J. Phycol. **47,** 144–151 (2011)

DOI: 10.1111/j.1529-8817.2010.00926.x

ISOLATION AND CHARACTERIZATION OF PARMALES (HETEROKONTA/HETEROKONTOPHYTA/STRAMENOPILES) FROM THE OYASHIO REGION, WESTERN NORTH PACIFIC[1]

Mutsuo Ichinomiya[2,*]

Tohoku National Fisheries Research Institute, 3-27-5 Shinhama-cho, Shiogama 985-0001, Japan

Shinya Yoshikawa[*], *Mitsunobu Kamiya*[*], *Kaori Ohki*[*]

Fukui Prefectural University, 1-1 Gakuen-cho, Obama 917-0003, Japan

Shinichi Takaichi[*]

Department of Biology, Nippon Medical School, Kosugi-cho 2, Nakahara, Kawasaki 211-0063, Japan

and Akira Kuwata[3,*]

Tohoku National Fisheries Research Institute, 3-27-5 Shinhama-cho, Shiogama 985-0001, Japan

A small siliceous species of marine phytoplankton, order Parmales (Heterokonta), was isolated and characterized for the first time with the aid of a fluorescent silicon tracer 2-(4-pyridyl)-5-([4-(2-dimethylaminoethylaminocarbamoyl)-methoxy]phenyl) oxazole (PDMPO). This dye was easily detected by clear fluorescence in newly produced silica cell plates. Our isolate was surrounded by eight smooth plates without any ornamentation, suggesting a similarity to *Triparma laevis* B. C. Booth. TEM observation showed the typical ultrastructure of photosynthetic heterokontophytes; with two chloroplast endoplasmic reticulate membranes, a girdle lamella, three thylakoid lamellae, and mitochondrion with tubular cristae. Molecular phylogenetic analyses of SSU rDNA and *rbc*L genes showed that the parmalean alga was within the bolidophycean clade of autotrophic naked flagellates and a sister group of diatoms. HPLC analysis detected chl *a*, $c_1 + c_2$, and c_3; fucoxanthin; and diadinoxanthin as major photosynthetic pigments, and a composition that is shared with Bolidophyceae and diatoms. Together, these data indicate a close evolutionary relationship between Parmales, Bolidophyceae, and diatoms. The PDMPO-staining procedure should accelerate isolation of other Parmales species, helping to establish their diversity and aiding quantitative study of their role in oceanic processes.

Key index words: **Bolidophyceae; diatoms; heterokonts; Parmales; PDMPO; phylogeny; stramenopiles; *Triparma laevis***

Abbreviations: **BI, Bayesian inference; EPMA, electron probe micro analyzer; ML, maximum likelihood; OTU, operational taxonomic unit; PDMPO, 2-(4-pyridyl)-5-([4-(2-dimethylaminoethylaminocarbamoyl)-methoxy]phenyl)oxazole**

The order Parmales (Heterokonta) is a group of marine phytoplankton species with small solitary cells that are generally 2–5 μm in diameter (Booth and Marchant 1987). The cells are surrounded by variously shaped plates, including round, rectangular, and triradiate, which are composed of silica (Silver et al. 1980). One early suggestion was that they were resting cysts of siliceous loricate choanoflagellates because of their association, comparable abundance, similarity in silicon chemistry, and cell-size range (Booth et al. 1980, Silver et al. 1980). However, Marchant and McEldowney (1986) observed a cup-shaped chloroplast, mitochondrion, nucleus, Golgi body, and the presence of only minimal storage materials in Parmales cells in natural populations prepared for TEM and concluded that Parmales were not resting cysts but vegetative phytoplankton cells. They also observed features typical for heterokont algae, including three thylakoid lamellae, a girdle lamella, and chloroplast endoplasmic reticulum. Parmalean algae have been reported from tropical to polar waters (Nisida 1986, Takahashi et al. 1986, Booth and Marchant 1987, Kosman et al. 1993), but they are especially abundant in polar and subpolar waters (Booth and Marchant 1987, Taniguchi et al. 1995, Komuro et al. 2005).

Booth and Marchant (1987) established a new order of Parmales, belonging to class Chrysophyceae, in which a considerable diversity of silica

[1]Received 30 October 2009. Accepted 30 June 2010.
[2]Author for correspondence: e-mail mutsuo@affrc.go.jp.
[3]Author for correspondence: e-mail: akuwata@affrc.go.jp.
[*]All authors contributed equally to this study.

architecture exists. Their taxonomic status was based on the structure and number of plates of the siliceous cell wall in samples collected from natural habitats (Booth and Marchant 1987, 1988, Bravo-Sierra and Hernández-Becerril 2003, Konno and Jordan 2007). The order Parmales was divided into two families, Triparmaceae and Pentalaminaceae. In the Triparmaceae, the cell wall consists of eight plates; this family comprises two genera, *Tetraparma* and *Triparma,* with three and five species, respectively. In the Pentalaminaceae, the cell wall consists of five plates; this family contains just one genus *Pentalamina*, with one species.

Taxonomic studies of Parmales have been restricted, so far, to environmental samples because these algae have never been successfully cultured. There are no molecular phylogenies and no information on the plasticity of cell morphology, such as the number of plates or plate ornamentation, and no data on photosynthetic pigment composition. Recently, studies of environmental molecular diversity of microbial eukaryotes using gene clone libraries found novel sequences in the Arctic Ocean that were sister to Bolidophyceae, and it was suggested that these represented the Parmales (Lovejoy et al. 2006).

