ISSN 1226-9999 (print)
ISSN 2287-7851 (online)

Morphology and molecular phylogeny of harmful dinoflagellate *Pseudocochlodinium profundisulcus* from Korean coastal area

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Contribution to Environmental Biology

- Resting cysts of harmful dinoflagellate *Pseudocochlodinium profundisulcus* are distributed in Korean coastal sediments.
- Korean and Chinese isolates of *P. profundisulcus* are conspecific.

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Received: 30 November 2023
Revised: 8 December 2023
Revision accepted: 19 December 2023

Abstract: The morphological features of germling cells were examined to identify an unspecified resting cyst (described as *Cochlodinium* cf. *polykrikoides*-like resting cyst) in the Korean coastal area. LSU rRNA gene sequences were also obtained from a strain established from the germling cells. The resting cysts isolated from Korean coastal sediment were characterized as being brown in color, having a large dark-red body, and fibrous lobed ornaments. The germling cells were ellipsoidal with an irregular outline and had an open comma-shaped ASC (apical structure complex), a wide and deep cingulum, and a deep sulcus. These morphological features were consistent with those of previously described harmful dinoflagellate *Pseudocochlodinium profundisulcus*. The molecular phylogeny revealed that the germling cells and *P. profundisulcus* were conspecific. Based on these morphological and phylogenetic data, this study documents the occurrence of *P. profundisulcus* in a Korean coastal area for the first time.

Keywords: HABs, *Cochlodinium*, *Polykrikos*, resting cyst, germination

1. INTRODUCTION

In Chinese coastal areas, dense blooms caused by an unknown dinoflagellate species occurred in April of 2006, September and October of 2009 and August of 2011, and the outbreaks of blooms caused serious economic losses in fisheries industries (Hu *et al.* 2021 and references therein). The organism responsible for the dense blooms was initially identified as *Cochlodinium germinatum* (Schütt) Schütt by Qi *et al.* (2009), and then this species was independently named as *Cochlodinium* sp. (Wang *et al.* 2011), and subsequently transferred to the genus *Polykrikos* as *Po. germinatus* (Schütt) Qui et Lin (Qiu *et al.* 2013). Recently, Hu *et al.* (2021) proposed this organism as a new genus and species, *Pseudocochlodinium profundisulcus* Zhang Hu, N. Xu, H. Gu, M. Iwataki, T. Takahashi, Y. Z. Tang & K. Matsuoka, based on detailed morphological observations and phylogenetic data of strains and resting cysts collected in China and Japan.

Many harmful dinoflagellates produce resistant resting cysts as part of their life cycle, and the resting cyst stage can play an important role in both the initiation and decline of harmful algal blooms (HABs) (e.g. Anderson 2012; Li *et al.* 2015; Hu *et al.* 2021). The blooms or harmful effects caused by *P. profundisulcus* have not yet been recorded in Korean coastal area, however morphologically similar resting cysts (described as

Cochlodinium cf. *polykrikoides*-like resting cyst) to that of *P. profundisulcus* described by Hu *et al.* (2021) have been reported (Park 2007; Shin *et al.* 2011). This indicates that *P. profundisulcus* may be considered as being potentially harmful in Korean coastal area.

Hence, the resting cysts were isolated from Korean coastal sediments, and the germling cells were identified by morphological observation and phylogenetic analysis. Based on the obtained results, this study documents for the first time the occurrence of harmful dinoflagellate *P. profundisulcus* in Korean coastal area.

2. MATERIALS AND METHODS

2.1. Sediment collection and culture establishment

Surface sediment samples were collected at a station in the Tongyeong coastal area, Korea (34°45′99″N, 128°12′36.00″E), using a gravity corer. The top 2 cm of the core samples were sliced and preserved immediately in dark and cool conditions at 4°C prior to further analysis. The samples were analyzed basically in accordance with the panning methods of Matsuoka and Fukuyo (2000); however, the sediment suspension in the present study was treated using screens with mesh sizes of 125 and 10 μm. *Cochlodinium* cf. *polykrikoides*-like resting cyst described by Matsuoka and Fukuyo (2000) were isolated using an inverted microscope (ECLIPSE Ti; Nikon, Tokyo, Japan) and its morphological features were recorded using a digital camera. The isolated cysts were inoculated into individual wells of 96 well tissue culture plates (SPL, Seoul, South Korea) filled with f/2-Si culture medium (salinity of 35) and were cultured at 20°C and ca 100 μmol photons m^{-2} s^{-1} cool-white illumination under a 14L : 10D photo-cycle. The germinated cells were transferred to individual wells of six well culture plates, and after sufficient growth the cells were transferred to a culture flask (70025; SPL Life Science, Korea) containing 25 mL sterile f/2-Si culture medium. A strain (LIMS-PS-3456) was established successfully and deposited in the Library of Marine Samples, Korea Institute of Ocean Science and Technology, and a specimen (MABIK PD00002407) was deposited in the National Marine Biodiversity Institute of Korea (MABIK).

