

A new diplopsalid species *Oblea acanthocysta* sp. nov. (Peridinales, Dinophyceae)

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Abstract: *Oblea acanthocysta* of the diplopsalid group is described as a new species from Omura Bay, West Japan. The motile cells are subspherical, have a large sulcal list at the left margin, and are characterized by the plate formula, Po, X, 3', 1a, 6'', 3c+t, 6s?, 5''', 2'''. The species resembles *Oblea torta* in shape and plate distribution, but is smaller and differs in the shape of plate 1' and position of plate 1a. Resting cysts of *O. acanthocysta* are spherical and pale brown in color, possess many hollow acuminate spines, and have a theropylic archeopyle. In the SSU rRNA gene sequences of three cells of *O. acanthocysta* and two cells of *O. torta*, no intraspecific base substitutions were detected within either species. Based on phylogenetic analyses, the *O. acanthocysta* clade is included in the diplopsalids clade together with other diplopsalid species such as *O. torta*, *Diplopsalis lebourae*, *Diplopsalopsis bomba* and *Gotoius ex-centricus*. The sequences of *O. acanthocysta* are different from those of *O. torta* in 154 base pair substitutions, but *O. acanthocysta* and *O. torta* have a very close phylogenetic relationship.

Key words: cyst–theca relationship, diplopsalid, *Oblea acanthocysta*, molecular phylogeny, thecate dinoflagellate

Introduction

The marine heterotrophic dinoflagellate genus *Diplopsalis* and some genera morphologically similar to *Diplopsalis* are known as 'the *Diplopsalis* group' (Abé 1936). The term 'diplopsalids' is used for this group in the present paper. The diplopsalids possess common characteristic features, i.e. the motile cells are simply globular or lenticular with a large sulcal list on the left side (e.g. Abé 1981; Dodge & Toriumi 1993), and the cysts have a brownish wall with a theropylic archeopyle (Matsuoka 1988; Lewis 1990; Dale et al. 1993). More than 10 genera including *Diplopsalis*, *Diplopsalopsis*, *Diplopelta* have been established within the diplopsalids, because the number of thecal plates is extremely diverse in comparison with the genus *Protoperidinium* (Bergh 1881; Stein 1883; Meunier 1910; Pavillard 1913; Abé 1941; Sournia 1986; Balech 1964; Loeblich Jr & Loeblich III 1970; Abé 1981; Dodge & Hermes 1981). Among such diplopsalid genera, the genus *Oblea* was established by Balech (1964) with the plate formula 3', 1a, 6'', 3c, 5''', 2'''' and now includes the following three species: *Oblea baculifera* Balech ex Loeblich Jr. et Loeblich III, *Oblea rotunda* (Lebour) Balech and *Oblea*

torta (Abé) Balech (Balech 1964).

Most diplopsalid cysts have a simple spherical body without ornamentation, and only the cysts of *Diplopelta parva* (Abé) Matsuoka and *Diplopelta symmetrica* Pavillard possess spines on the surface. Some species of the marine heterotrophic genus *Protoperidinium* likewise form round brown spiny cysts with a theropylic archeopyle (e.g. *Protoperidinium minutum* (Kofoid) Loeblich III and *Protoperidinium monospinum* (Paulsen) Zonneveld et Dale), although most spiny cysts of *Protoperidinium* have simple saphopylic archeopyles. The phylogenetic relationship between round brown spiny cysts and theropylic archeopyles is still unclear.

Molecular analysis is effective for identification and reconstruction of phylogenetic relationships, because it is often very difficult to confirm the validity of species based only on morphological examination. For several autotrophic species producing resting cysts, the morphology and phylogeny of both cysts and motile cells have been investigated (e.g. Bolch et al. 1999; Ellegaard et al. 2003). For heterotrophic species the single-cell PCR (Polymerase chain reaction) technique has been provided by Takano & Horiguchi (2004) and then adopted by Yamaguchi & Horiguchi (2005) and Matsuoka et al. (2006). This technique is important for reevaluating the systematic classification of modern and fossil dinoflagellates.

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In the present study, we describe a new species *Oblea acanthocysta* sp. nov. based on the morphological characters of both motile cell and cyst, and discuss its phylogenetic position using molecular phylogenetic analysis.

Materials and Methods

Sampling

Living cysts provided for incubation experiments were collected using a sediment trap deployed at the southern part of Omura Bay (32°55'N, 129°52'E), West Japan in December 2003. Motile cells were collected from Omura Bay and Shin-Nagasaki Harbor (32°48'N, 129°46'E) from May 2004 to August 2004. Both motile cells from field samples and those that germinated from the cysts were used for molecular analysis.