A major difficulty in obtaining a unialgal culture has been the small cell size of parmalean cells, which makes it almost impossible to distinguish them from other small phytoplankton in field samples under the light microscope. We have developed a new protocol to overcome this difficulty and identify Parmales cells by using a fluorescent dye, PDMPO (LysoSensorTM Yellow/Blue DND-160, Molecular Probes, Eugene, OR, USA) (Shimizu et al. 2001). PDMPO is incorporated into siliceous plankton cells and codeposited with silicon into the solid silica matrix of the newly produced cell walls. It fluoresces an intense yellowish green under UV excitation whenever silicic acid is polymerized forming biogenic silica. Using this PDMPO staining technique, a natural parmalean assemblage was successfully detected in the Oyashio region of the western North Pacific (Ichinomiya et al. 2010), making it possible to detect them without SEM observation. In the present study, we describe the first isolation and culture of a parmalean alga and report on its morphological, molecular, and pigment characteristics, which are important in establishing its taxonomic position.

MATERIALS AND METHODS

Isolation. A seawater sample was collected at 80 m from the Oyashio region (42°00′ N, 145°15′ E, July 2008) during the cruise of RV *Wakatakamaru* of the Tohoku National Fisheries Research Institutes. The sample was diluted from 10^1 to 10^4 with the filtered seawater added with PDMPO (final conc. 0.125 μM). Each 1 mL aliquot of this dilution series was transferred into a 48-well culture plate. The plate was incubated at 5°C under 100 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1} (14:10 light: dark photoperiod). After incubation, the plate was observed under UV excitation on an epifluorescence microscope (DM IRB, Leica Microsystems, Wetzlar, Germany). From the most diluted well, where the cells with PDMPO fluorescence were detected, the cells were transferred to a disposable plastic culture bottle. To purify the culture, this procedure was repeated three times. Finally, the obtained strain was maintained in modified f/2 medium (Guillard and Ryther 1962) with 100 μM nitrate and 100 μM silicic acid in the plastic bottle under the same light and temperature conditions as at isolation and deposited to the culture collection of National Institute of Environmental Science in Japan, with a culture number of NIES-2565.

EM observations. The culture was filtered through a polycarbonate membrane filter (0.6 μm pore size, Advantech, Tokyo, Japan) and air-dried at room temperature after rinsing with distilled water to remove salts. The filter was coated with Pt/Pd (E-1010 ion sputter, Hitachi, Tokyo, Japan) and examined by SEM (JSM-6390LV, JEOL, Tokyo, Japan). Procedures for preparing specimens for TEM (JEM-1210, JEOL) were the same as those used by Ohki and Fujita (1996). Thin sections were stained with uranyl acetate (saturated in 60% methanol, for 15–30 min) and lead citrate (Venable and Coggeshall 1965, for 1 min).

Energy-dispersive X-ray spectroscopy. Algal cells were fixed by glutaraldehyde (1%, final conc.) and collected on a polycarbonate membrane filter (pore size 0.2 μm, Advantech). Cells on the filter were air-dried at room temperature after rinsing with distilled water to remove salts and were then coated with gold. Distribution of silicon and oxygen was detected by an electron probe microanalyzer (EPMA JXA8900RL, JEOL). Analysis was performed at a magnification of ×4,000 and an accelerating voltage of 15 kV. Distributions of each element were expressed as values relative to the maximum.

Pigment analysis. The culture for photosynthetic pigment analysis was filtered onto GF/F filter (Whatman, Maidstone, UK). Pigments were extracted using acetone/methanol (7:2, v/v) and an ultrasonicator, with cell debris removed by centrifugation (2,700*g*; Tomy Seiko, Tokyo, Japan). After evaporation of the solvents, the extract was analyzed by an HPLC system equipped with μBondapak C_{18} column (8 mm × 100 mm, RCM type; Waters, Milford, MA, USA) eluted with methanol/water (9:1, v/v) for the first 14 min and then with 100% methanol (2.0 mL $\cdot$ min^{-1}). Furthermore, each carotenoid was purified with columns of silica gel (Merck, Darmstadt, Germany) and DEAE-Toyopearl 650 M (Tosoh, Tokyo, Japan) and finally collected from the HPLC (Takaichi et al. 2009).

We measured the absorption spectra of the pigments using an MCPD-3600 photodiode array detector (Otsuka Electronics, Osaka, Japan) attached to the HPLC apparatus. For quantitative analysis, the molar extinction coefficient of each carotenoid in the HPLC eluent at 450 nm was assumed to be the same. The relative molecular masses of the purified carotenoids were measured using an FD-MS, M-2500 double-focusing gas chromatograph-mass spectrometer (Hitachi) equipped with a field-desorption apparatus (Takaichi et al. 2009).