2.2. Light microscopy (LM)

Living cells of the strains were photographed at ×1,000 magnification using an ultra-high resolution digital camera (DS-Ri2; Nikon, Japan) on an upright microscope (ECLIPSE Ni; Nikon, Japan). Cell size was measured based on LM images. For fluorescence microscopy, approximately 2 mL of culture was transferred to a 5 mL microcentrifuge tube, and SYTOX® Green Nucleic Acid Stain (Molecular Probes, Eugene, OR, USA) was added at a final concentration of 1.0 μM. The cells were incubated in the dark at room temperature for 10 min. The cells were observed through a Dual excitation filter block FITC-TRITC specifications (excitation: BP 483−553, emission: BP 517−600) and photographed using a Digital Sight 10 digital camera on an upright microscope (ECLIPSE Ci; Nikon, Japan).

2.3. Scanning electron microscopy (SEM)

For SEM, 15 mL of mid-exponential batch culture was fixed by the addition of an equal volume of 1% osmium tetroxide (Sigma, St Louis, MO, USA) prepared in deionized water for 30 min at room temperature. The fixed cells were rinsed twice with deionized water and dehydrated in a graded ethanol series (10−99% in six steps) and a chemical preparation method using hexamethyldisilazane (HMDS) was employed to dry the germlings. The filters were mounted on stubs, coated with platinum-palladium, and were examined with a field emission scanning electron microscope (JSM 7600F; JEOL, Tokyo, Japan).

2.4. DNA extraction and sequencing

Genomic DNA was extracted from 2 mL of the culture using the DNeasy® Plant Mini Kit (QIAGEN Inc., Valencia, California USA), following the manufacturer's instructions. The partial LSU rRNA gene sequence was amplified using the primer pairs 25F1 and R2 (Yamaguchi and Horiguchi 2005; Takano and Horiguchi 2006). The PCR was conducted using a Thermal Cycler (Mastercycler® nexus; Eppendorf, Hamburg, Germany) at 95°C for 2 min, followed by 30 cycles of denaturation at 95°C for 20 sec, annealing at 55°C for 1 min, and extension at 72°C for 1 min. The reaction was completed with a final elongation at 72°C for 5 min. The PCR products were confirmed by 1% agarose gel electrophoresis. The PCR products were purified

