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Morphology, molecular phylogeny, and pigment characterization of an isolate of the dinoflagellate *Pelagodinium bei* from Korean waters

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The dinoflagellate genus *Pelagodinium* is genetically classified in distinct sub-clades and subgroups. However, it is difficult to determine whether this genetic diversity represents intra- or interspecific divergence within the genus since only the morphology of the type strain of the genus *Pelagodinium*, *Pelagodinium bei*, is available. An isolate associated with the genus *Pelagodinium* from Shiwha Bay, Korea, was recently cultured. This isolate was clustered with 3 to 4 strains from the Atlantic Ocean, Mediterranean Sea, and Indian Ocean. This cluster was distinct from the subgroup more closely associated with *P. bei*. The morphology of the isolate was analyzed using optical and scanning electron microscopy and was almost identical to that of *P. bei* except that this isolate had two series of amphiesmal vesicles (AVs) in the cingulum, unlike *P. bei* that has one series. When the pigment compositions of the isolate and *P. bei* were analyzed using high-performance liquid chromatography, these two strains had peridinin as a major accessory pigment and their pigment compositions were almost identical. In addition, the swimming behaviors of these two strains were very similar. The reexamination of the type culture of *P. bei* revealed two series in the cingulum as for the isolate. The new findings on the number of series of AVs in the cingulum, the pigment composition, and the swimming behaviors suggest that *P. bei* and the isolate are conspecific despite their genetic divergence. This study provides a basis to further understand the molecular classification within *Pelagodinium* combining genetic, morphological, pigment, and behavioral data.

Key Words: foraminifera; *Gymnodinium bei*; pelagic symbiont; Suessiaceae; Suessiales

INTRODUCTION

Marine dinoflagellates are ubiquitous and play diverse roles in marine ecosystems (Jeong et al. 2010, 2012, 2015). The dinoflagellate *Gymnodinium bei* Spero was a symbiont of the foraminifer *Orbulina universa* D'Orbigny (Spero 1987). While basic features such as the shape, dimensions, presence of an hypoconal flange and stalked pyrenoids penetrated by thylakoid lamellae, absence of trichocysts, and swimming behavior were recognized, detailed observations of the arrangement of the amphiesmal vesicles (AVs) was lacking (Spero 1987). Thereafter, various dinoflagellate symbionts of planktonic foraminifera sampled worldwide were characterized genetically and referred as "*G. bei*" (Gast and Caron 1996, Shaked and De Vargas 2006). However, these results suggested that "*G. bei*" was more closely related to the order Suessiales. Subsequently, a culture of a dinoflagellate isolated from

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Orbulina universa, collected off the coast of Puerto Rico in the Caribbean Sea, was established (Siano et al. 2010). Similar features to *G. bei* were observed on the collected isolate, suggesting that the cultured dinoflagellate was *G. bei* (Spero 1987). The morphological characterization of the dinoflagellate revealed various features. It had a single elongated apical vesicle (EAV) with a row of small knobs, a feature not present in the genus *Gymnodinium*. The presence of a type E extraplastidial eyespot, the arrangement of the AVs in series, and the absence of trichocyst confirmed its affiliation with other symbiotic dinoflagellates and certain genetically related non-symbiotic genera from Suessiales. The arrangement of the series of vesicles of the analyzed strain was unique within the Suessiales, and the pyrenoid ultrastructure was different from other symbiotic dinoflagellates. Furthermore, phylogenetic analysis based on the large subunit ribosomal RNA gene (referred to as LSU rDNA) established that *G. bei* clusters in an independent, well-supported clade within Suessiales with other sequences of symbiotic dinoflagellates extracted from planktonic foraminifera (Shaked and De Vargas 2006, Siano et al. 2010). This led to the reclassification of *G. bei* and its combination within the newly erected genus *Pelagodinium* Siano, Montresor, Probert et De Vargas as *Pelagodinium bei* (Spero) Siano, Montresor, Probert et De Vargas.

Extraction from pooled symbionts, combined host and symbionts, culture of symbionts established after microdissection, and bulk microplanktonic communities, followed by amplification or cloning, and sequencing have increased our knowledge on the distribution of symbionts associated with foraminifera around the world (Gast and Caron 1996, Shaked and De Vargas 2006, Siano et al. 2010, Decelle et al. 2012, Kok et al. 2014, De Vargas et al. 2015). The distribution of *Pelagodinium* is mainly based on molecular data. It has been reported in various areas of the Atlantic Ocean (Shaked and De Vargas 2006, Siano et al. 2010, De Vargas et al. 2015), Indian Ocean (Shaked and De Vargas 2006, De Vargas et al. 2015), western North Pacific Ocean (Fujiki et al. 2014, Kok et al. 2014), South Pacific Ocean (Shaked and De Vargas 2006, De Vargas et al. 2015), and Mediterranean Sea (Shaked and De Vargas 2006, Decelle et al. 2012, De Vargas et al. 2015).

In the phylogenetic analysis, the type sequence of *P. bei* clustered in clade P1, sister of clade P2, as defined by Shaked and De Vargas (2006) based on LSU rDNA (Siano et al. 2010). There is an important genetic diversity between and within clades P1 and P2 (Shaked and De Vargas 2006). As stated by Siano et al. (2010), only sequences identical to the type sequence should be attributed to *P. bei*. All other sequences should be designated as *Pelagodinium* sp. awaiting further characterization to verify their actual taxonomic status.

The increase of studies assessing the diversity and distribution in the field using molecular tools makes the detailed characterization of strains associated to the various sequences and their comparison with the type species particularly relevant. However, details of the sulcal area, the internal transcribed spacers and 5.8S ribosomal RNA gene (referred to as ITS rDNA), and the pigment composition of the type species *P. bei* were not assessed, which impaired the comparison between strains.

