

New records of five taxa of unarmored and thin-walled dinoflagellates from brackish and coastal waters of Korea

Hoon Choi¹, Minji Cho¹ and Sunju Kim^{1,2,*}

¹Division of Earth Environmental System Science, Pukyong National University, Busan 48513, Republic of Korea

²Department of Oceanography, Pukyong National University, Busan 48513, Republic of Korea

*Corresponding author

Sunju Kim

Tel. 051-629-6577

E-mail. sunkim@pknu.ac.kr

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Abstract: Unarmored and thin-walled dinoflagellates were collected from brackish and coastal waters of Korea from August 2019 to August 2021. A total of 10 species belonging to orders Sussiales and Gymnodiniales were isolated and established as clonal cultures. Of them, five species (*Biecheleria brevisulcata*, *Lepidodinium chlorophorum*, *Karlodinium decipiens*, *Kirithra asteri*, and *Wangodinium sinense*) are newly recorded in Korea and examined using a light microscope (LM) and a scanning electron microscope (SEM). Their molecular phylogeny was inferred from LSU rDNA sequences. Here, we present taxonomic information, morphological features, and molecular phylogenetic positions of these unrecorded dinoflagellate species.

Keywords: dinoflagellates, first record, Gymnodiniales, LSU rDNA, Suessiales

INTRODUCTION

Dinoflagellates are biflagellate, unicellular or colony-forming eukaryotes and constitute one of the main groups of marine and freshwater protists. The extant dinoflagellates including Ellobiopsea, Oxyrrhea, Syndinea, and Dinokaryota comprise 2377 species belonging to 259 genera (Gómez 2012). About half of the dinoflagellates are photosynthetic species representing marine important primary producers, with some of them responsible for toxic blooms (Smayda 1997; Anderson *et al.* 2012). Most photosynthetic dinoflagellates possess peridinin-containing plastids derived from a red alga via secondary endosymbiosis. Many dinoflagellates, however, have lost the plastid, and several have replaced or supplemented the peridinin plastid with new secondary or tertiary plastids, or kleptoplasts (Hehenberger *et al.* 2019). The family Kareniaceae are one such lineage, with some members (the genera *Karenia*, *Karlodinium*, and *Takayama*) harboring stable, genetically integra-

ted tertiary plastids derived from haptophyte algae with secondary plastids (Tengs *et al.* 2000). The species belonging to the genus *Lepidodinium* possess green-colored plastids that originated from core Chlorophyte via serial secondary endosymbiosis (Archibald 2009).

Dinoflagellates have a complex cell covering called an amphiesma, composed of a series of membranes, flattened amphiesmal vesicles (= alveoli). According to the presence or absence of cellulosic plates in the amphiesmal vesicles, dinoflagellates are either armored (= thecate) or unarmored (= athecate or naked) species. Most unarmored dinoflagellates belong to the order Gymnodiniales Apstein, which comprise about 600 species (about 25% of the described dinoflagellate species), mainly belonging to the large genera *Amphidinium*, *Cochlodinium*, *Gyrodinium*, and *Gymnodinium* (Reñé *et al.* 2015). The genera of unarmored species have recently been examined in detail and the main genera have been redefined (Daugbjerg *et al.* 2000; Flø Jørgensen *et al.* 2004) and this is being followed by studies on the

Table 1. List of unarmored and thin-walled dinoflagellates isolated in this study