Molecular phylogeny. Genomic DNA was extracted using FastDNA SPIN kit (MP Biomedicals, Solon, OH, USA). The SSU rRNA fragment was amplified using the eukaryote-specific primers SR1F (TACCTGGTTGATCCTGCCAG) and SR1R (CCTTCCGCAGGTTCACCTAC) (Nakayama et al. 1996). The *rbc*L gene was amplified as two fragments. At first, the latter parts of *rbc*L gene were amplified using primer sets rbcL5F (CACAACCATTCATGCG) and rbcSR (AASHDCCTTGTGTWAGTYTC) (Daugbjerg and Andersen 1997). A specific primer rbcLR1 (AGCCCAGATAGCAGCAGTT) was designed according to the sequences of the latter part of the *rbc*L gene. Then, the front part of the *rbc*L gene was amplified using primer sets rbcLF1 (AAGGAGGAADHHATGTCT) (Daugbjerg and Andersen 1997) and rbcLR1. The PCR products purified with

QIAquick gel extraction kit (Qiagen, Hilden, Germany) were directly sequenced using the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The partial SSU rDNA sequences from 74 operational taxonomic units (OTUs) were aligned using the ClustalW algorithm as implemented in BioEdit 7.0.9 (Hall 1999) and manually improved using secondary structure information in accordance with Wuyts et al. (2000). Positions with ambivalent homology assignment, the variable helices E23-1, E23-2, and 43 (see Wuyts et al. 2000), were removed, and finally, 1,660 aligned positions including gaps were used for phylogenetic analyses. The partial *rbc*L sequences from 56 OTUs were easily aligned due to fewer indels, and the final aligned data were 1,120 bp.

The obtained sequences were checked for congruency within multiple samples. Gaps were treated as missing data in any analysis, and the four haptophycean species were used as an outgroup. Maximum-likelihood (ML) analysis was performed using PAUP*4.0b10 (Swofford 2002). The TrN + I + G model and GTR + I + G model were selected for the SSU rDNA and *rbc*L sequence data, respectively, using Akaike information criterion in Modeltest 3.7 (Posada and Crandall 1998). Bootstrap analysis for ML was performed based on 100 replications of heuristic search with a nearest-neighbor interchanges algorithm generated from resampled data. For Bayesian inference (BI) analysis, the GTR + I + G model was selected for both sequence data, using MrModeltest 2.3 (Nylander 2004). Markov chain Monte Carlo iterations were conducted for 10,000,000 generations sampling every 100 generations, using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The sequences obtained were deposited in GenBank with the following accession numbers: SSU rDNA, AB546639; *rbc*L, AB546640. The alignment data are available from the authors on request.

RESULTS

Newly produced silica cell walls of parmalean algae fluoresced an intense yellowish green under UV excitation when PDMPO was added to the natural water samples (Fig. 1A). In the preliminary culture experiments, parmalean cells grew at 5°C, but not at 15°C. SEM observation revealed that cells were 2.4–3.6 μm and surrounded by three shield plates, one dorsal plate, one ventral plate, and three girdle plates with a single wing (Fig. 1B). The plates were smooth without any ornamentation, suggesting a similarity to *T. laevis* (Booth and Marchant 1987, Konno et al. 2007). Using energy-dispersive X-ray spectroscopy, clear signals that showed the presence of silicon and oxygen were also detected only in the places where cells existed (Fig. 1, C–E).

Under TEM observation, the cell consisted of a nucleus, chloroplast, mitochondrion, Golgi apparatus, and vacuole within silica plates with a thickness of ca. 80 nm (Fig. 2, A and B). The nucleus was located adjacent to the chloroplast and seemed to share a common bounding membrane with the chloroplast envelope (Fig. 2C). The chloroplast contained lamellae consisting of two to three adpressed thylakoids and a peripheral girdle lamella (Fig. 2D). The number of chloroplast envelopes was not clear, and a pyrenoid was not observed. Structures similar to a nucleoid underlay the girdle lamella