Cyst incubation experiment

The sediment trap sample was sonicated for 30 seconds and then sieved at 125 μm and retained on 20 μm sieves using filtered seawater. The material collected was stored at 4°C until the cyst incubation experiment started. Cysts were isolated with a micropipette and placed in multiple well plates (Asahi Techno Glass, Chiba, Japan) each well containing 1 mL of ESM medium (Watanabe et al. 2000). The multiple well plates were kept in a growth cabinet at 20°C and 24 hL with an irradiance of 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

Light microscopy

The cysts and excysted motile cells were observed under normal light and fluorescence microscopes (Olympus BX51, IX 70, Tokyo, Japan), and micrographs were taken with Olympus Camedia C-5060 and Olympus DP50 digital cameras. For investigation of the thecal tabulation, cells stained with 1mg/mL Fluorescent Brightener 28 (Sigma, St Louis, MO, U.S.A.) were observed with an Olympus BX51, IX70 fitted with a filter arrangement for ultraviolet excitation (330–385 nm) (Fritz & Triemer 1985).

Molecular analysis

SSU rDNA sequences were directly amplified from a single cell using the method of Takano & Horiguchi (2004). After microscopic observation, the cell was broken with a sharp glass rod and its contents were transferred to a 200- μL tube containing 10 μL distilled water. PCR amplification was carried out in a 20- μL reaction volume with the reagents according to the manufacturer's recommendation of KOD-Plus-DNA Polymerase (Toyobo, Osaka, Japan) on a GeneAmp 9600 PCR System (Perkin-Elmer, Foster City, USA). SSU rDNA was amplified using primers previously described and modified primers (Nakayama et al. 1996; Matsuoka et al. 2006). The PCR reactions were performed in two steps. The first round of PCR consisted of an initial denaturation at 95°C for 10 min, followed by 35 cycles of

95°C for 1 min, 55°C for 1 min, and 72°C for 3 min. The reaction was completed with a final elongation at 72°C for 10 min. The primer pair used in the first round of PCR to amplify the full length SSU rDNA was SR1 and SR12. The second round of PCR consisted of an initial denaturation at 95°C for 10 min, followed by 40 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s. The reaction was completed with a final elongation at 72°C for 10 min. The product of the first round of PCR was used as a template for the second round, where the following combinations of primer pairs were used: SR1 and SR5kaw (5'-ACTACGAGCT-TTTTAACCGC-3'), SR4 and SR9, and SR8kaw (5'-GGATTGACAGATTGATAGCT-3') and SR12. The PCR product was purified using a Microcon YM-100 Centrifugal Filter Device (Millipore, Billerica, MA, U.S.A.), and the cycle-sequencing reaction was performed using an ABI PRISM BigDye™ Terminator v3.1 Cycle Sequencing Kit (Perkin-Elmer) following the manufacturer's protocol. Sequencing was run on an ABI PRISM 377 Sequencer (Perkin-Elmer) with the PCR primer set and internal primers. The sequences can be obtained from DDBJ/EMBL/GenBank under accession numbers of AB273721–AB273725.

The sequences of dinoflagellates obtained from this study and DDBJ/EMBL/GenBank were aligned with referral to the secondary structure of the SSU rRNA molecule (The European Ribosomal RNA Database, <http://www.psb.uget.be/rRNA/index.html>) after automatic multiple alignment using the Clustal X 1.8 computer algorithm. Phylogenetic analyses included maximum-parsimony (MP), gamma-weighted neighbor-joining (NJ), and maximum-likelihood (ML) analyses using PAUP* 4.0b10 (Swofford 2002). The MP analysis was done using the heuristic search option with random addition of sequences (100 replicates) and a branch-swapping algorithm (TBR: tree bisection-reconnection). All characters were weighted equally and gaps were treated as missing data. For reconstruction of weighted NJ and ML trees, a suitable evolutionary model fitting the aligned multiple sequences and parameters were calculated by use of ModelTest 3.04 (Posada & Crandall 1998). Phylogenetic trees were constructed with the selected model by the hierarchical LRT, i.e. the GTR model with the proportion of invariable sites (I) and the shape parameter for among-site rate variation (G) calculated from the data (GTR+I+G). The parameters were as follows: assumed nucleotide frequencies A=0.26880, C=0.1810, G=0.24700, T=0.30010; substitution rate matrix with A-C=1.0000, A-G=3.9605, A-T=1.0000, C-G=1.0000, C-T=8.0840, G-T=1.0000; proportion of sites assumed to be invariable=0.2526, and rates for variable sites assumed to follow a gamma distribution with shape parameter=0.5734. The weighted NJ analysis was calculated using Tamura-Nei distance. The ML analysis was performed using the heuristic search option with a branch-swapping algorithm (TBR). Bootstrap values (Felsenstein 1985) were estimated for NJ (1000 replicates), MP (100 replicates) and ML trees (100

replicates), respectively.