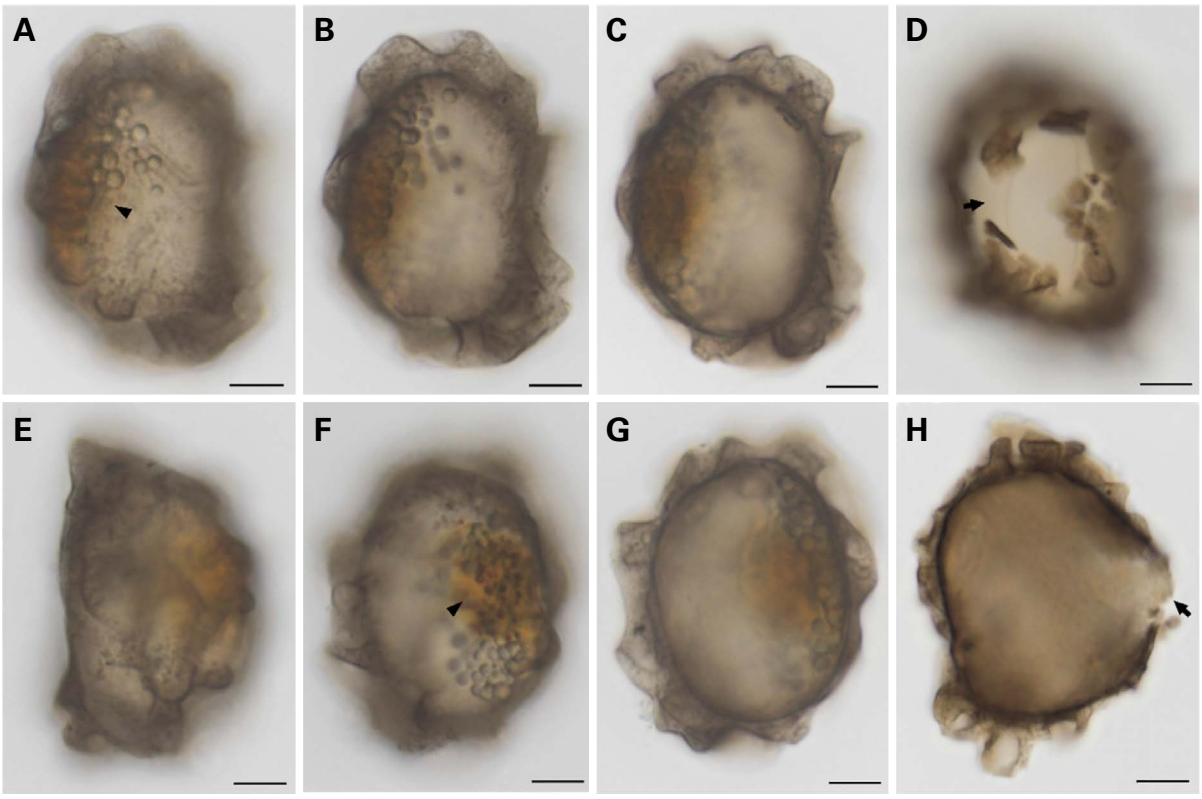

Fig. 1. Light microscopy micrographs of a resting cyst of *Pseudocochlodinium profundisulcus* collected from Korean coastal sediment. (A) Living cyst, showing granular contents (arrowheads). (B, C) Living cyst, showing lobed ornaments. (D) Empty cyst, showing the archeopyle (arrow). (E) Different view of the same specimen, showing lobed ornaments. (F, G) Same specimen in a different focus, showing a large dark-red body (arrowhead). (H) Different view of the same specimen, showing the archeopyle (arrow). Scale bars: 10 μm.

using the QIAquick PCR Purification Kit (QIAGEN). The cycle sequencing reaction was performed using the ABI PRISM BigDye^{TM} Terminator Ver. 3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA).

2.5. Sequence alignment and phylogenetic analysis

Sequences were viewed and assembled in DNABaser version 5.0 (http://www.dnabaser.com). Contigs were imported into Mafft online version 7.0 (http://mafft.cbrc.jp/alignment/server) and aligned using MEGA version 7.0 with default settings, excluding poorly aligned positions. The separate alignments were then checked using sequenceMatrix version 1.8 (Vaidya *et al.* 2011). For the analysis of LSU rRNA gene sequences the data set contained 49 taxa and consisted of 1,338 characters (including gaps introduced for alignment). *Alexandrium minutum* (GenBank accession number JF521634) was used as the out group. The GTR+G model of nucleotide substitution, with a gamma-distributed rate of variation across sites, was chosen. The assumed nucleotide frequencies were 0.2336 for A, 0.2147 for C, 0.2932 for G, and 0.2584 for T. The proportion of sites assumed to be invariable was 0.1660, and the rates for the variable sites were assumed to follow a gamma distribution with a shape parameter of 0.5480.

Phylogenetic trees for both datasets were constructed using maximum likelihood (ML) analyses and Bayesian inference. The ML analyses were performed using PhyML ver. 3.1 (Guindon *et al.* 2010). The starting tree was generated using BIONJ using optimization of the topology, branch lengths, and selected rate parameters. Bayesian inference was conducted on the LSU rRNA gene sequence alignments using MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003) under the TrN+G