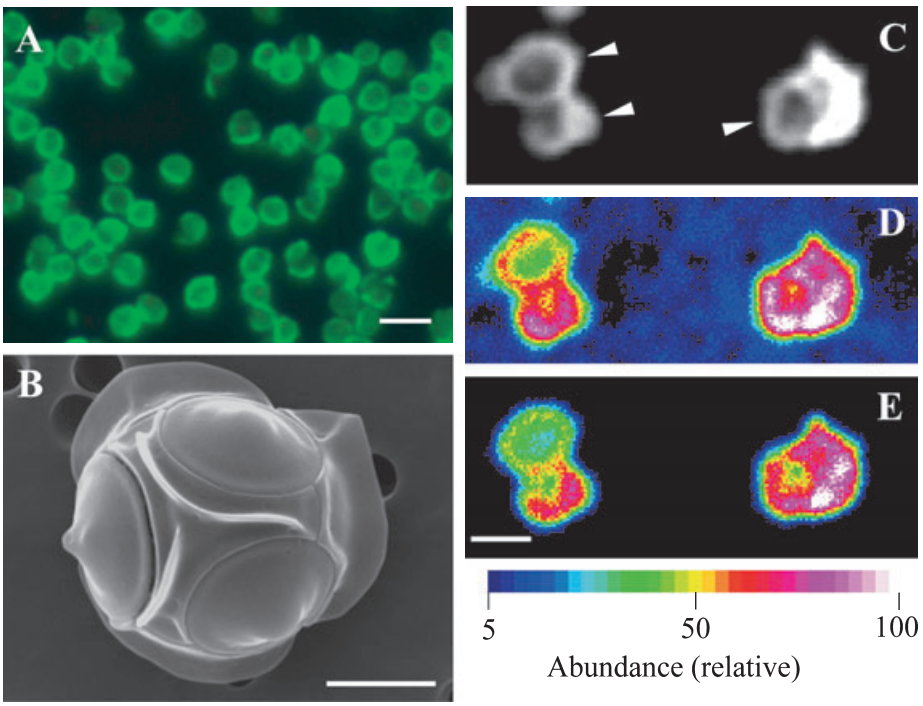

FIG. 1. (A) Parmales culture stained with the fluorescent dye PDMPO, which shows newly precipitated silica in actively dividing cells. Scale bar, 5 μm. (B) SEM microphotograph of the parmalean alga. Scale bar, 1 μm. (C) Scanning electron micrograph image of the sample used for EPMA analysis. Three cells are observed (arrowheads). (D) Distribution of oxygen. (E) Distribution of silicon. Abundance of oxygen and silicon is presented relative to the maximum value of each element. Scale bar, 2 μm. EPMA, electron probe micro analyzer; PDMPO, 2-(4-pyridyl)-5-([4-(2-dimethylaminoethylaminocarbamoyl)-methoxy]phenyl)oxazole.

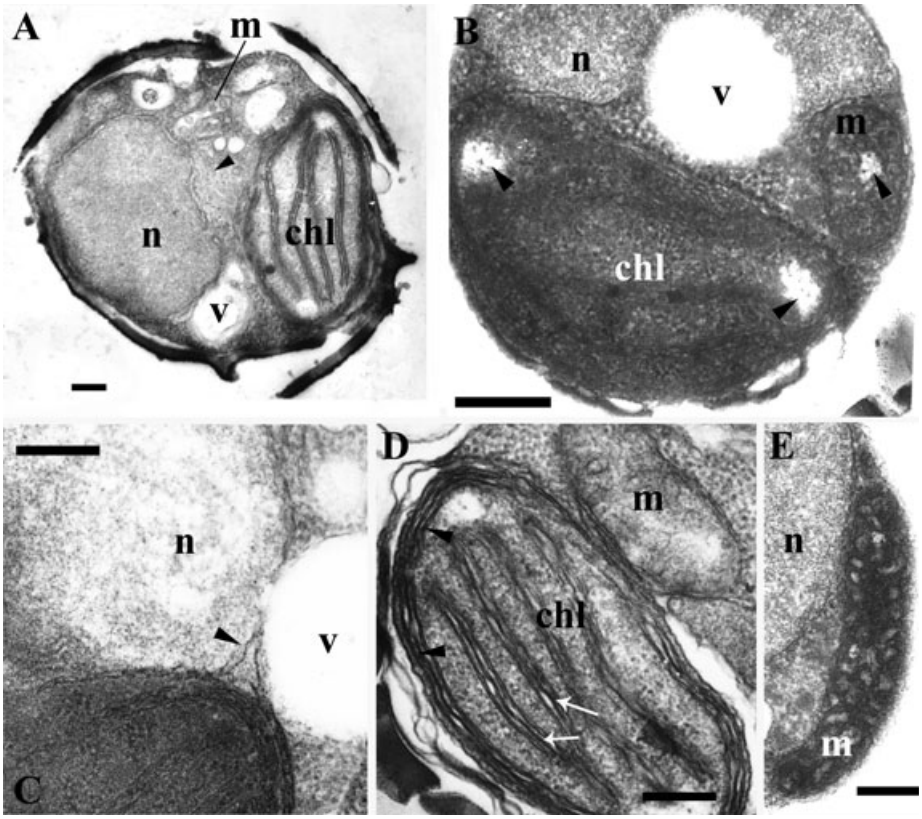

FIG. 2. (A) General ultrastructure of the parmalean alga observed under TEM. Cell with nucleus, chloroplast, mitochondrion, Golgi apparatus (arrowhead), and vacuole, surrounded by siliceous wall. Scale bar, 100 nm. (B) Morphology of the chloroplast and mitochondrion, including structures resembling nucleoids (arrowheads). (C) Image showing the nucleus closed to the chloroplast. The nucleus appears to share a common bounding membrane with the chloroplast envelope (arrowhead). Scale bar, 100 nm. (D) Chloroplast with appressed thylakoid (arrows) and girdle lamella (arrowheads). Scale bar, 200 nm. (E) Mitochondrion with tubular cristae. Scale bar, 200 nm. chl, chloroplast; m, mitochondrion; n, nucleus; v, vacuole.

(Fig. 2B). The mitochondrion contained tubular cristae (Fig. 2E).

Chl *a*, $c_1 + c_2$, and c_3 were identified based on their absorption spectra and the relative retention times on HPLC (Jeffrey et al. 1997). The carotenoids were also identified based on their absorption spectra, the specific retention times on HPLC, and their relative molecular masses. The composition of carotenoids was 71% fucoxanthin (mol% of total carotenoids), 21% diadinoxanthin, 4% diatoxanthin, 2% zeaxanthin, and 2% β-carotene.