Results

Oblea acanthocysta Kawami, Iwataki et Matsuoka sp. nov. (Figs. 1 and 2)

Diagnosis:

Vegetativae cellulae subsphaericae sine spinis et cornibus sunt, 25.0–38.0 μm longae et 30.0–40.0 μm latae. Formula laminarum: Po, X, 3', 1a, 6'', 3c+t, 6s?, 5''', 2'''. Nucleus sphaericus, in dorso hypothecae locatus. Cystae globatae badiae sunt. Cysta superfacie laeve est, ferens numerosae distributis temere aciculares, cavae spinae. Archeopyla theropylica. Parasuturae formatae est ad laminaris finices inter 1a/2'' (completus), 1a/3'' (completus), 1a/4'' (completus), 1a/5'' (completus), 2'/2'' (incompletus) et 3'/5'' (incompletus).

A species of *Oblea* whose motile cell is subspherical without spine or horn, is 25.0–38.0 μm long and 30.0–40.0 μm wide. Plate formula Po, X, 3', 1a, 6'', 3c+t, 6s?, 5''', 2'''. Nucleus spherical, located dorsally within the hypotheca. Resting cyst is round and brown. Cyst surface is smooth, bearing many acicular, hollow spines randomly distributed on surface. Archeopyle theropylic. Parasutures are formed at the plate boundaries of 1a/2'' (complete), 1a/3'' (complete), 1a/4'' (complete), 1a/5'' (complete), 2'/2'' (incomplete) and 3'/5'' (incomplete).

Holotype: Fig. 1.

Type locality: Omura Bay, Nagasaki, Japan (32°55'N, 129°52'E)

Etymology: Latin *acantho-*=spiny (from Greek *akantha*=spine), and Latin *cysta*=cyst (from Greek *kystis*); named with reference to the characteristic appearance of the hypothecopore.

Synonym: *Diplopelta parva* (auct. non Abé 1941) Matsuoka 1988, p. 100–101, plate 1

Non *Dissodium parvum* Abé 1941

Description:

The motile cells are subspherical and achromatic. They are 25.0–38.0 μm long, and 30.0–40.0 μm wide. The epitheca and hypotheca are nearly equal in length. The cingulum has a width of 1/5–1/6 the cell length (ca. 5 μm) and is bordered by narrow lists without hollow ribs. The cingulum has a displacement approximately 1/3 of its own width. The sulcus widens toward the antapex, and is broadest at 1/3 from its anterior end, especially where the right-hand margin bends sharply to the left (Fig. 1A). The nucleus is spherical, located dorsally within the hypotheca. The large sulcal list developed at the left margin extends far beyond the posterior end of the body (Fig. 1B). The thecal plates are smooth with trichocyst pores scattered on the surface (Fig. 1C). The tabulation is Po, X, 3', 1a, 6'', 3c+t, 6s?, 5''', 2'''. The apical pore plate is surrounded by a low collar rising from the anterior part of the 2' and 3' plates, and connects with the canal plate (Fig. 1D). The first apical plate is rhomboid and the widest part is lower than the mid point of