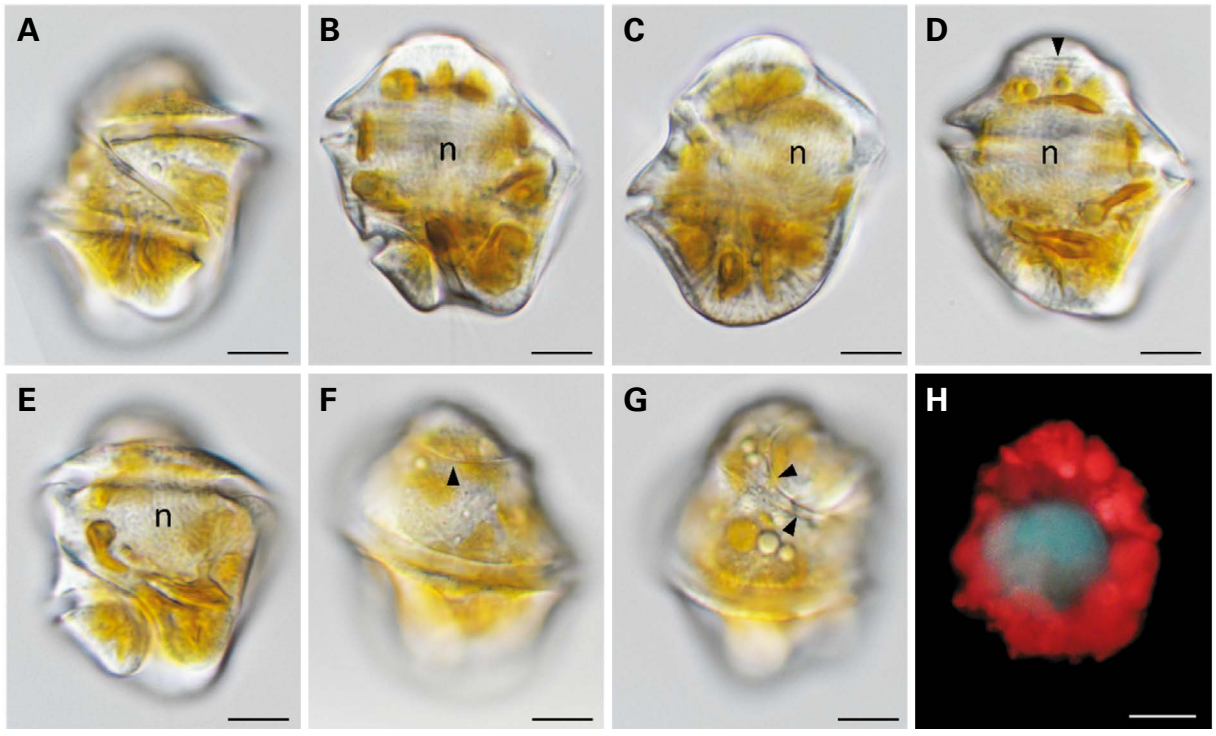

Fig. 2. Light and fluorescence micrographs of *Pseudocochlodinium profundisulcus* germinated from the resting cyst. (A) Surface focus of the ventral-left view, showing the cingulum and sulcus. (B) Deep focus of the left lateral view, showing the nucleus (n). (C) Deep focus of the right lateral view, showing the nucleus (n). (D) Dorsal view, showing the apical structure complex (ASC) and nucleus (n) (arrowhead). (E) Antapical-left lateral view, showing the nucleus (n). (F) Apical-left lateral view, showing the ASC and cingulum. (G) Surface focus of the ventral-right lateral view, showing the ASC (arrowheads). (H) Ventral view of a SYTOX Green-stained cell, showing the shape of the nucleus. Scale bars: 10 μm.

model taking into account six-class gamma and invariant sites. Four Markov chain Monte Carlo chains were run for 10 million generations, with sampling every 100 generations. A majority rule consensus tree was created in order to examine the posterior probabilities of each clade.

3. RESULTS AND DISCUSSION

3.1. Morphological features of a resting cyst and germling cell

Cochlodinium cf. *polykrikoides*-like resting cysts described by Matsuoka and Fukuyo (2000) were collected and germinated, however only one strain (LIMS-PS-3456) was established. The morphological features of a resting cyst and germling are shown in Figs. 1–3. The resting cyst in this study was ellipsoidal and brown in color, and size is 55.6 μm in length and 43.2 μm in width (Fig. 1). A large dark red body and granular contents were observed at the margin of the resting cyst (Fig. 1A–C, F and G). Cyst surface was characterized by fibrous lobed ornaments (Fig. 1A–C and E). These lobed ornaments were variable in size and arranged spirally on the cyst surface (Fig. 1E). Archeopyle was chasmic and occupied one-third of the cyst and its shape was unclear (Fig. 1D and E).

In this study, the morphological characteristics of the resting cyst were consistent with those of resting cysts from Korean, Japanese, Chinese and Russian coastal areas (Matsuoka and Fukuyo 2000; Orlova *et al.* 2004; Park 2007; Hu *et al.* 2021), and from sediments of ballast tanks (Shang *et al.* 2022). This cyst was previously regarded as the resting cyst of *Cochlodinium polykrikoides* (=*Margalefidinium polykrikoides*) or *Cochlodinium* cf. *polykrikoides* (e.g. Matsuoka and Fukuyo 2000; Orlova *et al.* 2004; Park 2007), and since then different morphological features of the resting cysts of *C. polykrikoides* was provided based on results of