Both molecular phylogenetic analyses based on SSU rDNA and *rbc*L sequences indicated that this alga was included within the bolidophycean clade (Figs. 3 and 4). The genetic distance (Tamura and Nei 1993) between this alga and the most closely related stains of *Bolidomonas pacifica* Guillou et Chrétiennot-Dinet was only 0.9% in the SSU rDNA region and 5.9% in *rbc*L gene. Among the six environmental clones examined in the SSU rDNA analysis, DH114-3A83 from the South Atlantic was closely related to this alga (Marande et al. 2009), being genetically 0.2% distance, while the others were placed as two sister clades to Bolidophyceae (Fig. 3).

DISCUSSION

This is the first report on isolation and characterization of Parmales. The ultrastructure of our isolate, including a girdle lamella, a plastid-nucleus membrane connection, and mitochondrion containing tubular cristae (Fig. 2), corresponds to that in other photosynthetic heterokontophytes, except that in Eustigmatophyceae a girdle lamella is lacking, and the Raphidophyceae and Synurophyceae lack a plastid-nucleus membrane connection (Andersen 2004). The pigment composition, mainly including fucoxanthin and diadinoxanthin as the major carotenoids, was also similar to that occurring in Bolidophyceae and diatoms (Bacillariophyceae) (Guillou et al. 1999, Andersen 2004), but different from Chrysophyceae, which does not possess diadinoxanthin/diatoxanthin-type xanthophyll pigments for photoprotection (Lichtlé et al. 1995). In addition to diadinoxanthin/diatoxanthin, diatoms possess violaxanthin/antheraxanthin/zeaxanthin-type xanthophyll pigments (Lohr and Wilhelm 1999), like the parmalean alga in this study. These results, therefore, strongly suggest that Parmales

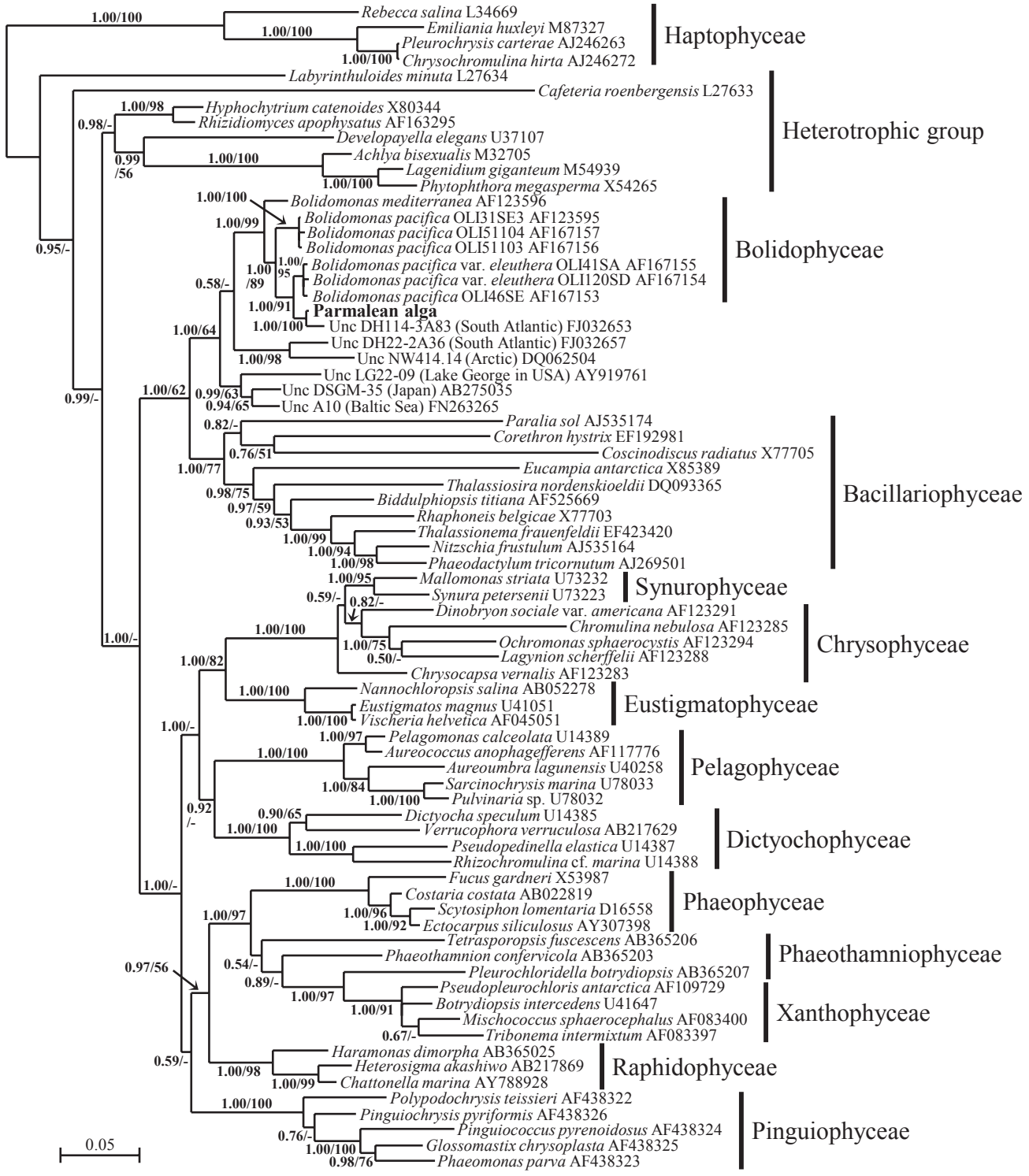

FIG. 3. Bayesian phylogenetic tree inferred from the SSU rDNA sequences. The corresponding posterior probabilities (left) and bootstrap values from maximum likelihood (>50%; right) are given on each branch. Accession numbers used in the analysis are given after species name.

should be taxonomically separated from Chrysophyceae.