Species	Strains	Locality	Sampling dates	Temp (°C)	Sal	Accession no.
GYMNODINIALES						
Ceratoperidiniaceae						
<i>Kirithra asteri</i>	KaLomme05	Manseongri, Yeosu (34°46'27"N, 127°44'50"E)	Apr. 29. 2021	-	-	OL699926
<i>K. sigma</i>	KsLomme01	Yongho, Busan (35°08'00"N, 129°06'55"E)	Dec. 23. 2020	13.5	33.7	OL699927
Gymnodiniaceae						
<i>Lepidodinium chlorophorum</i>	LcLomme01	Yongho, Busan (35°08'00"N, 129°06'55"E)	Dec. 23. 2020	13.5	33.7	OL699924
<i>Wangodinium sinense</i>	WsLomme01	Myeongseon island, Ulsan (35°23'08"N, 129°20'56"E)	Oct. 14. 2020	-	-	OL699923
Gymnodiniaceae insert. Sed						
<i>Grammatodinium tongyeonginum</i>	GtLomme01	Yongho, Busan (35°07'49"N, 129°07'02"E)	Aug. 31. 2020	25.0	30.2	OL699929
Kareniaceae						
<i>Karodinium decipiens</i>	KdeLomme01	Jangsaengpo, Ulsan (35°30'18"N, 129°23'15"E)	Aug. 11.2021	28.1	29.8	OL699925
SUESSIALES						
Suessiaceae						
<i>Ansanella granifera</i>	AgLomme01	Yongho, Busan (35°07'49"N, 129°07'02"E)	Dec. 02. 2019	14.9	32.2	OL691544
<i>Biecheleria brevisulcata</i>	BrbLomme01	Yongho, Busan (35°08'00"N, 129°06'55"E)	Aug. 02. 2019	22.2	32.2	OL699922
<i>Biecheleriopsis adriatica</i>	BaLomme01	Yongho, Busan (35°07'49"N, 129°07'02"E)	Aug. 13. 2019	28.8	31.5	OL691545
<i>Pelagodinium bei</i>	PbLomme01	Yongho, Busan (35°08'00"N, 129°06'55"E)	Apr. 09. 2020	14.1	33.4	OL699921

thin-walled species, the woloszynskioids (Lindberg *et al.* 2005).

The order Suessiales, so-called woloszynskioids, are thin-walled dinoflagellates and characterized by amphiesmal vesicles of intermediate number between armored and unarmored dinoflagellates (Fensome *et al.* 1993). The plates are not arranged in a plate pattern consistent with the Kofoidian tabulation system used to describe the arrangement of plates in other thecate dinoflagellates. This group of dinoflagellates has been the subject of much taxonomic revision over the last decade. Later, a serial of papers demonstrated that Suessiales are polyphyletic and now distributed in three families: Tovelliaceae, Borghiellaceae, and Suessiaceae based on eyespot structure (Lindberg *et al.* 2005; Moestrup and Daugbjerg 2007; Moestrup *et al.* 2009).

In the present study, we isolated planktonic dinoflagellates from the brackish and coastal waters of Korea and

determined the morphological features and molecular phylogenetic relationships inferred from LSU rDNA sequences. Five taxa of unarmored and thin-walled dinoflagellates were newly recorded in Korea.

MATERIALS AND METHODS

1. Sampling and culture collection

Samples were collected from surface to bottom waters by vertically towing using a 20- μ m mesh plankton net in brackish and coastal waters of Korea from August 2019 to August 2021 (Table 1). Water temperature and salinity were measured with a YSI instrument (YSI Inc., OH, USA). Net samples were stored at 20°C until microscopic observation. Dinoflagellate cells were isolated using a capillary micropipette under an inverted microscope (Axio Vert. A1;

Zeiss, Hallbergmoos, Germany), washed through serial drops of 0.2 μm filtered-seawater and transferred to a 96-well plate filled with the filtered seawater. The plate was incubated at 20°C under a photon flux density of 100–120 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on a 14 : 10 light-dark cycle. Following culturing of the isolated cells, clonal cultures were subsequently scaled up to 30 mL in F/2-Si medium and placed at the same culture condition as described above (Guillard and Ryther 1962). The clonal cultures were maintained by bi-weekly transfer to a fresh medium.

2. Light and electron microscopy

Cells fixed with non-acid Lugol solution (1% final concentration) were observed using an Axio Imager A2 (Zeiss) light microscope, equipped with epifluorescence and differential interference contrast optics. Light micrographs were obtained at $\times 1000$ magnification using an AxioCam HRC (Zeiss) photomicrographic system equipped with the microscope. Live cells were examined to determine the shape and location of nuclei after staining with 4'-6-diamidino-2-phenylindole (DAPI: 0.1 $\mu\text{g mL}^{-1}$ final concentration) under an epifluorescence microscope with ultraviolet light (excitation of 360 nm and emission of 460 nm).