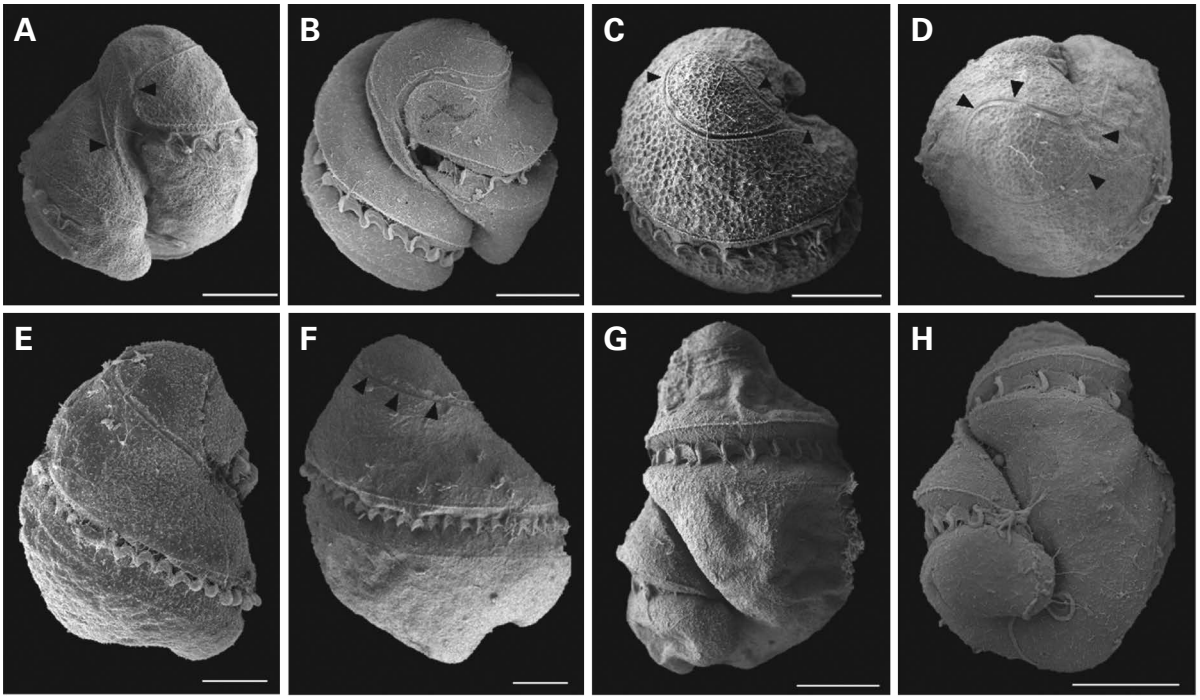

Fig. 3. Scanning electron micrographs of *Pseudocochlodinium profundisulcus* germinated from the resting cyst. (A) Ventral view, showing a comma-shaped apical structure complex (ASC) (arrowheads) and sulcus. (B) Ventral-apical view, showing the ASC, the transverse flagellum, cingulum, and sulcus. (C) Apical-right lateral view, showing the amphiesmal vesicle on the cell surface and the ASC (arrowheads). (D) Apical view, showing the ASC (arrowheads). (E) Right lateral view, showing the ASC and the cingulum. (F) Dorsal-right lateral view, showing the ASC (arrowheads) and cingulum. (G) Left lateral view, showing the cingulum. (H) Antapical-left lateral view, showing the cingulum and sulcal extension. Scale bars: 10 μm.

laboratory experiments and sediment sample analysis (Tang and Gobler 2012; Li *et al.* 2015). Recently, it has been clear that the previously described cysts belong to *Pseudocochlodinium profundisulcus* that was initially described as *C. germinatum* Schütt (Hu *et al.* 2021). According to Hu *et al.* (2021), the resting cysts of *P. fundisulcus* are morphologically characterized by ellipsoidal to ovoidal, reddish to dark brownish in color and irregular verrucate lobed ornaments. These morphological characteristics were also observed in the resting cyst isolated from Korean coastal sediments.

Germinated cells (strain LIMS-PS-3456) were ellipsoidal in shape, with irregular cell outline (Figs. 2, 3). Cells were yellowish in color, and the average size is 48.5 ± 7.76 μm in length and 40.2 ± 6.0 μm (n = 50) in width. Only single cells were observed in culture. In the sells, the apex is flattened (Fig. 2B, D). The cingulum and sulcus were deep and originated at one third of the length of the of the cell from the anterior end (Fig. 2A, E–G). The shape of the apical structure complex (ASC) was unclear however the line of the ASC was observed in LM (Fig. 2D, F, G). The nucleus was spherical and located near the center or slightly above center of the cell (Fig. 2B–D, H). Chloroplasts were distributed, peripherally (Fig. 2H).