The present molecular phylogenetic analyses suggest that Parmales is closely related to Bolidophyceae (Fig. 3), even though parmalean algae are clearly distinguishable from bolidophycean algae by the presence of siliceous cell walls and the absence of flagella (Guillou et al. 1999). Bolidophycean algae are

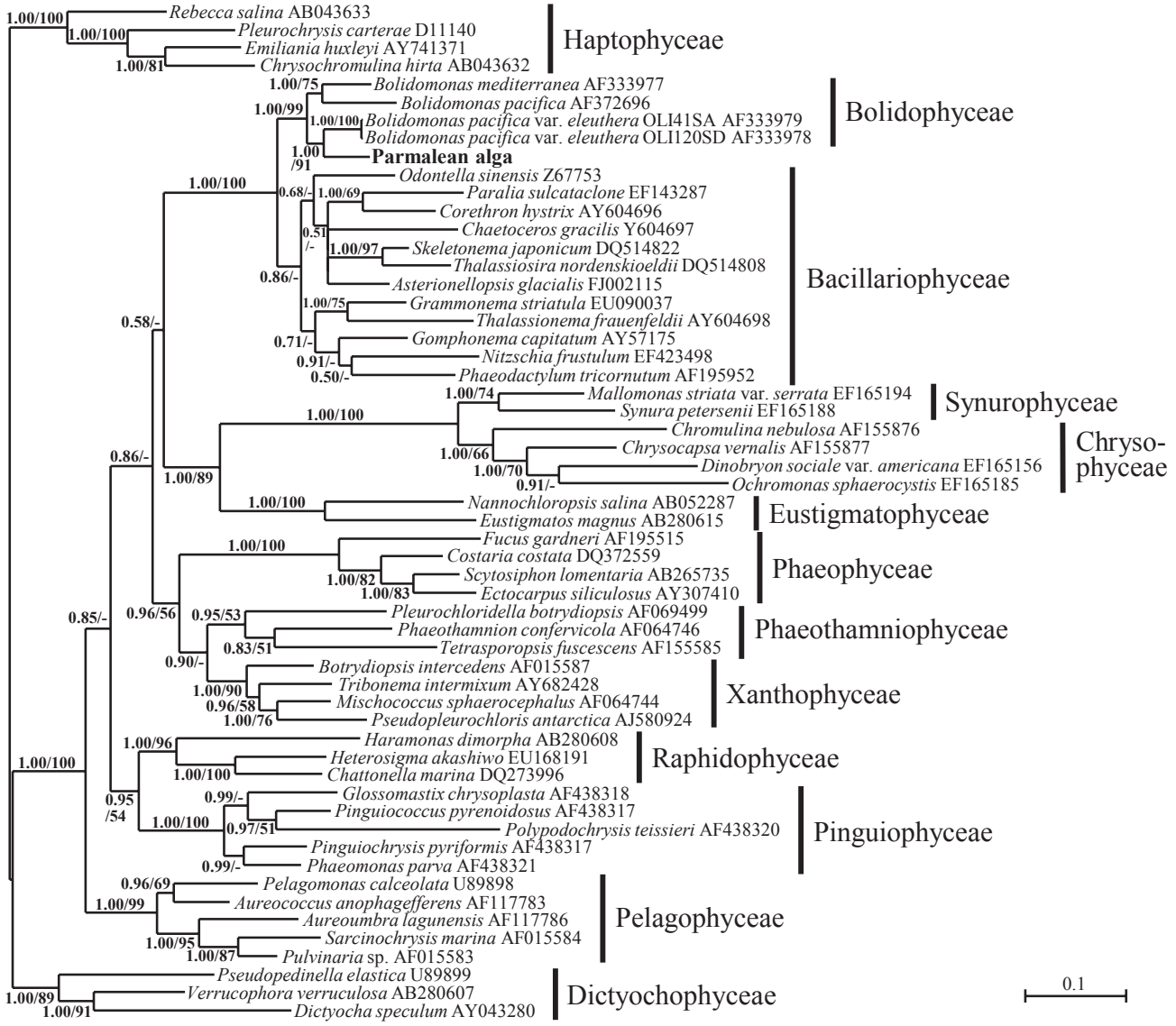

FIG. 4. Bayesian phylogenetic tree inferred from the *rbc*L sequences. The corresponding posterior probabilities (left) and bootstrap values from maximum likelihood (>50%; right) are given on each branch. Accession numbers used in the analysis are given after species name.

small, naked flagellates (1–1.7 μm) that have been recognized as a sister group of diatoms based on their molecular phylogeny (Guillou et al. 1999). Bolidophytes also share a two-amino-acid insertion in the *rbc*L gene with diatoms (Daugbjerg and Guillou 2001), and this insertion was observed in the parmalean strain (Fig. 4).