We recently established a clonal culture of a small dinoflagellate able to grow photosynthetically from Shiwha Bay, Korea, that was related to the genus *Pelagodinium*. The sequence of this strain was unreported. In this study, we describe the morphological and genetic features, and the swimming behavior of this isolate using optical and scanning electron microscopy (SEM), and phylogenetic analyses based on ITS and LSU rDNA. We also determined the pigment composition using high-performance liquid chromatography (HPLC). To clearly determine the identity of the isolate, we also reinvestigated the type culture of *P. bei* to complement the data previously available (Siano et al. 2010). This study provides a basis to further understand the molecular classification within the genus *Pelagodinium*.

MATERIALS AND METHODS

Isolation

The culture of the isolate from Korea was established from surface sediment samples collected on September 30, 2010 (depth, 11.5 m; surface temperature, 19.6°C; surface salinity, 11.7) from Shiwha Bay, Korea (37°18′ N, 126°36′ E). The surface sediment was collected from an Eckman grab (WILDCO; Wildlife Supply Company, Buffalo, NY, USA) and stored in the dark at 4°C until further analyses. To concentrate potentially viable cells, between 1 and 2 cm^3 of sediment were sieved through 100-μm and 15-μm Nytex meshes with filtered seawater. The 15-100 μm fraction was then transferred to a 100-mL beaker with filtered seawater. A manual vortex was applied and the suspended fraction was recovered. The remnant fraction was incubated in F/2-Si culture medium (Guillard and Ryther 1962) with a salinity of 32 at 20°C under a light-dark cycle 14 : 10 at a photon flux of 20 μmol m^{-2} s^{-1}. A cell swimming in the medium was isolated by micromanipu-

lation and a monoclonal culture was established after two serial single-cell isolations.

Type culture of *Pelagodinium bei*

The culture that was used to establish the holotype of the type species *P. bei* was obtained from the Roscoff Culture Collection (designation RCC #1491). The type culture was used to determine details not previously assessed for *P. bei* such as the morphology of the sulcal area based on SEM, the ITS rDNA, and the pigment composition based on HPLC analyses.

Optical microscopy

Cells were observed using a transmitted light inverted microscope (Zeiss Axiovert 200M; Carl Zeiss Ltd., Göttingen, Germany) at magnifications of ×50-1,000 to determine the general morphology and behavior. The measurements were determined with a Zeiss AxioCam MRc5 digital camera (Carl Zeiss Ltd.).

Scanning electron microscopy

For SEM, cells were fixed with 0.5-1% osmium tetroxide and rinsed in a dilution of 1 : 1 filtered seawater: distilled water. The cells were then washed in distilled water only. Cells were then subjected to a dehydration series in ethanol (10, 30, 50, 70, 90, and 100%). The cells were dried using a critical point dryer (CPD 030; BAL-TEC, Balzers, Liechtenstein). Finally, the cells were mounted on stubs, sputter coated with gold-palladium (SCD 005; BAL-TEC), and observed with an FE-scanning electron microscope (AURIGA; Carl Zeiss Ltd.).

Molecular characterization

For genetic analyses, a culture growing in the conditions described above was filtered through a polycarbonate membrane (25 mm, 3-μm pore size Whatman Nucleopore Track-Etch; GE healthcare, Buckinghamshire, UK) and resuspended by vortexing in distilled water in a 1.5-mL microtube (Scientific Specialties Inc., Lodi, CA, USA). The sample was subsequently centrifuged (WiseSpin CF-10 Microcentrifuge; DAIHAN Scientific Co., Ltd., Namyangju, Korea) at 7,500 ×g for 5 min at room temperature. The cells were immediately subjected to total DNA extraction using the AccuPrep Genomic DNA extraction kit (Bioneer Corp., Daejeon, Korea) according to the manufacturer's instructions.

Amplicons of the ITS and LSU rDNA were obtained. The polymerase chain reaction (PCR) final mix concentrations were as follows: 1× PCR f-Taq buffer (fTaq DNA polymerase; SolGent Co., Ltd., Daejeon, Korea), 0.2 mM of dNTP (fTaq DNA Polymerase; SolGent Co., Ltd.), 0.4 μM of each primer, 0.025 U μL^{-1} of f-Taq DNA polymerase (fTaq DNA polymerase; SolGent Co., Ltd.), and 1.5 mM of $MgCl_2$. A volume of 1.0 μL of the DNA extraction was used as template with a final reaction volume of 50 μL. The amplicons were obtained with the primer pairs ITSF2 and ITSR2 and 5.8 SF and LSUB (Litaker et al. 2003) with 54 and 50°C as annealing temperatures (AT), respectively. PCRs were conducted using a thermal cycler (Mastercycler ep, model 5341; Eppendorf AG, Hamburg, Germany) as follows: one activation step at 95°C for 2 min, followed by 35 cycles at 95°C for 20 s, AT for 40 s, 72°C for 1 min, and a final elongation step at 72°C for 5 min.

Positive and negative controls were used for all amplification reactions. The size of the amplicons was verified on a 1.0% agarose gel. The products were visualized under a UV lamp. The PCR products were purified using the AccuPrep PCR purification kit (Bioneer Corp.) according to the manufacturer's instructions. The purified PCR products were sent to the Genome Research Facility (School of Biological Science, Seoul National University, Korea) where they were sequenced on an ABI PRISM 3700 DNA Analyzer (Applied Biosystems, Foster City, CA, USA) with the primers used in the PCR.

The sequences of taxa used to construct the phylogenies were obtained from NCBI GenBank. The sequence of the isolate from Korea (strain HJ-2010), and the ITS rDNA sequence of the type species *P. bei* were deposited in GenBank (accession Nos. KP342301 and KP843723, respectively). Our new and reference sequences were aligned using CLUSTAL X v2.0 (Larkin et al. 2007). The alignments were inspected and refined manually using BioEdit v7.0.9.0 (Hall 1999). The aligned matrixes were then analyzed with PAUP v4.0b10 (Swofford 2002). Maximum likelihood was used as a phylogenetic method. The models of nucleotide substitution were determined with Modeltest v7.3 (Posada and Crandall 1998) based on the Akaike information criteria (Posada and Buckley 2004). A heuristic tree search was used to determine the optimal trees. The tree bisection-reconnection algorithm was used with 5 random additions of sequences. The characters were equally weighted and spaces in the alignment were treated as missing data. RAxML v7.0.4 (Stamatakis 2006) was used to calculate bootstrap values using the default algorithm with the general time reversible + Γ model of nucleotide substitution and 1,000 replicates.