For Scanning Electron Microscopy (SEM) examination, specimens of each clonal culture strain were prepared according to Choi and Kim (2021) and then examined using SEM (MIRA 3 LMH In-Beam Detector; TESCAN, Czech Republic).

3. DNA extraction, PCR, Sequencing

Genomic DNA was extracted using a Chelex extraction method (Kim and Park 2014) and the purity and quantity were determined using a NanoDrop ND-1000 system (Thermo Fisher Scientific, Waltham, MA, USA). PCR amplification was performed using the primers D1R-D3B (Nunn *et al.* 1996) for the LSU rRNA gene with commercially available PCR premix (Accu-Power PCR PreMix; BIONEER, Daejeon, Korea). The thermocycler program was as follows: initial 95°C for 3 min, followed by 39 cycles of denaturation at 95°C for 45 s, annealing at 52°C for 45 s, and extension at 72°C for 1 min, with a final extension at 72°C for 7 min. Amplification products were visualized on EcoDyeTM (SoleGent Co., Daejeon, Korea)-stained 1% agarose gels and purified using the ExoSAP-ITTM Express according to the manufacturer's instructions. Sequencing was carried out using an ABI model 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA), with the same

primers used for PCR in conjunction with a Big-Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems). ContigExpress (Vector NTI v. 10.1; Invitrogen, Grand Island, NY) was used to edit out low quality regions and to assemble the both end reads and the complete sequences were deposited in GenBank (Table 1).

4. Alignments and phylogenetic analyses

A total of 56 LSU rRNA gene sequences of dinoflagellates were retrieved from GenBank and aligned with our Korean strains using MAFFT v7.10 (Katoh and Standley 2013; Katoh *et al.* 2019). Unambiguously aligned region (1158 positions) was applied for further phylogenetic analyses. The syndinean dinoflagellates, *Syndinium turbo* Chatton and *Hematodinium perezii* Chatton & Poisson, were used as an outgroup. Maximum likelihood analysis was performed with RAxML 8.0 (Stamatakis 2014) using the GTRGAMMA evolution model and rapid bootstrapping of 2,000 replicates.

RESULTS AND DISCUSSION

1. Taxonomic summary

The unarmored and thin-walled dinoflagellates *Biechleria brevisulcata*, *Karlodinium decipiens*, *Kirithra asteri*, *Lepidodinium chlorophorum*, and *Wangodinium sinense* were newly recorded in brackish and coastal waters of Korea. We described the taxonomic information and provided the molecular phylogenetic relationships inferred from LSU rDNA sequences.

Class Dinophyceae Fritsch
Order Gymnodiniales Apstein
Family Ceratoperidiniaceae Loeblich III
Genus *Kirithra* Boutrup, Tillmann, Daugbjerg & Moestrup
K. asteri Boutrup, Tillmann, Daugbjerg & Moestrup

Family Gymnodiniaceae Lankester
Genus *Lepidodinium* Watanabe, Suda, Inouye, Sawaguchi & Chihara
L. chlorophorum (M.Elbrächter & E.Schnepf) Gert Hansen, Botes & Salas

Genus *Wangodinium* Z.Luo, Zhangxi Hu, Yingzhong Tang & H.F.Gu
W. sinense Z.Luo, Zhangxi Hu, Yingzhong Tang & H.F.Gu

Family Kareniaceae Bergholtz, Daugbjerg, Moestrup & Fernández-Tejedor

Genus *Karlodinium* J.Larsen

K. decipiens Salas & Laza-Martinez

Order Suessiales Fensome, F.J.R.Taylor, G.Norris, Sarjeant, D.I.Wharton, & G.L.Williams

Family Suessiaceae Fensome, F.J.R.Taylor, G.Norris, Sarjeant, D.I.Wharton, & G.L.Williams

Genus *Biecheleria* Moestrup, K.Lindberg & Daugbjerg

B. brevisulcata K.Takahashi & Iwataki

***Kirithra asteri* Boutrup, Tillmann, Daugbjerg & Moestrup (Fig. 1A–E)**

Reference: Boutrup *et al.* 2017 (Fig. 1)

Specimen examined. Table 1.