SEM photographs showed small amphiesmal vesicles on the cell surface and the sulcus deepening towards the antapical and the ASC (Fig. 3A–H). The sulcal extension on the epicone and the tranverse flagellum on the transverse furrow was clearly visible (Fig. 3A–C, E–H). The cingulum circled 1.5 times around the cell (Fig. 3B, E–H). The sulcus is narrow and deep, and circled 0.5 times, immediately diverging from the cingulum at the intersection of two furrows (Fig. 3A, B) and joining the cingulum near the antapical (Fig. 3G, H). A comma-shaped ASC that looped anticlockwise on the apex was observed, and the ASC started from the anterior end of the sulcal intrusion and ended almost at the right edge of the sulcal intrusion but did not contact it (Fig. 3A–D, F).

The morphological features of the germinated cells were completely consistent with those of Chinese iso-

lates of *P. profundisulcus* described by Hu *et al.* (2021); this species has an open comma-shaped ASC, a wide and deep cingulum, and a deep sulcus. However, Hu *et al.* (2021) observed two-celled chains of *P. profundisulcus* both in culture and field samples, whereas in our culture only single cells were observed. In addition, average cell sizes (48.5 ± 7.76 μm in length and 40.2 ± 6.0 μm in width) of Korean specimen were slightly larger than those of Chinese isolates (34.3 ± 3.1 μm in length and 31.8 ± 2.7 μm in width) (Hu *et al.* 2021), indicating that the size of *P. profundisulcus* can be variable among specimens.

Based on the historical descriptions, Hu *et al.* (2021) reviewed morphological similarities and differences of *Cochlodinium* species (including *Magalefidinium* species), *Polykrikos* and *Pheopolykrikos* species with *P. profundisulcus*, and concluded that the Chinses specimens of *P. profundisulcus* are distinguished from *Cochlodinium* species by an open comma-shaped ASC, a wide and deep cingulum and a deep sulcus, and from *Polykrikos* and *Pheopolykrikos* species by morphology of ASC, cingular displacement, sulcal torsion and cyst morphology. However, as recent studies have revealed that the traditional criteria for identification of unarmoured dinoflagellates, such as characteristics of cingulum and sulcus, are problematic and the cells with abnormal shapes were frequently observed (e.g. Shin *et al.* 2019a), the cingulum and sulcus to distinguish species should be treated with caution. Actually, both Korean and Chinese specimens have irregular cell outline.

3.2. Phylogenetic positions of germling cells

The accession numbers of LSU rRNA gene sequences for strain LIMS-PS-3456 and a germling were obtained and deposited in NCBI. Maximum-likelihood (ML) and Bayesian inference (BI) analyses yield similar phylogenetic trees. The ML tree based on LSU rRNA gene sequences showed that the germlings (accession numbers OR855909 and OR855908 for LIMS-PS-3456) form a larger clade with *Pseudocochodinium profundisucus* (MW811440, MW811439, MG874046, MG87 4047, MG874048 and MG874049), *Polykrikos germinatum* (JX967270, MF445291 and KF878934), and *Cochlodinium* cf. *germinatum* (EF616462) (ML bootstrap support 99, BI posterior probability 1.0) (Fig. 4). In the phylogenetic tree, the larger clade exhibited a sister relationship with a clade consisting isolates of *Polykrikos* species (*Po. schwartzii*, *Po. kofoidii* and *Po.* cf. *schwartzii*) and *Pheopolykrikos hartmanii*. *Po. tanit* and *Ph. hartmanii* was distant from other *Polykrikos* species (Fig. 4).