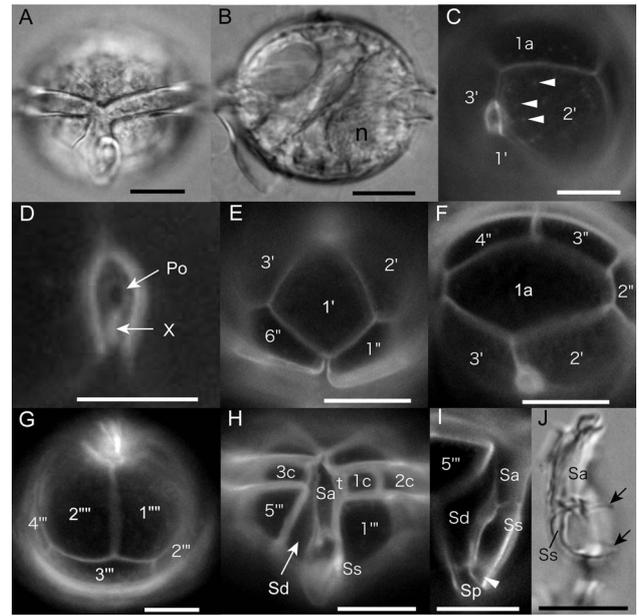


Fig. 1. Motile cells of *Oblea acanthocysta* sp. nov. A: Ventral view. B: Left-lateral view, showing the nucleus (n). C: Apical view, showing the trichocyst pores (arrowheads). D: Part of epitheca, showing the apical pore plate complex (Po, X). E: Rhombic first apical plate (1'). F: Anterior intercalary plate (1a). G: Antapical view. H: Sulcal plates, showing A-type of Sd. I: Sulcal plates, showing caspica-type of Sp and the small additional plate (arrowhead). J: Left-lateral view of sulcal plates, showing the additional plate with C-shape (arrows).

the plate (Fig. 1E). The single anterior intercalary plate is pentagonal due to contact with 2', 3', 2'', 3'', 4'' and 5'' plates, and located at the center of the dorsal surface (Fig. 1F). The cingulum is composed of three plates (1c–3c) and one transitional plate (t); two small cingular plates on the ventral side (1c and 3c) and the second cingular plate occupying most of the cingulum. The transitional plate is small and located between the 1c plate and the sulcus. The sulcus consists of four principal plates (Sa, Sd, Ss and Sp), and a few small additional plates (Figs. 1H, 1I, 1J). The right sulcal plate (Sd) directly contacts with the bottom of the 6'' plate (Fig. 1H). The posterior sulcal plate (Sp) is small and located in the most posterior portion of the sulcus (Fig. 1I). The additional plate with a C-shape extends into the cell from the dorsal side of the left sulcal plate (Fig. 1J). The small rectangular additional plate occupies the bottom margin of the flagellar pore (Fig. 1I). The right to upper marginal rim of the flagellar pore is narrow and has thickenings towards the inside of the cell (Fig. 1H, I). The exact number of additional plates is not determinable, because these plates are very small and do not show clear sutures between the main sulcal plates around the flagellar pore.

The postcingular plate series consists of five plates. The 2''' plate is larger than the 4''' plate, and therefore the 3''' plate is displaced towards the right (Fig. 1G). Two antapical plates are almost equal in size.

Dimensions:

Cell length 25.0–38.0 μm (\bar{x} =31.0 μm , n =23)

Cell width 30.0–40.0 μm (\bar{x} =34.8 μm , n =23).

The resting cysts are spherical and pale brown in color, with a central body diameter of 30.0–53.0 μm , (Figs. 2A–D). Cyst surface is smooth except for processes. Numerous spines, more than eighty in number, are randomly distributed over the surface. Spines are acicular and hollow, 1–8 μm in length, with slightly curved distal ends, and have bases that are circular in cross-section (Figs. 2A, 2B). Specimens bearing short spines were rarely found (Figs. 2C, 2D). The archeopyle is therapylic, formed by a zigzag principal suture, and encircles approximately 2/3 to 3/4 of the cyst. Traces of tabulation are evident in one cyst (Figs. 2C, 2D), revealing the archeopyle as following the boundaries of 1a/2" (complete), 1a/3" (complete), 1a/4" (complete), 1a/5" (complete), 2'/2" (incomplete) and 3'/5" (incomplete). These parasutures are always involved in archeopyle formation in the former 4 pairs and the sometimes involved in the latter 2 pairs.

Dimensions:

Diameter of central body 30.0–53.0 μm (\bar{x} =35.4), length of spines 1–8 μm (n =14).

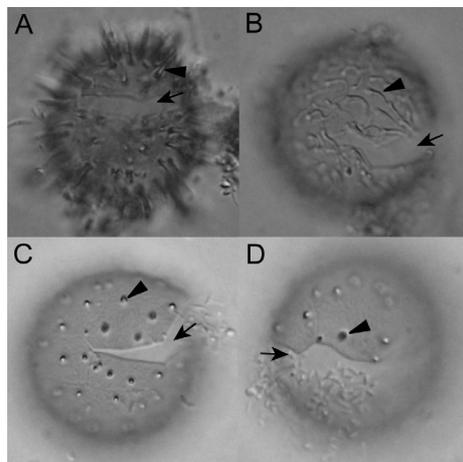


Fig. 2. Cysts of *Oblea acanthocysta* sp. nov. A: Dorsal view. B: Left-lateral view. C and D: Same cell with short spines. Showing the spines (arrowheads) and archeopyle (arrows).