Cells are 21.7–38.4 μm long (average 30.4 ± 0.6 ; $n = 30$) and 15.6–26.38 μm wide (average 21.9 ± 0.4 ; $n = 30$) and ovoid in shape with a conical epicone and hemispherical hypocone. The cingulum is descended with a displacement of about one to two cingulum widths. The sulcus widens posteriorly and almost reaches the antapex. Apical structure complex formed a complete circle. Within the circular apical structure complex is a central amphiesmal vesicle encircled by approximately eight amphiesmal vesicles. The nucleus is spherical and located in the hypocone. Chloroplasts are reticulated, radiating from 1–3 centrally located pyrenoids.

Distribution. Argentinian ocean shelf in Argentina (Hansen *et al.* 2007).

Site of collection. Specimens were collected from Marseongri of Yeosu, Republic of Korea ($34^{\circ}46'27''\text{N}$, $127^{\circ}44'50''\text{E}$) on April 29, 2021.

Voucher slide. NNIBRPR20167–NNIBRPR20168

***Lepidodinium chlorophorum* (M.Elbrächter & E.Schnepf) Gert Hansen, Botes & Salas (Fig. 1F–J)**

Basionym: *Gymnodinium chlorophorum* Elbrächter & Schnepf

Reference: Elbrächter and Schnepf 1996 (Figs. 5 and 6)

Specimen examined. Table 1.

Cells are 20.3–32.94 μm long (average 26.4 ± 0.5 ; $n = 30$) and 14.2–22.3 μm wide (average 18.0 ± 0.4 ; $n = 30$) and subglobular to ovoid in shape, dorsoventrally flattened. The cingulum is displaced by approximately one cingulum width. The sulcus is deeply excavated and extends onto the

episome. The nucleus is spherical and located in the epicone. Chloroplast is reticulated and bright green in color.

Distribution. Germany (Scholz and Liebezeit 2012), Netherlands (Veen *et al.* 2015), Gulf of California (Gárate-Lizárraga *et al.* 2014), Australia (McCarthy 2013), New Zealand (Rhodes *et al.* 2019).

Site of collection. Specimens were collected from Yongho Bay of Busan, Republic of Korea ($35^{\circ}08'00''\text{N}$, $129^{\circ}06'55''\text{E}$) on December 23, 2020.

Voucher slide. NNIBRPR20729–NNIBRPR20730

***Wangodinium sinense* Z.Luo, Zhangxi Hu, Yingzhong Tang & H.F.Gu (Fig. 1K–O)**

Reference: Luo *et al.* 2018 (Fig. 1A–D)

Specimen examined. Table 1.

Cells are 13.2–18.71 μm long (average 15.0 ± 0.3 ; $n = 30$) and 9.5–13.0 μm wide (average 11.1 ± 0.2 ; $n = 30$) and ovoid in shape. The epicone is rounded to conical and almost equal in size to the hypocone. The cingulum is wide and descended with a displacement of approximately two cingulum widths. The sulcus is deeply depressed and extends to near the antapex. The nucleus is spherical and located in the anterior side of the cell. Chloroplasts are located peripherally, green to brownish in color. A pyrenoid is consistently located in the hypocone.

Distribution. Xiamen Harbor, Lianyungang, and Beihai in China coastal sea (Luo *et al.* 2018).

Site of collection. Specimens were collected from Myeongseon island of Ulsan, Republic of Korea ($35^{\circ}23'08''\text{N}$, $129^{\circ}20'56''\text{E}$) on December 31, 2020.

Voucher slide. NNIBRPR20777–NNIBRPR20778

***Karlodinium decipiens* Salas & Laza-Martinez (Fig. 1P–T)**

Reference: de Salas *et al.* 2008 (Figs. 8A–C, and 9A)

Specimen examined. Table 1.

Cells are 14.1–29.8 μm long (average 21.9 ± 0.5 ; $n = 30$) and 14.0–21.6 μm wide (average 16.9 ± 0.3 ; $n = 30$), ellipsoidal in shape, dorsoventrally flattened. A straight apical groove extends to halfway down the dorsal epicone. The cingulum is wide and displaced by 1/3 of the cell length. The sulcus is narrow with a clearly visible tube-shaped structure in the intercingular region. The nucleus is large and located centrally in the cell, closer to the dorsal surface. Chloroplasts arranged into bands in a spiral in the epicone.