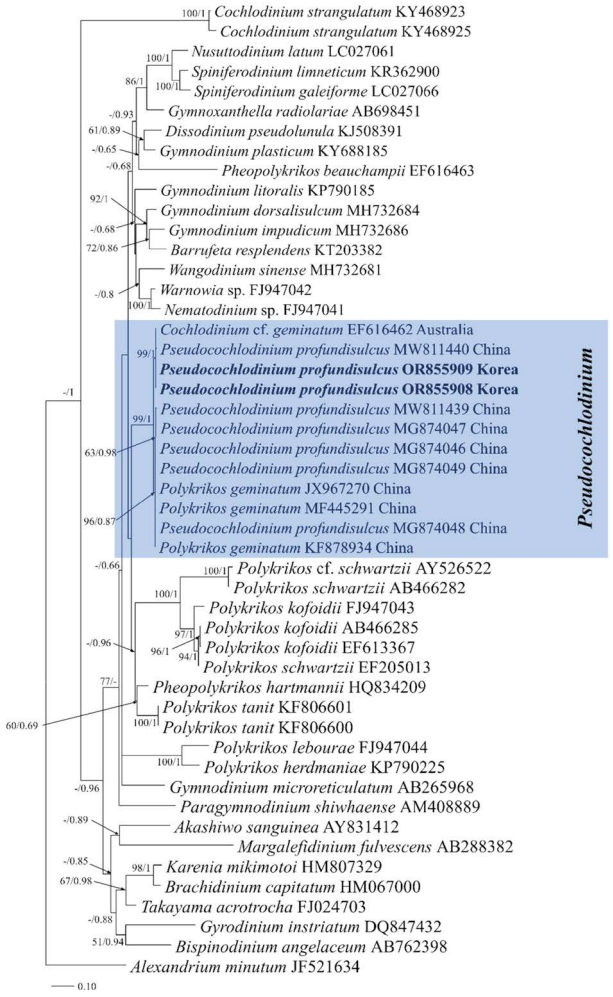

Fig. 4. Maximum likelihood (ML) tree showing the phylogenetic position of two *Pseudocochlodinium profundisulcus* germlings based on LSU rRNA gene sequences. *Alexandrium minutum* was used as the outgroup. The numbers on each node are bootstrap values (%), followed by the Bayesian posterior probability (PP). Only bootstrap values above 50% and a PP above 0.6 are shown. The GenBank accession number follows the taxon name. Scale bar = 0.1 nucleotide substitutions per site.

The molecular phylogeny indicates that the Korean and Chinese specimens can be conspecific. These specimens also formed a clade with an Australian specimen of *Cochlodinium* cf. *germinatum* (EF616462). Although clear morphological descriptions of Aus-

tralian specimen were not recorded, this specimen is probably conspecific with *P. profundisucus*. This clade is clearly distinct from the clade consisting isolates of *Polykrikos* species and *Ph. hartmanii*. This is supported by the morphological differences between those species. In particular, an open comma-shaped ASC and morphology of resting cyst are considered as key features to differentiate *P. profundisucus* from other allied species.

According to Hu *et al.* (2021), *P. profundisucus* has been regarded as harmful species and have a global distribution. Shang *et al.* (2022) suggested that *P. profundisucus* could be transported as resting cyst via ship's ballast water and sediments. This is widely regarded as a very important external cause for the dispersal of harmful species. In addition, Shin *et al.* (2019b, 2020) suggested that in Korean, Japanese and Chinese coastal areas the harmful species such as *Margalefidinium polykrikoides* and *Prorocentrum obtusidens* can be transported by Tsushima Current that is the branch of the Kuroshio Current (see Fig. 1 in Shin *et al.* 2019b). This may also apply to intrusion of *P. profundisucus* to Korean coastal area. Consequently, monitoring studies for *P. profundisucus* are necessary, although blooms or harmful effects caused by this species have not yet been recorded in Korean coastal area.

CRediT authorship contribution statement

JY Youn: Formal analysis, Data curation, Visualization, Writing-Original draft, Writing-Review & editing. **KH Han:** Investigation, Formal analysis, Visualization. **KY Kwak:** Formal analysis, Visualization. **HH Shin:** Conceptualization, Investigation, Formal analysis, Data curation, Writing-Review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

This work was supported by the management of Marine Fishery Bio-resources Center (2023) funded by the National Marine Biodiversity Institute of Korea (MABIK), the Korean Culture Collection of Microalgae and Collaboration Center (NRF-2022M3H9A1083416) and the KIOST project (PEA0121).

REFERENCES

Anderson DM, TJ Alpermann, AD Cembella, Y Collos, E Masseret and M Montresor. 2012. The globally distributed genus *Alexandrium*: Multifaceted roles in marine scosystems and impacts on human health. Harmful Algae 14:10–35. https://doi.org/10.1016/j.hal.2011.10.012

Guindon S, JF Dufayard, V Lefort, M Anisimova, W Hordijk and O Gascuel. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59:307–321. https://doi.org/10.1093/sysbio/syq010

Hu Z, N Xu, H Gu, Z Chai, K Takahashi, Z Li, Y Deng, M Iwataki, K Matsuoka and YZ Tang. 2021. Morpho-molecular description of a new HAB species, *Pseudocochlodinium profundisulcus* gen. et sp. nov., and its LSU rRNA gene based genetic diversity and geographical distribution. Harmful Algae 108:102098. https://doi.org/10.1016/j.hal.2021.102098

Li Z, MS Han, K Matsuoka, SY Kim and HH Shin. 2015. Identification of the resting cyst of *Cochlodinium polykrikoides* Margalef (Dinophyceae, Gymnodiniales) in Korean coastal sediments. J. Phycol. 51:204–210. https://doi.org/10.1111/jpy.12252

Matsuoka K and Y Fukuyo. 2000. Technical Guide for Modern Dinoflagellate Cyst Study. WESTPAC-HAB/WESTPAC/IOC. Asian Natural Environmental Science Center. Tokyo, Japan.