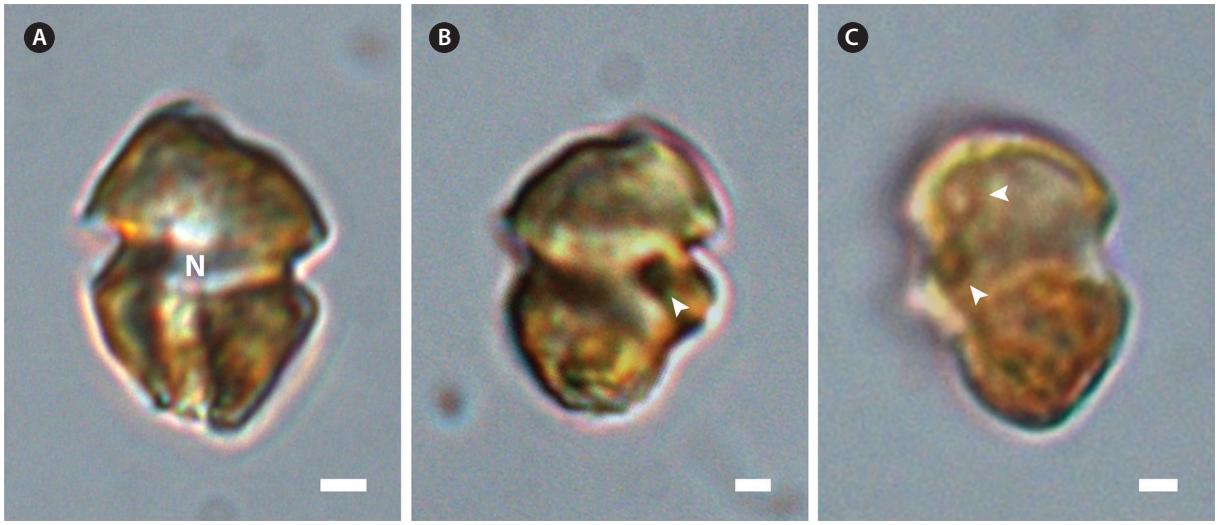

Fig. 1. Micrographs of the isolate taken using optical microscopy. (A) Ventral view illustrating the round to elliptical nucleus (N). (B) Side view. The arrowhead indicates the orange to red eyespot located in the sulcal area. (C) Side view. The arrowheads indicate pyrenoids. Scale bars represent: A-C, 1 μm.

The matrixes were also analyzed with MrBayes v3.2.3 (Ronquist and Huelsenbeck 2003) for Bayesian analyses. The models previously selected by Modeltest 7.3 were used. Four independent Markov chain Monte Carlo simulations were run simultaneously for 2,000,000 generations. Trees were sampled every 1,000 generations and the first 800 trees were deleted to ensure that the likelihood had reached convergence. A majority-rule consensus tree was created from the remaining 1,201 trees to examine the posterior probabilities of each clade.

Pigment composition

The pigments were analyzed using HPLC (LC-10A system; Shimadzu Co., Kyoto, Japan) as in Zapata et al. (2000). A volume of culture containing 2,000,000 cells growing in the conditions mentioned previously was used in the analysis. The culture was filtered through a 1.2 μm pore-sized GF/C filter. Three milliliters of 95% methanol were used for extraction and a Waters C8 column (150 × 4.6 mm, 3.5-μm particle size, 0.01-μm pore size; Waters Corporation, Milford, MA, USA) for separation. Pigments were identified by retention times and absorption spectra identical to those of authentic standards, and quantified against standards purchased from DHI Water & Environment (Hørsholm, Denmark).

RESULTS

Morphological characterization

The ranges (and mean ± standard deviation) of cell length and cell width of living cells from the Korean isolate were 7.9-14.1 μm (11.1 ± 1.3, n = 100) and 5.1-10.8 μm (8.3 ± 1.1, n = 100), respectively. The chloroplasts were golden-yellow (Fig. 1). The epicone and hypocone were similar in size (Fig. 1A & B). The epicone was typically round to elliptical, while the hypocone was either round or slightly asymmetrical in ventral view (Fig. 1). An eyespot was present (Fig. 1B). Pyrenoids were occasionally visible with light microscopy (Fig. 1C).

The cell surface of the isolate from Korea was ornamented with globular knobs and pores that were dispersed randomly (Fig. 2). A hypoconal flange was present, but not clearly expressed in all observed individuals (Fig. 2A). A single EAV ornamented with a row of globular knobs (Fig. 2B & C) was surrounded by a series of 3-4 quadrangular vesicles and a small squared vesicle (X vesicle) forming the apicals (series 1) (Fig. 2B & C). However, the apicals were constituted of 4 quadrangular vesicles only once. Another series of vesicles was posterior to the apicals and followed by an anterior intercalary and precingular series that were constituted of 5-8 (series 2), 0-3 (series 3), and 6-8 vesicles (series 4), respectively (Fig. 2D-I). The cingulum was wide, shallow and distinct, located in the median portion of the cell, descending,