Distribution. Spain, Southern Ocean, and Tasmania (de

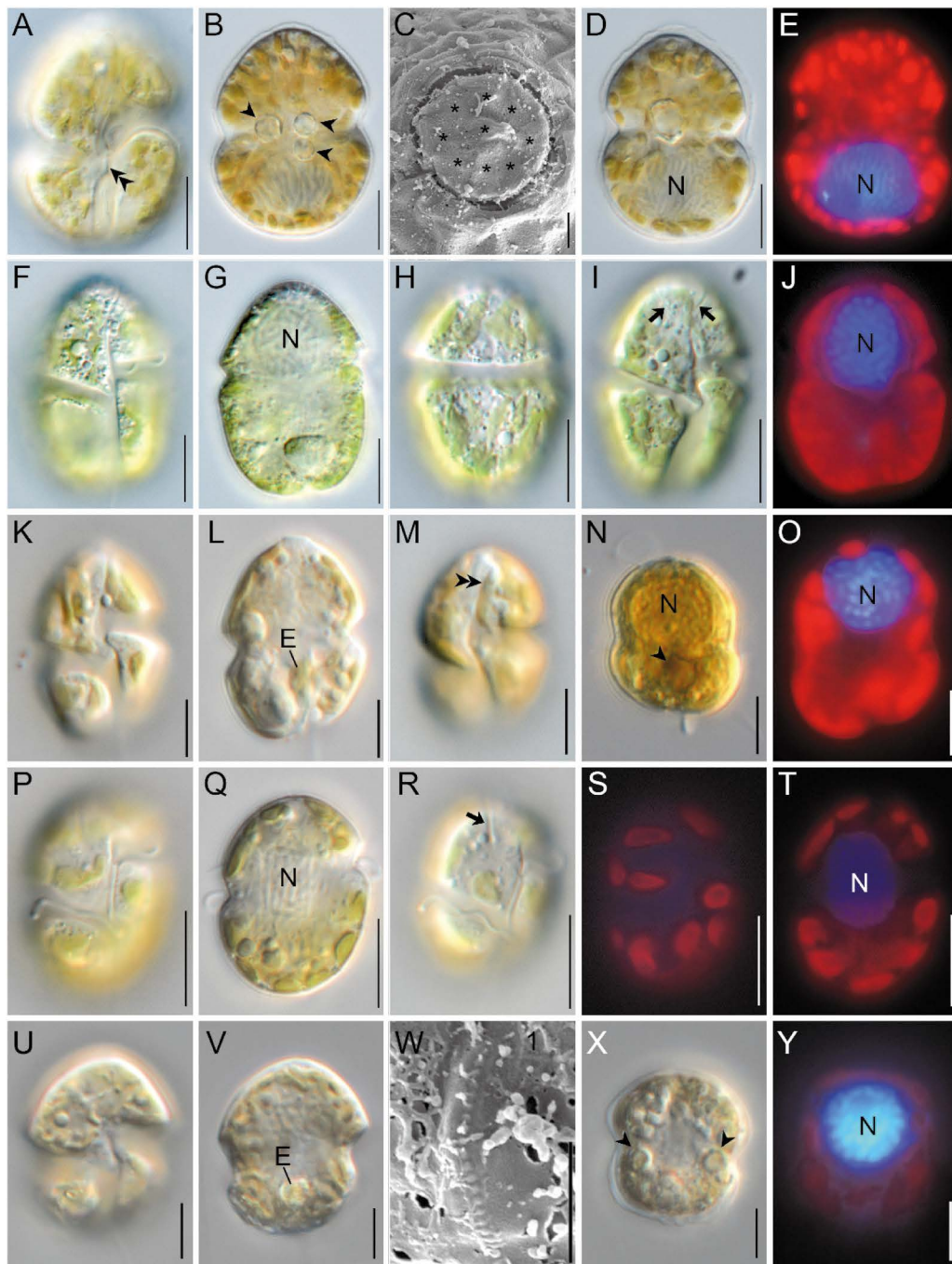


Fig. 1. Light and scanning electron micrographs of newly recorded unarmored dinoflagellates. (A–E) *Kirithra asteri*. (F–J) *Lepidodinium chlorophorum*. (K–O) *Wangodinium sinense*. (P–T) *Karlodinium decipiens*. (U–Y) *Biecheleria brevisulcata*. Ventral ridge with a delicate furrow (double arrowhead); Central amphiesmal vesicle encircled by approximately eight amphiesmal vesicles (asterisks); Cells showing deep sulcal intrusion into the epicone (open arrow); Nucleus (N); Eyespot (E); Pyrenoids (arrowhead); Apical groove (arrow). Scale bar, 10 μm in (A–J), 5 μm in (K–Y), and 1 μm in (C, W).

Salas *et al.* 2008), Japan (Benico *et al.* 2020), Australia (McCarthy 2013).

Site of collection. Specimens were collected from Jang-

saengpo of Ulsan, Republic of Korea (35°30'18"N, 129°23'15"E) on August 11, 2021.