Phylogenetic analysis

Three SSU rDNA sequences (ca. 1800 bp) of *O. acanthocysta* and two SSU rDNA sequences of *O. torta* were determined (Table 1). In the SSU rDNA sequences of three *O. acanthocysta* and two *O. torta* cells examined in this study, no intraspecific base substitutions were detected. Three sequences of *O. acanthocysta*, along with sequences from several other diplopsalid species retrieved from a database, were analyzed to assess their phylogenetic position among the dinoflagellates. Only the ML tree is shown in Fig. 5 because the MP, NJ and ML trees all gave similar results. Five species of the diplopsalid group, *O. acanthocysta*, *O. torta*, *Diplopsalis lebourae*, *Diplopsalopsis bomba* and *Gotoius excentricus*, formed a monophyletic clade within the dinoflagellates (bootstrap value ML/NJ/MP=100/100/100%). The diplopsalid clade is near the well-supported clade of the genus *Protoperidinium* (bootstrap value ML/NJ/MP=100/100/99%), but this relationship is not supported with high probability (bootstrap value ML/NJ/MP=69/56/<50%). The clade of the diplopsalid group is well supported with high probability. As expected from the morphological examination of motile cells, *O. acanthocysta* and *O. torta* are closely related (bootstrap value ML/NJ/MP=100/97/90%). Sequences of *O. acanthocysta* and *O. torta* differ in 154 base pair substitutions (8.75%).

Discussion

Taxonomy

The cell of *O. acanthocysta* is globular without horns and spines, has a cingulum encircling the equator of the cell, and has a large left sulcal list. These features are common to the diplopsalids. The plate morphology and tabulation are sometimes variable in germinated cells of other thecate dinoflagellates, however no unusual cells of *O. acanthocysta* were observed in the motile cells germinated from the cysts (n =15). *Oblea acanthocysta* morphologically resembles *Diplopsalis lebourae* (Nie) Balech in the epithecal tabulation and shape of the cell, although these two species are different in the number of antapical plates. While *O. acanthocysta* is also similar to *O. torta*, it differs notably in cell size and epithecal tabulation; *O. acanthocysta* (31.0 \times 34.8 μm on average) is smaller than *O. torta* (48.6 \times 57.4 μm on average) (Fig. 4); the anterior intercalary plate

Table 1. Sampling data and DDBJ/EMBL/GenBank accession numbers of *Oblea* analyzed in the present study.

Species	Isolate number	Specimen	Date	Site	Accession number
<i>Oblea acanthocysta</i>	1	Germinated cell	December 2003	Omura Bay	AB273721
<i>Oblea acanthocysta</i>	2	Germinated cell	December 2003	Omura Bay	AB273722
<i>Oblea acanthocysta</i>	3	Field sample	11 May 2004	Omura Bay	AB273723
<i>Oblea torta</i>	1	Field sample	20 July 2004	Omura Bay	AB273724
<i>Oblea torta</i>	2	Field sample	17 August 2004	Omura Bay	AB273725

of *O. acanthocysta* is less deflected than for *O. torta*; and the first apical plate of *O. acanthocysta* is wider than that of *O. torta* (Figs. 3A2, 3B2). The taxonomic significance of these morphological differences between *O. acanthocysta*