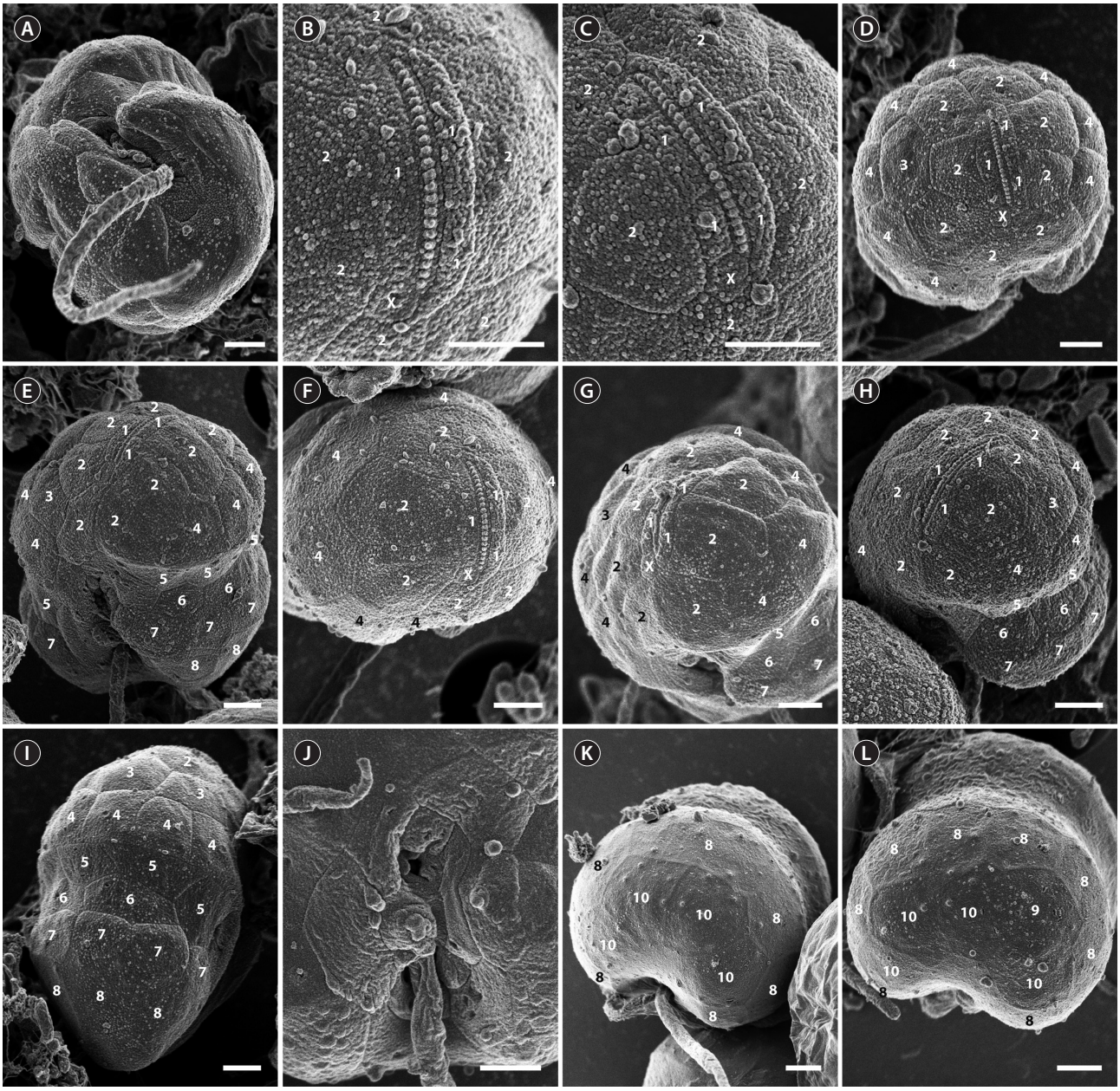

Fig. 2. Micrographs of the isolate taken using scanning electron microscopy. (A) Hypoconal flange. (B & C) Apical furrow. (D-G) Apical and ventral views. (H) Left view. (I) Right view. (J) Sulcal view. (K & L) Antapical views. X, small squared vesicle. The amphiesmal vesicles were numbered and assigned to their respective series. Scale bars represent: A-L, 1 μm.

and displaced by approximately its own width (Fig. 2E). The cingulum contained two series of AVs (series 5 and 6) (Fig. 2E & I). The number of series in the cingulum was reduced to one when approaching the sulcus, particularly on the right side (Fig. 2E & I). While the sulcus could be deep and narrow, it was in most cases shallow and wide enabling most of the AVs that constituted it to be seen (Fig. 2J). The sulcus contained 13 AVs (Fig. 2J). The hypocone was composed of a series of postcingular small vesicles (series 7) (Fig. 2E & G-I), anterior to another series of 6-8 hypoconal vesicles (series 8) (Fig. 2K & L), and 3-4 antapical vesicles (series 10) (Fig. 2K & L). An intercalary vesicle was sometimes observed preceding the antapicals (series 9) (Fig. 2L).

The SEM fixation of the type culture of *P. bei* from the Caribbean Sea revealed two series of AVs in the cingulum