Voucher slide. NNIBRPR21229–NNIBRPR21230

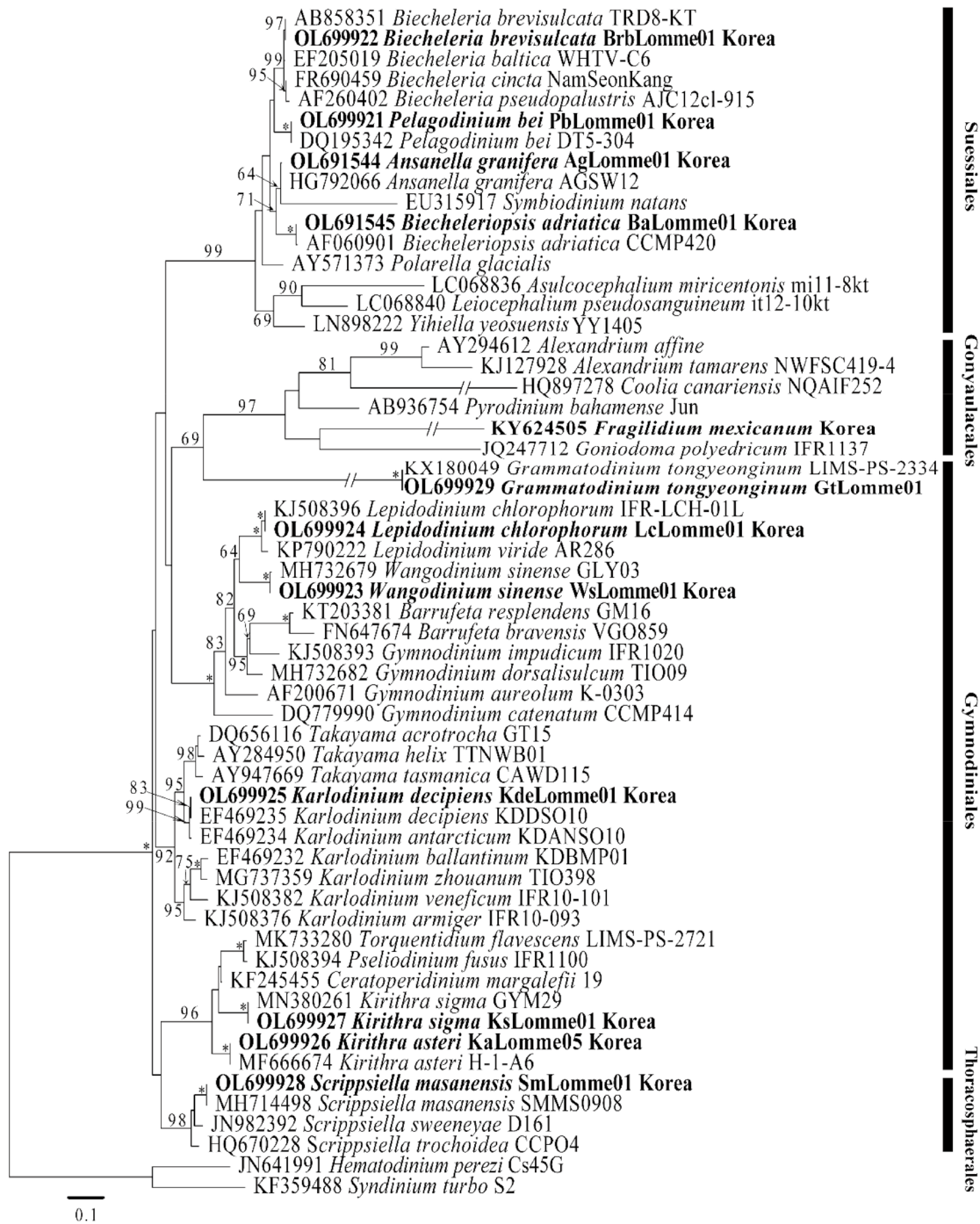


Fig. 2. Phylogenetic tree inferred from LSU rRNA gene sequences (1158 bp). Numbers above nodes represent ML bootstrap supports higher than 60%. Asterisk (*) indicates robust bootstrap supports (100%). Sequences obtained in this study are marked in bold.

***Biecheleria brevisulcata* K.Takahashi & Iwataki**
(Fig. 1U–Y)

Reference: Takahashi *et al.* 2014 (Figs. 1–3, 7 and 13)

Specimen examined. Table 1.

Cells are 11.2–15.2 μm long (average 13.0 ± 0.2 ; $n = 30$) and 9.7–13.9 μm wide (average 11.9 ± 0.2 ; $n = 30$) and spherical to ellipsoidal in shape. The epicone is wider than

the hypocone. The cingulum is median or slightly lower and displaced by one and half of cingulum widths. The sulcus is deep and widens towards the posterior end. About 20 globular knobs are present on the elongate apical vesicle. The spherical nucleus occupies most of the epicone. Chloroplasts are located peripherally, yellow-brown in color. Pyrenoids are surrounded by starch sheaths and two to five in number. An eyespot is present near the sulcus.

Distribution. Nagasaki (Takahashi *et al.* 2014) and Yamagata (Takahashi *et al.* 2015) in Japan.

Site of collection. Specimens were collected from Yongho Bay of Busan, Republic of Korea (35°08'00"N, 129°06'55"E) on August 02, 2019.

Voucher slide. NNIBRPR20817–NNIBRPR20818

2. Molecular phylogeny