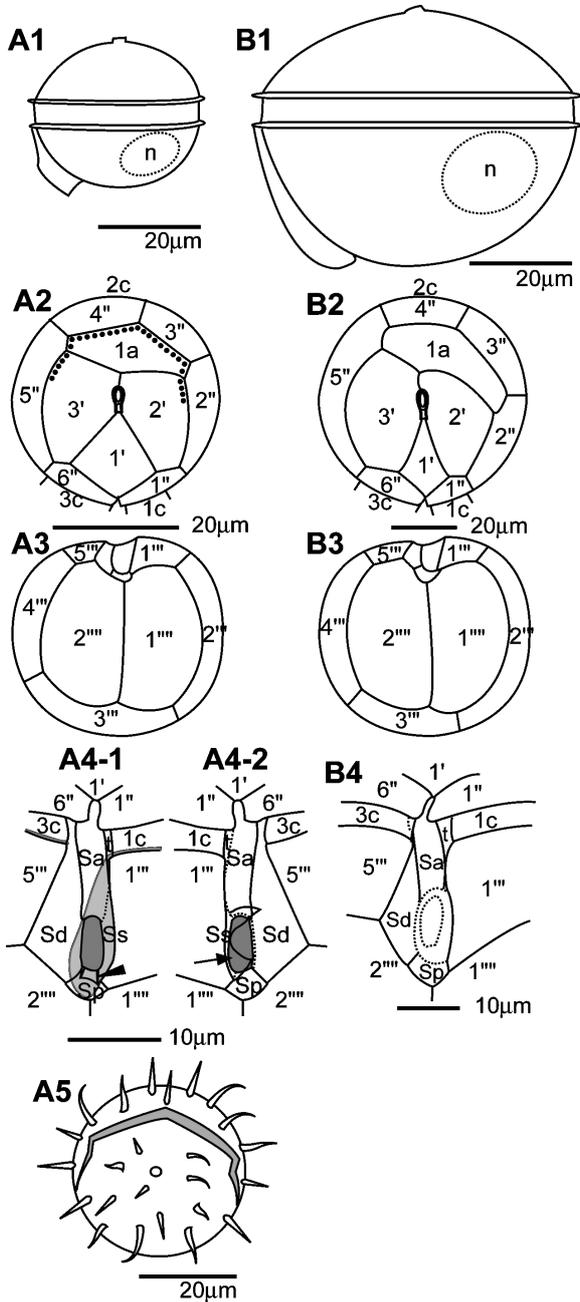


Fig. 3. Diagrams of thecal plate distribution and cyst. *Oblea acanthocysta* sp. nov. (A), *Oblea torta* (B). A1 and B1: Optical cross section from lateral side. A2 and B2: Epithelial tabulation with the position of archeopyle shown as the dotted line. A3 and B3: Hypothecal tabulation. A4-1, A4-2 and B4: Sulcal tabulation (A4-1 and B4 is ventral view, A4-2 is dorsal view), showing undeterminable suture with dotted lines. Showing the flagellar pore (dark gray), lists (light gray), the small additional plate (arrowhead), the additional plate with C-shape (arrow). A5: Cyst from polar view.

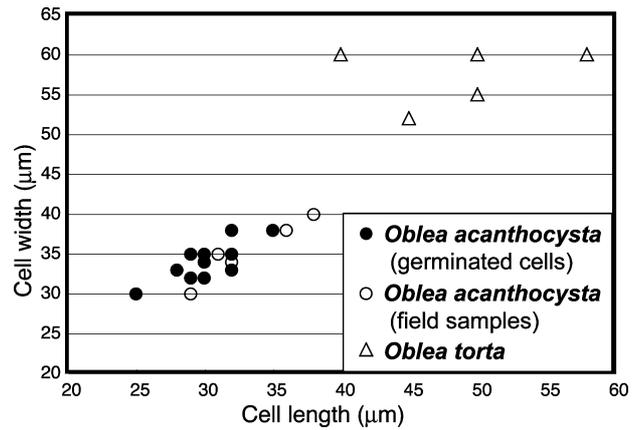


Fig. 4. Cell length vs. width for *Oblea acanthocysta* sp. nov. and *Oblea torta*.

The black circles show germinated cells of *Oblea acanthocysta* sp. nov., the white circles show plankton cells of *O. acanthocysta* and the white triangles show *Oblea torta*.