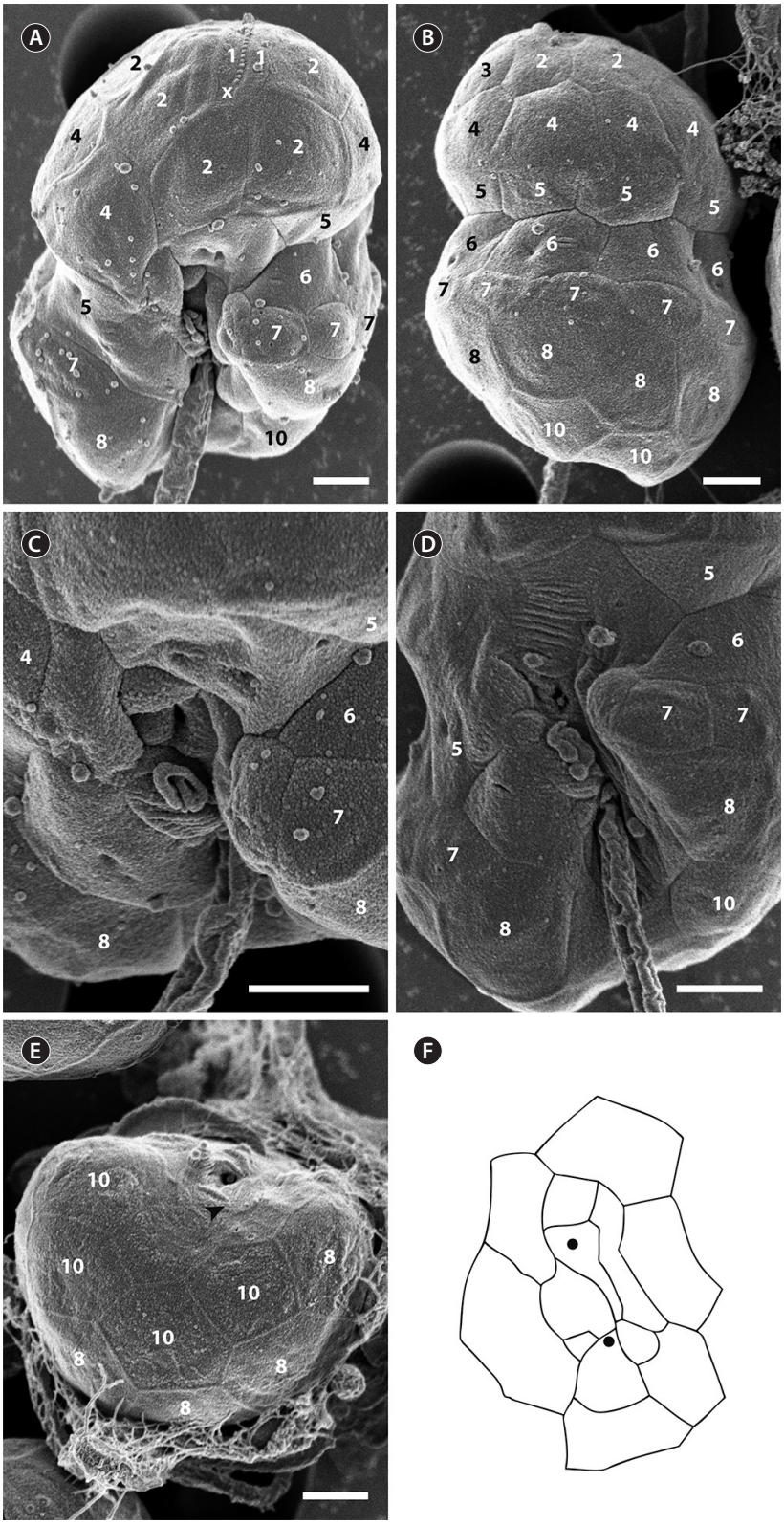

Fig. 3. Micrographs of the type culture of *Pelagodinium bei* (RCC #1491) taken using scanning electron microscopy and a schematized view of the sulcal area. (A) Ventral view. (B) Dorsal view. (C-E) Sulcal views. The arrowhead indicates a small amphiesmal vesicle located at the left side of the longitudinal flagellar pore. (F) Drawing of the sulcus. The black circles indicate the location of the flagellar pores of the transversal and longitudinal flagella. The amphiesmal vesicles were numbered and assigned to their respective series. Scale bars represent: A-E, 1 μm.