Lovejoy et al. (2006) suggests that some environmental DNA clones, including Clone NW414.14, from the Arctic Ocean and Beaufort Sea as sister to the bolidophytes might be Parmales. In the present study, among the six environmental clones showing high similarity to our isolate based on the SSU rDNA sequence, Clone DH114-3A83 from the South Atlantic was more closely related to our isolate than the other clones or bolidophycean algae, while Clone NW414.14 and four other clones were sister to the bolidophycean clade (Fig. 3). It is still unclear whether these clones are the other parmalean species or unknown organisms.

The present study also suggested a close relationship between Parmales and diatoms (Figs. 3 and 4), with both groups having silicified cell walls. In addition, extant centric diatoms, which have a diploid vegetative stage, switch to production of haploid naked flagellated male gametes (sperms) for sexual reproduction. From the life cycle of centric diatoms, we can hypothesize that parmalean and bolidophycean algae (or their common ancestor) might also have a life cycle that switches between silicified nonflagellated and naked flagellate stages. Mann and Marchant (1989) proposed that the diatom ancestor

could have been a haploid flagellate that formed a diploid silicified zygote, and mitotic division of the zygote might have taken place preferentially to give rise to the centric diatoms, which are the most ancient group of diatoms (Kooistra et al. 2007). Parmales studies may play a key role in understanding the early evolution of diatoms. More comprehensive analyses, including whole genome sequences, the pattern of cell division, and fossil records, will improve our understanding of the evolutionary relationships between Parmales, Bolidophyceae, and diatoms.

The present study provided important aspects on the taxonomic position of Parmales. However, further examination, including isolation of additional parmalean species, will be necessary for taxonomic rearrangement of the Parmales. PDMPO will help in this by enabling observation of living Parmales cells via epifluorescence microscopy without any fixation needed for SEM observation. The present PDMPO-staining and serial dilution procedure applied in this study should accelerate isolation of additional parmalean species, subsequently stimulating ecological studies on this group using specific molecular probes.

We thank Drs. D. Jewson, A. Taniguchi, and E. Wada for their critical reading of the manuscript. This study was partially supported by Grant-in-Aid for Scientific Research 19570092 (M. K.), 20580208 (K. O.), and 19580228 and 22657027 (A. K.); MAFF research fund ''Evaluation, Adaptation and Mitigation of Global Warming in Agriculture, Forestry and Fisheries: Research and Development'' (A. K.); Fukui Prefectural Fund for the Promotion of Science (K. O.); and the joint research project from the Wakasa Energy Research Center (K. O.). The EPMA analysis was carried out at the Wakasa Energy Research Center. We are grateful to Operator Jinro Nagatomo for his technical assistance. Special thanks are extended to two anonymous reviewers for their critical reading of the manuscript.

Andersen, R. A. 2004. Biology and systematics of heterokont and haptophyte algae. *Am. J. Bot.* 91:1508–22.

Booth, B. C., Lewin, J. & Norris, R. E. 1980. Siliceous nanoplankton. 1. Newly discovered cysts from the Gulf of Alaska. *Mar. Biol.* 58:205–9.

Booth, B. C. & Marchant, H. J. 1987. Parmales, a new order of marine chrysophytes, with descriptions of three new genera and seven new species. *J. Phycol.* 23:245–60.

Booth, B. C. & Marchant, H. J. 1988. Triparmaceae, a substitute name for a family in the order Parmales (Chrysophyceae). *J. Phycol.* 24:124.

Bravo-Sierra, E. & Hernández-Becerril, D. U. 2003. Parmales (Chrysophyceae) from the Gulf of Tehuantepec, Mexico, including the description of a new species, *Tetraparma insecta* sp. nov., and a proposal to the taxonomy of the group. *J. Phycol.* 39:577–83.

Daugbjerg, N. & Andersen, R. A. 1997. Phylogenetic analyses of *rbc*L sequences from haptophytes and heterokont algae suggest their chloroplasts are unrelated. *Mol. Biol. Evol.* 14:1242–51.

Daugbjerg, N. & Guillou, L. 2001. Phylogenetic analyses of Bolidophyceae (Heterokontophyta) using *rbc*L gene sequences support their sister group relationship to diatoms. *Phycologia* 40:153–61.

Guillard, R. R. L. & Ryther, J. H. 1962. Studies of marine planktonic diatoms I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. *Can. J. Microbiol.* 8:229–39.

Guillou, L., Chrétiennot-Dinet, M.-J., Medlin, L. K., Claustre, H., Loiseaux-de Goër, S. & Vaulot, D. 1999. *Bolidomonas*: a new genus with two species belonging to a new algal class, the Bolidophyceae (Heterokonta). *J. Phycol.* 35:368–81.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* 41:95–98.

Ichinomiya, M., Gomi, Y., Nakamachi, M., Ota, T. & Kobari, T. 2010. Temporal patterns in silica deposition among siliceous plankton during the spring bloom in the Oyashio region. *Deep-Sea Res. Part II Top. Stud. Oceanogr.* 57:1665–70.

Jeffrey, S. W., Mantoura, R. F. C. & Bjørnland, T. 1997. Data for the identification of 47 key phytoplankton pigments. *In* Jeffrey, S. W., Mantoura, R. F. C. & Wright, S. W. [Eds.] *Phytoplankton Pigments in Oceanography*. UNESCO Publishing, Paris, pp. 449–559.