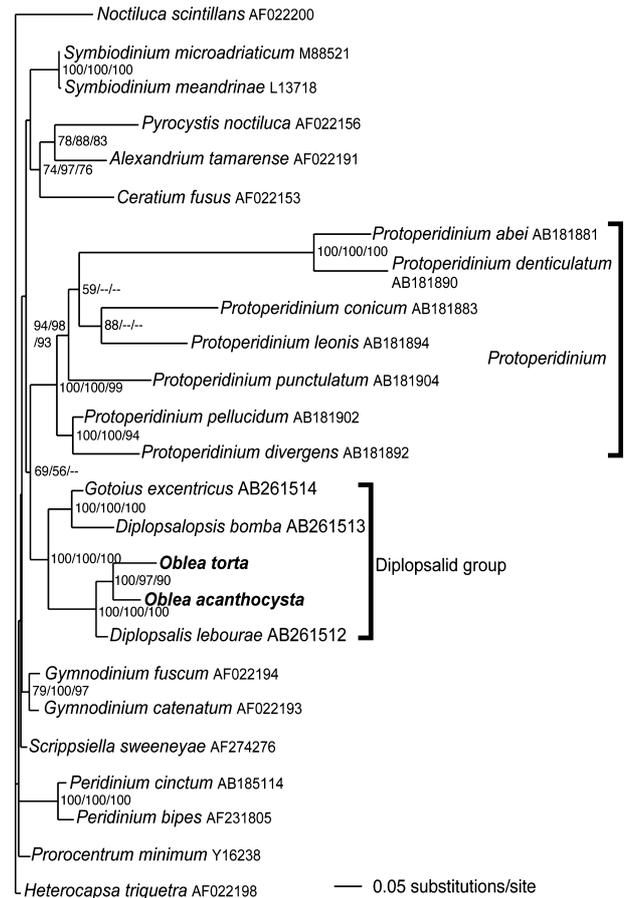


Fig. 5. Maximum-likelihood tree given by small subunit rDNA alignments of selected dinoflagellates. Species retrieved from GenBank shown with their accession number. Dinoflagellates analyzed in this study are indicated in bold letters. Numbers at nodes are bootstrap support percentages, which of more than 50% are shown maximum-likelihood (100 replicates); neighbor-joining (1000 replicates); maximum-parsimony (100 replicates), respectively.

and *O. torta* is also supported by the data of SSU rDNA sequences. In conclusion, we regard *O. acanthocysta* to be a new species.

The genus *Oblea* is defined as having a large anterior intercalary plate, an asymmetrical epitheca and hypotheca (Balech 1964) and the plate formula 3', 1a, 6'', 3c, 5''', 2'''''. The genus consists of three species: *Oblea baculifera*, *Oblea rotunda* and *Oblea torta* (Balech 1964). Before the establishment of this genus, *O. rotunda* had been described as *Peridiniopsis rotunda* by Lebour (1922). The large lenticular species, *O. torta*, was originally described as *Diplopsalis torta* by Abé (1941 and 1981), because it shares many morphological features with the genus *Diplopsalis* (e.g. cell shape, epithecal tabulation, and shape of the posterior sulcal plate) despite a different number of antapical plates. Abé (1941) considered the number of antapical plates in the diplopsalid group to be variable, and the shape of the posterior sulcal plate was regarded as being the most important taxonomic criterion. When *O. baculifera* was described by Balech (1964), a new genus *Oblea* was also proposed without the designation of a type species. Loeblich Jr. et Loeblich III (1966) later validated this genus by designating *O. baculifera* as the type species under the International Code of Botanical Nomenclature.

The four species now assigned to the genus *Oblea*, including *O. acanthocysta*, have a different arrangement in the first apical and the anterior intercalary plates. The cells of *O. baculifera* and *O. rotunda* are characterized by a transversally expanded anterior intercalary plate, which is deflected to the left of the sagittal line and additionally touches the first apical plate. Therefore, the first apical plate of these species is five sided (meta-type). On the contrary, *O. acanthocysta* and *O. torta* possess the four sided (ortho-type) first apical plate which does not touch an anterior intercalary plate, although leftward asymmetry of the anterior intercalary plate is maintained. The shape of the first apical plate is one of the significant characters for species identification in the genus *Oblea* as well as in the genus *Protoperidinium* as discussed by Yamaguchi & Horiguchi (2005). Therefore, we regard that the differences in the epithecal arrangement in these four species are intrageneric variations and we determine that *O. acanthocysta* is attributable to the genus *Oblea* based on its plate formula, although epi- and hypothecal tabulation is less asymmetric.

Round brown cysts with numerous spines on the surface were previously reported from the sediments of Omura Bay; these cysts being identified as belonging to *Diplopelta parva* (Abé) Matsuoka by association with germinated cells (Matsuoka 1988). The cyst type described by Matsuoka (1988) was spherical and pale brown, and covered with many spines. It had a peculiar archeopyle represented by a zigzag split. These features are identical to those of *O. acanthocysta*. It is evident from his description and drawings (Matsuoka 1988, fig. 2) that germinated cells from the cysts possess two anterior intercalary plates. The first anterior intercalary plate is very small and rectangular, and the

second anterior intercalary plate is broad and pentagonal. Although this feature is not clear in plate I of Matsuoka (1988), other morphological features including the size of cells are concordant with *O. acanthocysta*. Abé (1941) first described *Dissodium parvum* from Shimoda Bay in Japan. According to Abé's description and illustrations (Abé 1941, figs. 14–19), the motile cell of *D. parvum* is more globular compared to the cells assigned by Matsuoka (1988) to *Diplopelta parva*, and the first anterior intercalary plate of *D. parvum* is larger than that of Matsuoka's specimens. The present study reports that for Omura Bay, all the examined motile cells germinated from spherical light brown cysts covered with many aculeate and hollow spines, and with a peculiar zigzag archeopyle are of *O. acanthocysta* with an anterior intercalary plate. These lines of evidence strongly suggest that the specimens assigned by Matsuoka (1988) to *Diplopelta parva* in fact belong to the new species, *O. acanthocysta*.