All newly recorded unarmored species, *Lepidodinium chlorophorum*, *Wangodinium sinense*, *Karlodinium decipiens*, and *Kirithra asteri* in the order Gymnodiniales did not form a monophyletic group in LSU phylogeny. The Korean *L. chlorophorum* strain was identical with the strain (IFR-LCH-01L) from France (KJ508396) and closely related to *L. viride* (strain AR286) from Spain (KP790222). The Korean *W. sinense* strain was identical to that of the strain GLY03 from China (MH732679) and branched as a sister lineage of *Lepidodinium* species with a moderate bootstrap support (64%). The sequences of *K. decipiens* from Korea and the Southern Ocean tightly clustered with *K. antarcticum* with a high bootstrap support (99%) and formed a sister clade to other *Karlodinium* species. The newly recorded *K. asteri* from Korea was identical with the strain H-1-A6 from Argentina (MF666674). In LSU phylogeny, the unarmored dinoflagellate *Grammatodinium tongyeonginum* formed a long branch and distantly related to the other genera of Gymnodiniales in the LSU phylogeny, while *G. tongyeonginum* was closely related to *Cucumeridinium* species belonging to the order Gymnodiniales in SSU phylogeny (Li *et al.* 2017).

All sequences of thin-walled suessiaceae species including *Ansanella granifera* (AgLomme01), *Biecheleria brevisulcata* (BrbLomme01), *Biecheleriopsis adriatica* (BaLomme01), and *Pelagodinium bei* (PbLomme01) obtained in this study, clustered together, forming a monophyletic group with a high bootstrap support (99%). Sequences of *Biecheleria* species tightly clustered in a clade. The newly recorded *B. brevisulcata* from Korea was identical with the strain (TRD8-KT) from Japan (AB858351).

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