Komuro, C., Narita, H., Imai, K., Nojiri, Y. & Jordan, R. W. 2005. Microplankton assemblages at Station KNOT in the subarctic western Pacific, 1999–2000. *Deep-Sea Res. Part II Top. Stud. Oceanogr.* 52:2206–17.

Konno, S. & Jordan, R. W. 2007. An amended terminology for the Parmales (Chrysophyceae). *Phycologia* 46:612–16.

Konno, S., Ohira, R., Komuro, C., Harada, N. & Jordan, R. W. 2007. Six new taxa of subarctic Parmales (Chrysophyceae). *J. Nannoplankton Res.* 29:108–28.

Kooistra, W. H. C. F., Gersonde, R., Medlin, L. M. & Mann, D. G. 2007. The origin and evolution of the diatoms: their adaptation to a planktonic existence. *In* Falkowski, P. G. & Knoll, A. H. [Eds.] *Evolution of Primary Producers in the Sea*. Elsevier Academic Press, Amsterdam, pp. 207–49.

Kosman, C. A., Thomsen, H. A. & Østergaard, J. B. 1993. Parmales (Chrysophyceae) from Mexican, Californian Baltic, Arctic and Antarctic waters with the description of new subspecies and several new forms. *Phycologia* 32:116–28.

Lichtlé, C., Arsalane, W., Duval, J. C. & Passaquet, C. 1995. Characterization of the light-harvesting complex of *Giraudyopsis stellifer* (Chrysophyceae) and effects of light stress. *J. Phycol.* 31:380–87.

Lohr, M. & Wilhelm, C. 1999. Algae displaying the diadinoxanthin cycle also possess the violaxanthin cycle. *Proc. Natl. Acad. Sci. U. S. A.* 96:8784–89.

Lovejoy, C., Massana, R. & Pedrós-Alió, C. 2006. Diversity and distribution of marine microbial eukaryotes in the Arctic Ocean and adjacent seas. *Appl. Environ. Microbiol.* 72:3085–95.

Mann, D. G. & Marchant, H. J. 1989. The origins of the diatom and its life cycle. *In* Green, J. C., Leadbeater, B. S. C. & Diver, W. L. [Eds.] *The Chromophyte Algae: Problems and Perspectives*. Systematics Association Special Volume 38. Clarendon Press, Oxford, pp. 307–23.

Marande, W., López-García, P. & Moreira, D. 2009. Eukaryotic diversity and phylogeny using small and large-subunit ribosomal RNA genes from environmental samples. *Environ. Microbiol.* 11:3179–88.

Marchant, H. J. & McEldowney, A. 1986. Nanoplanktonic siliceous cysts from Antarctica are algae. *Mar. Biol.* 92:53–57.

Nakayama, T., Watanabe, S., Mitui, K., Uchida, H. & Inouye, I. 1996. The phylogenetic relationship between the Chlamydomonadales and Chlorococcales inferred from 18SrDNA sequence data. *Phycol. Res.* 42:47–55.

Nisida, S. 1986. Nanoplankton flora in the southern ocean, with special reference to siliceous varieties. *Mem. Natl. Inst. Polar Res. Spec. Issue* 40:56–68.

Nylander, J. A. A. 2004. *MrModeltest v2*. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden.

Ohki, K. & Fujita, Y. 1996. Occurrence of a temperate cyanophage lysogenizing the marine cyanophyte *Phormidium persicinum*. *J. Phycol.* 32:365–70.

Posada, D. & Crandall, K. A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14:817–18.

Ronquist, F. & Huelsenbeck, J. P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–74.

Shimizu, K., Del Amo, Y., Brzezinski, M. A., Stucky, G. D. & Morse, D. E. 2001. A novel fluorescent silica tracer for biological silicification studies. *Chem. Biol.* 8:1051–60.

Silver, M. W., Mitchell, J. G. & Ringo, D. L. 1980. Siliceous nanoplankton. II. Newly discovered cysts and abundant choanoflagellates from the Weddell Sea, Antarctica. *Mar. Biol.* 58:211–17.

Swofford, D. L. 2002. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods).* version 4.0b10. Sinauer, Sunderland, Massachusetts.

Takahashi, E., Watanabe, K. & Satoh, H. 1986. Siliceous cysts from Kita-no-Seto Strait, north of Syowa Station, Antarctica. *Mem. Natl. Inst. Polar Res. Spec. Issue* 40:84–95.

Takaichi, S., Maoka, T. & Mochimaru, M. 2009. Unique carotenoids in the terrestrial cyanobacterium *Nostoc commune* NIES-24: 2-hydroxymyxol 2′-fucoside, nostoxanthin and canthaxanthin. *Curr. Microbiol.* 59:413–19.

Tamura, K. & Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* 10:512–26.

Taniguchi, A., Suzuki, T. & Shimada, S. 1995. Growth characteristics of Parmales (Chrysophyceae) observed in bag cultures. *Mar. Biol.* 123:631–38.

Venable, J. H. & Coggeshall, R. 1965. A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.* 25:407–8.

Wuyts, J., De Rijk, P., Van de Peer, Y., Pison, G., Rousseeuw, P. & De Wachter, R. 2000. Comparative analysis of more than 3000 sequences reveals the existence of two pseudoknots in area V4 of eukaryotic small subunit ribosomal RNA. *Nucleic Acids Res.* 28:4698–708.