Round, brown, spiny cysts possessing a slit-like archeopyle have been reported from some autotrophic gymnodinioids (e.g. *Pheopolykrikos hartmannii* (Zimmermann) Matsuoka et Fukuyo) and heterotrophic protoperidinioids (e.g. *Diplopelta symmetrica* Pavillard, *Protoperidinium minutum* (Kofoid) Loeblich III, and *Protoperidinium monospinum* (Paulsen) Zonneveld et Dale). The cyst of *D. symmetrica* resembles that of *O. acanthocysta* in having a zigzag-slit archeopyle, but differs from the latter in process morphology. The processes of *D. symmetrica* cysts described by Dale et al. (1993) are very slender and hair-like. However, the processes of *O. acanthocysta* cysts are thicker than those of *D. symmetrica*. Two different cyst types for *P. minutum* have been reported, one by Wall & Dale (1968) and the other by Fukuyo et al. (1977). The cyst of *P. minutum* sensu Wall & Dale has processes with flat-topped distal ends, whereas the cyst of *P. minutum* sensu Fukuyo et al. (1977) is covered by hair-like spines densely distributed on the surface. Cysts of *O. acanthocysta* can be distinguished from both cyst types by their process morphology. The cysts of *P. monospinum* also resemble those of *O. acanthocysta* in having thick spines and a zigzag-slit archeopyle. However, *P. monospinum* cysts have two spine morphologies on the same specimen; one is larger, hollow and capitate, bifurcated or double-branched at the distal ends, and the other is solid, acuminate, and scattered on the cyst surface (Zonneveld & Dale 1994). These characteristics have never been recognized in *O. acanthocysta*. The fossil genus *Echinidinium* is a spheroidal and brownish-pigmented cyst with randomly dispersed spines (Zonneveld 1997; Head et al. 2001). The cyst of *O. acanthocysta* resembles *Echinidinium granulatum* Zonneveld ex Head. *E. granulatum* with a granulate cyst surface, whereas the cyst surface of *O. acanthocysta* is smooth.

Molecular phylogeny

The SSU rDNA sequences of the germinated cells were

determined only after these cells had been observed carefully to ascertain their plate formula. The plankton cells were obtained from the location where cysts were collected in Omura Bay, West Japan. The results of SSU rDNA analysis revealed no base substitution in the three sequences of *O. acanthocysta*, including two germinated cells and a planktonic cell. These results, based on molecular and morphological analysis, show that the morphology of germinated cells of *O. acanthocysta* was not irregular but maintained the original plate formula for this species. The SSU rDNA sequences of *O. acanthocysta* were compared with those of *O. torta*, because *O. torta* morphologically resembles *O. acanthocysta* except for cell size. The sequence of *O. acanthocysta* was found to differ in 8.75% of the SSU rDNA (ca. 1800 base pair) of *O. torta*. Therefore, *O. acanthocysta* is clearly a distinct and independent species from *O. torta* based on both morphology and SSU rDNA data. This implies that cell size is an important taxonomic character in this case.

The phylogenetic position of *O. acanthocysta* is located in the diplopsalid group which forms a single clade among the dinoflagellates. *Oblea acanthocysta* is closely related to *O. torta* based on the present phylogenetic trees, and these results are supported by morphological features as discussed above. These diplopsalids and the genus *Protoperidinium* are sister groups, although this is weakly supported by bootstrap probability (Fig. 5). The present SSU rDNA sequence data are not sufficient to clarify the phylogenetic relationship of the genus *Oblea*, because genetic information on the two remaining species, *O. baculifera* and *O. rotunda*, is unavailable at present. Further investigation is therefore required to establish whether the genus *Oblea* represents a single clade or not.

Based on the round brown spiny morphology of its cyst, *O. acanthocysta* is assumed to be related to the genus *Protoperidinium*, because some species of the genus *Protoperidinium* have similar spiny cysts—such as *P. minutum* and *P. monospinum*. However, *O. acanthocysta* in fact belongs to the diplopsalid group and forms a monophyletic group with *O. torta* based on our molecular analysis. Evidently, the presence of spines on the cyst surface is newly added to the features documented for the diplopsalids. In addition, according to Matsuoka et al. (2006), the theropylic archeopyle exists in the cysts of both the diplopsalids and the genus *Protoperidinium*, and that the group with round brown cysts with a theropylic archeopyle is polyphyletic. SSU rDNA sequence data for species with round brown spiny cysts with a theropylic archeopyle is necessary to understand the relationship between cyst morphology and phylogeny in the diplopsalids and the genus *Protoperidinium*.

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