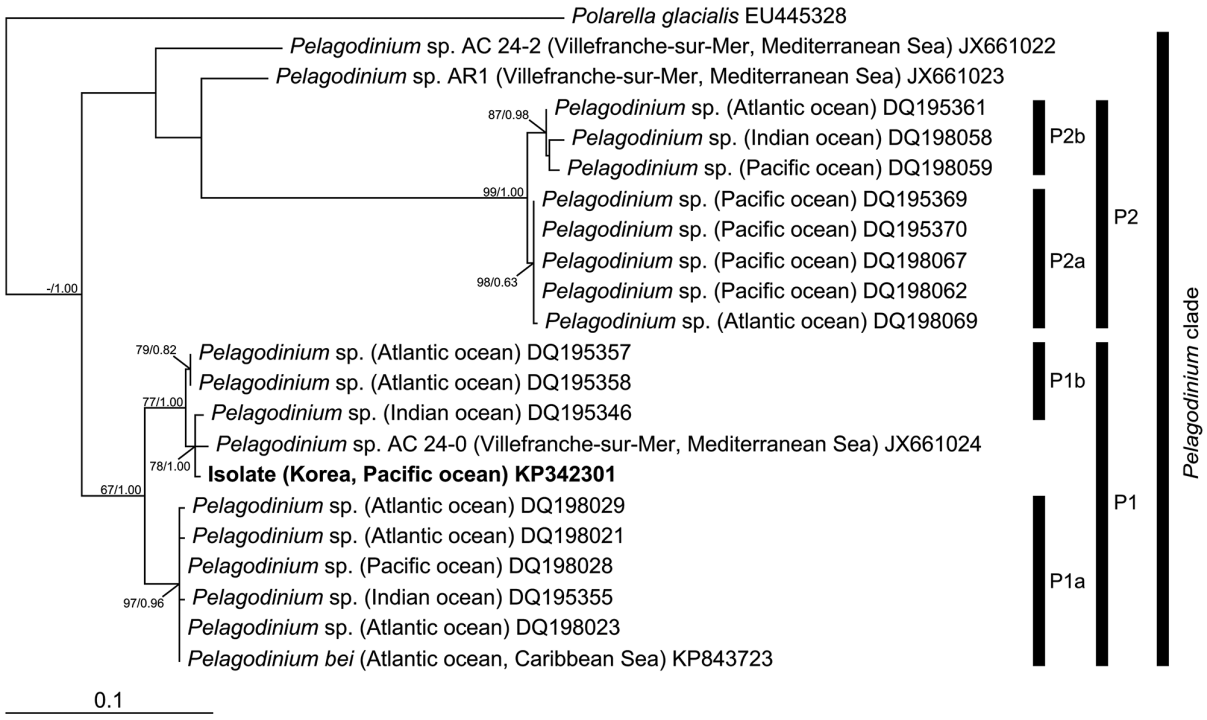

Fig. 4. Maximum likelihood (ML) phylogenetic tree based on 595 aligned nucleotides of the nuclear internal transcribed spacer rDNA using the GTR + G model with *Polarella glacialis* as an outgroup taxon. Alignment length includes gaps. The parameters were as follows: assumed nucleotide frequencies A = 0.1893, C = 0.2283, G = 0.2600, and T = 0.3224; substitution rate matrix with G-T = 1.0000, A-C = 0.5967, A-G = 1.7246, A-T = 0.9218, C-G = 0.3420, C-T = 3.2104; proportion of invariable sites = 0.0000 and rates for variable sites assumed to follow a gamma distribution with shape parameter = 0.2878. The numbers at the nodes of the branches indicate the ML bootstrap (left) and Bayesian posterior probability (right) values; only values ≥ 50% or 0.5 are shown.

with a reduction to one series at the proximity of the sulcus (series 5 and 6) (Fig. 3A & B) as it has been previously observed for the isolate. The sulcal area was composed of 13 AVs (Fig. 3C-F) and was similar in morphology to the sulcus of the isolate from Korea.

Molecular characterization

In the phylogenies based on ITS rDNA (Fig. 4) and LSU rDNA (Fig. 5), the isolate from Korea clustered with other sequences associated to the genus *Pelagodinium* (Figs 4 & 5). The localities from which the sequences were obtained were not related to their phylogenetic grouping. The isolate clustered within the sub-clade P1 as defined by Shaked and De Vargas (2006), along with *P. bei*. The sub-clade P1 was subdivided in subgroups also defined by Shaked and De Vargas (2006). The isolate was more closely related to the subgroup P1b, while *P. bei* was more closely related to the subgroup P1a (Figs 4 & 5). However, the subgroups within the sub-clade P1 were not always clearly supported.

Pigment composition

The isolate contained chlorophyll *a* and accessory pigments such as chlorophyll *c*2, peridinin, diadinoxanthin, diatoxanthin, and β-carotene (Fig. 6A). The pigment composition of *P. bei* was very similar (Fig. 6B). Zeaxanthin and alloxanthin were detected, but not in both the isolate from Korea and *P. bei*. These pigments were not well represented and might not have been detected in the analyses. The main accessory pigment was peridinin.

Swimming behavior

The isolate usually swam fast in a straight line. It stopped quickly, changed direction at different angles, and backtracked repetitively. These behaviors were also observed previously for *P. bei* (Siano et al. 2010).