Orlova TY, TV Morozova, KE Gribble, DM Kulis and DM Anderson. 2004. Dinoflagellate cysts in recent marine sediments from the east coast of Russia. Bot. Marina 47:184–201. https://doi.org/10.1515/BOT.2004.019

Park JS. 2007. An environmental analysis of distribution of dinoflagellate cysts in the southwest sea of Korea and a taxonomic study through germination. Ph.D. dissertation. Chonnam National University. Gwangju, Korea.

Qi Y, L Ou, Y Li and S Lu. 2009. Taxonomy differentiation of harmful algal bloom causative species in genus *Cochlodinium* in Chinese coastal waters. pp. 15–18. In: Proceedings of the Fifteen Academic Conference and Celebration of 30-year Anniversary for Chinese Society of Phycology. Jinan, China.

Qiu D, L Huang, S Liu, H Zhang and S Lin. 2013. Apical groove type and molecular phylogeny suggests reclassification of *Cochlodinium geminatum* as *Polykrikos geminatum*. PLoS One 8:e71346. https://doi.org/10.1371/journal.pone.0071346

Ronquist F and JP Huelsenbeck. 2003. MrBayes 3: Bayesian

phylogenetic inference under mixed models. Bioinformatics 19:1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Shang L, X Zhai, W Tian, Y Liu, Y Han, Y Deng, Z Hu and YZ Tang. 2022. *Pseudocochodinium profundisulcus* resting cysts detected in the ballast tank sediment of ships arriving in the ports of China and north America and the implications in the species geographic distribution and possible invasion. Int. J. Environ. Res. Public Health 19:299. https://doi.org/10.3390/ijerph19010299

Shin HH, YH Yoon, YO Kim and K Matsuoka. 2011. Dinoflagellate cysts in surface sediments from southern coast of Korea. Estuaries Coasts 34:712–725. https://doi.org/10.1007/s12237-011-9373-y

Shin HH, Z Li, D Réveillon, GA Rovillon, KN Mertens, P Hess, HJ Kim, JH Lee, KW Lee, DK Kim, BS Park, JI Hwang, MH Seo and WA Lim. 2020. *Centrodinium punctatum* (Dinophyceae) produces significant levels of saxitoxin and related analogs. Harmful Algae 100:101923. https://doi.org/10.1016/j.hal.2020.101923

Shin HH, Z Li, KW Lee and K Matsuoka. 2019a. Molecular phylogeny and morphology of *Torquentidium* gen. et comb. nov. for *Cochlodinium convolutum* and allied species (Ceratoperidiniaceae, Dinophyceae). Eur. J. Phycol. 54:249–262. https://doi.org/10.1080/09670262.2018.1550213

Shin HH, Z Li, MH Seo, MY Soh, WA Lim and JW Park. 2019b. Harmful dinoflagellate *Prorocentrum donghaiense* Lu is widely distributed along the East China Sea and Korean coastal area. Ocean Sci. J. 54:685–691. https://doi.org/10.1007/s12601-019-0028-4

Takano Y and T Horiguchi. 2006. Acquiring scanning electron microscopical, light microscopical and multiple gene sequence data from a single dinoflagellate cell. J. Phycol. 42:251–256. https://doi.org/10.1111/j.1529-8817.2006.00177.x

Tang YZ and CJ Gobler. 2012. The toxic dinoflagellate *Cochlodinium polykrikoides* (Dinophyceae) produces resting cysts. Harmful Algae 20:71-80. https://doi.org/10.1016/j.hal.2012.08.001

Vaidya G, DJ Lohman and R Meier. 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics 27:171–180. https://doi.org/10.1111/j.1096-0031.2010.00329.x

Wang Z, S Jiang, YG Gu, W Kang, J Feng, L Lin, J Shao and K Zhang. 2011. Effects of *Cochlodinium bloom* in Pearl River Estuary in China on the growth of other harmful algal bloom species. J. Shenzhen Univ. Sci. Eng. 28:553–558.

Yamaguchi A and T Horiguchi. 2005. Molecular phylogenetic study of the heterotrophic dinoflagellate genus *Protoperidinium* (Dinophyceae) inferred from small subunit rRNA gene sequences. Phycol. Res. 53:30–42. https://doi.org/10.1111/j.1440-183.2005.00370.x