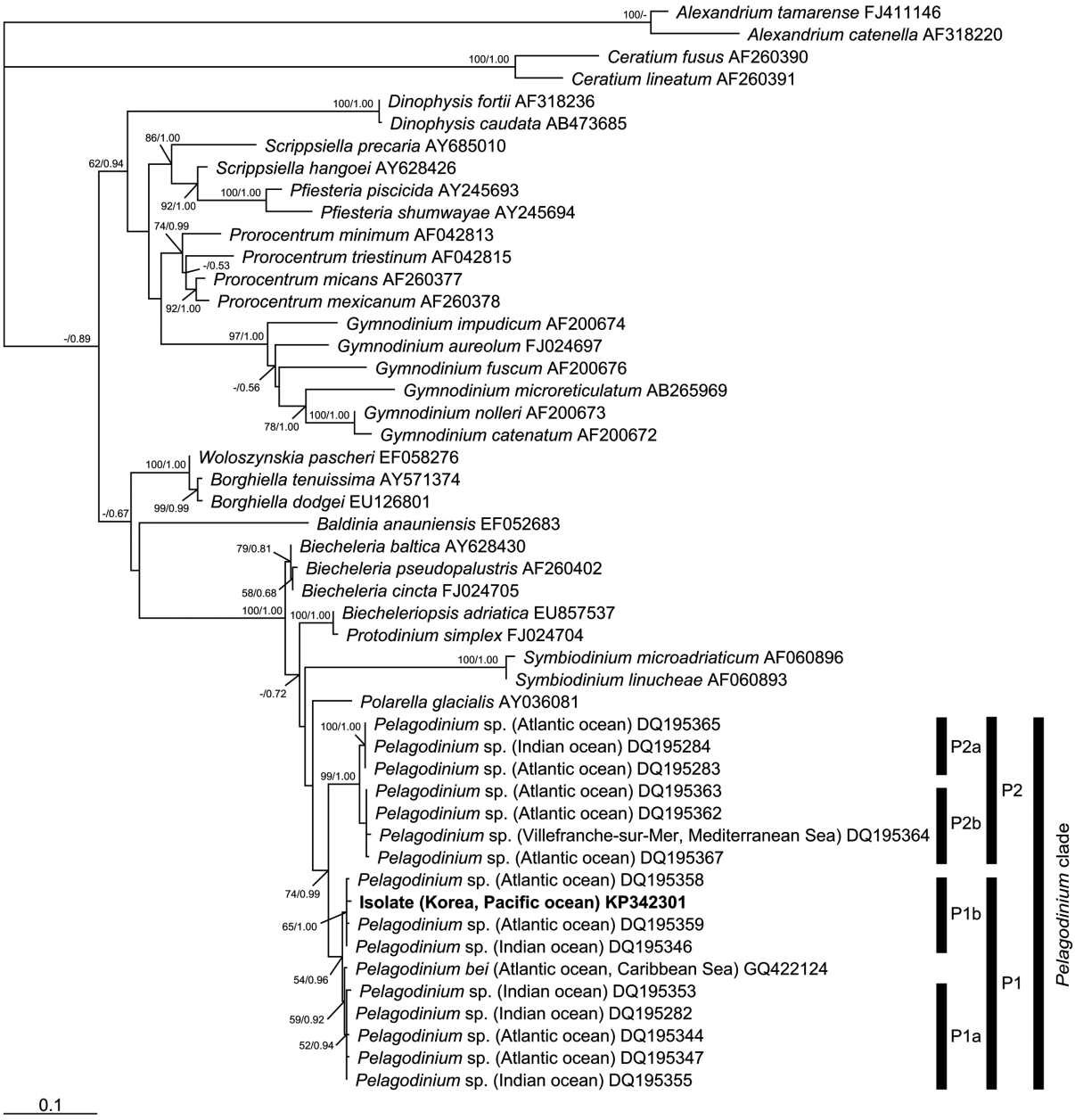

Fig. 5. Maximum likelihood (ML) phylogenetic tree based on 558 aligned nucleotides of the nuclear large subunit rDNA using the TIM + I + G model with *Alexandrium tamarense*, *A. catenella*, *Ceratium fusus*, and *C. lineatum* as outgroup taxa. Alignment length includes gaps. The parameters were as follows: assumed nucleotide frequencies A = 0.2404, C = 0.1639, G = 0.2942, and T = 0.3014; substitution rate matrix with G-T = 1.0000, A-C = 1.0000, A-G = 2.2082, A-T = 0.7906, C-G = 0.7906, C-T = 6.5313; proportion of invariable sites = 0.1258 and rates for variable sites assumed to follow a gamma distribution with shape parameter = 0.7930. The numbers at the nodes of the branches indicate the ML bootstrap (left) and Bayesian posterior probability (right) values; only values ≥50% or 0.5 are shown.

DISCUSSION

The identification of dinoflagellates in the order Suessiales is difficult because many species in this order are small and fragile and SEM fixation does not always result in a clear distinction of the AVs. Thus, classification based on genetic characterizations has been used within several genera such as *Pelagodinium* and *Symbiodinium*. Two sub-clades subdivided into subgroups in *Pelagodinium* and nine clades in *Symbiodinium* have been suggested based on genetic characterization (Rowan and Powers 1992, Shaked and De Vargas 2006, Pochon and Gates

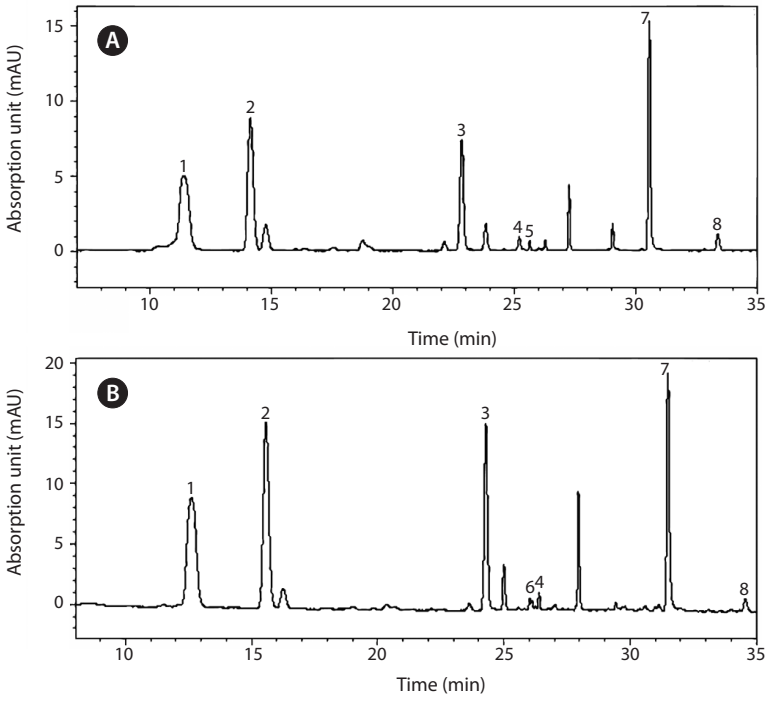

Fig. 6. Pigment composition of the isolate from Korea and *Pelagodinium bei* derived by high performance liquid chromatography. (A) Chromatogram of the isolate from Korea. (B) Chromatogram of the type culture of *P. bei* (RCC #1491). 1, Chlorophyll *c*2; 2, Peridinin; 3, Diadinoxanthin; 4, Diatoxanthin; 5, Zeaxanthin; 6, Alloxanthin; 7, Chlorophyll *a*; 8, β-carotene.

2010, Siano et al. 2010). There have been some efforts on providing detailed morphology of *Symbiodinium* and several new species have been established (Hansen and Daugbjerg 2009, Jeong et al. 2014*b*, LaJeunesse et al. 2015, Lee et al. 2015). However, detailed morphology of only one strain of *Pelagodinium*, the type culture used to describe *P. bei*, has been reported. Therefore, it is difficult to determine if the genetic classification represents intra- or interspecific variability within the genus *Pelagodinium*.

This study is the first to report the detailed morphological, behavioral, and pigment characterization of a strain of *Pelagodinium* closely associated with subgroup P1b, showing a clear similarity with known features of *P. bei* more closely associated with subgroup P1a (Table 1). This suggests that the isolate and *P. bei* are conspecific.

The observations of *P. bei* revealed new morphological features. The cingulum of *P. bei* was previously described with only one series of AVs in the cingulum (Siano et al. 2010). However, the new observations on both strains of *P. bei* revealed that the cingulum contains two series of AVs on the majority of its length with a reduction to one series of AVs at the proximity of the sulcus. The size of the cingular AVs are very similar to those of the postcingular series of small vesicles. Therefore, it is unclear if the postcingular row of AVs in *P. bei* should be included within the cingulum. This should be addressed when a more comprehensive view of the diversity within Suessiales is available. This important feature that was consistent between strains of *P. bei* should be carefully considered when further strains belonging to other sub-clades and subgroups are compared within the genus *Pelagodinium*. While *P. bei* appeared previously peculiar within Suessiales regarding its cingulum, it now appears more closely related to several other species (Kremp et al. 2005, Moestrup et al. 2008, 2009*a*, 2009*b*, Siano et al. 2009, Kang et al. 2011, Balzano et al. 2012, Luo et al. 2013, Daugbjerg et al. 2014, Jeong et al. 2014*a*, Takahashi et al. 2014).

The sulcal features were useful in determining the taxonomic identity within the same species since they are consistent between strains of *P. bei*. However, the observation of the sulcal area can be impaired by SEM fixation and the observation of all AVs is difficult. Therefore, sulcal features should be considered when possible to properly assess the diversity within *Pelagodinium*.

P. bei use chlorophyll *a* as the main pigment and peridinin as the main accessory pigment. The presence of peridinin as the main carotenoid pigment is consistent with strains of *P. bei* from Korea and the Caribbean Sea.

However, peridinin also appears to characterize several species from Suessiales such as *Symbiodinium* (Venn et al. 2006), *Baldinia anauniensis* (Hansen et al. 2007), *Biecheleria baltica* (Kremp et al. 2005), *Biecheleriopsis adriatica* (Jang et al. 2015), *Polarella glacialis* (Montresor et al. 2003), and *Ansanella granifera* (Jeong et al. 2014*a*). Diadinoxanthin, another carotenoid pigment, was also well represented in *P. bei* and *Symbiodinium* (Venn et al. 2006), *B. adriatica* (Jang et al. 2015), *P. glacialis* (Montresor et al. 2003), and *A. granifera* (Jeong et al. 2014*a*). Based on the available data, well-represented secondary accessory pigments appeared consistent among strains of *P. bei*, but

Table 1. Comparison between the isolate from Korea and *Pelagodinium bei* from the Caribbean Sea

Features	Isolate	*Pelagodinium bei*
AP length (μm) (living cells)	7.9-14.1 (11.1 ± 1.3)	8.8-11.4 (10.0 ± 0.8)[a]
Cell width (μm) (living cells)	5.1-10.8 (8.3 ± 1.1)	6.0-7.5 (6.6 ± 0.4)[a]
Epicone	Round to elliptical	Round to elliptical[a]
Hypocone	Slightly asymmetrical or round	Slightly asymmetrical[a]
Chloroplast	Golden-yellow	Golden-yellow[a]
Pyrenoids	Present	Present[a]
Apical furrow	Present	Present[a]
Cingulum	Wide and shallow Descending Displaced by one cingulum width	Wide and shallow[a] Descending[a] Displaced by one cingulum width[a]
Sulcus	Shallow and wide or deep and narrow	Deep and narrow[a]
Hypoconal flange	Present (not always well expressed)	Present[a]
Postcingular series of small vesicles	Present	Present[a]
Number of epiconal series	3-4	4[a]
Number of cingular series	1-2 (mainly 2)	1[a]-2[b] (mainly 2)
Number of hypoconal series	3-4	3-4[a,c]
Number of apical vesicles surrounding the furrow	3-4 + X	3 + X[a]
Number of vesicles surrounding the apicals	5-8	7[a]
Number of anterior intercalary vesicles	0-3	2-3[a]
Number of precingular vesicles	6-8	8[a]
Number of cingular vesicles	Not count	Not count[a]
Number of sulcal vesicles	13	13[b]
Number of postcingular small vesicles	Not count	16-20[a]
Number of hypoconal vesicles surrounding the postcingulars	6-8	8[a]
Number of posterior intercalary vesicles	0-1	0-1[a]
Number of antapical vesicles	3-4	3-4[a]
Pigments	Chlorophyll *a*, chlorophyll *c*2, peridinin, diadinoxanthin, diatoxanthin, β-carotene, and zeaxanthin[d]	Chlorophyll *a*, chlorophyll *c*2, peridinin, diadinoxanthin, diatoxanthin, β-carotene, and alloxanthin[b,d]
Swimming behavior	Swam fast in a straight line, stopped quickly, changed direction at different angles, and backtracked repetitively	Swam fast in a straight line, stopped quickly, changed direction at different angles, and backtracked repetitively[a]
References	This study	Siano et al. (2010)[a] This study[b]

The numbers of cingular series and sulcal vesicles of *P. bei* were re-examined and its pigment composition was analyzed in this study.
AP, anteroposterior.
[a]Siano et al. (2010).
[b]This study.
[c]Posterior intercalary series was not considered for *P. bei* (Siano et al. 2010).
[d]Zeaxanthin and alloxanthin were not well represented and might not have been detected in the analyses.

were not peculiar to the genus *Pelagodinium*.

The swimming pattern is consistent between strains of *P. bei* (Siano et al. 2010, this study) and appears to diverge in some aspects from other species of Suessiales by using repetitive backtracking (Jakobsen et al. 2006, Moestrup et al. 2009*b*, Siano et al. 2009, Lee et al. 2014, Jang et al. 2015). However, the swimming behavior can be highly variable within the same species (Moestrup et al. 2009*b*, Jang et al. 2015). Therefore, more detailed observations of strains are required to determine the importance of particular swimming behaviors as distinctive characters for the genus *Pelagodinium*.

In conclusion, this study established detailed features of an isolate associated with the genus *Pelagodinium*. While every sequences associated with the genus *Pelagodinium* should be complemented by morphological data, the results of this study suggest that sequences associated with the sub-clade P1, including both the subgroups P1a and P1b, belong to *P. bei*. Furthermore, new features of *P. bei* regarding the cingulum, sulcus, and pigment composition were revealed. The recognition of these features is critical for understanding the diversity within *Pelagodinium*. These results also provide a basis to further understand the molecular classification within the genus *Pelagodinium* combining genetic, morphological, pigment, and behavioral data. Further work on strains associated with the sub-clade P2 will be required in the future.

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